Rate of Lesion Expansion in Leaves as a Parameter of Resistance to *Xanthomonas campestris* pv. *oryzae* in Rice

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ABSTRACT

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Lesions on five rice (*Oryza sativa*) cultivars clip inoculated with three strains of *Xanthomonas campestris* pv. *oryzae* at two plant ages appeared 4–5 days after inoculation and continued expanding until leaf senescence. A quadratic model accounted for 99% of the variation in the measured values. Mean lesion length and mean daily rate of lesion expansion were highly correlated. Younger plants and susceptible cultivars had higher rates of increase than older plants and moderately susceptible cultivars, respectively. The rate of expansion decreased somewhat with time for strains PXO71 and PXO99 and increased or remained the same for strain PXO86. There were no major differences among cultivars for the change in the rate of lesion expansion over time. Because of this, there were no major changes in the relative levels of resistance of the six cultivars over time. Therefore, multiple scorings are not expected to give extra information about this factor. The observed differences between strains for the change in the rate of lesion development over time may indicate a characteristic of strains of *X. c. oryzae* worth further study.

Response in rice (*Oryza sativa* L.) to infection by *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye, the causal organism of bacterial blight, varies from highly resistant to highly susceptible depending on host and pathogen genotype. A hypersensitive response is not known in this host-pathogen system (4,11), and a range of lesion sizes is usually found. Resistance is usually assessed 14 or 21 days after plants are inoculated with a concentrated bacterial suspension (7,8). Cultivars are considered as resistant, moderately resistant, or moderately susceptible to each specific strain of the pathogen based on a comparison with a susceptible cultivar (3,8). Although this division is arbitrary and lacks clear-cut distinctions, high levels of resistance have been shown to be inherited by race-specific major genes called *Xa* genes (2,7). In contrast, in some cultivars, moderate resistance has been associated with an incomplete reaction of known major genes to certain strains (16) and in other cases to be quantitatively inherited (14).

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After clip inoculation (5), bacteria of virulent strains move via the vascular system downward toward the leaf base and lesions appear 4–5 days after inoculation. Bacterial blight lesions resulting from clip inoculation are distinct lesions, often as wide as the leaf itself, and the length of lesions can be easily and accurately measured. If repeated measurements of lesion size are made on a single leaf, the daily rate of lesion expansion can be calculated. Lesions may expand at a uniform rate throughout the infectious period, and cultivars can be compared for their mean rates of lesion expansion. However, lesion expansion could also change in rate during the infectious period, as for example, if lesions slowed expansion later in the infectious period. If cultivars also differ in changes in the rate of expansion of lesions, assessment of symptom development at different days after inoculation would lead to different conclusions regarding the relative resistance of various cultivars.

Such interactions were sought by closely following the progress of lesions on various cultivars at two plant ages inoculated with three strains of *X. c. oryzae*. Such a comparison can offer insight into the way in which lesions develop and possibly show differences in the host-pathogen interactions between seemingly similar cultivars.

MATERIALS AND METHODS

The rate of lesion expansion was measured on five cultivars—Cisadane, IR40, IR28, BR51-282-8, and TN1. The first four cultivars possess the *Xa-4* gene for resistance and are also moderately resistant to strains virulent to plants with the *Xa-4* gene, whereas TN1 does not have the *Xa-4* gene and is susceptible to all strains used. Seeds were pregerminated on moist filter paper, and when the first leaf was 1–2 cm long, seeds were transferred to 33 × 26 × 11 cm plastic trays filled with lowland soil. Each tray contained six plants of each of the five cultivars. Within the tray, cultivars were planted in rows in a randomized block design. Trays were randomized on greenhouse or phytotron tables. Nitrogen fertilizer (ammonium sulfate 21-0-0) was applied twice during growth.

Bacterial strains were stored in skim milk suspensions at −20 C until needed. Inoculum was prepared from 72-hr cultures on peptone-sucrose agar (PSA) slants grown at 30 C. The inoculum preparations were always one transfer removed from storage. Each slant culture was suspended in 20 ml of distilled water per slant and adjusted to an OD of 1 (590 nm), giving a suspension of about 1×10^9 cfu/ml. The uppermost fully extended leaf of the main tiller of each plant was inoculated by the method of Kauffman et al (5), in which a scissor is dipped in freshly prepared inoculum and 1–2 cm of the leaf tip is clipped off.

