Field Distribution of Wheat Stripe Rust Latent Infection Using Real-Time PCR

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Abstract

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Stripe rust of wheat, caused by *Puccinia striiformis* f. *tritici*, is of worldwide significance. Quantification of latent infection level is critical to estimate the potential for disease epidemics. In this study, field distribution of latent infection and the corresponding observed disease were studied in two growing seasons from 2009 to 2011 in Gangu, Gansu Province and Shangzhuang, Beijing, China. A previously developed real-time polymerase chain reaction (PCR) assay was applied to obtain the molecular disease index (MDX) to quantify the level of latent infection. At 1 to 3 weeks after leaf sampling, the observed disease indices (DX) were assessed in the corresponding experimental

sites. The computer software SURFER showed that the spatial distribution patterns of MDX had a linear relationship with DX in field plots with $P = 0.01$. The aggregation levels of MDX correlated with those of DX in the fields. The disease foci which were correctly detected for latent infections with the real-time PCR for the Gangu and Shangzhuang field plots were 71.4 and 85.7%, respectively. The triadimefon fungicide treatment focused on the detected latent infection foci reduced both the initial inoculum and disease development, resulting in an average reduction in disease area in the field plots of 73 to 81%.

The fungal pathogen *Puccinia striiformis* f. *tritici* Erikss. causes stripe rust of wheat, a devastating disease with worldwide distribution (5,6,13,21,25). Disease management relies on host resistance and fungicide applications. Prediction of disease development trends during the growing season would provide important information for decision making on disease management. Disease prediction relies on the accurate estimation of initial inoculum or disease level early in a season to predict epidemics (27).

In autumn or early spring, infections of *P. striiformis* f. *tritici* in leaves usually exist in a latent phase and such infections usually serve as an inoculum source to initiate epidemics during the season (5,24,27). The latent period can be several days to weeks or as long as several months in the winter, depending on weather conditions at different geographic locations (28). Obviously, estimation of the latent infection level is important to estimate inoculum potential and assist in timely decision making on disease management. Control of the latent infection foci before symptom appearance may significantly reduce inoculum potential and cost of fungicides. Because *P. striiformis* f. *tritici* is an obligate fungal pathogen, incubation of leaves with latent infections is needed to promote symptom appearance. A reasonable number of plants and at least 7 to 10 days of incubation under restricted conditions are needed to estimate latent infection level. Thus, this traditional method is time consuming and labor intensive. Pan et al. (14) established a real-time polymerase chain reaction (PCR) assay to quantify the latent infection level of *P. striiformis* f. *tritici* in leaves but its potential field application needs to be confirmed with field experiments.

In this study, we conducted multi-season field experiments in two geographic locations in China: Gangu county of Gansu Prov-

ince and Shangzhuang experimental center of China Agriculture University, Beijing. In the mountainous region of Gangu, *P. striiformis* f. *tritici* overwinters in volunteer wheat (28). These populations infect the winter wheat planted in early autumn at lower elevations. At these lower elevations, *P. striiformis* f. *tritici* overwinters in infected leaves that may serve as primary inoculum the following spring. As these populations expand, their inoculum is dispersed eastward into neighboring wheat-producing regions. Thus, the latent infection level in the fields in both autumn and early spring in this region is important for estimating the inoculum potential which may influence the regional disease epidemics in China through long-distance disease spread (28). On the other hand, Beijing belongs to another epidemic zone where winter is cold and summer is hot. Here, *P. striiformis* f. *tritici* is unlikely to survive between seasons (28). The epidemics on seedlings in autumn are unusual and those in the spring season rely on the exogenous inoculum coming from southern regions. These latent spring infections can serve as a source of initial inoculum for epidemics later in the season.

The objectives of this study were to (i) determine the field spatial distributions of latent infection of wheat stripe rust and the predictability of using the established real-time PCR assay to determine the spatial distribution patterns of the disease index in the field plot, and (ii) estimate the efficacy of fungicide control by focusing on the determined latent infection foci in the fields to reduce disease development.

