MODELING THE TEMPORAL PROGRESS OF VERTICILLIUM WILT EPIDEMICS IN COTTON

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Abstract

Cotton (*Gossypium hirsutum* L.) is an economically important crop in the Texas High Plains. Reduced yield and fiber quality induced by Verticillium wilt, caused by *Verticillium dahliae* Kleb., is capable of causing substantial losses. Use of partially resistant cultivars is most commonly used for management of the disease; however, other production practices such as irrigation, reduced seeding rates and crop rotation are known to affect the disease. A small plot field experiment was conducted using a split-plot design with three replications at the Texas Tech University Quaker Farm. Whole plots consisted of seeding rate (1, 2, 3, 4, 5 or 6 seeds ft^{-1}) and the cultivars Deltapine 1212 B2RF, NexGen 4111 RF, Fibermax 2484 B2F, and All-Tex Nitro-44 B2RF served as sub-plots. Results indicated that seeding rate and cultivar affected disease incidence. Medium (3-4 seeds ft^{-1}) and high (5-6 seeds ft^{-1}) seeding rates significantly ($P < 0.05$) decreased the foliar disease incidence compared to the low seeding rates (1-2 seeds ft^{-1}). Area under the disease progress curve (AUDPC) showed a similar trend as the final foliar disease incidence rating. The partially resistant cultivars NexGen 4111 RF, Fibermax 2484 B2F, and All-Tex Nitro-44 B2RF exhibited lower foliar disease incidence (5.8%, 3.3%, and 6.1%) relative to the susceptible control Deltapine 1212 B2RF (10.8%). In addition, among the partially resistant cultivars, Fibermax 2484 B2F was superior to others in view of Verticillium wilt resistance. Five growth models (exponential, monomolecular, Gompertz, logistic, and Weibull) were used to simulate the temporal disease development and evaluate the effectiveness of the management practices on retarding disease epidemic rate. The Gompertz model was identified as the model with the highest coefficient of determination (R^2) of 0.843. High seeding rates and partially resistant cultivars not only decreased the disease incidence significantly ($P < 0.05$) but also slowed the rate of disease increase.

Introduction

Verticillium wilt of cotton (*Gossypium hirsutum* L.), caused by *Verticillium dahliae* Kleb., is an important vascular wilt disease in temperate and subtropical regions of the world. This disease was first reported in Arlington, Virginia (Carpenter, 1914). The most common pathogenic type of *V. dahliae* was the defoliating type in the Texas High Plains (Puhalla and Hummel, 1983; Mathre et al., 1966). Cotton infected by *V. dahliae* shows foliar symptoms throughout the growing season when the environment (cool and wet conditions) is favorable (Karademir et al., 2012). Premature foliar chlorosis and necrosis, vascular discoloration in stems and roots are characteristics in most hosts (Fig. 1). Vascular discoloration is an iconic indicator of infection by *V. dahliae*. Specifically, chlorosis of leaf tissues along the margins and between the major veins appears in the early stage. Later, these areas become more intensely yellow, and finally turn necrotic and/or defoliate.

Figure 1. Chlorosis and necrosis on cotton leaves infested with *Verticillium dahliae* (left) and vascular discoloration in stems compared to the healthy tissues (middle and right).

Cotton is one of the most economically important crops grown in the Texas High Plains, and this region produced about 25% of U.S. cotton in 2013 (NASS, 2013). The economic loss induced by Verticillium wilt, therefore, is important. Verticillium wilt of cotton was responsible for significant losses throughout the 1970s and 1980s in the Texas High Plains. The pathogen populations were disrupted by widespread use of partially resistant cultivars in the 1990s (Paymaster HS-26). However, Verticillium wilt has become the major cause of yield losses and reductions in fiber quality in the region due to picker type cotton cultivars planted (Zhang et al., 2012). In some years with severe disease, Verticillium wilt may result in reductions in net returns greater than $400 per acre (Woodward and Wheeler, 2011).

