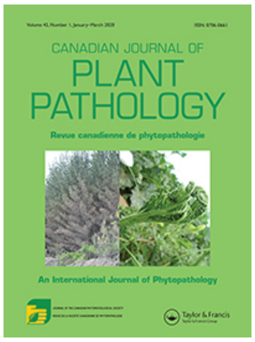

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Epidemiology/Épidémiologie

Spore concentrations of *Blumeria graminis* f. sp. *tritici* in relation to weather factors and disease development in Gansu, China

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Abstract: Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is an important disease in China. In this study, airborne spore concentrations were monitored using spore samplers and real-time PCR during 2013– 2015 at three locations in Gangu, Gansu province of China. The effects of meteorological variables on spore concentration were analyzed and models were developed for forecasting dynamics of airborne conidia and disease progress. The temperature, solar radiation and rainfall were positively correlated to spore concentrations and disease development. Although there was a negative correlation between relative humidity and spore concentration, relative humidity was positively related with disease development. Stepwise regression models were obtained for predicting the dynamics of airborne conidia based on the positive degree days and accumulation of rainfall (R^2 = 0.31, P < 0.01), the positive degree days (R^2 = 0.16, P = 0.04) and mean temperatures (R^2 = 0.24, P = 0.01) at three locations, respectively. Disease forecasting models were developed based on various meteorological variables and spore concentration, the most important variables being temperature and spore concentration. This knowledge can improve the disease forecasting of wheat powdery mildew in Gangu and help develop better disease control strategies.

Keywords: forecasting models, meteorological factors, spore concentration, spore sampler, wheat powdery mildew

Résumé: L'oïdium du blé, causé par *Blumeria graminis* f. sp. *tritici* (*Bgt*), est une maladie importante en Chine. Dans cette étude, les concentrations de spores aéroportées ont été suivies à l'aide d'échantillonneurs de spores et de la PCR en temps réel, de 2013 à 2015, à trois sites dans le district de Gangu, dans la province du Gansu, en Chine. Les effets des variables météorologiques sur la concentration des spores ont été analysés et des modèles ont été développés pour prédire la dynamique des conidies aéroportées et la progression de la maladie. La température, l'ensoleillement et la quantité de pluie étaient positivement corrélés aux concentrations de spores et au développement de la maladie. Bien qu'il y eût une corrélation négative entre l'humidité relative et la concentration de spores, l'humidité relative était positivement associée au développement de la maladie. Des modèles utilisant la régression séquentielle ont été obtenus pour prédire la dynamique des conidies aéroportées en se basant sur les degrés-jours positifs et l'accumulation de pluie (R^2 = 0,31, P < 0,01), les degrés-jours positifs (R^2 = 0,16, P = 0,04) et les températures moyennes (R^2 = 0,24, P = 0,01) à trois sites, respectivement. Les modèles de prévision de la maladie ont été basés sur diverses variables météorologiques et concentrations de spores, les plus importantes étant la température et la concentration de spores. Ces connaissances peuvent contribuer à améliorer la prédiction de l'occurrence de l'oïdium du blé dans le district de Gangu et à développer de meilleures stratégies de lutte contre la maladie.

Mots clés: Oïdium du blé, concentration de spores, facteurs météorologiques, modèles de prévision, échantillonneur de spores

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Introduction

Blumeria graminis f. sp. *tritici* (*Bgt*), the causal agent of wheat powdery mildew, can cause devastating yield losses in wheat production worldwide (Marone et al. 2013; Zeng et al. 2014). Wheat may be infected by *Bgt* at all growth stages. The severity of this disease has increased in China since the 1970s, and expanded from local to entire wheat planting regions (Huo et al. 2002). This has been considered to be the result of increasing acreage of susceptible cultivars, disease conducive cultural and environmental conditions, and improper field management (Cao et al. 2015). Particularly in 1990 and 1991, a severe epidemic of wheat powdery mildew occurred, causing severe yield losses (Liu and Shao 1994).

Blumeria graminis f. sp. *tritici* (*Bgt*) is an obligate biotrophic pathogen, and thus needs the living host to complete its life cycle. Over-summering and over-wintering, when host tissue is unavailable and environmental conditions are unfavorable, are important stages for *B. graminis* f. sp. *tritici* to complete its life cycle. In summer, although the volunteer wheat seedlings may act as hosts for the pathogen after wheat harvest (Te Beest et al. 2008), the high temperatures limit the growth and spread of *Bgt* in some wheat-growing areas.

