Eur J Plant Pathol (2011) 131:483–496
DOI 10.1007/s10658-011-9824-0

Spatiotemporal effects of cultivar mixtures on wheat stripe rust epidemics

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Accepted: 20 June 2011 / Published online: 12 July 2011

Abstract The use of cultivar mixtures is increasingly practical in wheat stripe rust management. Field experiments with wheat cultivar mixtures were conducted to determine their effects on temporal and spatial patterns of stripe rust epidemics in three regions. In the Beijing and Gangu fields, where the epidemics were caused by artificial inoculation, disease incidence and the area under the disease progress curve (AUDPC) of the cultivar mixtures were significantly lower ($P<0.05$) than those of the susceptible pure stands. We defined the relative effectiveness of cultivar mixture on disease development related to that in pure stands (*REM*). The results demonstrated that in many treatments of mixtures of susceptible cultivar with resistant cultivars at various ratios in different locations, their effects on disease reduction were positive ($REM<1$). The reduction of epidemic rate in cultivar mixtures expressed in either early season or late season depended on the initial pattern of disease and cultivar mixture treatments. Semivariograms were used to determine the spatiotemporal patterns of disease in the Gangu field. The spatial analysis showed clear spatial patterns of the disease in all four directions of the fields on susceptible pure stands but not on cultivar mixtures. The results implied that the mechanisms of cultivar mixture on disease management might include the interruption of disease spatial expansion and a physical barrier to pathogen inoculum by resistant cultivars.

Keywords Disease progress · *Puccinia striiformis* · Resistance · Semivariogram

Introduction

Stripe rust, caused by *Puccinia striifomis* f.sp. *tritici* (*Pst*), is one of the most destructive diseases of wheat in China. The pandemics in 1950, 1964, 1990, and 2002 caused yield losses up to 6.0, 3.0, 1.8, and 1.3 million tons, respectively (Li and Zeng 2002). From 2000, stripe rust generally occurred in 40–53 million hectares in China causing 5%–10% yield losses. It can cause >30% losses in severe epidemics. In China, the use of host resistance and fungicides are two major strategies in stripe rust management (Wan et al. 2007).

Pst possesses unique features of its life cycle in China. The pathogen has to oversummer in areas

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where the highest average daily temperature in summer is lower than 22°C. Thus, the pathogen cannot oversummer in most wheat-growing areas in China due to high temperatures, and can only oversummer in the mountainous areas in western China. The inoculum can then disperse over long distances to eastern areas in autumn to cause epidemics. In the mountainous regions, the pathogen can not only oversummer at high elevation but also overwinter at low elevation to complete its life cycle locally. In such areas, a huge number of wheat cultivars with various resistance genes, agronomic features and productivities were grown to fit the environments and cultural conditions. Based on their unique features and roles in interregional epidemics, 15 epidemiological regions have been classified in China (Zeng and Luo 2006, 2008). Deployment of resistant cultivars with various resistance backgrounds in different wheat-growing areas, including complete qualitative resistance to some races, partial resistance and quantitative durable resistance, is an important strategy in stripe rust management in China.

Cultivar mixtures have been applied in agricultural crop production, although single genotype implementation still dominates modern cropping systems (Mundt 2002). Cultivar mixtures are still not commonly used in wheat production to control stripe rust in China, although they have been used in some countries as one of the disease control strategies (Wolfe 1985; Mundt 2002). Recently, this strategy has been emphasized by Chinese governmental agencies and researchers to control some important diseases, such as rice blast in some rice growing regions (Zhu et al. 2000; Zhu et al. 2005) and wheat stripe rust in areas where *P. striiformis* can oversummer to complete its life cycle.

Cultivar mixtures can reduce disease intensity and improve production (Wolfe 1985; Zhu et al. 2000; Mundt 2002), especially for some foliar diseases of small grains, such as cereal rusts and powdery mildews (Wolfe 1985; Garrett and Mundt 2000; Cowger and Mundt 2002; Cox et al. 2004) and potato late blight (Garrett et al. 2001; Andrivon et al. 2003; Finckh et al. 2007). The reduction of disease intensity due to cultivar mixtures could range from 4% to 89% (Smithson and Lenne 1996). Studies on effects of cultivar mixtures on disease epidemics have focused on the mechanisms of disease temporal development, disease control and yield increase. Possible mechanisms in disease reduction by cultivar mixtures may include: i) dilution of inoculum due to the presence of resistant plants, ii) a physical barrier created by the resistant plants, and iii) the potential of induced resistance (Garrett and Mundt 1999; Mundt 2002). The detailed information of these mechanisms is still needed. For instance, we need to know: i) whether the rate of epidemic development may change during the growing season in the cultivar mixtures, thus affecting temporal disease development; ii) whether different climatic conditions in various geographic locations can affect the efficacy of cultivar mixtures; and iii) whether the cultivar mixtures can affect the spatial distribution pattern of disease in the fields that may correlate with the temporal development of disease. Compared to the number of studies on effects of cultivar mixtures on disease temporal development and control, few studies have considered in the effects of cultivar mixtures on the spatial pattern of disease epidemics.

In this study, field experiments on cultivar mixtures were conducted in three geographic locations in China. The objectives of this study were: (i) to determine the effects of cultivar mixtures on progression of stripe rust epidemics; (ii) to determine the period of time when the cultivar mixtures affect disease epidemics; and (iii) to characterize the spatial patterns of stripe rust epidemics in cultivar mixtures.

Materials and methods

Location selection

Fields at three locations, Gangu (Gansu province, northwest of China), Yanting (Sichuan province, southwest of China) and Beijing (north of China), with different climatic conditions, crop management and stripe rust epidemics were selected. Gangu is one of the key areas influencing interregional wheat stripe rust epidemics in China (Zeng and Luo 2006) and serves as a bridging area where *Pst* can oversummer to complete its life cycle, and where wheat stripe rust occurs annually. Yanting is a location with high humidity year round, where *Pst* can overwinter and wheat stripe rust frequently occurs with high disease intensity. Beijing is a relatively dry area where stripe rust occurs occasionally with low intensity, and *Pst* cannot oversummer and is unlikely to overwinter (Zeng and Luo 2006).

Field experimental design

In the 2007–2008 growing season, the fields at Gangu and Yanting consisted of 36 and 21 plots, respectively. Each plot was 4.5 m long and 4.5 m wide. Each plot had 21 rows with 0.2 m spaces between rows. Plots were separated by 0.5 m. In the 2008–2009 growing season, the fields of Gangu and Beijing consisted of 21 and 27 plots, respectively. Plots were 4.4 m long and 4.7 m wide with 22 rows in the Gangu field; and 6 m long and 5 m wide with 24 rows in the Beijing field. The distances between rows in each plot was 0.2 m and between plots was 0.5 m in each field. The experimental design of the Gangu and Beijing fields in the 2009–2010 growing season was the same as that in the 2008–2009 growing season.