Management of Verticillium wilt includes cultural practices, resistant cultivars, fungicidal applications, biological controls, and soil disinfestations, to minimize the loss derived from Verticillium wilt (Stapleton and DeVay, 1995; Lang et al., 2012). However, Verticillium wilt of cotton is difficult to control due to the broad host range of the pathogen, the long viability of microsclerotia, and the inability of fungicides to affect the pathogens once they enter the xylem. The management tactics of cultural practices and partially resistant cultivars, which were more flexible and environmental friendly compared to others, were given more focus. Wheeler et al. (2010), for example, found the Verticillium wilt incidence was negatively related to the increasing of seeding rate by conducting small plot experiments in the Southern High Plains of Texas. Chawla et al. (2012) reported that Verticillium wilt incidence increased from 8% to 58% for a susceptible cotton cultivar and from 0% to 5% for the partially resistant cotton cultivar over a 3-year microplot study in Lubbock County of the Texas High Plains. In this study, management strategies of both partially resistant cultivars and high seeding rates are used to reduce Verticillium wilt of cotton.

One of the major objectives of plant pathology research is to expand our understanding of temporal disease dynamics in the host crop (Van Maanen and Xu, 2003; Xu, 2006), which can be used to evaluate the effectiveness of management strategies for disease. Growth models are popular statistical methods to analyze the temporal disease development. The commonly used nonlinear regression growth models are exponential, monomolecular, logistic, Gompertz, and Weibull (Gottwald et al., 1989; Campbell and Madden, 1990). With the exponential model, the epidemic rate is proportional to the increasing number in an exponential or logarithmic progression. The monomolecular model simulates the progress of a growth condition in which the rate of growth at certain time is proportional to the resources available. The logistic is the most commonly used asymptotic model form (Winsor 1932; Fresco 1973; Hunt 1982; Zeide 1993; Heinen 1999). Whereas, the Gompertz model is a model having a sigmoid type of behavior and is found to be very useful in biological study. However, the Gompertz model is not the same as the logistic model, which is symmetric about its point of inflexion. The Weibull model is used as a probability density and statistical distribution function (Weibull, 1951). Abundant research has been focused on final disease incidence assessments for comparing management strategies. Little information on epidemiological aspects of Verticillium wilt in cotton is available. The objectives of this study were to: (1) evaluate the effectiveness of different seeding rates and partially resistant cultivars for the Verticillium wilt management; and (2) model the temporal development of Verticillium wilt incidence and compare the effect of two management tactics (seeding rate and resistance) on selected model rate parameters.

Materials and Methods

Field experiment

A field experiment was conducted at Texas Tech University Quaker Farm in Lubbock County in 2015. The field had a long (>7 year) history of cotton production and was naturally infested with *V. dahliae*. Treatments were arranged in a split-plot design with three replications. Whole-plots consisted of 6 seeding rates, which were 1, 2, 3, 4, 5, and 6 seeds ft^{-1}, whereas, the cultivars Deltapine (DP) 1212 B2RF (susceptible control), NexGen (NG) 4111 RF, Fibermax (FM) 2484 B2F, and All-Tex (AT) Nitro-44 B2RF (partially resistant cultivars) served as sub-plots. Plots were 35 feet long with 5 feet alley between. Each plot consisted 2 rows with 40-inches space. The trial was planted on June 4^{th}, 2015. To achieve the seeding rates of 1 to 6 seeds $foot^{-1}$, the planter with cone unites was used and 35, 70, 105, 140, 175, 210 seeds were added to cones for each row, respectively. Irrigation utilized subsurface drip. The fertilization practices were based on the Texas A&M AgriLife Extension Service recommendations.

Plant stand and foliar symptoms were monitored throughout the cotton growing season. Incidence of plants with foliar wilt symptoms was measured for each plot at a 7-day interval from July 28 until September 29, 2015 with a total of 10 measurements. Foliar disease incidence was the number of plants with Verticillium wilt symptoms on leaves or defoliation divided by the total number of plants in each plot. Plant heights were measured on September 2, 2015.

Nonlinear regression by growth models

The integrated equations of the five growth models were used to fit the disease progress curve for each plot (Table 1). The variable y represents foliar disease incidence, t denotes time in weeks, parameter r indicates epidemic rate and y_0 represents foliar disease incidence at the beginning of the season. The parameters were estimated and subject to analysis of variance to determine the epidemic rates of four cultivars at six seeding rates. Coefficient of determination (R^2) of predicted disease incidences against observed values was used to determine the appropriateness of five statistical models through analysis of variance.

Table 1. Integrated equations and parameter staring values used in nonlinear regression analysis of disease progress.