Gangu County of Gansu Province is a mountainous area with altitudes ranging from 800 to 2400 m. The diversity of topographical and climatic features and distribution of wheat cultivation from low to high altitudes in this area favour completion of the powdery mildew pathogen's life cycle and disease development (Cao et al. 2011; Li et al. 2013). Powdery mildew has been an increasingly serious wheat disease in Gangu. It has been found that the conidia of *Bgt* play an important role in infection and spore dispersal in Gangu, and the role of ascospores as the primary inoculum is limited (Cao et al. 2011). Also, the conidia of *Bgt* have the ability for long-distance dispersal (Wingen et al. 2013), and Gangu is located at the western edge of the largest wheat-growing region in China. So, the *Bgt* survival over summer in this region has the potential to provide primary inoculum to the major wheat-growing areas in China. Hence, it is important to forecast and control the disease in Gangu as disease control in this area affects powdery mildew management elsewhere in China.

Each plant disease needs at least three necessary elements: a pathogen, a susceptible host and a favourable environment (Scholthof 2007). The development of weather-based disease forecasting models has been reported for many plant pathogens (Pietravalle et al. 2003; Bondalpati et al. 2012; Copes 2015), including for powdery mildew disease (Daamen and Jorritsma 1990; Holb and Füzi 2016). Liu and Shao (1998) demonstrated that weather factors, including temperature, sunshine duration and rainy days had a strong relationship with the occurrence and epidemiology of wheat powdery mildew, and rainfall was the most important factor related to epidemics of the disease.

Dispersal and deposition of conidia, which are influenced by meteorological factors, determine the spore concentration in the air and influence disease severity (Granke et al. 2014). Significant correlations exist between spore concentration and many meteorological factors (Troutt and Levetin 2001; Burch and Levetin 2002), and such factors are useful in predicting the number of fungal spores in the air (Bruno et al. 2007). Pakpour et al. (2015) found a negative correlation between the fungal spore concentration in the air and rainfall. Cao et al. (2012) monitored airborne conidia of *Bgt* and analyzed relationships between airborne inoculum with weather conditions and disease index (DI). From these analyses, forecasting models were built based on meteorological factors and spore concentration under artificial inoculation conditions (Cao et al. 2015). However, as opposed to other wheat production regions, Gangu is an area where wheat powdery mildew can occur naturally and frequently in the spring. Specific environmental conditions may explain this phenomenon. It is thus necessary to determine the dynamics of spore concentrations of *Bgt* in the Gangu region and to develop models to predict disease severity based on spore concentrations and weather conditions.

Using spore samplers to monitor airborne inoculum is an applicable approach in epidemiological research and Burkard spore samplers can be used to collect airborne spores (Aylor 1993; Kennedy and Wakeham 2015). The traditional approach to quantify spore concentration is to count the number of spores using a microscope, which is time consuming and labour intensive. However, the method of real-time quantitative PCR (*q*PCR) has the feature of high-efficiency and has been widely used in epidemiological studies to quantify plant pathogen spores for the prediction of disease development (Luo et al. 2007; Dedeurwaerder et al. 2011; Meitz-Hopkins et al. 2014; West and Kimber 2015).

The disease epidemic of wheat powdery mildew is closely related to the availability of inoculum source and meteorological factors. It is well known that the regional conditions significantly influence the disease severity (Junk et al. 2016). Therefore, monitoring the dynamics of airborne conidia and meteorological variables are helpful to forecast the disease development and provide management recommendations for the local areas, and other regions where wheat powdery mildew could be also influenced by these airborne conidia as inoculum source.

The objectives of this study were to: (i) monitor the annual dynamics of the airborne conidia of *Bgt* using the spore samplers and real-time PCR over 2013– 2015 in Gangu; (ii) analyze the effects of meteorological factors on spore concentration and disease development; and (iii) implement disease forecasting models based on meteorological variables and spore concentration.

Materials and methods

Sampling location

This study was conducted in Gangu County, a mountainous area where *Bgt* was thought to be able to complete its annual life cycle. It is split by Weihe River into three parts: the south mountain (SM), middle valley (VL) and the north mountain (NM). Spore sampling and disease surveys were done at three sites, which were 1730 m above sea level (ASL) at the SM (location SM, 105.36 235° E and 34.67 403° N), 1289 m ASL in the valley (location VL, 105.29 144° E and 34.76 281° N), and 1558 m ASL at the NM (location NM, 105.28 709° E and 34.78 568° N), respectively. Wheat was planted around these three sites and the varieties were relatively consistent. The fields at SM and NM had no irrigation system while those at VL used slight irrigation. No fungicides were applied on any of these fields. Natural infections of wheat powdery mildew occurred annually at all the three sites due to the existence of volunteer seedlings at different altitudes.

Spore sampler and sample collection

One Burkard 'Multi-vial Cyclone Samplers' (Burkard Manufacturing Co. Ltd., UK) for field operation was used at each site. The spore samplers were placed approximately 3 m above ground, and about 100 m from the nearest wheat fields in order to avoid the contamination by splash-dispersed conidia during the period of rain. Spore samplers were placed at a distance from the fields to collect airborne samples, which could reflect the spore concentration in a region rather than a single field. The spore samplers could collect fungal spores efficiently into 1.5 mL Eppendorf tubes. The air throughput of spore sampler was 16.5 L/minute, or 23.76 m^3 per day. The spore sampler ran 24 h for each sample tube, and four samples were collected per week (every Tuesday, Thursday, Saturday and Sunday). These sample tubes were replaced manually every 2 weeks. The spore samplings were conducted from March 2013 to December 2015 at the SM, from April 2013 to December 2015 at the NM and VL, respectively. Spore sampler tubes were stored in −20°C before processing.