Because of different characteristics of climatic and wheat growing conditions, different wheat cultivars with various resistance levels were used at each of the three locations (Table 1). Cultivar mixture treatments at each location included single cultivars as pure stands and mixtures of immune (I) or resistant (R) cultivar with a susceptible (S) or moderately susceptible (MS) cultivar in various proportions (Table 1). Experiments at each location were arranged as a complete block design with three replications.

Inoculation

In the Gangu and Beijing fields each year, 15 seeds of susceptible cultivar Mingxian169 (MX169) were sown in the centre of each plot. Two races of *P. striiformis*, CYR32 and CYR33 (Su11-14), were used for inoculation. They are highly virulent to almost all the commercial varieties in China with frequencies in the population of 29.50% and 26.72% in 2008 and 21.6% and 23.7% in 2009. Urediniospores of races CYR32 and CYR33 were mixed in the same proportions to make spore suspensions in 0.05% agar of 0.1 mg/ml in 2008 and 0.17 mg/ml in 2009 and 2010. At the 4-leaf stage, each plant of MX169 (S) was inoculated by spraying about 5 ml of the spore suspension. Inoculations in the Gangu field were performed on 31 March 2008, 31 March 2009 and 22 April 2010. Inoculations in the Beijing field were performed on 26 March 2009 and 6 April 2010. The inoculated plants were covered with clear plastic bags for about 15 h overnight. Symptoms of wheat stripe rust appeared about 12 days after inoculation, and 15 leaves of these diseased plants were maintained per plot to serve as an initial disease focus in each plot.

In the Yanting field, the wheat stripe rust epidemics were caused by natural infections. A diseased leaf was first found in a plot of cultivar 95–71 but not in other plots on 21 January 2008. Rust developed slowly due to the low temperature from January to February.

Disease assessment

In the inoculated fields, five subplots were defined in each plot: one was around the centre with a 10 cm diameter; the other 4 subplots (100 cm^2 each) were set at positions 1.5 m away in 4 directions—north, south, east and west from the centre (five-point sampling). Disease incidence and severity (the percentage of leaf area with lesions from the top-three leaves of each of 33 plants) were periodically assessed at each subplot. In the Gangu field, disease assessment was performed on five occasions from 4 May to 2 June in 2008, from 5 May to 29 May in 2009 and from 13 May to 2 June in 2010. In the Beijing field, five disease assessments from 2 May to 31 May 31 in 2009 and six disease assessments from 3 May to 5 June in 2010 were performed. In the Yanting field, five subplots (about 10×10 cm) in each plot were randomly selected. In each subplot, 100 leaves were randomly sampled to assess disease incidence and severity every 7 days from 1 March 1 to 14 April.

In order to characterize the spatiotemporal patterns of stripe rust, additional disease assessments were performed in a subset of plots in the Gangu field in three consecutive growing seasons in the plots sowed with a single susceptible cultivar, or a mixture of a susceptible and a resistant cultivar at different ratios. Each plot consisted of m rows (m=21, 22 and 21 in 2008, 2009 and 2010, respectively). Each row was divided into a certain number of sections depending on the field. In each section, a 10-cm (2007–2008 and 2009–2010 growing seasons) or 20-cm (2008–2009 growing season) row was marked successively. Thus, each plot consisted of m rows and n sections per row (n=45, 22 and 44 in 2008, 2009 and 2010, respectively) for a specific field, and these $m \times n$ grids were used in disease assessment for spatiotemporal analysis. On each recording date, the number of diseased

Table 1 Field experimental design in three different geographical locations of China used in this study. Different mixtures of wheat cultivars with various susceptibilities to *Puccinia striiformis* races CYR32 and CYR33 were used from 2008 to 2010. Different inoculation methods and field practices were implemented in different locations

Location	Latitude and longitude	Growing season	Cultivar		Reaction to race of *P. striiformis*[a]		Cultivar mixture and corresponding proportion	Seed sowing date/ density (g/ m^2)	Irrigation	Previous crop / fertilization	Pesticide application	Initiation of epidemics
			Name (abbreviation)	Height (cm)	CYR32	CYR33						
Gangu	34°45′ N 105°17′ E	2007–2008	9362-10 (A)	90–95	I	I	100% A, 100% B,	Oct.19 / 22.5	Irrigation once in spring	Pepper / none	Only insecticide was used to control aphids	Artificial inoculation
			9220-42 (B)	90–95	R	R	100% C, 100% D,					
			Lantian13 (C)	85–90	MS	MS	A: C=2: 1, A: D=2: 1,					
			Shi917 (D)	90	S	S	B: C=2: 1, B: D=2: 1, A: C=1: 1, A: D=1: 1, B: C=1: 1, B: D=1: 1					
		2008–2009 and 2009–2010	9220-12 (E)	90–100	R	R	100%E, 100%F,					
			Lantian13 (F)	85–95	MS	MS	100%G, E: F=1: 1,	Oct. 16/ 24	Irrigation once in spring	Winter wheat/ compound fertilizers used before seed sowing	No fungicides application	Artificial inoculation
			Lantian6 (G)	85–90	S	S	E: F=5: 1, E: G=1: 1, E: G=5: 1	Oct. 13/ 24				
Yanting	31°15′ N 105°43′ E	2007–2008	Mianmai39 (H)	85	R	R	100% H, 100% I,	Nov. 7 / 15	No	Rice / compound fertilizers used before seed sowing		Natural infection
			Chuanmai28 (I)	80–90	MS	MS	100% J, H: I=2: 1,					
			95–71 (J)	85	S	S	H: J=2: 1, H: I=1: 1, H: J=1: 1					
Beijing	40°08′ N 116°10′ E	2008–2009 and 2009–2010	Nongda211 (K)	75–80	R	R	100%K, 100% L,	Oct. 1/ 15	Irrigation once in spring	Maize/ compound fertilizers used before seed sowing	Only insecticide was used to control aphids	Artificial inoculation
			9428 (L)	80–85	MS	MS	100% M, K: L=5: 1,	Sept. 26/ 10				
			0045 (M)	75–80	S	S	K: M=5: 1, K: L=3: 1, K: M=3: 1, K: L=1: 1, K: M=1: 1					

[a] I, R, MS, and S represent the reaction types of a cultivar as immune, resistant, moderate susceptible, and susceptible to stripe rust of the corresponding race of *P. striiformis*, respectively (Wan et al. 2004). Cultivars 9220–42 and 9220–12, bred from the same parents, had a similar reaction to *P. striiformis*.

leaves and disease incidence on the three top leaves of plants, and the average disease severity for each section was assessed. The spatial sampling was done for a reduced number of treatments. These included the pure stand of Shi917 (S) and the mixtures of 9362–10 (I) with Shi917 (S) at ratios of 1:1 and 2:1 assessed on 4, 11, 17 and 26 May 2008; and those for the pure stand Lantian6 (S) and the mixtures of Lantian6 (S) with 9220–12 (R) at ratios of 1:1 and 1:5 assessed on 5, 11, 19 and 25 May 2009 and on May 13, 18, 28 and June 2, 2010, respectively.

