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A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene

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Summary

• Continuous planting of crops containing single disease resistance (*R*) genes imposes a strong selection for virulence in pathogen populations, often rendering the *R* gene ineffective. Increasing environmental temperatures may complicate *R*-gene-mediated disease control because high temperatures often promote disease development and reduce *R* gene effectiveness. Here, performance of one rice bacterial blight disease *R* gene was assessed in field and growth chamber studies to determine the influence of temperature on *R* gene effectiveness and durability.

• Disease severity and virulence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) populations were monitored in field plots planted to rice with and without the bacterial blight *R* gene *Xa7* over 11 yr. The performance of *Xa7* was determined in high- and low-temperature regimes in growth chambers.

• Rice with *Xa7* exhibited less disease than lines without *Xa7* over 11 yr, even though virulence of *Xoo* field populations increased. *Xa7* restricted disease more effectively at high than at low temperatures. Other *R* genes were less effective at high temperatures.

• We propose that *Xa7* restricts disease and *Xoo* population size more efficiently in high temperature cropping seasons compared with cool seasons creating fluctuating selection, thereby positively impacting durability of *Xa7*.

Introduction

Climate change presents considerable challenges for plant disease management (Coakley *et al.*, 1999; Garrett *et al.*, 2006). While the specific effects of climate change on plant diseases will depend on the plant–pathogen system in question, the impact of some changes, such as temperature and precipitation, can be predicted for several plant species from empirical studies (reviewed by Coakley *et al.*, 1999). For example, increased temperature results in higher susceptibility of wheat and oats to rust diseases, while forage species become more resistant to certain fungi (Coakley *et al.*, 1999). The impact of increased temperatures associated with climate change on plant disease are speculated to be through both the host plant and the pathogen. These proposed mechanisms for effects, as reviewed by Coakley *et al.* (1999) and Garrett *et al.* (2006), include alterations of plant architecture to create more suitable microclimates for pathogen colonization and disease development; changes in the function and effectiveness (ability to restrict pathogen multiplication and colonization) of plant disease resistance; and increased abiotic stresses on plants, which would affect susceptibility to plant pathogens.

Higher temperatures are predicted to accelerate the breakdown of plant disease resistance through higher disease pressure and/or altered resistance gene efficacy in many host–pathogen systems. Higher disease pressure is coincident with elevated pathogen populations, and this, in turn, increases the probability that a mutation will allow the pathogen population to overcome a single resistance (*R*) gene, the primary sources of resistance in many crop breeding programs. There are examples of *R* genes that are either more or less effective at high temperatures (Dyck & Johnson, 1983; Browder & Eversmeyer, 1986; Kolmer, 1996; Wang *et al.*, 2001; Eizenberg *et al.*, 2003; Uauy *et al.*, 2005). In the *Puccinia recondite*–wheat pathosystem, some, but not all, wheat resistance genes are more effective at higher temperatures, and this temperature effect is highly

isolate-dependent (Dyck & Johnson, 1983; Browder & Eversmeyer, 1986; Kolmer, 1996) The wheat stripe rust *R* gene *Yr36* confers resistance to a broad spectrum of races of the pathogen *P. striiformis* f. sp. *tritici* at high temperatures (25–35°C), while at low temperatures (15°C), wheat with *Yr36* is susceptible to the fungus (Uauy *et al.*, 2005). The recent cloning of *Yr36* may facilitate understanding of how the gene function is influenced by temperature (Fu *et al.*, 2009).

Deciphering the interconnectivity of temperature, pathogen population evolution and *R* gene effectiveness will require an understanding of how *R* genes function and how that function is affected by high temperature. In general, *R* genes directly or indirectly interact with the products of pathogen effector genes (also called avirulence or *avr* genes), and this interaction signals a disease resistance cascade (for review, see Chisholm *et al.*, 2006). Effector proteins function as critical components of a pathogen's virulence, and thus plants have evolved to recognize the effectors as a trigger for defense. This suggests that a mutation in an effector gene can result in a loss of *R* gene recognition and increased host range, but their importance to virulence means that mutation can also result in reduced pathogenic fitness (Leonard & Czochor, 1980; Leach *et al.*, 2001; Burdon & Thrall, 2003). Pathogenic fitness, in this context, refers to aggressiveness to a susceptible host, and ability to persist in the population. Thus, the more important an effector gene is to a pathogen's fitness, the more difficult it will be for the pathogen to adapt to overcome the corresponding plant *R* gene (Leach *et al.*, 2001). While there are field and laboratory studies that support the prediction that *R* gene durability correlates with the importance of pathogen effectors to a pathogen's fitness (reviewed in Leach *et al.*, 2001), there are no studies that integrate the influence of climate, particularly high temperatures, with pathogen population evolution and *R* gene function or durability.