DNA extraction

Spore sampler tubes were processed to extract DNA using PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., USA) by following the protocols from the manufacturer. First, 500 μL upper part of reagent solution in PowerBead Tubes was transferred into the Eppendorf tubes containing the spore samples. The Eppendorf tubes were shaken on the Vortex Genie (Scientific Industries, USA) for 2 min to mix the spore suspension. The spore suspension was then transferred into the PowerBead Tubes, added 60 μL Solution C1, preheated at 60°C until dissolved and inverted several times for mixing. The mixture in the PowerBead Tubes was then processed with a FastPrep-24 Instrument (MP Biomedicals, LLC, USA) twice each for 40 s at 6.0 m/s with an interval of 5-min incubation in ice. The subsequent steps were operated following the instruction from the manufacturer. Finally, DNAs were suspended in the TE buffer and stored in −20°C for later use.

Quantification of spore concentration

The DNA of *Bgt* in the DNA extracts was quantified via quantitative real-time PCR (*q*PCR) using a set of primers and *Taq*Man probe specific to *Bgt* based on the ribosomal DNA internal transcribed spacersequence (Li et al. 2015). The primers were *Bgt*-F (5′ – CCGTAACAACCTCTCAAG – 3′) and *Bgt*-R (5′ – CAACCTGAGCAATTAAGGA – 3′), and the corresponding probe was *Bgt*-P (5′ – HEX – TATTGGGACTCGCTGCCTC – BHQ1 – 3′). A 157 bp amplicon was targeted.

The *q*PCR was conducted according to the method established and optimized previously (Li et al. 2015). The amplifications were performed with a Bio-Rad MyiQ^{TM}2 Two Colour Real-Time PCR Detection System (Bio-Rad Laboratories, USA) in a 20 μL volume containing 2.00 μL Buffer (10×; Mg^{2+} Free), 3.20 μL $MgCl_2$ (25 mM), 2.00 μL dNTP (2.50 mM), 0.30 μL each primer (10 μM), 0.25 μL probe (10 μM), 0.60 μL r*Taq* (5 U/μL), 2.00 μL DNA template (the DNA extracts from samples) and 9.35 μL ddH_2O. All of the reagents were purchased and the primers and probe synthesised from TAKARA (TAKARA Biotechnology (Dalian) Co., LTD., China). The procedure was initiated with denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 94°C for 20 s, annealing at 55°C for 30 s, extension at 72°C for 30 s. The fluorescence signal from the probe was recorded at 72°C during the extension in every cycle. In each *q*PCR reaction, *Bgt* DNA with concentration of 100 ng/μL was used as positive control and the ddH_2O was used as negative control. Three replicates were used for each sample, and the mean Ct value was calculated.

The number of spores of *Bgt* was calculated for each sample based on the corresponding average Ct value by using the corresponding standard curve. The equation of standard curve was: $y = -0.28x + 10.66$ ($R^2 = 0.99, P < 0.01$), where x is average Ct value and y is $\log_{10}$ (number of spores) (Li et al. 2015). Then, the spore concentration (number of spores per cubic metre) was obtained by dividing the number of spores per day by the air flow rate of the spore samplers (23.76 m^3 per day) for each sampling date.

Disease assessment

Seven to nine fields within 1 km distance from each spore sampler were selected for disease assessment. Assessments of powdery mildew were conducted at 3– 10 times during each sampling site at each wheat-growing season from March to June. In each field, five plots were randomly selected for investigation on each disease assessment date. A total of 20 plants (about 100 leaves) were randomly selected to record the number of diseased leaves and disease severity at each plot. Disease incidence was calculated by dividing the number of diseased leaves by the total number of surveyed leaves. The severity was assessed using a 0– 9 scale system (Sheng and Duan 1991) and the growth stage of wheat was recorded according to Zadoks et al. (1974). The DI was calculated as DI = disease incidence × average disease severity × 100. The area under the disease progress curve (AUDPC) was calculated with the following formula:

$$\mathrm{AUDPC} = \sum_{i}^{n-1}\left[\left(\frac{x_i + x_{i+1}}{2}\right)(t_{i+1} - t_i)\right]$$

where n is the total number of disease assessments, x_i is the DI at ith day, x_i is the date at ith day.

Meteorological data

Meteorological data of each day in Gangu County were obtained from the National Meteorological Information Centre (NMIC), China Meteorological Administration. Daily wind direction of the maximum wind speed, daily average wind velocity, daily solar radiation, daily average temperature, daily maximum temperature, daily minimum temperature, daily average humidity, daily minimum humidity and daily total rainfall were recorded.