Temporal analysis

Area Under the Disease Progress Curve (AUDPC) was calculated for each replicate of each plot as:

$$AUDPC = \sum_{t=1}^{k-1} [(X_{i+1} + X_i)/2][T_{i+1} - T_i] \quad (1)$$

where, k is the total number of disease assessments, and X_i and X_{i+1} are the disease incidence at dates i (T_i) and date i+1 (T_{i+1}), respectively.

The relative effectiveness of a cultivar mixture in reducing disease development relative to those in pure stands (*REM*) for each treatment was estimated as:

$$REM = \frac{DIM}{\sum_{i=1}^{n} DIP_i{*}R_i} \quad (2)$$

where, *DIM* is the final disease incidence in a season for a cultivar mixture treatment, DIP_i is the final disease incidence for the *i*th cultivar in pure stands, R_i is the proportion (0–1) of the *i*th cultivar in the mixture, and n is the number of cultivars in the mixture assessed, here n=2. Thus, a cultivar mixture reduced the disease incidence when *REM*<1.

Temporal development of the disease was determined for each plot. Logistic models (Madden et al. 2007) (SAS ver. 8.0, SAS Institute, Cary, NC, USA) were fitted to the disease incidence data. In order to determine whether the epidemic rate differed in different periods, the whole growing season was divided into early and late periods for each field (Table 2). The epidemic rate (r) was estimated for each treatment in each time period. The observed disease incidence at the initial time of each time period was used as the initial y_0 in the regression analysis for each treatment. The maximum incidence was set to 1 for each regression. When there were only two assessments, $r = (\ln(y_2/(1-y_2)) - \ln(y_1/(1-y_1)))/(t_2 - t_1)$, in which *ln* represents the natural logarithmic function and y_2 and y_1 are disease incidence at times t_2 and t_1 respectively.

The comparisons in estimated r value for each replicate of each plot were made between cultivar mixtures and the corresponding pure stands in each field. In this analysis, covariance analysis (Madden et al. 2007) by using the CONTRAST method of GLM procedure of SAS was implemented to determine the significance of difference in mean epidemic rate between mixture treatments.

Spatial analysis

Geostatistical analysis (GS+version; Gamma Design Software Plainwell, MI) was used to determine the effect of cultivar mixtures on the spatial pattern of wheat stripe rust. Semivariograms, the plots of semivariance ($\gamma(h)$) versus lag distance (h), were used to determine existence of spatial dependence in disease development in a specific field (Liebhold et al. 1993). Semivariance is the average squared difference in values between pairs of samples separated by a given distance (h). The presence or absence of isotropic and anisotropic patterns was determined by examining ominidirectional and directional semivariograms in four directions: 0, 45, 90 and 135° for all 3 blocks of a treatment, where 0° is the direction within rows and 90° is the direction across rows. The semivariance versus lag distance for a given direction for each plot was plotted.

Results

Effect of cultivar mixture on stripe rust epidemics

Results from the Gangu field for the three consecutive growing seasons showed that for the treatments of cultivar Lantian13 (MS) mixed with 9220–42 (R) and with 9362–10 (R), and cultivar Shi917 (S) or Lantian6 (S) mixed with 9220–42 (R) or with 9362–10 (R), the incidence and AUDPCs were significantly lower than those on Lantian13 (MS), Lantian6 (S) and Shi917 (S) at P<0.05. Moreover, the higher the proportion of the resistant or immune cultivars present in a mixture, the less severe stripe rust occurred

Table 2 Comparisons in epidemic rate of wheat stripe rust among the different cultivar mixture treatments and their component pure stands in the Gangu field for different time periods of three consecutive growing seasons

Mixture component and ratio	Epidemic rate (±SE)[a]			R^2	Mixture component and ratio	Epidemic rate (±SE)			R^2
Gangu, 2007–2008 growing season	Before May 17	After May 17	Whole growing season		*Gangu, 2007–2008 growing season*	Before May 11	After May 11	Whole growing season	
Lantian13 (MS)	0.176±0.019 a	0.095±0.031 a	0.142±0.007 a	0.980[c]	Shi917 (S)	0.218±0.035 a	0.092±0.044 a	0.135±0.034 a	0.973[c]
Lanitan13: 9362–10 (1:1)	0.048±0.013 b	0.049±0.018 a	0.063±0.008 b	0.960[c]	Shi917: 9362–10 (1:1)	0.102±0.037 bc	0.082±0.006 a	0.089±0.003 b	0.995[c]
Lanitan13: 9362–10 (1:2)	0.026±0.024 b	0.096±0.038 a	0.060±0.005 b	0.927[c]	Shi917: 9362–10 (1:2)	0.056±0.026 c	0.093±0.015 a	0.089±0.011 b	0.959[c]
Lanitan13: 9220–42 (1:1)	0.060±0.023 b	0.085±0.029 a	0.076±0.012 b	0.852[b]	Shi917: 9220–42 (1:1)	0.189±0.066 ab	0.066±0.009 a	0.089±0.008 b	0.959[c]
Lanitan13: 9220–42 (1:2)	0.051±0.039 b	0.128±0.030 a	0.088±0.004 b	0.798[b]	Shi917: 9220–42 (1:2)	0.057±0.011 c	0.050±0.011 a	0.055±0.006 b	0.975[c]
9220-42 (R)	0.063±0.024 b	0.072±0.004 a	0.069±0.016 b	0.980[c]	9220-42 (R)	0.041±0.025 c	0.076±0.011 a	0.069±0.016 b	0.980[c]
9362-10 (I)	–	–	–	–	9362-10 (I)	–	–	–	–
Gangu, 2008–2009 growing season	Before May 11	After May 11	Whole growing season	R^2	*Gangu, 2008–2009 growing season*	Before May 11	After May 11	Whole growing season	R^2
Lantian13 (MS)	0.864±0.076 ab	0.224±0.018 a	0.262±0.008 a	0.835[b]	Lantian6 (S)	0.974±0.010 a	0.218±0.078 a	0.258±0.007 a	0.867[b]
Lantian13:9220-12(1:1)	0.939±0.031 a	0.219±0.024 a	0.244±0.003 ab	0.872[b]	Lantian6:9220-12(1:1)	0.844±0.024 ab	0.135±0.004 a	0.253±0.004 ab	0.872[b]
Lantian13:9220-12(1:5)	0.946±0.052 a	0.205±0.060 ab	0.239±0.009 ab	0.891[b]	Lantian6:9220-12(1:5)	0.785±0.058 b	0.140±0.013 a	0.236±0.006 bc	0.865[b]
9220-12 (R)	0.838±0.098 b	0.110±0.010 b	0.231±0.002 b	0.758[b]	9220-12 (R)	0.838±0.098 ab	0.110±0.010 a	0.231±0.002 c	0.758[b]
Gangu, 2009–2010 growing season	Before May 23	After May 23	Whole growing season	R^2	*Gangu, 2009–2010 growing season*	Before May 23	After May 23	Whole growing season	R^2
Lantian13 (MS)	0.249±0.022 a	0.242±0.035 c	0.302±0.023 a	0.935[c]	Lantian6 (S)	0.415±0.079 a	0.235±0.083 a	0.340±0.037 a	0.875[b]
Lantian13:9220-12(1:1)	0.046±0.023 bc	0.382±0.026 ab	0.249±0.033 bc	0.890[b]	Lantian6:9220-12(1:1)	0.296±0.117 ab	0.229±0.119 a	0.305±0.013 a	0.802[b]
Lantian13:9220-12(1:3)	−0.023±0.023 c	0.403±0.030 a	0.223±0.029 c	0.861[b]	Lantian6:9220-12(1:3)	0.023±0.061 c	0.309±0.044 a	0.182±0.017 b	0.841[b]
Lantian13:9220-12(1:5)	0.023±0.023 c	0.268±0.071 bc	0.169±0.024 c	0.882[b]	Lantian6:9220-12(1:5)	0.106±0.054 bc	0.206±0.004 a	0.163±0.022 b	0.849[b]
9220-12 (R)	0.000±0.000 c	0.139±0.000 c	0.079±0.009 c	0.770[b]	9220-12 (R)	0.000±0.000 c	0.139±0.000 a	0.079±0.009 c	0.770[b]