Model	Expression	Parameter[a]	Starting value	Parameter constraint	Estimation method
Exponential	$y = y_0 \exp(rt)$	r	0.3		Levenberg-Marquardt
		y_0	0.0008		
Monomolecular	$y = 1-(1-y_0) \exp(-rt)$	r	0.004		Sequential quadratic programming
		y_0	0	$y_0 \geq 0$	
Logistic	$y = 1/[1 + \exp\{-[\ln(y_0/(1-y_0)) + rt]\}]$	r	0.3		Sequential quadratic programming
		y_0	0.0006	$0<y_0<1$	
Gompertz	$y = \exp[-b \exp(-rt)]$	r	0.1		Levenberg-Marquardt
	$(b = -\ln y_0)$	b	12.0		
Weibull	$y = 1-\exp[-\{(t-a)/b\}^c]$	a	0.004	a≤8	Sequential quadratic programming
		b	29.0		
		c	4.2		

[a] y represents foliar disease incidence, t denotes time in weeks, r indicates epidemic rate parameter, and y_0 represents foliar disease incidence at the beginning time.

Data processing and analysis

The area under disease progress curve (AUDPC) was calculated from the foliar disease incidence data for each plot directly as the formula described by Campbell and Madden (1990). Statistical analyses were performed using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Plant stand, plant height, foliar disease incidence, AUDPC, and R^2 were subjected to analysis of variance and means separated using Fisher's Protected Least Significant Difference (LSD) at a probability level of $P < 0.05$.

Results and Discussion

Cotton plant growth parameters

Analysis of variance indicated a highly significant difference of plant stands among six seeding rates ($F_{5, 48} = 185.0$, $P < 0.01$) (Table 2). Plant stands increased as seeding rates increased from 1 to 6 seeds ft^{-1} as expected, while there were no differences between stands of cultivars ($F_{3, 48} = 1.1$, $P = 0.354$). There was no interaction of plant stands between seeding rate and cultivar ($P > 0.05$).

Plant heights of those not exhibiting wilt symptoms were significantly different among seeding rates and cultivars ($P < 0.01$). Cotton cultivar may perform differently with regard to plant height when seeded at different rates. Plant height decreased as seeding rates increased (Table 2), which may be due to the effects of crop growth competitions. Plant heights of cultivar NG 4111RF were significantly higher than other cultivars. Interaction between seeding rate and cultivar was not observed for plant heights ($P > 0.05$). Paired samples t-test was used to compare heights of plants with and without wilt symptom in each plot. The wilted plants were significantly shorter (24.7 inches) than plants without foliar symptoms (28.3 inches) ($P < 0.01$).

Table 2. Plant stand, plant height, final foliar disease incidence of cotton cultivars and seeding rates.

Factor (level)	**Plant stand (plants ft^{-1})**	**Plant height (inch)**	**Foliar disease incidence (%)**	**AUDPC**
Seeding rate[a] (seeds ft^{-1})				
1	0.74 f	30.4 a	9.4 ab	33.0 ab
2	1.49 e	28.9 b	12.5 a	46.5 a
3	2.23 d	27.5 c	6.2 bc	23.3 b
4	2.87 c	27.4 c	3.9 bc	16.1 b
5	3.42 b	26.8 cd	4.3 bc	19.4 b
6	4.23 a	26.1 d	2.7 c	11.4 b
Cultivar[a]				
NG 4111RF	2.47 a	29.6 a	5.8 b	22.7 b
FM 2484B2F	2.58 a	27.1 b	3.3 b	12.1 b
DP 1212B2RF	2.40 a	27.3 b	10.8 a	43.5 a
AT Nitro-44B2RF	2.54 a	27.4 b	6.1 b	21.5 b

[a] values within a column followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Fishers Protected LSD.

Disease incidence

There was a significant difference of final foliar disease incidence among seeding rates ($F_{5, 48} = 3.861$, $P = 0.005 < 0.05$). Disease incidence was negatively correlated with stand. The lower seeding rates resulted in establishing inadequate plant stands and higher disease incidence (9.4 % and 12.5% for 1 and 2 seeds foot^{-1}, respectively) (Table 2). Incidence of wilt also significantly differed among cultivars ($F_{3, 48} = 4.0$, $P = 0.013 < 0.05$). Partially resistant cultivars (NG 4111RF, FM 2484B2F, and AT Nitro-44B2RF) exhibited lower disease incidence compared to the susceptible control DP 1212B2RF (Table 2). Among the partially resistant cultivars, FM 2484B2F had the lowest incidence of Verticillium wilt. No interaction of seeding rate and cultivar was observed for foliar disease incidence ($P > 0.05$).