Leaves were numbered 4 days after inoculation, and the lesion length on each leaf was recorded on alternate days, beginning on day 5 and continuing until the leaf began to senesce. Six leaves were measured per cultivar per strain per replicate.

In the first experiment (April–June 1986), the expansion of lesions caused by three strains in two plant ages was tested under greenhouse conditions. Seeds were sown on day 0 and day 20, and plants were inoculated when 60 and 40 days old, respectively. Strains of race 2 (PXO86), race 4 (PXO71), and race 6 (PXO99) were used. Races 2 and 6 are virulent on plants with the *Xa-4* gene. Race 4 is incompletely virulent on plants with this gene (6,12) and cultivars with the *Xa-4* gene are moderately resistant to strain PXO71. Three trays (replicates) of each strain × growth stage combination were measured.

In a second experiment (April–June 1987), lesion expansion after inoculation with race 2 strain PXO86 was compared with that after inoculation with race 6 strain PXO99 in plants grown in the

phytotron (29/21 C, 70–90% RH, natural light). Plants were inoculated 55 days after seeding. Three trays (replicates) were tested per strain.

The relationship between lesion length and time was fitted by both a linear and a quadratic model for each plot, using an iterative least squares method to minimize the sum of squares of the residuals of the fitted quadratic curves (13). The predicted values for each day were compared with the observed values and the correlation coefficient between the two sets of values was calculated per plot.

RESULTS

At least 95% of the variation in the measured values was predicted by a linear model, whereas more than 99% was predicted by a quadratic model in all cases. The quadratic model was chosen to compare the treatments. This model allows for gradual change in the daily rate of lesion expansion over time.

Lesions extended significantly faster on younger plants than on older plants (Table 1). Lesions on younger plants increased at a mean rate of 1.55 cm per day between days 4 and 16 after inoculation. Lesions on older plants increased at a mean rate of 0.88 cm per day between days 4 and 24 after inoculation.

Lesions enlarged more rapidly on the susceptible cultivar TN1 than on those with moderate resistance. The difference between plants of the two age groups for the difference between TN1 and the other cultivars for rate of lesion expansion led to a significant age × cultivar interaction ($0.01 < P < 0.05$). The rate of lesion expansion of the most resistant cultivar was 1.25 cm day^{-1} (51%) slower than the most susceptible cultivar among young plants, and 1 cm day^{-1} slower (38%) than the most susceptible cultivar among older plants (Table 1). The range betwen the susceptible and the moderately resistant cultivars was also greater among older plants than among younger plants (Table 2). Mean lesion length of the most resistant cultivar was 11.5 cm (55%) shorter than the susceptible cultivar among younger plants and 18.3 cm (40%) shorter than TN1 among older plants.

On the younger plants, all three strains caused similar rates of lesion expansion on TN1 (Table 1) and similar lesion lengths 14 days after inoculation (Table 2), indicating equal aggressiveness. On older plants, PXO99 was less aggressive than PXO86 and PXO71. This led to a significant age × strain interaction for the mean rate of lesion expansion ($P < 0.01$). The lower mean rate of lesions on plants inoculated with strain PXO71, as compared with those inoculated with strains PXO99 and PXO86, resulted from the incomplete virulence of this strain on plants with the *Xa-4* gene for resistance. This was reflected in a significant cultivar × strain interaction for the difference between the susceptible cultivar TN1 and the other four cultivars (Table 1).

The daily rate of lesion expansion was highly correlated with the mean lesion length, averaged over all days ($r = 0.93$** for both younger and older plants) and for the mean lesion length on the representative days 14 or 22 after inoculation ($r = 0.96$ and 0.98 for younger and older plants, respectively, $P < 0.01$).

Coefficients for the quadratic term of the fit line varied from +0.052 to −0.077 over all treatments. No significant differences between cultivars were found for this value, indicating equal change in the rate of lesion expansion over time for all cultivars. However, significant differences were found between all three strains at both plant ages, as well as a significant interaction for age × strain ($P < 0.01$). On younger plants, PXO99 and PXO71 slowed the rate of expansion, having mean coefficients of −0.067 and −0.032, respectively, whereas PXO86 increased its rate over time, by +0.015 cm day^{-2}. This slowing of lesion expansion was not attributable to a lack of fresh leaf tissue. Less than 10% of lesions on TN1, and even fewer on the moderately resistant cultivars, had reached the leaf sheath by the time measurements were stopped.