[a] Mean and corresponding standard error from three replicates calculated from logistic equation. The mean values with the same letter in a column for each treatment of each location indicate no significant difference at P=0.05, and [b] and [c] denote the significance of the regression at 0.05 and 0.01 levels, respectively.

(Fig. 1). For mixtures of Lantian6 (S) or Shi917 (S) with 9220–12 (R) or 9220–42 (R), *REM* was <1, except for the mixture at a 1:1 ratio in the 2008–2009 growing season. For mixtures of Lantian13 (MS) with resistant 9220–12 (R), *REM* was >1 except for the mixture at 1:1 ratio in three growing seasons. However, *REM* was <1 when Lantian13 (MS) was mixed with 9362–10 (I) (Fig. 3a–c).

In the Yanting field in 2007–2008, where the epidemics were initiated by natural infection, 95–71 (S) or Chuanmai28 (MS) mixed with Mianmai39 (R) did not lead to a significant reduction in the incidence and AUDPC ($P>0.05$) (Fig. 2a, b). Mixture of 95-71 (S) with Mianmai39 (R) and of Chuanmai28 (MS) with Mianmai39 (R) also did not reduce disease more than that expected ($REM \geq 1.0$).

In the Beijing field, no significant differences in disease incidence and AUDPC between cultivar mixtures and the pure stand of susceptible cultivars were detected (data not shown) in the 2008–2009 growing season due to the dry weather conditions. In the 2009–2010 growing season, the disease incidence and AUDPC of cultivars 0045 (S) or 9428 (MS) mixed with Nongda211 (R) were each significantly lower than that of the corresponding susceptible pure stand at $P<0.05$ (Fig. 2c-d), except for the mixture of 9428 (MS) and Nongda211 (R) in the ratio of 1:1. Furthermore, all mixtures of 0045 (S) with Nongda211 (R) had $REM<1$. However, *REM* was >1 in the mixture of 9428 (MS) with Nongda211 (R) (Fig. 3e).

Temporal development of the disease

Logistic models fitted the experimental data well. In the Gangu field in the 2007–2008 growing season, the whole-season epidemic rates of all cultivar mixtures were all significantly lower ($P<0.05$) than those of the pure stands Shi917 (S) and Lantian13 (MS) (Table 2). For the 2008–2009 growing season, there were no significant differences in whole-season epidemic rates between Lantian13 (MS) and the mixtures (Table 2). However, the whole-season epidemic rate of the mixture of Lantian6 (S) with 9220–12 at ratio of 1:5 was significantly lower than that of Lantian6, but not for the 1:1 mixture ratio (Table 2). In the 2009–2010 growing season, the whole-season epidemic rates of the mixture of Lantian13 (MS) with 9220–12 at all three ratios were significantly lower than that of Lantian13 (Table 2). Similarly, the whole-season epidemic rates (in 2008 and 2010) of the cultivar mixture of Lantian6 with 9220–12 at ratios of 1:3 and 1:5 were significantly lower than that of Lantian6, but not for the 1:1 mixture ratio (Table 2). In comparison with susceptible pure stands, when mixtures significantly reduced the whole-season epidemic rates, these reductions were usually positively related to the reduction of epidemic rate early in the season except for the mixture of Shi917 (S) with 9220–42 (R) at a 1:1 ratio in the 2007–2008 growing season. Thus, the reductions of the whole-season epidemic rate most likely resulted from the reductions of epidemic rate in the early season.

In the Yanting field in the 2007–2008 growing season, the epidemic rates for 95–71 (S) and Chuanmai28 (MS) were 0.394 and 0.428 logits per day, respectively. Comparatively, the epidemic rates for all cultivar mixtures were significantly lower ($P<0.05$) than those for the susceptible cultivars, ranging from 0.119 to 0.198 logits per day during the whole growing season (Table 3). The epidemic rates were significantly lower for the mixtures of 95–71 (S) with Mianmai39 (R) and the mixtures of Chuanmai28 (MS) with Mianmai39 (R) during the late period of the growing season (after 21 March) than those for susceptible cultivars 95–71 (S) and Chuanmai28 (MS) (Table 3).

In the Beijing field, the epidemic rates for 0045 (S) mixed with Nongda211 (R) at various ratios during the early and late periods of the season and whole growing season were significantly lower than those for 0045 (S) except for the mixture ratio 1:1 early in the season. However, the epidemic rates for the 9428 (MS) and its mixture with Nongda211 (R) showed no significant differences for the late period or for for the whole season (Table 3).