Materials and Methods

Gangu field plots. In Gangu, four experimental field plots were identified in each of 2 years. In the 2009–10 growing season, two field plots, GF1-1 and GF1-2, were selected in Wang Village (105.33°E and 34.68°N) at an elevation of 1,690 m. Two other fields (GF1-3 and GF1-4) were selected in Dong Village (105.35°E and 34.15°N) at an elevation of 1,770 m. The distance between these two locations is 59 km. In the 2010–11 growing season, two field plots (GF2-1 and GF2-2) were selected at Wang Village and designated as GF1-3 and GF1-4, respectively. Two other field plots, GF2-3 and GF2-4, were selected at Dong Village. Each field was divided into 36 subplots (Table 1). Table 1 provides additional information on the experimental design. The fields were rain fed and the soil fertility was low. A moderately resistant wheat ('Linfeng 157') was planted in this field.

Natural infections of stripe rust occurred in this area. In the 2009–10 season, leaf sampling was conducted at wheat growth

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stage GS22 (26; Table 1) when no wheat stripe rust symptoms were visible. In each subplot, 30 leaves were randomly collected as a replicate and processed in bulk to extract DNA, the real-time PCR was run (14), and molecular disease index (MDX) was obtained as described below. The disease incidence (DI; number of diseased leaves/total number of leaves assessed) and disease severity (DS; visually assessed average leaf area with symptoms/average total leaf area) of the corresponding subplots were assessed 9 days after leaf sampling, when wheat stripe rust symptoms appeared. The disease index (DX; 28) was then calculated as $DX = DI \times DS \times 100$. Thus, for each subplot, we obtained latent infection level as *P. striiformis* f. *tritici* DNA quantity and corresponding DX data.

The experiments were continued in autumn of the 2010–11 growing season (Table 1). The MDX was obtained for each subplot (see data analysis below) and used to draw the maps of field distribution of latent infection of wheat stripe rust (see data analysis below) for the field plots GF2-1 and GF2-3. The fungicide treatment, triadimefon 250WP (Meibang, China) was then applied to the selected latent infection foci in these two field trials on 27 October 2010. In each detected latent infection focus, triadimefon 250WP at 374.8 g ha⁻¹ was applied with water in a final volume of 1,000 liters ha⁻¹ with a back sprayer in each subplot. No-fungicide controls were arranged in the field trials GF2-2 and GF2-4. The DI and DS in the subplots of each field were then assessed after the appearance of wheat stripe rust symptoms in the control plots. Disease assessment was conducted two more times on the dates listed in Table 1.

Shangzhuang field plots. At Shangzhuang (116.46°E and 39.92°N, at an elevation of 56 m above sea level), three field plots (SF1-1, SF1-2, and SF1-3) were selected during the 2009–10 season and four field plots (SF2-1, SF2-2, SF2-3, and SF2-4) were selected during the 2010–11 season. Similarly, each field was divided into a different number of subplots (Table 2). Additional

information on the experimental design is provided in Tables 2. Flood irrigation was used in these field plots. A moderately resistant wheat, ‘Jing0045’, was planted in this field.

Due to the dry weather in spring in this region, disease promotion was performed by planting the susceptible wheat ‘Mingxian169’ in the center of each field (100 seeds) and inoculation was conducted on 16 April 2010 by spraying 30 ml of spore suspension (5×10^4 spores/ml) on leaves of these susceptible plants. The inoculated plants were covered with plastic sheets overnight to maintain high humidity. These inoculated plants started to show stripe rust symptoms on 28 April 2010 and were removed from the fields on 2 May 2010. The leaf sampling was conducted on 5 May 2010 at growth stage GS22 (26; Table 2), and 30 leaves were randomly collected as a replicate.

The experiments were continued in spring of the 2011 season (Table 2). Inoculation, leaf sampling, and processing were conducted as described for the 2010 season. Similarly as in Gangu, the fungicide treatment was applied in the selected latent infection foci in field plots SF2-1 and SF2-3 on 10 May 2011, and the nontreated controls were field plots SF2-2 and SF2-4. Disease assessment was continued on dates shown in Table 2.

DNA extraction from plant leaf tissues. To extract DNA from sampled wheat leaves, 1-cm-long sections were removed. The cetyltrimethylammonium bromide method following the previously published protocols (2), with minor modification (14,30), was applied to obtain DNA for PCR. The extracted DNA was dissolved in 100 µl of distilled water and diluted 60× and 500× for real-time PCR amplification of *P. striiformis* f. *tritici* DNA and wheat DNA, respectively.