AUDPC was highly correlated with the final disease during evaluation time ($R^2 = 0.976$). There were significant differences among seeding rates and cultivars for AUDPC ($P < 0.05$). The medium (3-4 seeds ft^{-1}) and high (5-6 seeds ft^{-1}) seeding rates had lower disease values compared to the low seeding rates (1-2 seeds ft^{-1}) (Table 2). Partially resistant cultivar FM 2484B2F had a reduced AUDPC relative to the susceptible cultivar DP 1212B2RF (Table 2). In general, both AUDPC and foliar disease incidence on (give the date) could be used to assess the effectiveness of cotton cultivar and seeding rates on Verticillium wilt.

Nonlinear regression analysis of temporal disease progress

The number of disease progress curves that can be fitted varied by models. The plots of which the disease progress curves can be fitted by all 5 models were subject to analysis of variance. The total number of plots was 55. The coefficient of determination (R^2) of nonlinear regression by the Gompertz model was significantly higher compared to monomolecular model ($P < 0.05$) (Fig. 2). The R^2 of Gompertz model (averaged 0.843) was also higher relative to exponential (0.812), logistic (0.812), and Weibull (0.821) models, even though no significant difference was found. The Gompertz model, therefore, was identified as the best fitting model in this study for the following epidemic rate analysis.

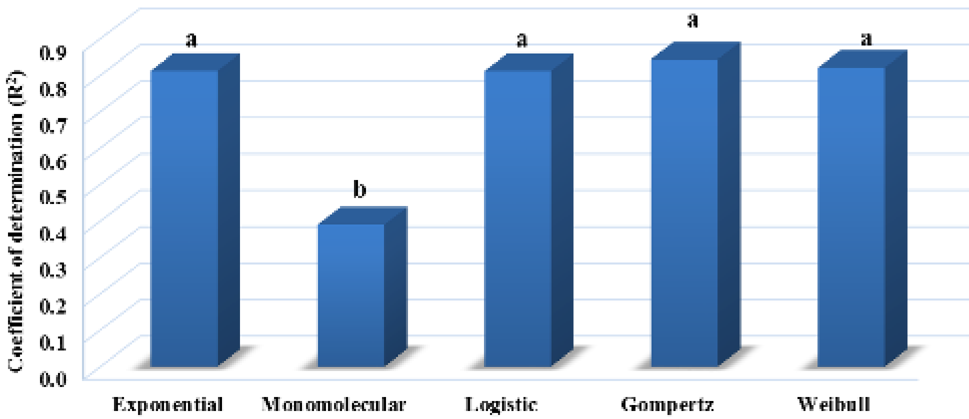

Figure 2. Coefficient of determination of nonlinear regression by five statistical models for each disease progress curve. The models tested were exponential, monomolecular, logistic, Gompertz, and Weibull. The total number of curves that can be fitted by all models was 55. The same lowercase letter above the bar was not significantly different at $P < 0.05$.

Disease progress curves simulated by Gompertz model

Disease incidence for each seeding rate was combined across all four cultivars, and disease incidence for each cultivar was combined across all six seeding rates. Disease incidences of foliar wilt symptoms for each seeding rate and cultivar were plotted against time in weeks and fitted by Gompertz model using R software (Figs. 3 and 4). The observed disease incidence data fitted the Gompertz curve closely. As shown in Figures 3, the R^2 values were all above 0.87. The Gompertz model simulated foliar disease incidences at different seeding rate have a good agreement with the field observed values. The model predicted disease incidences of different cultivars also matched very well against the field observed values with $R^2 > 0.92$ (Fig. 4).