Spatial analysis

In the 2007–2008 growing season in the Gangu field, consistent patterns in semivariance over the lag distance were observed for all the three treatments and on all the observation dates. The semivariance for the pure stand treatment Shi917 (S) increased almost linearly with increasing lag distance up to 2.0 m and then decreased nearly linearly on all four assessment dates (Fig. 4). However, for both treatments of 9362–10 (I) mixed with Shi917 (S) at 1:1 and 2:1, no such

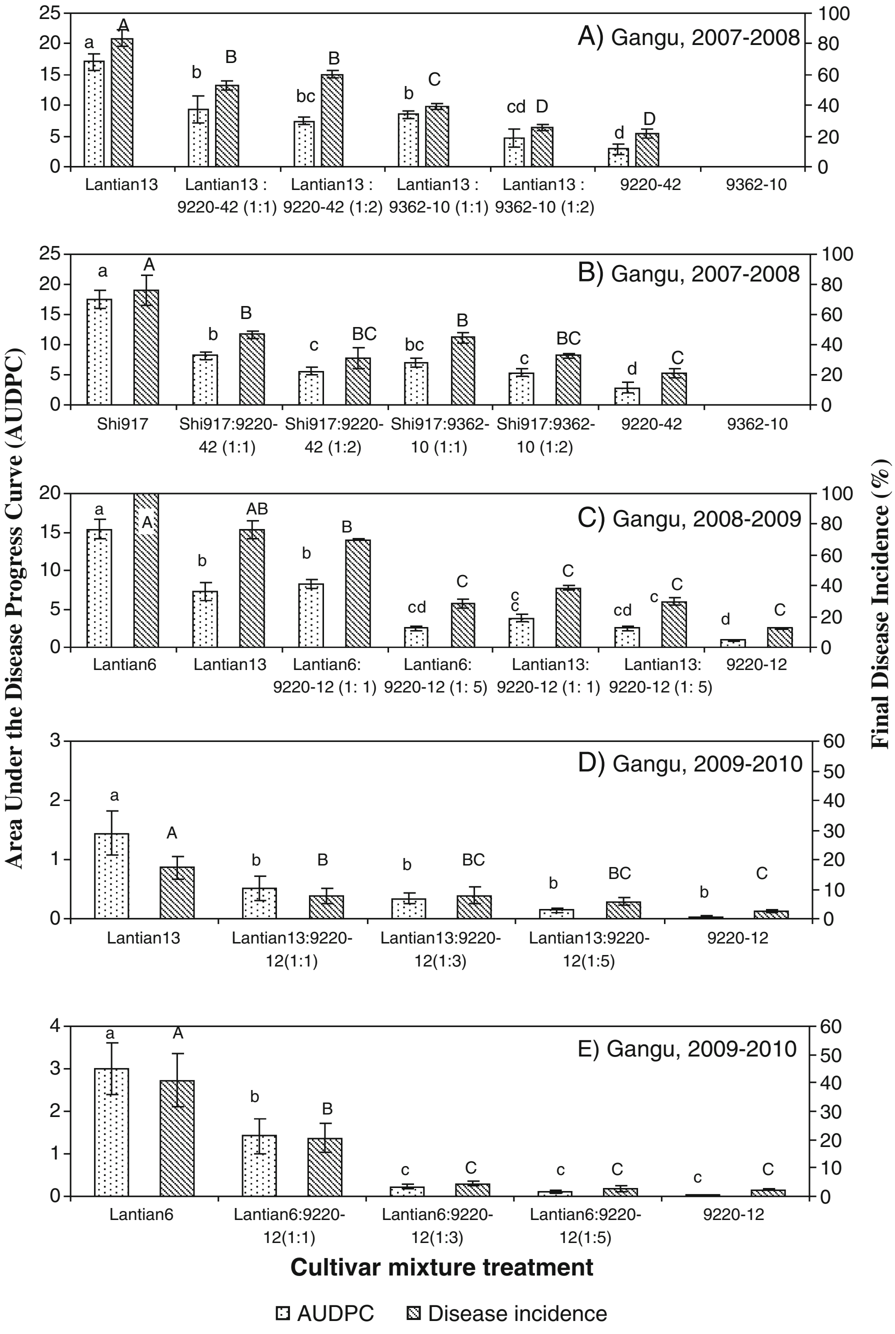

Fig. 1 Comparisons in the area under the disease progress curve (AUDPC) of wheat stripe rust epidemics and the final disease incidence among susceptible pure stands and cultivar mixtures. The experiments were conducted in the fields located in Gangu (Gansu Province) in three growing seasons from 2008 to 2010. Each bar represents the mean value from three replications. Footnote: The bars with the same lower case letter indicate no significant difference in AUDPC at $P=0.05$ among the cultivar mixture treatments, and the bars with the same upper case letter indicate no significant difference in disease incidence at $P=0.05$ among the cultivar mixture treatments

relation was observed on any disease observation dates, since the semivariance did not correlate with the lag distance for these two treatments (Fig. 4).

A slight difference in disease spatial pattern was observed in the 2008–2009 season. The semivariance in the pure susceptible stand increased with increasing lag distance in all four directions early in the season when the disease was assessed 39 and 41 days after inoculation. But no clear spatial dependences were observed for all directions early but not late in the season. Comparatively, the mixture treatments of Lantian6 (S) with 9220–12 (R) at both ratios of 1:1 and 1:5 showed no consistent trend in the relationship between lag distance and semivariance on all four assessment dates, except possibly on day 49 after inoculation for the 1:1 ratio (Fig. 4).