Real-time PCR assay. The following primer pair specific to *P. striiformis* was used: Pst-F (5′-TAAGGTGTCTGATACCGTGTTCG-3′)/Pst-R (5′-GCTAGTTTACGGAGATCAGAGT-3′). The following wheat-specific primer pair was also used: TAG2315F

Table 1. Summary of experimental design in Gangu, China, field plots during the 2009–10 and 2010–11 growing seasons^a

Field variable	Field name							
	2009–10 season				2010–11 season			
	GF1-1	GF1-2	GF1-3	GF1-4	GF2-1	GF2-2	GF2-3	GF2-4
Field area (m ²)	108	108	144	144	144	144	108	108
Number of subplots per field	36	36	36	36	36	36	36	36
Subplot area (m × m)	1.5×2	1.5×2	2×2	2×2	2×2	2×2	1.5×2	1.5×2
Variety	Lintao157	Lintao157	Lintao157	Lintao157	Lintao157	Lintao157	Lintao157	Lintao157
Seed-sowing date	26-09-09	26-09-09	14-09-09	14-09-09	28-09-09	28-09-09	16-09-09	16-09-09
Planting density (seeds/m ²)	450	450	450	450	450	450	450	450
Leaf sampling date	19-03-10	19-03-10	19-03-10	19-03-10	19-10-10	19-10-10	19-10-10	19-10-10
Fungicide treatment date	...	...	...	...	27-10-10	...	27-10-10	...
Disease assessment date	28-03-10	28-03-10	28-03-10	28-03-10	11-11-10	11-11-10	11-11-10	11-11-10
	...	...	...	...	21-11-10	21-11-10	21-11-10	21-11-10
	...	...	...	...	01-12-10	01-12-10	01-12-10	01-12-10

^a Dates given in day-month-year format (e.g., 26-09-09 = 26 September 2009).

Table 2. Summary of experimental design in Shangzhuang, China, field plots during the 2009–10 and 2010–11 growing seasons^a

Field variable	Field name							
	2009–10 season				2010–11 season			
	SF1-1	SF1-2	SF1-3	SF2-1	SF2-2	SF2-3	SF2-4	
Field area (m ²)	36	36	36	36	36	36	36	
Number of subplots per field	36	36	36	36	36	36	36	
Subplot area (m × m)	1×1	1×1	1×1	1×1	1×1	1×1	1×1	
Variety	Jing0045	Jing0045	Jing0045	Jing0045	Jing0045	Jing0045	Jing0045	
Seed-sowing date	30-09-09	30-09-09	30-09-09	01-10-10	01-10-10	01-10-10	01-10-10	
Planting density (seeds/m ²)	420		420	420	420	420	420	
Leaf sampling date	05-05-10	05-05-10	05-05-10	08-05-11	08-05-11	08-05-11	08-05-11	
Fungicide treatment date	...	...	...	10-05-11	...	10-05-11	...	
Disease assessment date	16-05-11	16-05-11	16-05-11	14-05-11	14-05-11	14-05-11	14-05-11	
	...	...	...	19-05-11	19-05-11	19-05-11	19-05-11	
	...	...	...	24-05-11	24-05-11	24-05-11	24-05-11	

^a Dates given in day-month-year format (e.g., 26-09-09 = 26 September 2009).

(5'-CAGAAAGCGAGTGGAAAGATGAAAG-3')/TAG2473R (5'-GCAAGGAGGACAAAGATGAGGAA-3') (14).

Real-time PCR amplifications were performed using the SYBR Green I fluorescent dye. Amplifications were conducted in a 20- μ l volume containing 10 μ l of SYBR Premix Ex Taq (TAKARA Corp., Dalian, China), 0.4 μ l of ROX Reference Dye (50 $\times$), 1 μ l of template DNA, 1 μ l of either forward or reverse primer (2 μ M each), and 7.6 μ l of distilled H₂O. The following conditions were used for real-time PCR with primer pair Pst-F/Pst-R: an initial preheat for 30 s at 95°C, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s. The following condition was used to amplify the fragment of wheat genomic DNA using the primer pair TAG2315F/TAG2473R: an initial preheat for 30 s at 95°C, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 67°C for 20 s, and extension at 72°C for 30 s. After each run, the corresponding melting curve was analyzed for each sample by determining the curve peak position to confirm the specific PCR product. By using the primer pair Pst-F/Pst-R and *P. striiformis* f. *tritici* DNA, the peak of melting curve was at 85.5 to 86°C, with a range of 84 to 87°C, whereas, using the primer pair TAG2315F/TAG2473 and wheat DNA, the peak of the melting curve was at 83.8 to 84.3°C with a range of 81.5 to 85°C.