Understanding the interaction of temperature and *R* gene function and durability is complicated in systems with multiple crops per year, such as rice, where temperatures can vary between being conducive and nonconducive for disease by season. Standard definitions of resistance gene durability include the continuing effectiveness of the gene when environmental conditions are conducive to disease for long periods of time (Johnson, 1984), but seasonal breaks in disease conduciveness can make the useful life of resistance genes longer in practice. Changing environmental conditions can impose a fluctuating selection pressure on pathogen populations (Lynch, 1987; Hairston & Dillon, 1990). Indeed, seasonality may produce regular fluctuations in environmental conditions, or oscillations, allowing for a reversal in the direction of selection (Gibbs & Grant, 1987; Grant & Grant, 2002). While not yet investigated, the influence of such fluctuating selection on *R* gene durability will likely vary with *R* gene, and will depend on the speed of pathogen evolution to races that can overcome resistance, as influenced by changes in infection cycles and the availability of suitable microclimates for disease development.

We have been studying the factors influencing the durability of plant *R* genes in rice to the bacterial blight (BB) pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). In a previous field study, the cost of pathogenic fitness for *Xoo* was shown to be an important factor contributing to the durability of one rice BB *R* gene, *Xa7* (Vera Cruz *et al.*, 2000). The effectiveness of the *Xa7* gene was correlated with mutations in *Xoo* that resulted in reduced pathogenic fitness, as measured by reduced aggressiveness on susceptible rice cultivars (Bai *et al.*, 2000; Vera Cruz *et al.*, 2000). These mutations occurred specifically in the pathogen effector gene *avrXa7*, which is important to pathogenic fitness (Bai *et al.*, 2000; Ponciano *et al.*, 2004). By contrast, mutation of the effector gene *avrXa10* did not affect pathogenic fitness, and the corresponding *R* gene *Xa10* was rapidly overcome in the field (Bai *et al.*, 2000; Vera Cruz *et al.*, 2000). Thus, selection on the pathogen population imposed by the *Xa7* gene, but not the *Xa10* gene, resulted in reduced pathogenic fitness (Bai *et al.*, 2000; Ponciano *et al.*, 2004), and the cost of pathogenic fitness to the pathogen was one factor in effectiveness of the *Xa7* gene (Vera Cruz *et al.*, 2000).

Although the costs of pathogenic fitness affected pathogen population structure when under *R*-gene-imposed selection, other factors are likely to also influence an *R* gene's durability. In the BB–rice system, temperature is a likely influence because there are typically two cropping seasons in tropical regions, a cool season and a hot season (Peng *et al.*, 2004). At the International Rice Research Institute (IRRI) farms in the Philippines, for example, between 1979 and 2003, cool-season temperatures averaged a maximum of 30°C and a minimum of 22.4°C (extreme temperatures of 31.4 and 21.0°C), and the hot-season temperatures averaged a maximum of 31.3°C and a minimum of 24.4°C (extreme temperatures of 35.7 and 16.4°C) (Centeno *et al.*, 1995; Peng *et al.*, 2004). High temperature is conducive to BB development in field situations, and disease severity is particularly high in the hot season (Yamada *et al.*, 1979; Horino *et al.*, 1982; Ou, 1985; Ezuka & Kaku, 2000). By contrast, little disease is observed in the cool season.

In our previous experiments, after 3 yr continuous planting with *Xa7* in the same field sites (six cropping seasons), we measured an increase in a *Xoo* population that could cause disease on rice with *Xa7* (loss of *R* gene recognition) without loss of pathogenic fitness (aggressiveness function). This group of strains, designated race 9b, exhibited a wide variation in their aggressiveness to rice, including rice with *Xa7* (Ponciano *et al.*, 2004). The detection of the race 9b strains in the fields raised the possibility that the *Xoo* population was evolving to one that can overcome resistance controlled by *Xa7* while maintaining fitness on susceptible hosts.

In this study, we monitored the structure of the field *Xoo* population for an additional 9 yr (total 11 yr) to determine if the *Xoo* strains that had lost *avrXa7* function but maintained pathogenic fitness would break down resistance conferred by *Xa7*. We show that, although the *Xoo* population virulent to *Xa7* dominated the field population, *Xa7*-directed resistance remained effective. The differences between disease pressure observed in the hot (conducive) and cool (nonconductive) rice cropping seasons and the observations from other host–pathogen systems that some *R* genes differ in their effectiveness at high temperatures led us to hypothesize that *Xa7* may be more effective at high temperatures, and that this is one factor contributing to the relative durability of the *R* gene. As a first step to testing this hypothesis, we demonstrate in this study that *Xa7* is more effective at high than at low temperatures relative to other BB *R* genes. We speculate that the greater effectiveness of *Xa7* in the hot season may slow the evolution to more aggressive races of *Xoo* in the field. A novel aspect of this work is that, because field plots were established and maintained at one epidemiological location with continuous rice crops during both growing seasons in the Philippines from 1993 to 2004, long-term analysis of the impacts of the *Xa7* gene on evolution of *Xoo* populations under fluctuating selection pressures imposed by temperature was possible.