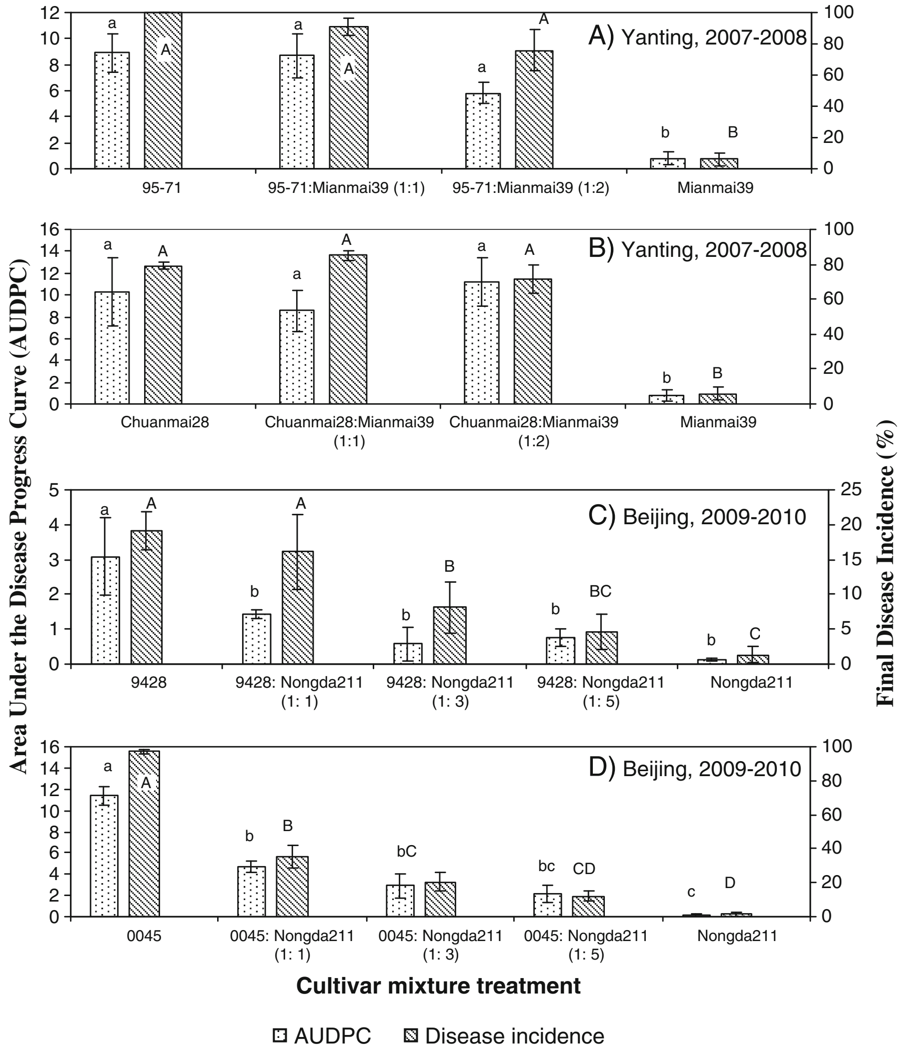

Fig. 2 Comparisons in the area under the disease progress curve (AUDPC) of wheat stripe rust epidemics and the final disease incidence among susceptible pure stands and cultivar mixtures. The experiments were conducted in the fields located in Yanting (Sichuan Province) (**a**, **b**) in the 2007–2008 growing season and in Beijing (**c**, **d**) in the 2009–2010 growing season. Each bar represents the mean value from three replications. Footnote: The bars with the same lower case letter indicate no significant difference in AUDPC at $P=0.05$ among the cultivar mixture treatments, and the bars with the same upper case letter indicate no significant difference in disease incidence at $P=0.05$ among the cultivar mixture treatments

Similar to the 2007–2008 growing season, in the 2009–2010 season the semivariances increased almost linearly with increasing lag distance up to 2.0 m and then decreased nearly linearly on all the four assessment dates on the pure stand Lantian6 (S) (Fig. 4). However, such spatial dependence was not found in any of the 4 assessment dates on the mixtures of Lantian6 (S) with 9220–12 (R) at ratios of 1:3 and 1:5, and a much weaker pattern was observed at the ratio 1:1 (Fig. 4).

Discussion

Applications of cultivar mixtures have been successfully used in disease management (Wolfe 1985; Zhu et al. 2000; Mundt 2002; Cox et al. 2004; Andrivon et al. 2003). Disease reductions caused by cultivar mixtures may be expressed in both temporal and spatial aspects in the field. Concerning temporal disease development, this study provides evidence showing that wheat cultivar mixtures often reduced wheat stripe rust epidemics in the fields when compared with susceptible pure stands. This effect was observed as a significant reduction of disease incidence, AUDPC and the epidemic rate in most cases (Beijing and Gangu regions) but with some exceptions (Yanting regions). However, this reduction in the disease level was not always better than a simple average of disease levels among susceptible and resistant cultivars in the mixture (i.e. *REM* was not always less than 1).