The standard curves with 10 $\times$ serial dilutions were generated by using DNA standards for *P. striiformis* f. *tritici* and wheat with known concentrations established by Pan et al. (14). Both standards for *P. striiformis* f. *tritici* and wheat DNA were used in each run with the unknown samples. The equation for *P. striiformis* f. *tritici* DNA using the primer pair Pst-F/Pst-R was $y = -0.2891x + 9.1852$ ($r^2 = 0.9946$, $P < 0.001$), where x is the cycle threshold (Ct) value and y is the corresponding log₁₀ DNA quantity in picograms. The equation for wheat DNA using the primer pair TAG2315F/TAG2473 was $y = -0.48x + 16.11$ ($r^2 = 0.9957$, $P < 0.01$), where x

is the Ct value and y is the corresponding log₁₀ DNA quantity in nanograms.

Data analysis. The previously defined MDX (14) was applied as MDX = quantity of *P. striiformis* f. *tritici* DNA (picograms)/quantity of wheat DNA (nanograms) for each sample. The subplot data of both MDX and DX were analyzed by using the computer program SURFER (Surfer 8.0; Golden Software, Golden, CO) to show the spatial distribution of both MDX and DX for each field plot, from which the foci of MDX and DX could be identified. For each field plot in the first season, the replicated subplot data of MDX versus the corresponding DX were used in linear regression analysis with the GLM procedure of SAS (version 9.1; SAS Institute, Cary, NC).

Geostatistics were applied to determine the aggregation levels of both MDX and DX in the field plots. The variance-to-mean (VM) ratio (9) was measured to determine the aggregation level in the field plots as $VM = S^2/X$, where S^2 is the sample variance and X is the sample mean. When $VM < 1$, $VM = 1$, and $VM > 1$, the distributions were considered as a regular spatial pattern, a random pattern, and an aggregated pattern, respectively. The index of dispersion, D , as the ratio of the observed variance of the variable (MDX or DX) among the sampling units to the expected binomial variance (10), was also analyzed. The situations when $D > 1$, $D = 1$, and $D < 1$ were considered as an aggregation pattern, a random pattern, and an under-dispersed pattern (20), respectively. Here, $(N - 1) \times D$ has a χ^2 distribution under the null hypothesis of randomness with $N - 1$ degrees of freedom (where N is number of subplots in each field plot). Both VM and D were calculated for Gangu and Shangzhuang field plots for two seasons.

To evaluate the efficacy of the fungicide treatment on latent foci of wheat stripe rust, each field spatial distribution map of MDX or DX generated with SURFER was used to determine the latent

Fig. 1. Field distributions of molecular disease index (MDX) for wheat stripe rust in four field plots: **A1**, GF1-1; **B1**, GF1-2; **C1**, GF1-3; and **D1**, GF1-4. The corresponding stripe rust disease index (DX) for these field plots is shown in plates **A2**, **B2**, **C2**, and **D2**, respectively. Experiments were conducted in Gangu, Gansu Province of China during spring 2010.

infection area and diseased area, respectively, by placing the corresponding distribution map on a small-grid-scale sheet (10 by 10 grids/m²) to calculate the field latent infection area or disease area. In the nontreated control field plots, the disease area was usually greater than the latent infection area. Thus, the corrected proportion of disease area (*C*) was calculated as $C = (Pd - Pl)/Pl$, where *Pd* is the proportion of the disease area (disease area divided by the total area of the field as shown with DX), and *Pl* is the proportion of latent infection area (the area with detected latent infection divided by the total area of the field as shown with MDX). For each field, the average *C* (CA) was calculated based on the data from the corresponding field plots. For the fungicide-treated fields, the corrected latent infection area (CLA) was calculated as $CLA = LA(1 + CA)$, where *LA* was the latent infection area detected in a field plot.

The fungicide control efficacy was then estimated as $P_r = (CLA - DAT)/CLA \times 100$, where *P_r* is the percentage of reduction in disease area, and *DAT* is the disease area with fungicide treatment in the field. The average *P_r* was calculated for the field plots having fungicide treatment. Additionally, for the 2010 autumn season in Gangu and 2011 spring season in Shangzhuang, the DX values versus the corresponding disease assessment dates were used to draw the disease progress curve, from which the area under the disease progress curve (AUDPC) was calculated for each field plot at the two locations using an SAS program generated by the authors.