Materials and Methods

Field experimental design and disease assessment

Experiments were conducted in neighboring field plots located in Calauan, the Philippines, during the years 1993–95, 1998–99 and 2002–04 as described previously (Vera Cruz *et al.*, 2000). During both the cool and hot seasons, rice lines were planted in a randomized complete block design using 13 × 13 m plots with three (1998–99) or four (1993–95 and 2002–04) replications. In 1993–95, rice with the *Xa7 R* gene (IRBB7) was present in 20% of the plots, and the remaining 80% of the plots were planted with near-isogenic rice lines that did not contain *Xa7*, including the recurrent susceptible parent, IR24. In 1998–99, 33% of the plots contained *Xa7*, and in 2002–04, 60% of the plots contained *Xa7*. Data on the monthly average temperatures were collected from 1993 to 2004 (Sheehy *et al.*, 2005). Disease estimates and leaf samples were collected in a W pattern throughout each plot, with either seven (1993–95) or five (1998–2004) collection sites per W pattern (Vera Cruz *et al.*, 2000). Disease estimates and leaf samples were taken from IRBB7 and IR24 (lacks *Xa7*) plots in the hot season only, as little disease is observed during the cool season (Ou, 1985; Ezuka & Kaku, 2000). Estimates of disease, including incidence (occurrence of symptoms) and disease severity (% diseased leaf area, % DLA), were assessed and bacteria were isolated from sampled symptomatic tissue as previously described (Oña *et al.*, 1998; Vera Cruz *et al.*, 2000). Differences in disease incidence and severity were evaluated in an analysis of variance (ANOVA) using Proc Mixed in SAS statistical software (SAS Institute, version 9.2, Cary, NC, USA).

Xoo population structure

Changes to *Xoo* populations in plots planted to rice line IR24, which does not contain *Xa7*, were monitored by assessing the race structure and aggressiveness patterns for strains collected from these plots over three time frames (1993–95, 1998–99, and 2002–04). Up to 15 bacterial strains per plot per replication per year were randomly selected and the race of each strain was determined by inoculation to a set of seven rice differential lines, each containing a different BB *R* gene or no known *R* genes (IR24, susceptible control) (Kauffman *et al.*, 1973; Ogawa *et al.*, 1991). Changes in the *Xoo* population structure in terms of both aggressiveness (to susceptible host) and virulence (to resistant host) over time were measured by calculating the mean lesion lengths for all strains collected within a single year after inoculation to IR24 and IRBB7, respectively. *Xoo* race 3 and races 9b and 9a/c are differentiated by lesion lengths on IRBB7 as follows: race 3, 0–4.9 cm; race 9b, 5–17 cm; and race 9a/c, > 17 cm. To identify differences in *Xoo* population aggressiveness and virulence, the mean lesion lengths were evaluated using ANOVA in Proc Mixed.

Effect of temperature on *R* gene function

To determine the effect of temperature on various *R* genes, near-isogenic rice lines IRBB3, IRBB4, IRBB5, IRBB7, and IRBB10, which contain *Xa3*, *Xa4*, *xa5*, *Xa7* and *Xa10*, respectively, as well as the recurrent susceptible parent IR24 (Ogawa *et al.*, 1991), were grown in a glasshouse for 11 wk. Near-isogenic lines were used to minimize differences owing to genetic background and so that all effects could be associated directly with the *R* gene in question. One week before inoculation, the plants were transferred to growth chambers with 70% RH and day : night temperatures of 35 : 27 and 29 : 21°C, respectively. *Xoo* strains (races) used for inoculation induced resistance in combination with each *R* gene, and were PXO61 (race 1), PXO79 (race 3), PXO71 (race 4), and PXO112 (race 5) (Mew, 1987). Flag leaves were inoculated with bacteria (concentration was adjusted to *c.* 5×10^8 CFU ml^{-1}) at 85 d after sowing (das) using a pin-prick method as described in (Horino *et al.*, 1982; Ou, 1985). Lesion lengths were measured 21 d after inoculation (dai). Experiments were performed using a split-plot design with temperature applied at the whole-plot level and variety–isolate combinations applied at the subplot level (three replications, two plants

per replicate). The experiment was performed three times and differences in lesion length from isolates causing resistant interactions for each *R* gene were assessed using SAS Proc Mixed. In a separate experiment, IRBB7 and IR24 plants grown at 35 : 29 and 29 : 21°C were inoculated using a leaf clip assay (Kauffman *et al.*, 1973) with two race 3 isolates (PXO340 and PXO368). Plants were inoculated at 45 das, and lesion lengths measured at 14 dai. Lesion lengths from both isolates on a single line were combined and analyzed for significant differences using ANOVA (SAS Proc Mixed).