Data analysis

Degree day is a method for analyzing the effect of accumulated temperature on disease development (Coakley and Line 1981). The optimal temperature for germination of *Bgt* conidial and for occurrence of disease are 10– 17°C and 14– 20°C, respectively (Last 1953). So, 16°C of daily average temperature ($\overline{T}$) was considered as the threshold temperature to calculate the accumulated temperature in this study. The positive degree days (PDD) and negative degree days (NDD) were calculated by the following formulas, respectively (Coakley and Line 1981):

When $\overline{T} > 16°C$, $PDD = \sum(\overline{T} - 16°C)$; and $\overline{T} < 16°C$, $NDD = \sum|\overline{T} - 16°C|$.

The meteorological variables used in this study included average wind velocity, average temperature, average maximum temperature, average minimum temperature, average solar radiation, accumulated solar radiation, average humidity, average minimum humidity, average rainfall, accumulated rainfall, rain days, PDD, NDD for the different periods.

For determining the effect of meteorological factors on spore concentration, the correlations of 10-day average spore concentration with all the above meteorological variables during the corresponding 10-day period were analyzed by Spearman's non-parametric correlation, because the airborne spores did not display normal distribution by normality test. The Pearson's correlation coefficients were used to evaluate the correlations of disease development indices, including incidence, DI and AUDPC with accumulated spore concentration (ASC) and all the meteorological variables of the period between investigations at three locations in spring. The airborne conidia forecasting models based on meteorological factors were constructed and the incidence, DI and AUDPC forecasting models based on meteorological factors and accumulated spore concentration (ASC) were constructed using multiple stepwise regression analysis with 3 years of data at three different locations, respectively. A total of 23, 19 and 23 disease observations of both disease incidence and DI were used for correlation analyses and model developments at SM, VL and NM, respectively. Nineteen disease observations of AUDPC based on different investigation periods were used for correlation analyses and model developments at three location sites, respectively. The models and corresponding variables were selected among all of the parameters selected from the multiple stepwise regression analysis conducted with the Statistical Product and Service Solutionspackage version 20.0 (IBM, USA). All tests were two-tailed, with $P < 0.05$ and $P < 0.01$ considered statistically significant.

Results

Annual dynamics of spore concentrations

The patterns of annual dynamic of *Bgt* spores were similar during 3 years at each location in Gangu, and spores could be detected year-round, including during winter and summer when no wheat was grown (Fig. 1). Each year, at the three locations, spore concentrations increased from March to reach the maximum in May or June, which is similar to the disease progress during wheat-growing seasons. The highest spore concentrations were observed in May and June at all locations. At SM and VL in 2013, after the wheat crop was harvested, the spore concentrations dropped rapidly until late August and early September, and increased again to reach about 12 spores/m^3. The daily spore concentration had a large variation from near 0 to above 200 spores/m^3 among different years and different locations. In 2014, the spore concentrations at the three locations were higher than in 2013 and 2015, and higher at the SM and NM sites than at VL in 2015.

Temporal progress of wheat powdery mildew

For the 3 years, overall development of powdery mildew at the three locations was similar in the spring (Fig. 2). DI increased from early April to May, decreased slightly in early May due to little precipitation, and increased again towards the final disease assessments at SM in 2013 and 2014. However, the DI in 2015 showed an increase from early April till late May. At VL, the DI reached the highest level in the middle of May, and then declined until the final disease assessment in 2013. As for NM, an unusual progress curve of powdery mildew was recorded in 2014, with