Results

Gangu field plots. In GF1-1, a focus of latent infection at a higher level and two small foci with lower infection levels were identified (Fig. 1A1), which matched the DX distribution (Fig. 1A2). In GF1-2, latent infections were detected (Fig. 1B1), which developed to several disease foci around the area (Fig. 1B2). Moreover, when the smaller scale of MDX map was used in Figure 1B1,

the distribution of latent infection matched that of DX (*data not shown*). The GF1-3 showed that five major latent infection foci were detected (Fig. 1C1) and three of them developed into major disease foci plus one with lower DX values (Fig. 1C2). Quite similar distributions between MDX (Fig. 1D1) and DX (Fig. 1D2) in GF1-4 were observed. The corresponding linear regressions between MDX and DX for these four field plots were all significant at $P = 0.01$, with r^2 values of 0.8136, 0.8845, 0.5928, and 0.9661 for GF1-1, GF1-2, GF1-3, and GF1-4, respectively.

In autumn 2010, GF2-2 showed a latent infection focus (Fig. 2B1) which developed to the disease focus at exactly the same location (Fig. 2B2). In GF2-1, three latent infection foci were identified (Fig. 2A1) and treated with the fungicide, which resulted in a clear distribution pattern for DX (Fig. 2A2). Similarly, a relatively severe latent infection focus was identified (Fig. 2D1), plus three foci with lower infection levels in GF2-4 (Fig. 2D1). Two of the latent infection foci developed to clear disease foci with low DX levels (Fig. 2D2), while the others did not (Fig. 2D2). Two major latent infection foci were identified in GF2-3 (Fig. 2C1) and treated with the fungicide, which resulted in a clear disease distribution (Fig. 2C2). Compared with the areas of the disease distribution in GF2-2 and GF2-4, the average *P_r* in GF2-1 and GF2-3 was 72.9%. The AUDPCs of GF2-1 and GF2-3 were 1.15 and 1.58, respectively, while those of GF2-2 and GF2-4 were 5.18 and 10.53, respectively.

Shangzhuang field plots. In spring 2010, the field distribution patterns of MDX matched the field distribution patterns for disease in all three field plots (Fig. 3). The corresponding linear regressions between MDX and DX were all significant at $P = 0.01$, with r^2 values of 0.7423, 0.5784, and 0.8584 for SF1-1, SF1-2, and SF1-3, respectively.

In spring 2011, the detected latent infection foci (Fig. 4B1) all developed to the disease foci with various disease levels in SF2-2 (Fig. 4B2). For SF2-1, the identified latent infection foci were

Fig. 2. Field distributions of the molecular disease index (MDX) for wheat stripe rust in four field plots: **A1**, GF2-1; **B1**, GF2-2; **C1**, GF2-3; and **D1**, GF2-4. The corresponding stripe rust disease index (DX) for these field plots is shown in plates **A2**, **B2**, **C2**, and **D2**, respectively. Experiments were conducted in Gangu, Gansu Province of China in autumn 2010. Field plots GF2-1 and GF2-3 were treated with the fungicide triadimefon 250WP at 374.8 g ha⁻¹ based on the latent infection foci detected in A1 and C1. Field plots GF2-2 (B1 and B2) and GF2-4 (D1 and D2) were nontreated plots.

treated with the fungicide (Fig. 4A1), which resulted in a clear distribution pattern for DX (Fig. 4A2). Similarly, SF2-4 showed several latent infection foci (Fig. 4D1), which developed into disease foci (Fig. 4D2). In SF2-3, several latent infection foci were treated with the fungicide (Fig. 4C1), which resulted in a clear distribution pattern for DX (Fig. 4C2). Compared with the areas of the disease spatial distribution in SF2-2 and SF2-4, the average P_r

in SF2-1 and SF2-3 was 81.1%. The AUDPCs of field plots SF2-1 and SF2-3 were 0.69 and 0.76, respectively. Comparatively, the AUDPCs for field plots SF2-2 and SF2-4 were 2.49 and 2.62, respectively.