To comprehensively evaluate the effects of different temperature regimes (35 : 31 and 29 : 21°C) on interactions with *Xa7*, strains of race 9a/c without *avrXa7* (PXO346, PXO2684), race 3 with *avrXa7*$^+$ (PXO1865, PXO368), and race 9b with partially functional *avrXa7* (PXO348, PXO0314, PXO350, PXO354, PXO355, PXO356, PXO360 and PXO554) were used to inoculate IRBB7 and IR24 at 45 das as previously described (Kauffman *et al.*, 1973). A split-plot design was used, with temperature applied at the whole-plot level, and variety–isolate applied at the subplot level (two replicates, with two plants per replicate). The experiment was performed twice. Lesion lengths were measured at 3, 6, 9, 12, 15, and 18 dai. Area under the disease progress curves (AUDPC) were calculated (Madden *et al.*, 2007) and significant differences compared using ANOVA (SAS Proc Mixed).

Results

Evolution of *Xoo* populations on a susceptible host over 11 yr

To estimate the impact of deploying rice with *Xa7* over 11 yr on the race structure of *Xoo* field populations, the race type of *Xoo* isolates from the susceptible host (IR24) was determined. IR24 acts as a susceptible 'trap' plot as it will produce disease with all indigenous *Xoo* races that are present in the local population, and the *Xoo* population on IR24 reproduces in the absence of selection from any *R* gene being deployed in other plots. In 1993 and 1994, *Xoo* strains in the field plots were predominantly race 3 (Fig. 1). Race 2 strains, which dominated in the population in the Philippines before 1993 (Mew *et al.*, 1992; Vera Cruz *et al.*, 1996), were only detectable in early samples, and were not detected on IR24 after 1999. Race 9 strains were distinguished into two functional subgroups (race 9b, and 9a/c) based on the function of the *Xoo avrXa7* gene (Mew *et al.*, 1992; Vera Cruz *et al.*, 2000). Both subgroups were isolated from the susceptible host IR24, with race 9b populations significantly increasing (starting in 1995), and then stabilizing as the dominant component of the population (Fig. 1). Concurrently, the prevalence of race 3 (recognized by *Xa7*) decreased. Significant increases in the proportion of

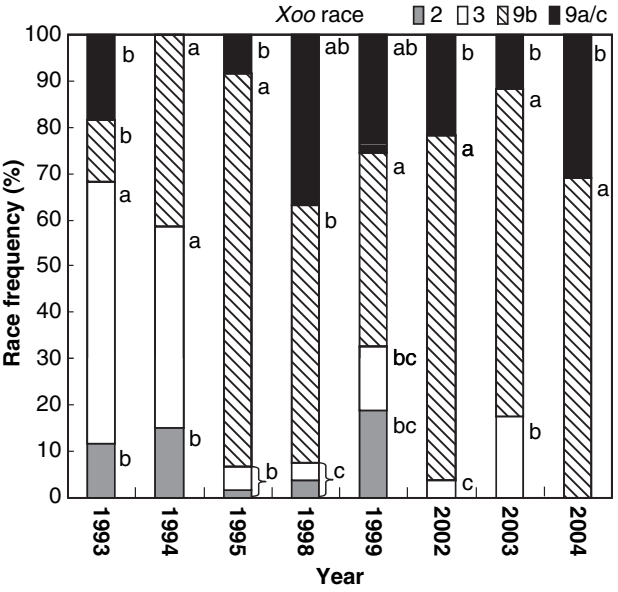

Fig. 1 Frequency (% of total population) of *Xanthomonas oryzae* pv. *oryzae* races (2, 3, 9b, 9a/c) in the population isolated from rice with no *R* gene (IR24) in field sites located in Calauan, the Philippines, over 11 yr (22 cropping seasons). Lower-case letters indicate significant differences in race frequency within each year with separations calculated based on an *F*-test in SAS Proc Mixed (P = 0.05).

race 9a/c (22.5%; not recognized and therefore virulent to *Xa7* as a result of mutation of *avrXa7* function) were detected starting in 1998 (Fig. 1). By 2004, race 9, including both subgroups (9b and 9a/c), was the dominant race in the field, with the incidence of subgroup 9a/c increasing to 31.1% of the population. Thus, from 1993 to 2004, the *Xoo* population structure at these field sites changed significantly from dominance by strains that are controlled by *Xa7* (race 3) to a population dominated by strains able to cause disease (virulent) on rice with *Xa7* (race 9), as indicated in a mean separation test (least significance difference) of the yearly means (P = 0.05).

Virulence and aggressiveness increased in the *Xoo* population

In previous studies, a loss of *Xoo avrXa7* gene function resulted in reduced aggressiveness (less disease) to susceptible rice hosts, suggesting reduced pathogenic fitness (Bai *et al.*, 2000; Vera Cruz *et al.*, 2000). Because the *Xoo* field population changed to being primarily composed of strains able to cause disease on rice with *Xa7* (race 9) (Fig. 1), we asked if the overall population was more or less aggressive to IR24. *Xoo* strains were collected from each year of the study, and aggressiveness was determined by measuring the disease lesion length on IR24 and IRBB7 (Fig. 2). Lesion lengths provide a measure of pathogen colonization of the rice tissues (Barton-Willis *et al.*, 1989). In 1993, the mean