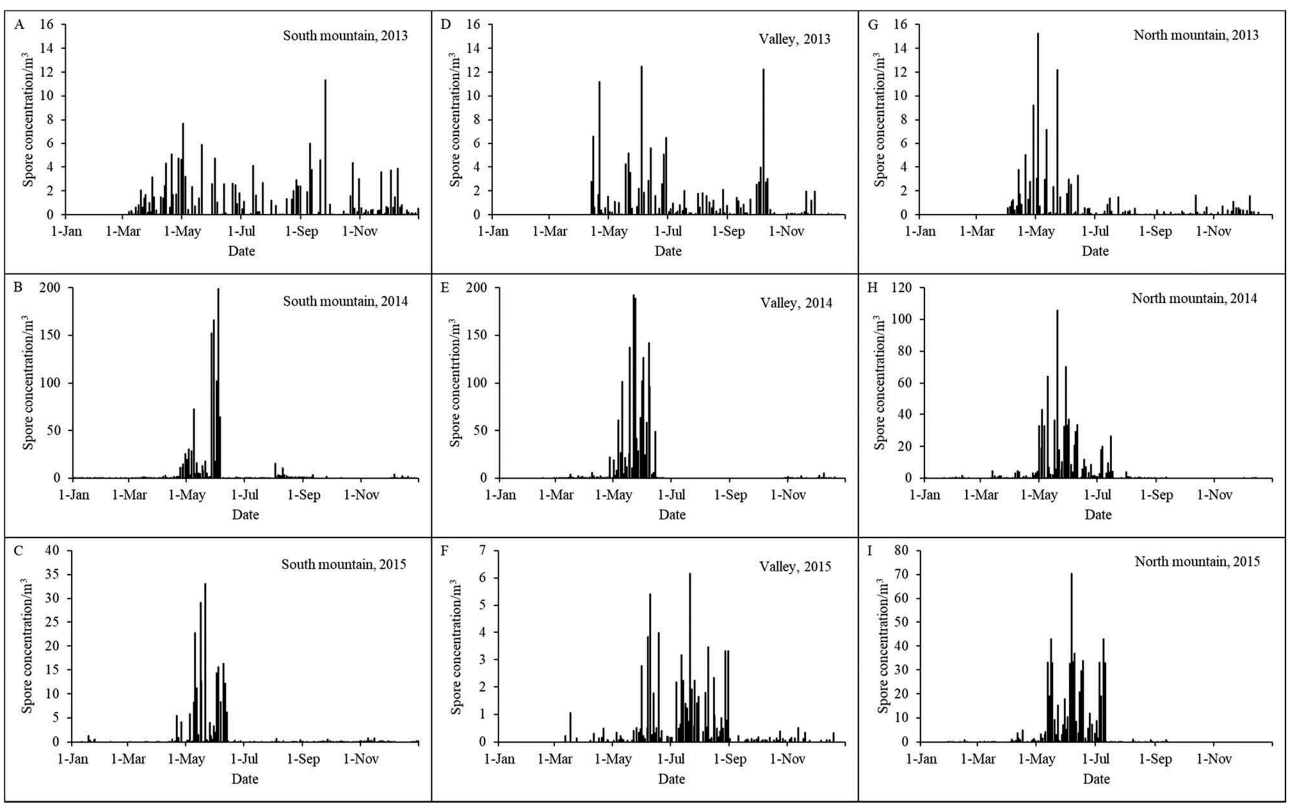

Fig. 1 The annual dynamics of airborne conidia of *Blumeria graminis* f. sp. *tritici* (*Bgt*) in Gangu. (a– c) 2013, 2014 and 2015 at south mountain; (d– f) r2013, 2014 and 2015 at valley; (g– i) 2013, 2014 and 2015 at north mountain. The periods with missing data due to spore sampler failure included 24 October– 1 December 2014 for south mountain, 25 December 2013– 14 February 2014, 23 June– 27 October 2014 and 20 February– 11 March 2015 for valley, and 12 September– 1 December 2014 and 14 September– 31 December 2015 for north mountain.

a long period decline in DI from middle April to early June, as well as that of SM in 2013 and 2014.

Correlations between spore concentration and meteorological factors

In most cases, significant correlations (P < 0.05 or P < 0.01) were detected between the spore concentrations and meteorological variables at three locations (Table 1). There were significant positive correlations between 10-day average spore concentration and 10-day variables of average temperature (P < 0.05 at SM, P < 0.01 at VL and NM), average maximum temperature (P < 0.01 at SM, VL and NM), average minimum temperature (P < 0.01 at SM, VL and NM), PDD (P < 0.05 at SM, P < 0.01 at VL and NM), NDD (P < 0.01 at SM, VL and NM), average solar radiation (P < 0.01 at SM, VL and NM) and accumulated rainfall (P < 0.05 at SM, P < 0.01 at VL and NM) at the same periods. At SM, the average wind velocity, rain days, average humidity and average minimum humidity showed no significant effect on 10-day average spore concentration. At VL, the 10-day average spore concentration showed a positive significant correlation with average wind velocity at P < 0.05. Rain days had no significant effect on 10-day average spore concentration. However, average humidity and average minimum humidity showed a significant negative correlation with 10-day average spore concentration at P < 0.01. At NM, the relationship between 10-day average spore concentration and average wind velocity and rain days showed significant positive