On plants inoculated 60 days after sowing, lesions of all strains slowed expansion somewhat when lesion expansion was followed until leaf senescence. However, PXO99 and PXO71 had similar coefficients, −0.033 and −0.019, respectively, whereas PXO86 had a significantly smaller coefficient, −0.007 cm day^{-2}. This difference between PXO99 and PXO71, on the one hand, and PXO86 on the other, was even greater when lesion expansion was fit for only those days also measured on 40-day-old plants, days 4–16. In this case, PXO99 and PXO71 had coefficients of −0.063 and −0.023, respectively, whereas PXO86 had a coefficient of +0.015 cm day^{-2}. Lesion expansion slowed on all cultivars when inoculated with PXO99 (Fig. 1A). Expansion of lesions of PXO86 was constant on the moderately resistant cultivars and slowed slightly in TN1 after about 20 days after inoculation (Fig. 1B).

When the experiment was repeated in the phytotron under cooler conditions, lesions expanded at an average of 1.65

Table 1. Daily rate of lesion extension (cm day^{-1}) measured on five rice cultivars and two plant ages after clip inoculation[x] of leaves with three strains of *Xanthomonas campestris* pv. *oryzae*

	Days after sowing[y]							
	40				60			
Cultivar	PXO99	PXO86	PXO71	Mean[z]	PXO99	PXO86	PXO71	Mean[z]
IR28	1.35	1.63	0.98	1.32 b	0.56	0.90	0.44	0.61 c
IR40	1.53	1.59	0.88	1.33 b	0.62	0.91	0.39	0.63 c
Cisadane	1.39	1.85	0.66	1.30 b	0.81	0.83	0.51	0.72 bc
BR51-282-8	1.61	1.51	0.73	1.28 b	0.81	1.20	0.48	0.85 b
TN1	2.46	2.54	2.60	2.53 a	1.20	1.86	1.78	1.61 a
Mean[z]	1.67 b	1.82 a	1.17 c	1.55	0.92 b	1.25 a	0.85 b	0.89

[x] Inoculum concentration 1×10^9 cfu/ml.
[y] Rate determined for days 4–16 or days 4–24 after inoculation, respectively, for 40- and 60-day-old plants. Values are means of three replications, six plants per replication mean.
[z] Standard error of the mean for strains is 0.062 and 0.040 for 40- and 60-day-old plants, respectively. Standard error of the mean for cultivars is 0.084 and 0.057 for 40- and 60-day-old plants, respectively. Values in a row (strain) or columns (cultivars) followed by different letters differ as determined by Bonferroni's test for inequalities ($P = 0.05$).

Table 2. Lesion lengths (cm) measured on five rice cultivars of two plant ages after clip inoculation[x] of leaves with three strains of *Xanthomonas campestris* pv. *oryzae*

	Days after sowing[y]							
	40				60			
Cultivar	PXO99	PXO86	PXO71	Mean[z]	PXO99	PXO86	PXO71	Mean[z]
IR28	15.9	15.9	11.1	14.3 b	11.5	16.2	9.0	12.3 c
IR40	17.3	15.9	9.4	14.2 b	13.2	15.5	7.3	12.0 c
Cisadane	16.4	20.0	7.7	14.7 b	16.9	15.0	9.9	13.9 c
BR51-282-8	18.8	16.2	8.6	14.6 b	16.8	21.2	10.0	16.0 b
TN1	27.9	23.5	25.7	25.7 a	25.2	32.6	33.0	30.3 a
Mean[z]	18.8 a	17.8 a	12.6 b	16.4	18.8 b	22.0 a	16.0 b	18.9

[x] Inoculum concentration 1×10^9 cfu/ml.
[y] Lesion lengths recorded 14 or 22 days after inoculation for 40 and 60 days after sowing, respectively. Values are means of three replications, six plants per replication mean.
[z] Standard error of the mean for strains is 0.673 and 0.868, for cultivars is 0.850 and 1.002, for 40 and 60 days after sowing, respectively. Values in a row (strain) or column (cultivar) followed by different letters differ significantly ($P = 0.05$).

cm per day, as compared with 0.88 cm per day for the older group of plants in the previous experiment. A linear model described 94–99% of the observed variation in the dependent variable, whereas a quadratic model described 99% or more in all but four of the 36 plots. Mean lesion length over days 5–20 after inoculation was highly correlated ($r = 0.93$, $P < 0.01$) with the mean daily rate of lesion expansion.