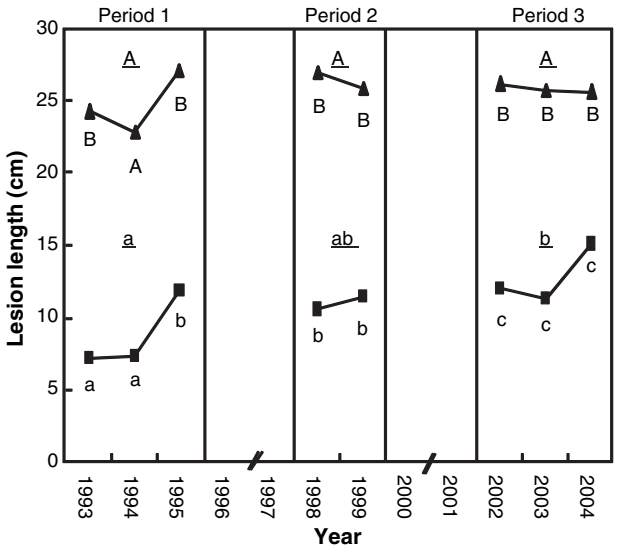

Fig. 2 The *Xanthomonas oryzae* pv. *oryzae* population increases in virulence to rice with *Xa7* (IRBB7; rectangles) and maintains aggressiveness to IR24 (triangles) over three time periods from 1993 to 2004. Mean comparisons were done for each period (underlined) and year within a period. Upper-case letters are for comparisons with IR24, while lower-case letters are for IRBB7 and indicate population means that are significantly different based on an *F*-test in SAS Proc Mixed ($P = 0.05$).

lesion lengths of the *Xoo* population on IRBB7 and IR24 were 4.6 and 20.5 cm, respectively, which is consistent with a functional *Xoo avrXa7* gene (Mew *et al.*, 1992) in the predominant race 3 population. Between 1995 and 2004, the mean lesion length of the *Xoo* population on rice with *Xa7* significantly increased from 10.5 to 15.1 cm. The population's aggressiveness to IR24 also increased between 1993 and 1995 and then remained stable through 2003, with a mean lesion length of *c.* 26 cm (Fig. 2). This increase in aggressiveness in the overall pathogen population is associated with a decrease in race 3 and an increase in race 9 in the population (Fig. 1).

Disease incidence and severity

To determine if the increase in virulent race 9 strains in the *Xoo* population correlated with an increase in disease on rice with *Xa7*, we assessed disease development in the rice plots over the 11 yr period. Disease incidence (% of plants showing lesions) and severity (% DLA) were monitored on rice with and without *Xa7* during the hot seasons of 1993–95, 1998–99, and 2002–04 (Table 1). Although both disease incidence (48–94%) and severity (5–32%) were consistently high on rice without *Xa7*, both indicators of disease were significantly lower on IRBB7 than on the susceptible IR24, for all years included in the study (Table 1); this indicates that resistance conferred by *Xa7* was still effective during each year of the study. Even though the proportion of the pathogen population virulent to *Xa7* (race 9) was as high as 100% in 2004 (Fig. 1), BB incidence and severity remained low for IRBB7 (Table 1). Thus, although race 9 strains became the predominant population, *Xa7* continued to effectively restrict BB disease in the field. These results also suggested that additional factors outside pathogenic fitness contributed to the continued effectiveness of *Xa7*.

Table 1 Significant terms from an ANOVA of the disease severity (% diseased leaf area) and disease incidence (% occurrence of symptoms) of bacterial blight in field plots from the hot season (1993–2004)

	Disease severity		Disease incidence	
Year	IR24	IRBB7	IR24	IRBB7
1993	32.04a	0.68b	94.44a	15.76b
1994	21.12a	0.35b	89.74a	14.93b
1995	5.68a	0.15b	66.67a	7.64b
1998	26.37a	1.68b	50a	3.33b
1999	18.33a	2ba	48.33a	8.33b
2002	4.5a	0.43b	84.5a	44.1b
2003	6.88a	2.63b	73.65a	46.15a*
2004	5.2a	0.57a	82.23a	19.27b

Values are means of four replications (1993–95; 2002–03) or three replications (1998–99, 2004). Means within a row by year followed by a different letter are significantly different ($\alpha = 0.05$) based on an *F*-test in SAS Proc Mixed.
*Not significantly different ($\alpha = 0.05$), $P = 0.0579$.

Xa7-mediated resistance is more effective at high temperatures

Although race 9 strains (virulent to *Xa7*) are predominant in the field plots, rice varieties with *Xa7* continued to exhibit less disease than IR24 (Table 1). During the initial analysis (in 1998) of *Xoo* population race structure, we tested field strains on IR24 and IRBB7 in March/April (average temperature 33°C) and January (average temperature 29°C) under screenhouse conditions. A two-tailed *t*-test, assuming unequal variances, showed that the field strains caused shorter lesions on IRBB7 when screened in months with higher temperatures than in months with cooler temperatures ($P = 9.0 \times 10^{-14}$) (Fig. 3). This suggested that the *Xa7* gene was more effective at high temperatures than at low temperatures.