Geostatistical analysis (Table 3) demonstrated that the aggregation levels of MDX correlated well with those of DX, and the corresponding correlation coefficients for VM and D between

Fig. 3. Field distributions of the molecular disease index (MDX) for wheat stripe rust in three field plots: **A1**, SF1-1; **B1**, SF1-2; and **C1**, SF1-3. The corresponding stripe rust disease index (DX) for these field plots is shown in plates **A2**, **B2**, and **C2**, respectively. Experiments were conducted in Shangzhuang experimental center of China Agricultural University, Beijing, in spring 2010.

MDX and DX were 0.88 and 0.91 ($P < 0.01$), respectively. The field plots of GF1-1, GF1-2, SF1-1, SF1-2, and SF1-3 showed high aggregation levels for both MDX and DX, while others exhibited under-dispersion (Table 3).

Discussion

The real-time PCR assay was shown to be a valuable approach for quantifying latent infection levels and estimating potential disease intensity for wheat stripe rust in the field. The field distribution of latent infection determined by the PCR assay matched the stripe rust field distributions. The average focus-targeting rates for Gangu and Shangzhuang were 71.4 and 85.7%, respectively. By targeting latent infection foci with fungicide applications, the initial inoculum could be effectively reduced, lessening the chance for a rust epidemic.

MDX was used as a measurement of latent infection level which was quantified with real-time PCR. Measuring both *P. striiformis* f. *tritici* and host DNAs, the MDX value takes into consideration not only the DI but also the DS. Good correlations between MDX and DX from this study demonstrated the usefulness of MDX to estimate potential disease intensity in the near future. However, disease development from latent infections in the field depends on many factors such as environmental (temperature, humidity, and solar radiation) and biological (host resistance and pathogen virulence) effects on disease development. In Gangu during spring 2011, latent infection levels were very low and very little disease developed. Thus, it seems impossible to obtain a standard and universal relationship between quantities of MDX and DX applicable to all fields. However, the approach can at least be used to determine whether latent infections exist in leaves. Because the major factor affecting appearance of stripe rust from latent infection is temperature (24), latent infection level can most likely be considered as the maximal potential of disease level in the near future. If this variable was combined with other approaches in which environmental and biological effects on disease are considered, the prediction of future disease intensity could be more accurate and applicable.

Improvement of the methodology used in this study to fit the needs for large-scale samplings and regional disease prediction will require additional work. To estimate initial inoculum intensities on a regional scale, appropriate sampling time, sampling method, and sample size need to be investigated further. The ultimate goal of this study was to establish an approach to quickly process large samples and accurately estimate possible inoculum intensity early in a season for large areas.

Using the information of detected MDX to estimate potential DX assumes that there is no exogenous inoculum. In other words, no new infections were assumed to occur during the prediction period. In the Shangzhuang field plots, the correlations in field distribution between MDX and DX were higher than those from the Gangu field plots. The weaker correlation at Gangu field plots may be due to exogenous inoculum. Thus, the variation or inaccuracy in estimation of DX by using MDX might become greater if new infections occur in the fields. The greatest potential for using this molecular approach is to estimate the latent infection levels just before and after winter in the locations where the epidemics get initiated.

The real-time PCR assay was effective at determining latent infection levels. The latent infection level can even be obtained on the same day as leaf sampling, making it possible to quickly obtain results. The unit cost per sample can also be reduced by processing larger samples. Thus, establishment of a standard system to routinely process field samples and to rapidly provide necessary information at the regional level might be possible.

The real-time PCR had been widely used to efficiently identify the plant pathogens (17) from samples of soil (1,3,7), air (8,19), seeds (11), and plant tissues (4,12,15,16,22) to quantify the inoculum or infection level. For instance, the real-time PCR assays were used to determine the correlations between DNA concentration of *Fusarium graminearum* in grain samples and the DI (18,22), to

quantify the rice leaf blast pathogen *Magnaporthe grisea* in rice leaf tissues and to screen for host resistance (15), to quantify the DNA of the pathogen *Phaeocryptopus gaumannii* in needles of Douglas-fir early in the first year of colonization (23), to assess DS and quantify the development of fungal pathogens *Alternaria brassicicola* and *Botrytis cinerea* in *Arabidopsis thaliana* (4), and to quantify the infection level of *Verticillium* wilt diseases in olive root tissues caused by different *Verticillium dahliae* pathotypes (12). However, most studies did not focus on determining the spatial distribution of latent infection in the fields. This study provides an example in this area.