As in the previous experiment, significant differences were found between cultivars for the mean lesion length and for the daily rate of expansion but not for changes in this rate. Significant differences between strains were not found for the daily rate of lesion expansion. A significant difference was again found between strains for the change in daily rate of lesion expansion. Lesions on plants inoculated with strain PXO99 slowed growth over the measurement period whereas lesions on plants inoculated with PXO86 increased the rate of expansion, as was found in the previous experiment.

DISCUSSION

A quadratic equation better fit the observed lesion expansion than a simple linear equation. Baw (1) also found a quadratic model appropriate to describe lesion expansion in 40- to 50-day-old TN1 plants but that a linear relationship sufficiently described the lesion expansion in booting stage plants. Large differences were found between cultivars for the coefficient of the linear term of the fit equation, but there were few differences between cultivars for the coefficient of the quadratic term. Therefore, although the lesions expanded on the different cultivars at different rates, few significant interactions over time are to be expected. Comparisons between cultivars varying from moderately resistant to highly susceptible can therefore be made any time after symptom appearance, although such a comparison will probably be most meaningful in the period of rapid, more or less linear growth, before leaf senescence affects measurements. No extra information is to be expected by measuring lesions on two or more separate occasions and using a parameter based on the lesion growth in this interval. The greater rate of increase seen in the phytotron experiment, as compared with the greenhouse experiment, could have been attributable to the cooler conditions in the phytotron or to the slightly younger age and different nutritional status of the plants.

The incompletely virulent race 4 strain PXO71 was distinguishable from strains PXO86 and PXO99, both virulent on plants with the *Xa-4* gene for resistance, by the lower mean rate of lesion expansion. The latter two strains were distinguished from each other by the difference in the change in the rate of expansion over time. Strain PXO99 began with a rapid rate that slowed over time, whereas strain PXO86 kept expanding at a constant rate, or even speeded up, depending on the plant age and the number of days lesion expansion was followed. This characteristic difference between the two strains was found to be repeated in both experiments and should be further studied. A consequence of this difference in expansion rate is that lesions of PXO99 covered more of the leaf faster than lesions of PXO86, especially in young plants. Because leaves of young plants tend to be shorter, the damage done by PXO99 to these leaves is proportionately more extensive, although the final lesion length of both strains was often equal on cultivars.

Baw (1) and Yoshimura et al (16) used a logistic and a Gompertz model to describe the expansion of bacterial blight lesions. Lesions were expressed as a percentage of the leaf area or of the leaf length, respectively. Logistic and Gompertz models are two sigmoid curves often used to describe the development of a disease in a field. This is assumed to first increase its rate of spread as inoculum builds up and then later to slow development as healthy plant tissue becomes scarce. These two conditions cannot be transferred to the monocyclic infection process studied here. There is no indication that lesions increase logarithmically in length shortly after symptoms appear. While lesions on older plants slowed growth slightly, lesions on young plants infected with strain PXO86 appeared to increase in growth rate.

As stated earlier, there is some indication that lesions, especially on older plants, slow but do not stop growth. The lesion is not usually at the leaf base when the rate of expansion decreases. In the case where the leaf is fully affected, a situation found in young leaves of highly susceptible cultivars, a lesion can extend further down the leaf sheath, although this is not usually measured. The tendency to slow lesion expansion identified here was already noticeable about 10 days after inoculation (Fig. 1A). Morinaka et al (9) found no difference in suscep-