To confirm that *Xa7* is more effective at high temperature and to determine if the effect is unique to *Xa7*, performances of five BB *R* genes in high- (35°C day : 27°C night) and low-temperature (29°C day : 21°C night) regimes were compared in growth chamber studies (Fig. 4a). Four of the five genes examined – *Xa3*, *Xa4*, *xa5*, and *Xa7* – were affected by temperature in some way. *Xa3*, *Xa4*, and *xa5* were less effective at high temperatures (as indicated by longer lesions) than at low temperatures (Fig. 4a). In particular, *Xa4* exhibited much longer lesions at high

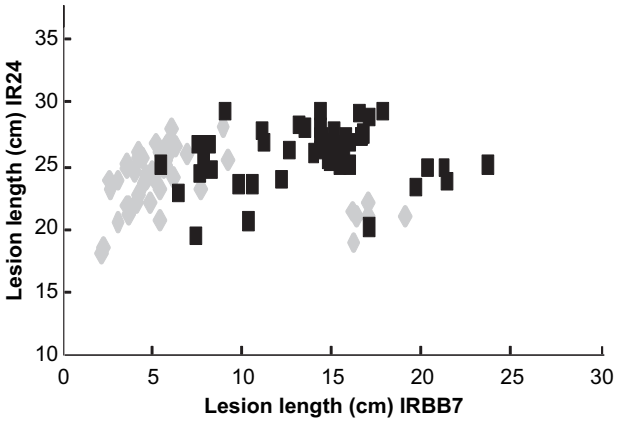

Fig. 3 Mean lesion lengths for a population of *Xanthomonas oryzae* pv. *oryzae* strains that were inoculated to IRBB7 and IR24 at two different time periods (January 1998 (black squares) and March/April 1998 (gray diamonds)). Average daytime temperatures were 29 and 33°C, respectively. Lesions were generally shorter on IRBB7 during the higher temperatures (April). Population means were significantly different using a two-tailed *t*-test ($P < 0.05$).

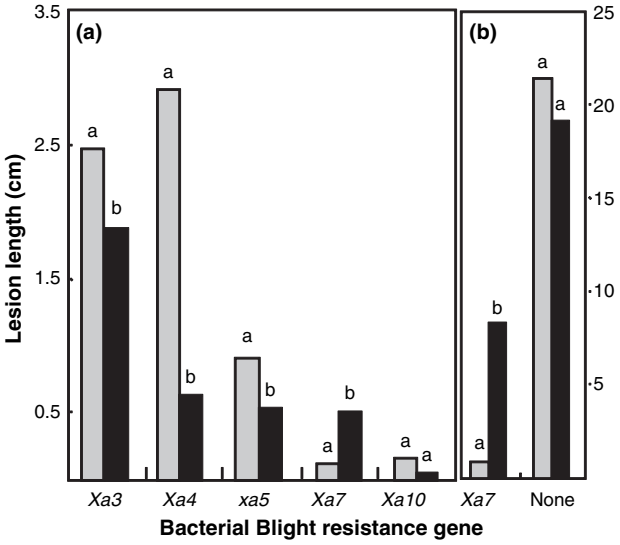

Fig. 4 *Xa7* is more effective in higher (gray bars) than in lower (black bars) temperature regimes. (a) Leaves of rice near-isogenic lines with *R* genes *Xa3*, *Xa4*, *xa5*, *Xa7*, and *Xa10* were inoculated with strains of *Xanthomonas oryzae* pv. *oryzae* that result in resistant interactions using a pin-prick method and grown in high- (35 : 27°C, day : night) and low-temperature regimes (29 : 21°C, day : night). (b) Leaves of rice near-isogenic lines containing the *Xa7* gene (IRBB7) and no *R* gene (cultivar IR24) were inoculated using a leaf clip method grown in high- (35 : 29°C, day : night) and low-temperature (29 : 21°C, day : night) regimes. Lower-case letters indicate significant differences between temperature regimes within a single *R* gene interaction ($P = 0.05$).

temperatures. By contrast, *Xa7* was more effective (produced shorter lesions) at the high temperatures. The increased effectiveness of *Xa7* at higher temperatures was confirmed using a second inoculation assay, the leaf clip inoculation method (Kauffman *et al.*, 1973) (Fig. 4b). In these experiments, there was no significant difference in the amount of disease on the near-isogenic susceptible host IR24.