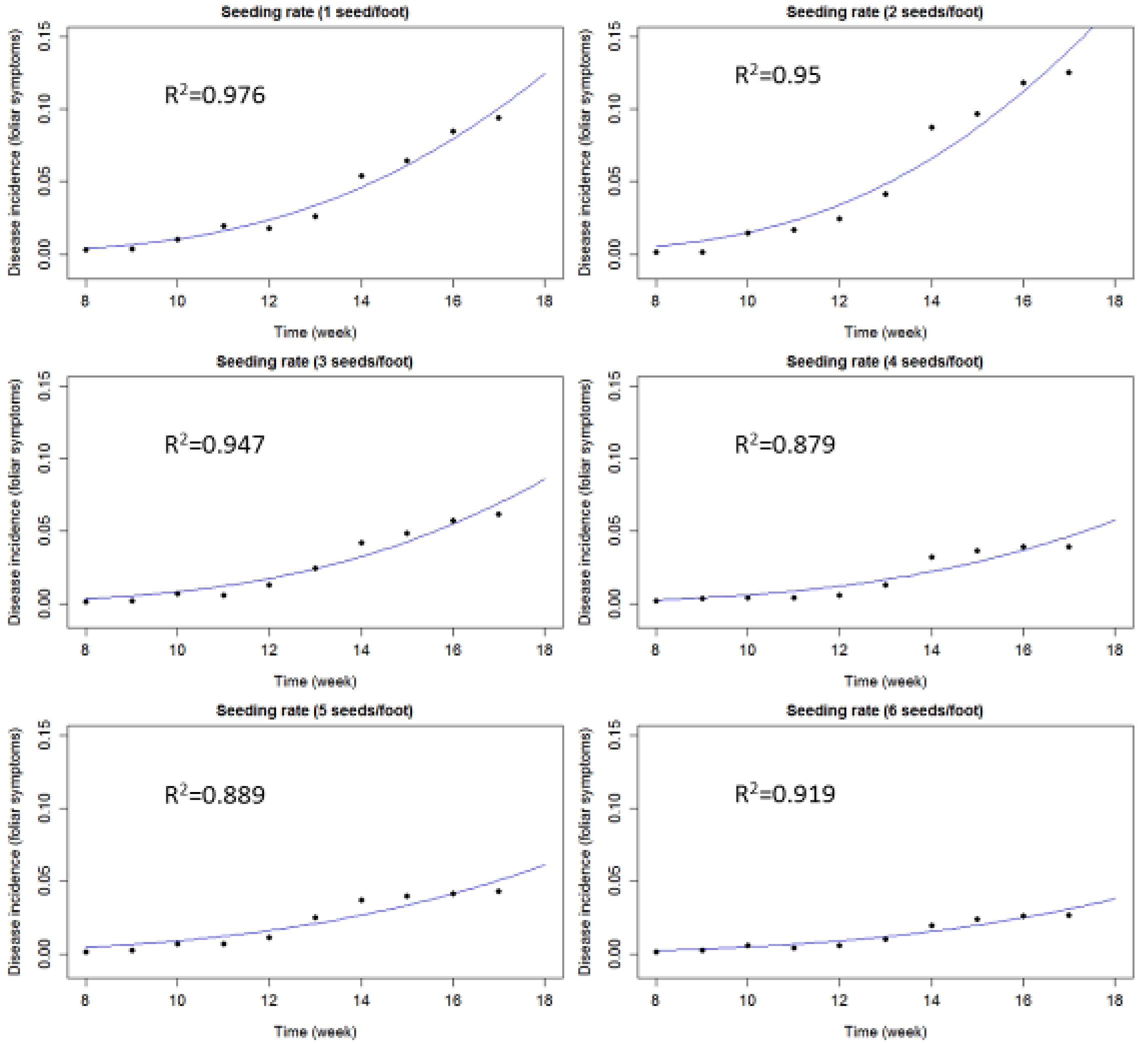

Figure 3. Foliar disease incidence progress of each seeding rate and fitting curve with Gompertz model.