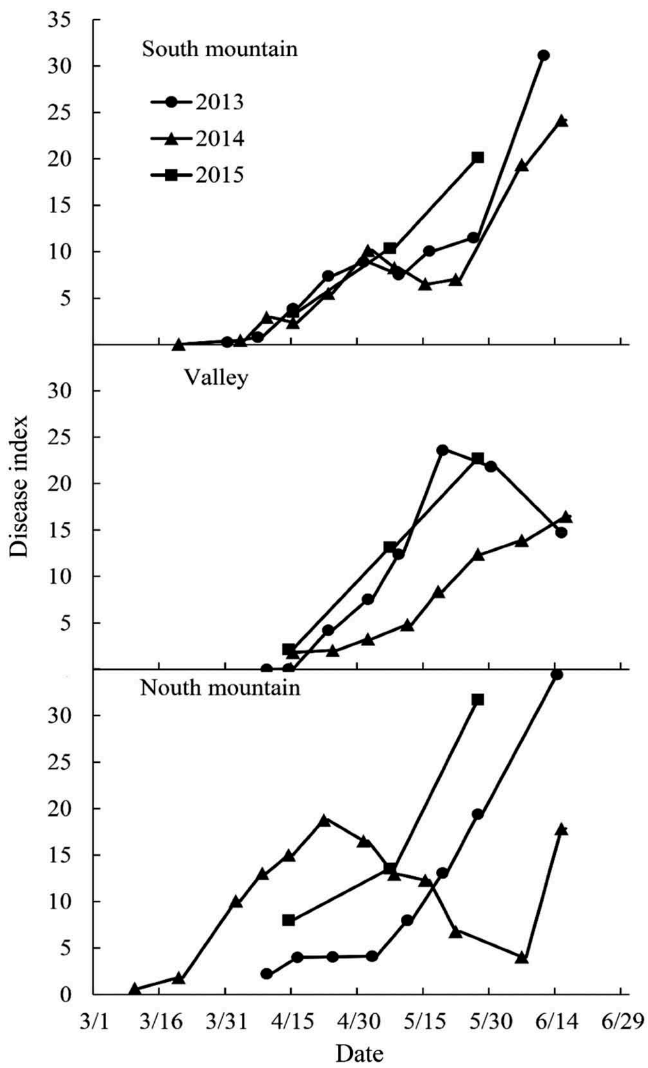

Fig. 2 The disease indices of wheat powdery mildew at three locations in Gangu.

Table 1. Spearman correlation coefficients between 10-day average spore concentration and meteorological variables during the same periods at three locations in Gangu during 2013– 2015.

Location	AWV	AT	MaxT	MinT	ASR	ASR'	AH	AMH	AR	AR'	RD	PDD	NDD
South mountain	0.02	0.25*	0.28**	0.22*	0.30**	0.31**	– 0.10	– 0.18	0.21*	0.22*	0.11	0.25*	– 0.29**
Valley	0.26*	0.57**	0.59**	0.52**	0.46**	0.45**	– 0.31**	– 0.34**	0.29**	0.29**	0.21	0.55**	– 0.56**
North mountain	0.30**	0.46**	0.49**	0.43**	0.40**	0.39**	– 0.19	– 0.22	0.31**	0.31**	0.31**	0.41**	– 0.51**

AWV = average wind velocity; AT = average temperature; MaxT = average maximum temperature; MinT = average minimum temperature; ASR = average solar radiation; ASR' = accumulated solar radiation; AH = average humidity; AMH = average minimum humidity; AR = average rainfall; AR' = accumulated rainfall; RD = rain days; PDD = the positive degree days; NDD = the negative degree days
*: P < 0.05, **: P < 0.01.

correlation at $P < 0.01$. Average humidity and average minimum humidity had no significant effect on 10-day average spore concentration.

Correlations between disease development and meteorological factors and spore concentration

There were significant correlations between the disease progress expressed as incidence, DI and AUDPC and temperature-related variables, including average temperature, average maximum temperature, average minimum temperature, PDD and NDD for the period between investigations at SM ($P < 0.01$), VL ($P < 0.01$ or $P < 0.05$) and NM ($P < 0.01$ or $P < 0.05$ except incidence with average temperature, average maximum temperature and PDD) (Table 2). The average rainfall ($P < 0.05$), accumulated rainfall ($P < 0.01$) and rain days ($P < 0.01$) of the period between investigations showed significant correlations on incidence and DI at NM. The accumulated solar radiation ($P < 0.05$) of the period between investigations showed significant correlations on incidence and DI at SM and NM. Furthermore, the accumulated spore concentration of the period between investigations showed a positive and significant correlation with incidence ($P < 0.05$), DI ($P < 0.01$) and AUDPC ($P < 0.01$) at SM and DI ($P < 0.05$) and AUDPC ($P < 0.01$) at NM, and had no significant correlation with that of VL.

Prediction models of spore concentration and disease development

Multiple stepwise regression modelling approach was used to select models to predict the dynamics of airborne conidia (Table 3) based on meteorological variables. For predicting the spore dynamics, PDD was an important factor in the models for predicting the spore concentration at SM and VL. And 10-day average temperature was used to estimate the dynamic of *Bgt* conidia at NM. All of the three models were significant at $P < 0.05$ (Table 3).

As shown in the models predicting disease development based on meteorological variables and accumulated spore concentration (Table 4), temperature-related variables and accumulated spore concentration corresponding with the disease investigations were crucial factors

Table 2. Pearson correlation coefficient between wheat powdery mildew, spore concentration and meteorological variables at three locations in Gangu during 2013– 2015.