Fig. 4. Field spatial distributions of the molecular disease index (MDX) for wheat stripe rust in four field plots: **A1**, SF2-1; **B1**, SF2-2; **C1**, SF2-3; and **D1**, SF2-4. The corresponding stripe rust disease index (DX) for these field plots is shown in plates **A2**, **B2**, **C2**, and **D2**, respectively. Experiments were conducted in Shangzhuang experimental center of China Agricultural University, Beijing, in spring 2011. Field plots SF2-1 and SF2-3 were treated with the fungicide triadimefon 250WP at 374.8 g ha⁻¹ based on latent infection foci detected in A1 and C1. Field plots SF2-2 and SF2-4 served as nontreated controls.

Table 3. Values of variance-to-mean (*VM*), index of dispersion (*D*), and the corresponding χ^2 used to analyze aggregation levels of molecular disease index (MDX) and disease index (DX) of wheat stripe rust in different field plots of two locations in this study^a

Season	Field plot	MDX			DX		
		<i>VM</i>	<i>D</i>	χ^2	<i>VM</i>	<i>D</i>	χ^2
2009–10	GF1-1	9.81	2.30	125.91	1.72	2.60	91.09
2009–10	GF1-2	1.84	0.55	23.37	1.00	1.51	53.00
2009–10	GF1-3	0.17	0.15	2.13	0.17	0.26	9.24
2009–10	GF1-4	0.38	0.11	4.82	0.29	0.44	15.57
2010–11	GF2-1	1.63	0.49	20.79	0.03	0.04	1.52
2010–11	GF2-2	2.76	0.84	35.47	0.11	0.17	6.02
2010–11	GF2-3	0.79	0.24	10.01	0.05	0.07	2.72
2010–11	GF2-4	0.48	0.14	6.10	0.08	0.12	4.29
2009–10	SF1-1	7.78	2.36	99.20	2.92	4.40	154.06
2009–10	SF1-2	7.53	2.28	95.78	1.63	2.47	86.62
2009–10	SF1-3	4.92	1.48	62.57	1.32	1.98	69.33
2010–11	SF2-1	0.62	0.19	7.88	0.02	0.04	1.42
2010–11	SF2-2	1.16	0.35	14.82	0.04	0.05	2.06
2010–11	SF2-3	0.58	0.18	7.42	0.03	0.05	1.73
2010–11	SF2-4	0.57	0.17	7.19	0.04	0.06	2.11

^a MDX and DX were significantly correlated ($P < 0.01$) for *VM* (correlation coefficient = 0.88) and *D* (correlation coefficient = 0.91). MDX = quantity of *Puccinia striiformis* f. *tritici* DNA (picograms)/quantity of wheat DNA (nanograms) for each sample. DX = DI $\times$ DS $\times$ 100, where DI = disease incidence = number of diseased leaves/total number of leaves assessed and DS = disease severity = average area with symptoms/average total leaf area. The *VM* ratio was measured to determine the aggregation level in the field plots as $VM = S^2/X$, where S^2 is the sample variance and X is the sample mean. *D* was analyzed as the ratio of the observed variance of the variable (MDX or DX) among the sampling units to the expected binomial variance. Under the null hypothesis of randomness, $(N - 1) \times D$ has a χ^2 distribution, where N is number of subplots in each field plot.

There is considerable potential for using this approach to aid in disease management in some regions of China (28), where the overwintering local inoculum plays an important role in spring epidemics. The initial inoculum potential can be determined just before the winter by determining the MDX values. Therefore, the growers may have enough time to prepare for disease management early the next spring or even treat the severe latent infection foci in the autumn. Early disease control can save time and costs through more timely disease management, especially at early low initial inoculum levels. For the regions where *P. striiformis* f. *tritici* can overwinter and infects seedlings in the autumn, early, efficient, and accurate determination of latent infection level plus timely disease management could significantly reduce the amount of potential inoculum. Although this study demonstrated the efficacy of disease management by fungicide application in latent infection foci early in the season, more intensive studies are needed which should involve multi-year, multi-location experiments under different disease intensity levels.

In terms of long-distance disease spread, significant reduction of inoculum in source regions could prove to be an important regional disease management strategy (28). Latent infection level has the potential to be combined with disease forecast models (29) to assess the possible risks of regional disease epidemics based on both disease levels and climatic and weather information.

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