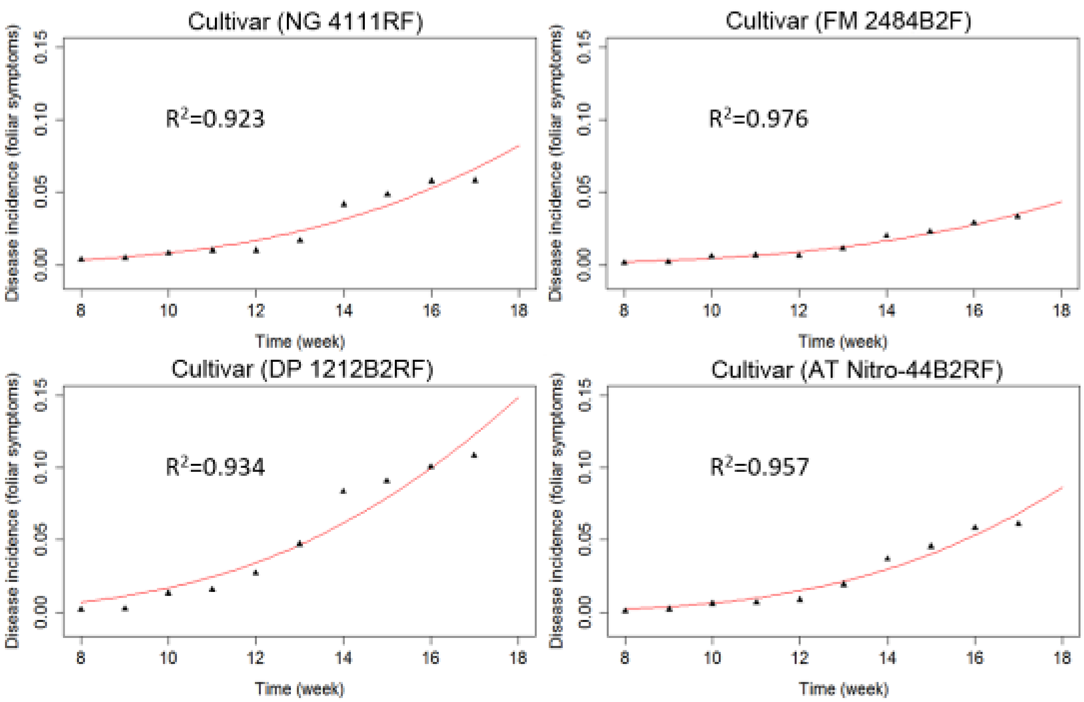

Figure 4. Foliar disease incidence progress for each cultivar and fitting curve with Gompertz model.

The linearized form of the Gompetz model can be expressed as $-\ln(-\ln y) = -\ln(-\ln y_0) + rt$. The derivative of the linearized form of the Gompetz model is r, which is representing the slope of the Gompetz model. Van der Plank (1963) used the r "apparent infection rate", which was based on the appearance of disease symptoms, to describe the rate of disease increase per unit of disease and had the units of proportion per unit of time. In this study, r was also used to indicate the disease epidemic rate as Van der Plank (1963) study. The epidemic rate (r) decreased as seeding rates increased (Table 3). The disease incidence of susceptible cultivar DP 1212B2RF developed more rapidly than other partially resistant cultivars in this study.

Table 3. The epidemic rate (r) and initial disease incidence ($b = -\ln y_0$) simulated by Gompertz model for each seeding rate and cultivar[a]

Seeding rate (seeds ft^{-1})	***r***	***b***
1	0.097	12.0
2	0.108	12.4
3	0.083	10.9
4	0.072	10.4
5	**0.065**	9.0
6	**0.060**	9.7
Cultivar		
NG 4111RF	0.081	10.8
FM 2484B2F	0.067	10.5
DP 1212B2RF	**0.095**	10.5
AT Nitro-44B2RF	0.090	12.3

[a] Gompertz model expression, integrated form: $y = \exp[-b \exp(-rt)]$; $b = -\ln y_0$
Linearized form: $-\ln(-\ln y) = -\ln(-\ln y_0) + rt$

Summary

High seeding rates (3-6 seeds ft^{-1}) and partially resistant cultivars (NexGen 4111 RF, Fibermax 2484 B2F, and All-Tex Nitro-44 B2RF) significantly reduced incidence of Verticillium wilt of cotton. The Gompertz model was selected as the best fitting model for the temporal disease progress simulation. The epidemic rate of the disease decreased as the seeding rate increased. The susceptible cultivar Deltapine 1212 B2RF had rapid disease development relative to the partially resistant cultivars in this study. The disease process depends on the combined effect of pathogens, host susceptibility, and various environment factors. Inoculum density, cultivar tolerance, soil and air temperature, precipitation, and irrigation could all play key roles in disease development and subsequent economical loss.

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