The possible influence of bacterial genotype on increased function of *Xa7* at high temperatures was tested by inoculating several *Xoo* strains with nonfunctional (race 9a/c), partially functional (race 9b), or functional (race 3) *avrXa7* alleles to IRBB7 and IR24. We used the AUDPC as a measure of the rate of disease development that includes lesion length measurements over time (Madden *et al.*, 2007). At high temperatures, the amount of disease on IRBB7 induced by all *Xoo* isolates with functional or partially functional *avrXa7* alleles was reduced (average AUDPC, 74.2) relative to low temperatures (average AUDPC, 142.9) (Fig. 5a, upper case letters). Because the *avrXa7* allele varies among these bacterial strains (Bai *et al.*, 2000; Ponciano *et al.*, 2004), and this can contribute to differences in AUDPC on *Xa7*, we tested if there was an effect of temperature on the *avrXa7* gene in particular strains. In this study, we did not directly partition the effect of temperature on the pathogen *avr* effector gene vs the rice *R* gene; however, there was no temperature × strain × variety interaction ($P = 0.857$), nor a temperature × strain interaction ($P = 0.899$), suggesting that temperature does not have an impact on the function of the pathogen *avrXa7* gene. Additionally, there was no difference in AUDPC ($\alpha = 0.05$) when each strain was compared with itself at either temperature or in each of the varieties tested (data not shown). The two race 9a/c strains (no *avrXa7* function) (Ponciano *et al.*, 2004) cause about the same amount of disease at both temperatures (Fig. 5a,b). Race 3 and race 9b strains cause less disease than race 9a/c strains on IRBB7 independent of temperature, and at high temperature there was no difference in AUDPC caused by most race 3 and 9b strains. These data confirm that *Xa7* is more effective at higher temperatures. Furthermore, the data suggest that, at the high temperature, the *R* gene more efficiently recognizes pathogen strains in which the *avrXa7* alleles either are partially mutated or have complete function, as these strains cause intermediate responses at the low temperature (as compared with isolates that have completely lost *avrXa7* function at low temperatures) (Fig. 5a).

An ANOVA did not reveal differences in AUDPC at the two temperature regimes on the susceptible host (IR24, Fig. 5b). However, a sign test was performed to determine if the mean AUDPCs for the low- and high-temperature regimes differed, and it indicated that, when each of the 13 isolates was evaluated as a separate trial, significantly longer lesions were produced at higher temperatures than were produced at lower temperatures ($P = 0.0001$) on susceptible hosts, supporting the previous studies (Ou, 1985; Ezuka & Kaku, 2000).

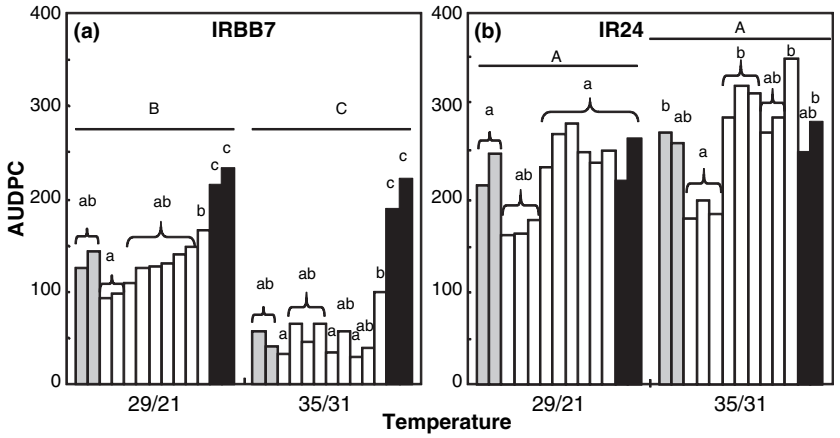

Fig. 5 *Xa7* is more effective in higher (35 : 31°C, day : night) than in lower (29 : 21°C) temperature regimes. Area under the disease progress curves (AUDPC) for rice cultivar IRBB7 (a) and cultivar IR24 (b) after inoculation with 13 strains of *Xanthomonas oryzae* pv. *oryzae*, representing race 3 (functional *avrXa7*; gray bars), race 9b (variation in *avrXa7* function; white bars) and race 9a/c (nonfunctional *avrXa7*; black bars). Inoculation was by the leaf clip method. Lower-case letters indicate significant differences ($P < 0.05$) among the strains within a single temperature regime. Upper-case letters indicate significant differences among all the strains comparing the two different temperature regimes within a cultivar.

Discussion

One factor influencing the durability of the BB *R* gene *Xa7* is the fitness penalty associated with the loss of the corresponding pathogen effector gene function (*avrXa7*) (Vera Cruz *et al.*, 2000). This would imply that the *Xoo* population would slowly develop increased virulence to *Xa7* only after extended deployment of the *R* gene. Over the 11 yr (1993–2004) of our field study, the population of *Xoo* isolated from the susceptible host (IR24) increased in virulence to rice with *Xa7* (Fig. 1). This change was attributed to the increase in the proportion of *Xoo* races 9b and 9a/c, which have reduced or caused complete loss of *avrXa7* function, respectively, and are virulent to rice with *Xa7* (Ponciano *et al.*, 2004; Fig. 1). The *Xoo* field population from 1995 onwards also caused longer lesions on rice with or without *Xa7* relative to earlier years (Fig. 2). The increase in aggressiveness was attributed to the increase in predominantly race 9b *Xoo* strains that had partially lost *avrXa7* function, but had not lost pathogenic fitness. What is most intriguing is that, despite the increased pathogen virulence to *Xa7*, disease severity on IRBB7 remained low during the 11 yr period and that resistance remained effective (Table 1). These results confirmed that pathogenic fitness cost is only one factor in pathogen evolution to virulence, and pointed to the need to explore other factors that might also influence *R* gene durability.