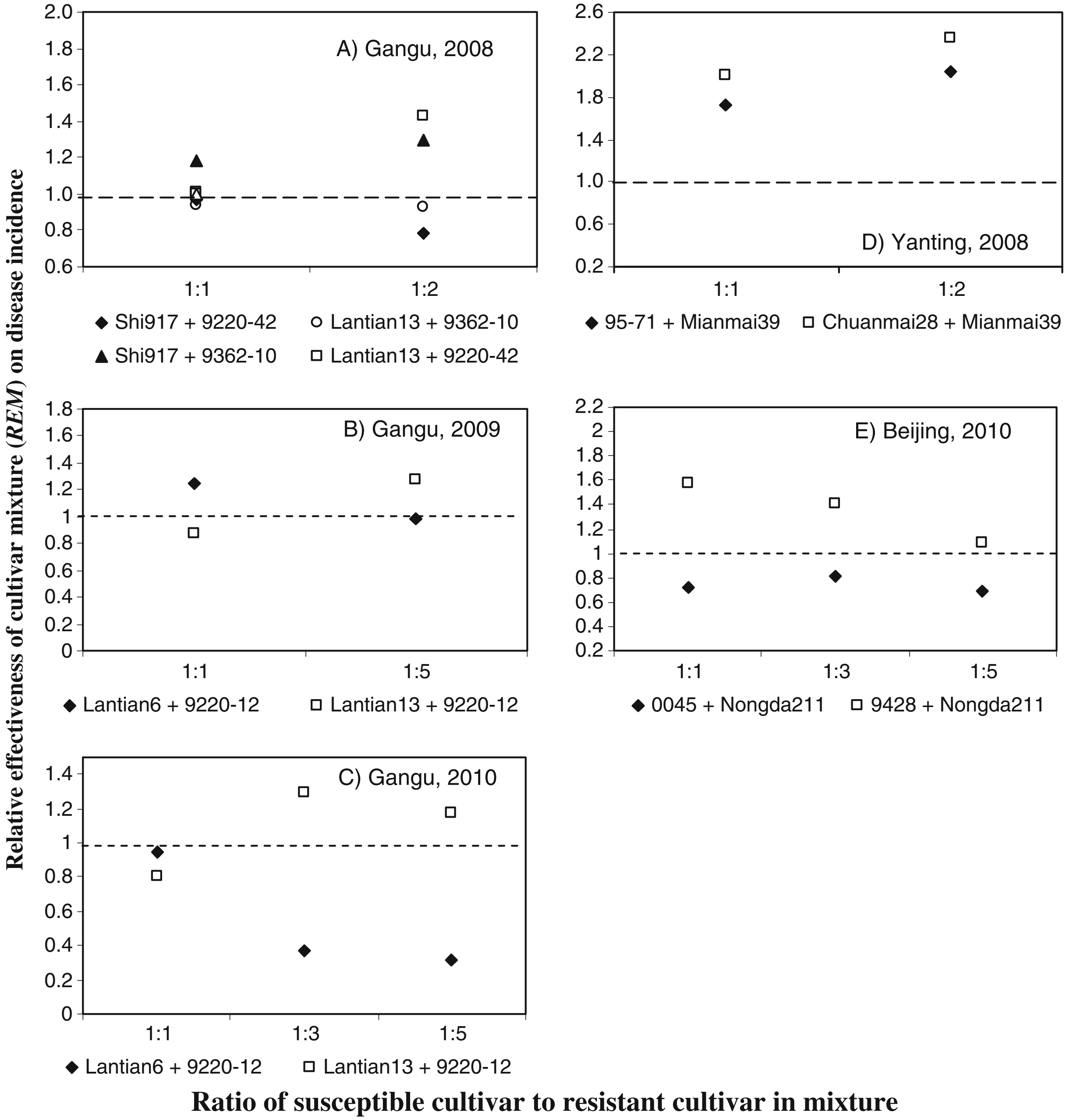

Fig. 3 Relative effectiveness of cultivar mixture on stripe rust disease incidence (*REM*) (see the text for the corresponding definitions) for different cultivar mixture treatments. Data were obtained from field experiments conducted in three growing seasons in Gangu (Gansu Province) from 2008 to 2010 (**a**–**c**), Yanting (Sichuan Province) (**d**) in the 2007–2008 growing season and Beijing (**e**) in the 2009–2010 growing season in China. Different cultivar mixtures were used in each field

Simulations have shown that the effect of cultivar mixtures on reduction of disease was influenced by the initial pattern of disease (Xu and Ridout 2000). In this study, the mixture effect was higher when the disease was initiated by artificial focal inoculation (Beijing and Gangu) rather than that initiated naturally (Yanting). For instance, in the Gangu field in 2007–2008 growing season, the disease incidence was decreased by 53.3% in mixture of resistant cultivar 9220–42 with the susceptible Shi917 at 1:1 against

Table 3 Comparisons in epidemic rate of wheat stripe rust among the different cultivar mixture treatments and their component pure stands in the Beijing and Yanting fields for different time periods

Mixture component and ratio	Epidemic rate (±SE)[a]			R^2	Mixture component and ratio	Epidemic rate (±SE)			R^2
Yanting, 2007–2008 growing season	Before March 21	After March 21	Whole growing season		*Yanting, 2007–2008 growing season*	Before March 21	After March 21	Whole growing season	
Chuanmai28 (MS)	0.187±0.075 a	0.468±0.011 a	0.428±0.007 a	0.986[c]	95-71 (S)	0.480±0.104 a	0.382±0.023 a	0.394±0.0049 a	0.971[c]
Chuanmai28: Mianmai39 (1:1)	0.170±0.012 a	0.205±0.010 b	0.198±0.009 b	0.924[c]	95-71: Mianmai39 (1:1)	0.241±0.085 ab	0.127±0.010 b	0.140±0.0191 b	0.899[b]
Chuanmai28: Mianmai39 (1:2)	0.244±0.006 a	0.161±0.016 b	0.173±0.016 b	0.988[c]	95-71: Mianmai39 (1:2)	0.230±0.026 b	0.101±0.042 b	0.119±0.0320 bc	0.968[c]
Mianmai39 (R)	0.159±0.043 a	0.028±0.019 b	0.050±0.008 b	0.886[b]	Mianmai39 (R)	0.159±0.043 b	0.028±0.019 b	0.050±0.0076 c	0.886[b]
Beijing, 2009–2010 growing season	Before May 16	After May 16	Whole growing season	R^2	*Beijing, 2009–2010 growing season*	Before May 16	After May 16	Whole growing season	R^2
9428 (MS)	0.273±0.074 a	0.095±0.042 ab	0.173±0.013 a	0.790[b]	0045 (S)	0.365±0.055 a	0.302±0.07 a	0.298±0.025 a	0.788[b]
9428:Nongda211(1:1)	0.207±0.052 ab	0.102±0.030 ab	0.133±0.009 a	0.983[c]	0045:Nongda211(1:1)	0.241±0.014 ab	0.134±0.02 b	0.188±0.022 b	0.901[c]
9428:Nongda211(1:3)	0.070±0.054 c	0.193±0.046 a	0.126±0.027 a	0.820[b]	0045:Nongda211(1:3)	0.180±0.066 bc	0.134±0.02 b	0.171±0.015 b	0.902[c]
9428:Nongda211(1:5)	0.139±0.070 abc	0.093±0.019 ab	0.124±0.019 a	0.714[b]	0045:Nongda211(1:5)	0.168±0.040 bc	0.071±0.01 b	0.116±0.012 bc	0.846[b]
Nongda211 (R)	0.073±0.060 bc	0.040±0.020 b	0.050±0.030 b	0.990[c]	Nongda211 (R)	0.073±0.060 c	0.040±0.02 b	0.050±0.030 c	0.990[c]

[a] Mean and corresponding standard error from three replicates calculated from logistic equation. The mean values with the same letter in a column for each treatment of each location indicate no significant difference at P=0.05, and [b] and [c] denote the significance of regression at 0.05 and 0.01 level, respectively.