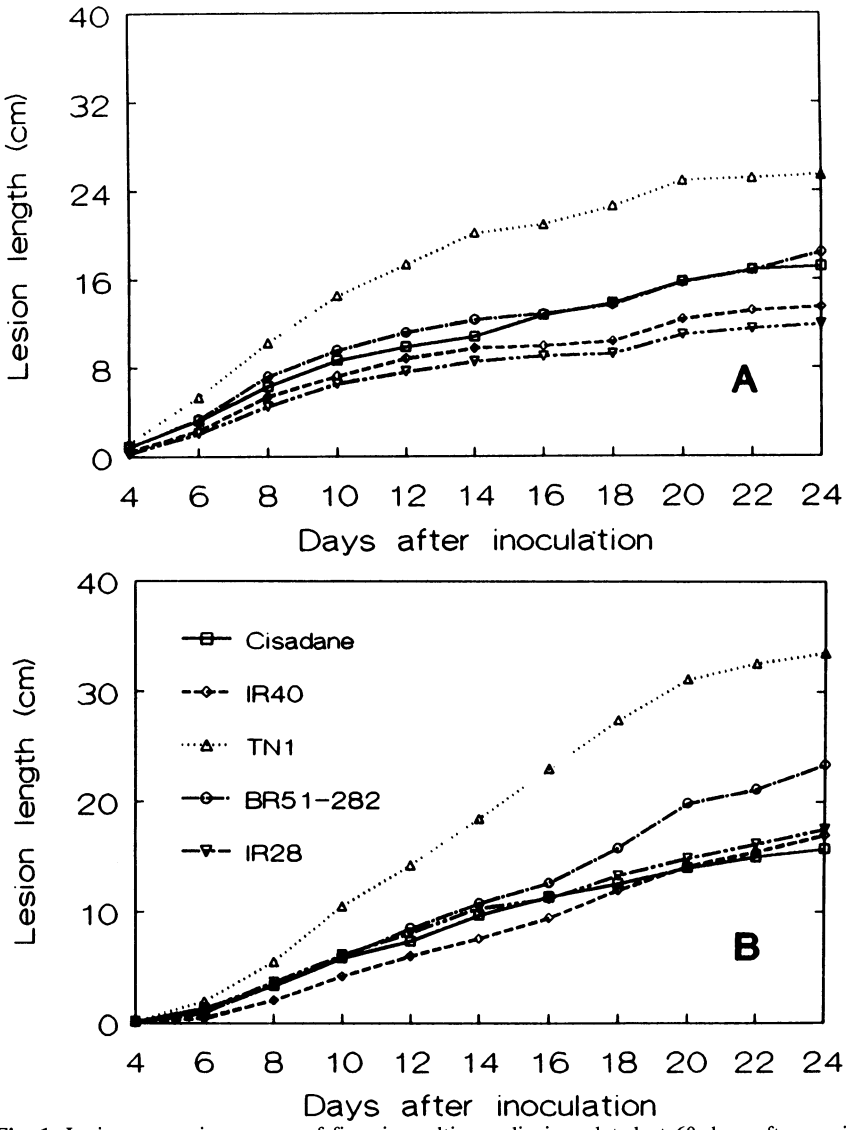

Fig. 1. Lesion expansion curves of five rice cultivars clip inoculated at 60 days after sowing with **(A)** race 6 strain PXO99 and **(B)** race 2 strain PXO86 of *Xanthomonas campestris* pv. *oryzae*.

tibility between the tip and the midleaf section of leaves after clip inoculation. We found similar results with TN1 plants (M. Koch, *unpublished*). However, Yamanaka et al (15) found that the leaf base was slightly less susceptible than the upper section based on the number of successful prick inoculations. Because lesions are found to slow shortly before leaf senescence, it is possible that the changes in the internal environment related to senescence adversely affect the bacterial growth and multiplication. Some strains may be more sensitive to these changes than others.

Lesions on more resistant cultivars did not begin to slow growth earlier than lesions on highly susceptible cultivars. This is in agreement with Baw (1), who found that lesions did not stop expansion for up to 3 wk, even lesions of a highly resistant reaction on plants with the *Xa-4* gene. Ogawa et al (10) also report that on plants with the *Xa-4* gene for resistance, lesions continued extending for up to 22 days after inoculation without a noticeable change in rate. Yoshimura et al (16), however, reported that lesions of avirulent race 2 strains on cultivars with the *xa-5* gene stopped growth by 18 days after inoculation. Ogawa et al (10) reported that lesions of strain-cultivar combinations involving the *Xa-3* gene for resistance slowed or even stopped expansion 18–20 days after inoculation. In some cases, browning was seen to develop in these lesions. They used these two characteristics, which they attributed to the presence or absence of the *Xa-3* gene for resistance, to separate segregating plants into resistant and susceptible groups. Lesion length was not used as a selection characteristic in these cases, and some plants selected as resistant had longer lesions than plants selected as susceptible. Conclusions concerning slowing of lesion expansion may be related to the specific resistance and virulence genes being studied.

For comparison of genotypes varying in resistance from moderately resistant to highly susceptible, a single measurement of lesion length, well timed to fall in the period of rapid, more or less linear growth, will adequately distinguish differences in levels of resistance. No extra information is gained by using a parameter based on lesion expansion. This period will be longer on plants older than 55 or 60 days than on younger plants, and comparisons between cultivars may give a larger range from susceptible to moderately resistant. Strains of *X. c. oryzae* appear to differ for their ability to continue colonization of rice leaves after about 10 days after clip inoculation.

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