Location	Index	ASC	AWV	AT	MaxT	MinT	ASR	ASR'	AH	AMH	AR	AR'	RD	PDD	NDD
South mountain	Incidence[a]	0.50*	– 0.20	0.74**	0.73**	0.62**	0.16	0.42*	0.31	0.23	0.03	0.21	0.39	0.67**	– 0.65**
	DI[b]	0.58**	– 0.07	0.83**	0.81**	0.76**	– 0.04	0.43*	0.28	0.26	0.08	0.23	0.32	0.90**	– 0.63**
	AUDPC[c]	0.65**	– 0.29	0.89**	0.85**	0.84**	– 0.01	0.33	0.17	0.17	0.00	0.09	0.21	0.90**	– 0.64**
Valley	Incidence[a]	0.27	– 0.20	0.82**	0.76**	0.71**	– 0.20	0.38	0.38	0.33	0.03	0.19	0.40	0.73**	– 0.72**
	DI[b]	0.34	– 0.22	0.79**	0.75**	0.66**	– 0.19	0.43	0.37	0.31	0.12	0.30	0.47*	0.74**	– 0.65**
	AUDPC[c]	0.22	– 0.14	0.81**	0.75**	0.66**	– 0.20	0.41	0.29	0.33	0.02	0.16	0.11	0.93**	– 0.62*
North mountain	Incidence[a]	0.38	– 0.16	0.38	0.35	0.45*	0.05	0.44*	0.47*	0.25	0.50*	0.67**	0.61**	0.26	– 0.41*
	DI[b]	0.47*	– 0.09	0.51*	0.45*	0.55**	– 0.02	0.42*	0.41	0.28	0.42*	0.55**	0.48*	0.56**	– 0.46*
	AUDPC[c]	0.81**	– 0.26	0.67**	0.61**	0.66**	– 0.02	0.17	0.13	0.19	0.00	0.06	0.17	0.68**	– 0.55*

ASC = accumulated spore concentration; AWV = average wind velocity; AT = average temperature; MaxT = average maximum temperature; MinT = average minimum temperature; ASR = average solar radiation; ASR' = accumulated solar radiation; AH = average humidity; AMH = average minimum humidity; AR = average rainfall; AR' = accumulated rainfall; RD = rain days; PDD = the positive degree days; NDD = the negative degree days; [a] Incidence, [b] DI and [c] AUDPC represent different evaluation indices of disease severity, respectively.
*: P < 0.05, **: P < 0.01.

Table 3. *Blumeria graminis* f. sp. *tritici* airborne spore concentration prediction models at three locations.

Location	Prediction model	R^2	P
South mountain	$Y = 0.39$ PDD – 0.18 AR + 4.14	0.31	<0.01
Valley	$Y = 0.45$ PDD + 1.57	0.16	0.04
North mountain	$Y = 1.13$ AT – 9.37	0.24	0.01

PDD = the positive degree days; AR = accumulated rainfall of 10-day period; AT = average temperature of 10-day period.

for disease forecasting at three locations. Also, rainfall and number of rainy days were also significant variables in the prediction models at VL and NM locations. Average wind velocity, corresponding with the disease investigations, was a significant factor related to disease at NM. The R^2 of all models were above 0.50 and statistically significant at $P < 0.0001$ (Table 4).

Discussion

Airborne inoculum of *B. graminis* f. sp. *tritici* was detected year-round, and the dynamics of airborne conidia showed obvious annual trends during monitoring over 3 years. Spore concentrations increased from March to reach the highest concentration in May or June, corresponding to wheat growth stages from GS 30 to GS 77 (Zadoks et al. 1974), which was in accordance with the development of disease. These observations suggest that airborne spore concentrations during this period were mainly from local wheat fields. Furthermore, our study also revealed that airborne conidia are present during the non-growing season of wheat, particularly from late August to early September. It was inferred that the airborne inoculum during the non-growing season came from the volunteer seedlings grown at higher altitude, which were infected by *Bgt* bridging the fall seedlings by providing a host for the pathogen over the summer in Gangu. There was no significant difference between disease occurrence and main peaks of spore concentration for the three locations. The greatest difference in the dynamics of *Bgt* was that spores were present over a longer period at SM and VL in 2013 from March to December. Lower spore concentrations could explain this difference; however, low concentrations of spores were also observed at VL in 2015. Although southeast wind was monitored at the maximum ratio in this study (data not shown), Gangu is a mountainous area and the complex topography could influence wind direction, hence influencing the dispersal of spores. No clear and directed transmission of *Bgt* among the three locations was found.

In this study, spore concentration and disease severity in Gangu were significantly influenced by meteorological factors, including temperature, humidity, wind and solar radiation. Almost all of the meteorological factors showed significant correlations with 10-day average spore concentration. Increasing relative humidity led to increasing disease development, but it had a negative effect on airborne spore spread, where smaller number of spores could be trapped. This was consistent with the results from a previous study (Cao et al. 2015). Although the infections of *Bgt* could occur at different humidity conditions, the increasing humidity promotes the infection of *Bgt* on wheat and cause more severe disease development. Thus, humidity plays an important role in disease development. However, the spores in the air fall easily during high relative humidity days, thus causing low spore counts. Also, latent period of wheat powdery mildew could be influenced by climatic factors. Therefore, the correlation of spore concentration and humidity may also be influenced by changes of latent period caused by complex climate.