An *R* gene is only considered to be durable after it has been effective while deployed over a long time, in several environments, and under conditions conducive to disease development (Johnson, 1984). In the Philippines, rice is grown in two seasons with different environmental growing conditions, a dry, cool season and a wet, hot season. Because environment is predicted to influence *R* gene durability, we tested the impact of one environmental factor, high temperature, on *Xa7* function. We found that *Xa7* functions more effectively at higher temperatures, that is, it restricts the development of lesions (shorter lesions) on leaves compared with lower temperatures (Figs 4,5). The average maximum temperature during the hot season at the field site from 1979 to 2003 was *c.* 31.1°C, with high temperature extremes of > 35°C (Peng *et al.*, 2004; Sheehy *et al.*, 2005). We predict that if only *Xa7* had been deployed in the plots, and the susceptible IR24 plants had not been there to serve as a reservoir for the race 9b populations, the greater effectiveness of *Xa7* at high temperatures would have reduced the race 9b populations much more over the 11 yr period.

We propose that *Xoo* field populations developing on *Xa7* experience fluctuating selection pressure in the shift from the hot to cool seasons. During the hot season, the abiotic environment is more conducive to disease. The greater effect of *Xa7* during the hot season can have a complicated effect on selection for virulence in *Xoo*: selection for virulent individuals within the population is stronger than during the cool season because of the greater effect of *Xa7*, but population size is reduced compared with the hot season potential. The pathogen numbers that would carry over into the cool season would be substantially reduced. Thus, we suggest that when the cool season crop is planted, the reduced initial inoculum load, the presence of *Xa7* (even if reduced in effectiveness), and the cool, nonconducive temperatures would restrict disease development and pathogen numbers further. The effect of these two alternating forms of selection on the *Xoo* population across years is likely to increase the effective life of *Xa7*. Unfortunately, we were

not able to measure the amount of inoculum at the beginning of the cool season, nor could we measure disease in that season, because little or no disease was observed in the cool season.

While the BB *R* gene *Xa7* was more effective when incubated at high temperatures, the opposite was true for BB *R* genes *Xa3*, *Xa4*, and *xa5*. These differential responses of *R* genes to temperature may provide additional information when predicting *R* gene durability. In our study, *Xa10* resistance was not affected by temperature. This, combined with the fact that the corresponding *avrXa10* effector gene in *Xoo* was found to be unimportant for pathogenic fitness (Bai *et al.*, 2000), may explain why *Xa10* was not durable in the field (Vera Cruz *et al.*, 2000). Intriguingly, although *Xa4* was much less effective at higher temperatures, it confers durable resistance in many countries (Mew, 1987; Bonman *et al.*, 1992). This *R* gene does not exhibit a classic hypersensitive response (Guo *et al.*, 1993) and is predicted to exhibit a quantitative rather than a qualitative type resistance (Li *et al.*, 2001), which may influence its durability. Because it has not yet been cloned, the contribution of the *avrXa4* gene to pathogenic fitness, if any, is currently unknown.

The biological basis for enhanced activity of *Xa7* at high temperatures is not known. The rice blast *R* gene *Pib* is upregulated at high temperatures, and it is predicted that the increased production of the *R* gene product enhances defense response (Wang *et al.*, 2001). Increased amounts of the Xa7 protein at high temperatures could account for enhanced recognition of the wild-type and mutant AvrXa7 effector proteins. Alternatively, protein–protein interactions involving *Xa7* may be affected by high temperatures. These hypotheses cannot be tested currently as the *Xa7* gene has not been cloned.

Previous work demonstrated that the fitness cost imposed on the pathogen to overcome resistance can influence *R* gene durability (Vera Cruz *et al.*, 2000). We have shown that another likely factor influencing *R* gene durability is the enhanced effectiveness of an *R* gene at high temperature. While we have only tested the cost of pathogenic fitness (Vera Cruz *et al.*, 2000) and the effect of temperature, it is likely that other factors, such as humidity and nutrition, as well as host genetic background and developmental attributes (Mazzola *et al.*, 1994; Cao *et al.*, 2007; Iyer-Pascuzzi *et al.*, 2008), will influence *R* gene durability, and that not all factors will affect all *R* genes or have an equal effect on them (Burdon & Thrall, 2003). We predict that temperature affects *R* gene durability by causing variable (fluctuating) selection on the pathogen population between the cool and hot growing seasons. Given the myriad ways that climate change will affect plant disease management (Garrett *et al.*, 2006), it is important to include both environmental factors, such as temperature, and pathogenic fitness parameters in the development of predictive models that will accurately identify durable *R* genes before their deployment.

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