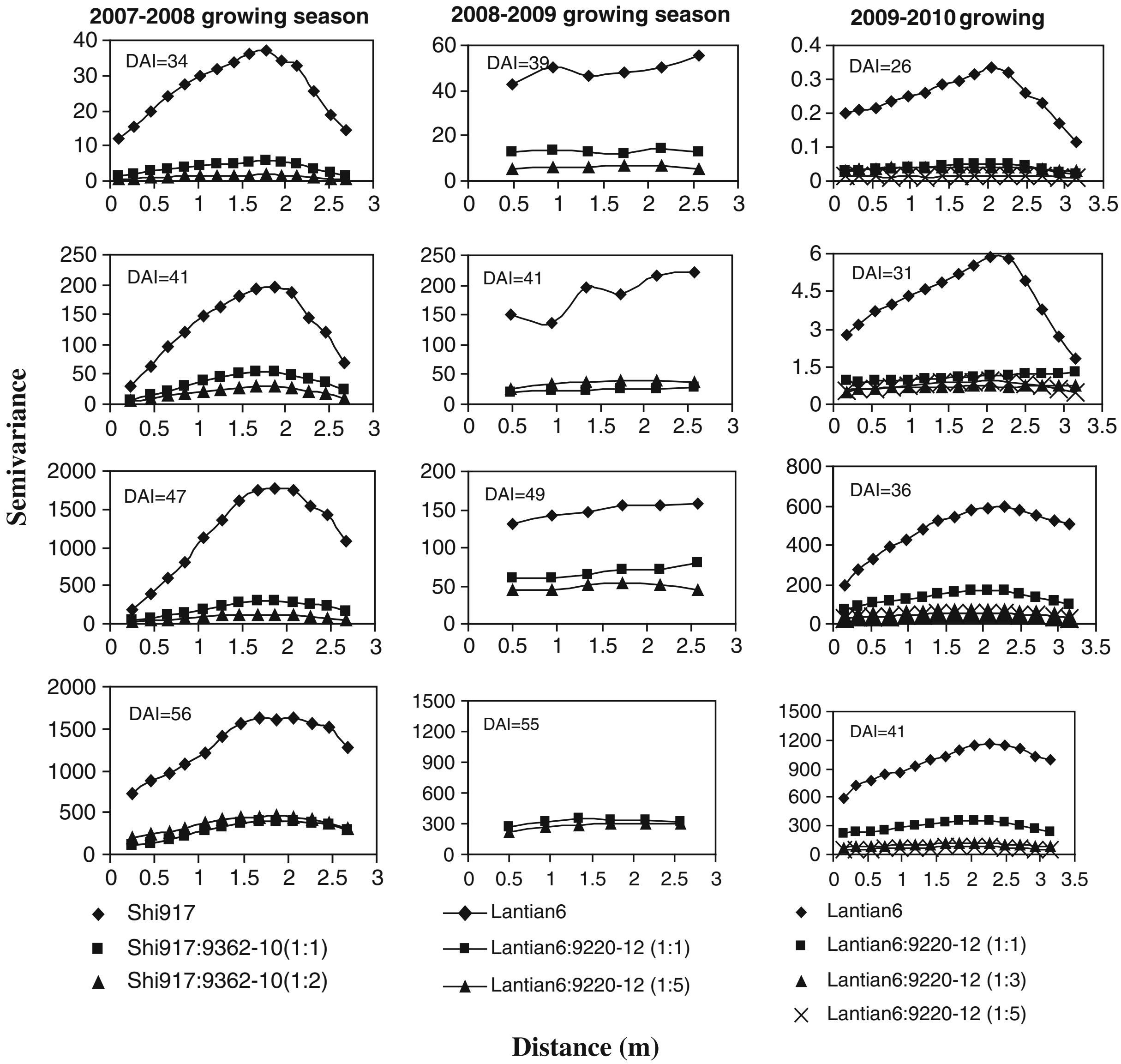

Fig. 4 Semivariograms (semivariance of incidence of wheat stripe rust against lag distance) for a pure susceptible cultivar and mixtures of the susceptible cultivar with a resistant cultivar. Footnote: Each plot corresponds to one assessment date (expressed as days after inoculation) made in one of three growing seasons from 2008–2010 in the Gangu field experiments (dates when incidence was too close to 100% are not presented). Analyses are presented for omnidirectional semivariograms because all directional semivariograms were very similar

the pure stands of Shi917 (S) in the final disease assessment. However in the Yanting field, the disease incidence was decreased by only 2.7% in the mixture of resistant cultivar Mianmai39 with the susceptible of 95–71 at 1:1 in comparison with the pure stands 95–71 (S).

Modelling studies (Mundt and Brophy 1988; Xu and Ridout 2000) and field experiments (Mundt et al. 1996; Andrivon et al. 2003; Cox et al. 2004; Didelot et al. 2007) have shown that the rate of disease increase in mixtures can be reduced significantly, compared to those on susceptible pure stands, but the period of time when cultivar mixture can affect epidemic development has still not been determined. In this study, the rates of stripe rust development of most mixtures were significantly less than those of susceptible pure stands. The time period when the mixtures significantly affected the epidemics was again influenced by initial disease. In Gangu and Beijing where the diseases was initiated by artificial focal inoculation, the reduction of epidemic rate mainly occurred early in the season. Such reductions were also correlated with the reductions in the whole-season epidemic rates for all but one of the 15 mixture treatments. In Yanting where the disease was naturally initiated, any such reduction mainly occurred late in the season.

When the epidemics were initiated by focal inoculum, there were few primary infection foci in the early period of the epidemics. Resistant plants in the mixtures reduced disease spread by acting as a barrier to reduce new infections. However, as the epidemics developed and more infections occurred, such an effect decreased. In Yanting, the effect of mixture on disease reduction appeared to be not significant at first but became significant later in the season. When the number of potential infection sites approached the maximum (Yanting region), the effect of mixtures on reduction of the disease was reduced. The findings were consistent with those from Mundt (2002) and modelling results (Xu and Ridout 2000).

Regarding the spatial aspect, resistant cultivars in mixtures are known to reduce disease spread through two mechanisms: they can act as a physical barrier to inhibit the spread of the disease, and the inoculum is diluted (Chin and Wolfe 1984; Xu and Ridout 2000; Mundt 2002). Spatial analysis in this study confirmed these findings: wheat stripe rust did not show large (2 m) disease clusters in mixtures, contrary to susceptible pure stands. Since pure stand and mixture treatments were allocated randomly to the field plots, these results indicated that the cultivar mixture disrupted the spatial rust spread or reduced its range to a spatial distance smaller than the sampling size (10 or 20 cm). No matter what spatial patterns were observed in pure stand treatments, such spatial dependence was not observed for any mixture treatments either early or late in the season. Thus, it can be concluded that the mixture prevented the development of any spatial pattern, probably through the limitation of disease spread.

Cultivar mixtures have been used in some countries as one of the disease control strategies (Wolfe 1985; Mundt 2002) and have been promoted more in China (Zhu et al. 2000). Here, we showed that the relative effect of the mixture was not always satisfactory for disease control (i.e. the disease level in mixtures was not always less than the average of the susceptible and resistant pure stands) and that the efficiency, as well as spatial and temporal effects on the disease, of the mixture may depend on factors such as the initial pattern of the disease and climatic conditions. The effect of mixtures can be strengthened when combined with appropriate application of fungicides to reduce disease epidemics (Garrett et al. 2001) and the combination of these control methods should be further studied. Pathogen population dynamics in the cultivar mixtures, such as changes in frequencies of different races, need to be studied as well to determine the optimal proportions of cultivars in the mixtures to balance the proportions of races, which is important in wheat stripe rust management. Knowledge on the spatial and temporal effects of cultivar mixtures on disease development could be used in disease management in combination with consideration of environmental factors. This study showed that for different regions where the level of initial inoculum of wheat stripe rust differs, strategies for using cultivar mixtures could be different.

Acknowledgments This study was supported by the Special Fund for Agro-Scientific Research in the Public Interests (200903035) and the National Natural Science Foundation of China (31071642). The authors gratefully thank Jie-bin Guo and Lei Zhao of China Agricultural University, and She-lin Jin, Man-ku Guo, Ming-an Jin, Shi-qin Cao, and Hui-sheng Luo, Institute of Plant Protection of Gansu province, for their assistance in the field experiments at Gangu. The authors also thank Yue-fu Liu and his colleagues, Yanting Plant Protection Station, for their help with the field trials at Yanting.

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