Table 4. Wheat powdery mildew prediction models in Gangu.

Location	Indice	Prediction model	R^2	P
South mountain	Incidence[a]	$Y = 4.63$ AT – 36.65	0.54	<0.0001
	DI[b]	$Y = 0.38$ PDD – 0.02 ASC + 3.51	0.87	<0.0001
	AUDPC[c]	$Y = 10.11$ PDD – 0.45 ASC + 66.25	0.88	<0.0001
Valley	Incidence	$Y = 6.30$ AT – 64.04	0.63	<0.0001
	DI	$Y = 1.76$ AT + 1.23 RD – 22.64	0.70	<0.0001
	AUDPC	$Y = 10.38$ PDD – 0.12 ASC + 26.42	0.90	<0.0001
North mountain	Incidence	$Y = 1.27$ AR – 24.82 AWV + 76.79	0.54	<0.0001
	DI	$Y = 0.17$ PDD + 0.26 AR + 5.27	0.50	<0.0001
	AUDPC	$Y = 3.18$ ASC + 34.12 MLT – 95.10	0.75	<0.0001

AT = average temperature for the period between investigations; PDD = the positive degree days; ASC = accumulated spore concentration for the period between investigations; RD = rain days for the period between investigations; AR = accumulated rainfall for the period between investigations; AWV = average wind velocity for the period between investigations; MLT = mean low temperature for the period between investigations; [a] Incidence, [b] DI and [c] AUDPC represent different evaluation indices of disease severity, respectively

Conidia of wheat powdery mildew are small and light, and can easily be carried by airflow and travel with it (Cao et al. 2012). Wind is one of the important factors determining spore dispersal, and wind speed contributes to spore bursts (Burch and Levetin 2002). Theoretically, there are two effects for wind on the dynamic of spore concentration in the air. The conidia on the plant can be detached and released in the air by wind, and wind could transport these spores to adjacent plants or fields. Our study showed that the 10-day average spore concentration was positively correlated with average wind velocity at three locations.

There have been previous reports about the effect of solar radiation on the development of powdery mildew, which showed that sunlight was negatively correlated with the development of disease (Liu and Shao 1998; Zahavi et al. 2001; Austin and Wilcox 2012). However, we found that there were significantly positive correlations between spore concentration and the development of disease with accumulated solar radiation in Gangu, which was inconsistent with previous studies. On the one hand, sunlight can cause an increase of temperature in exposed tissue, which may be an important factor influencing disease severity (Downey et al. 2006). On the other hand, mean 10-day temperature in Gangu is usually not at the optimum for the development of wheat powdery mildew in early spring. Thus, increasing solar radiation is an effective way to raise the foliage temperature and thus relieves the limitation to disease development caused by low temperature.

In this study, the best forecasting models to predict wheat powdery mildew development in spring and early summer at three locations in Gangu were using stepwise regression analysis with accumulated spore concentrations and meteorological variables. It showed that accumulated spore concentration and 10-day temperature variables, including average temperature, average maximum temperature and PDD played important roles in most of the forecasting models, and rainfall and mean wind velocity also appeared in part of models. This was different from the study by Cao et al. (2015), who demonstrated that spore concentration was the most important of all of the variables studied, including weather. A prime difference of both studies is the type of disease occurrence. Natural infection of wheat powdery mildew was investigated in this study, while artificial inoculation was considered in previous study. The mountainous environment made the source of inoculum that caused wheat powdery mildew in Gangu more complex, which might be from the local fields or adjacent regions. Overall, the spore concentration and meteorological factors were equally important to predict the development of wheat powdery mildew in Gangu.

Undoubtedly, microclimate plays an important role in disease development, so it is perhaps of little surprise that this is a crucial factor for disease predictions. Although geographic distance of three locations was close, the microclimate was different because of topography and elevation, causing the specific features of disease development and the differences in variables used for forecasting models among the three locations. Gangu area plays an important role in wheat stripe rust in China (Li and Zeng 2002). Therefore, it is of great significance to accurately predict the disease development of stripe rust in Gangu. The meteorological data that were used to establish models in this study came from the NMIC of China Meteorological Administration, which did not represent the specific microclimate at these three locations. However, it was difficult to monitor the microclimate at each location and each altitude of Gangu due to the complexity of topography. There was little difference in DI among three locations in our study, which implied that the wheat powdery mildew was rarely affected by microclimates in Gangu. Therefore, it is possible to predict wheat powdery mildew development in Gangu using the meteorological data from NMIC of China Meteorological Administration.

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