The Effects of Temperature and Wetness Period on the Development of Spinach White Rust

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ABSTRACT

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Experiments were conducted in controlled environments to determine the influence of temperature and duration of wetness on development of white rust of spinach. Plants of the susceptible cv. Kent were exposed to temperatures of 6 to 28°C and interrupted wetness periods that totaled 3 to 84 h following inoculation. Disease severity was assessed following further incubation in a greenhouse at 20 to 30°C. Disease was observed at all temperatures and increased with wetness duration. The optimum temperature for disease development ranged from 12 to 18°C. Only 3 h of wetness were required for disease development at 12 to 22°C. A minimum wetness period of 6 to 12 h was required for disease development at suboptimal temperatures. A multiple regression model describing the response surface of arcsine square root transformed disease severity was developed that had significant quadratic wetness effects, cubic temperature effects, and interaction between temperature and wetness. The resulting polynomial model provided a good fit to the observed data, accounting for 89% of the variation in transformed disease severity.

Additional keywords: *Albugo occidentalis*, epidemiology, oomycete, *Spinacia oleracea*

Albugo occidentalis Wilson, is an obligate oomycete pathogen that causes white rust, an economically important foliar disease of spinach (*Spinacia oleracea* L.) in production areas of the United States east of the Rocky Mountains (5). White rust is a serious problem for spinach production in the Arkansas River Valley of Oklahoma, where it was first reported in 1943 (4,18). The disease causes substantial yield losses through a reduction in the quality and marketability of fresh and processed spinach (2).

Symptoms of white rust begin as chlorotic lesions on the upper leaf surface. As the lesions develop, small white pustules (sori) containing sporangia are produced on the underside of the leaf and occasionally on the upper leaf surface (16,17). The pustules often are so abundant that nearly the entire leaf surface is covered. Primary infection is thought to occur from soilborne oospores that are splashed on plants by rainfall or overhead irrigation, or by airborne sporangia (6,8,22). It is

speculated that oospores germinate and infect plants through open stomata (17). However, there have not been any descriptions of oospore germination in *A. occidentalis*, and their role in the disease cycle has not been defined (5). Secondary infection results from airborne sporangia discharged from pustules (16). Sporangia can germinate directly, but usually germinate indirectly to produce six to nine biflagellate zoospores (16,17).

Specific temperatures and durations of free surface moisture are required for infection and subsequent disease development by many plant pathogenic fungi (3,11,14,19). Severe epidemics of white rust in Texas are favored by cool nights with heavy dew alternating with warm, dry sunny days (16). Temperatures of approximately 22°C are optimal for the production of sporangia (5). Sporangia germinate indirectly at temperatures from 2 to 25°C with the optimum ranging from 12 to 16°C (17). The optimum temperature for zoospore release is 12°C (17).

Free surface moisture is required for the indirect germination of sporangia, and may be a useful parameter for predicting infection periods and subsequent disease (17). Dainello and Jones (7) used continuous hours of leaf wetness to prompt fungicide applications and achieved efficient disease control when fungicides were applied following 12 continuous hours of leaf wetness.

White rust is managed through crop rotation, partial genetic resistance, and fungicide programs (7,15). For efficient disease

management with fungicides, knowledge of the environmental conditions required for disease development would be beneficial. However, temperature and the duration of leaf wetness requirements for disease development have not been defined (17).

It may be possible to forecast white rust by verifying the 12-h wetness threshold and incorporating other variables that are required for sporangial germination, such as temperature. The objective of this study was to quantify the effects of temperature and the duration of leaf wetness on the development of white rust of spinach. A brief report of this work has been published (21).

MATERIALS AND METHODS

Pathogen maintenance and inoculation. Cv. Kent spinach seedlings were grown in a greenhouse for 40 to 60 days at 20 to 30°C in plastic pots (10 cm diameter) containing a soilless growing medium (65% peat moss, 20% vermiculite, 10% perlite, and 5% coarse sand). Plants were watered as needed, and nutrients were supplied by applying liquid fertilizer weekly (0.029, 0.013, and 0.025 g/liter N-P-K, respectively).

An isolate of *A. occidentalis*, obtained from diseased leaves collected in a field in Bixby, OK, was maintained on spinach plants. Oospores were extracted from leaves as previously described (24) and assayed for infectivity. Oospores failed to germinate or cause disease following incubation of inoculated plants during an extended 3-week period of wetness at 12°C. Therefore, sporangia were used in all subsequent inoculations. Sporangial suspensions were prepared by agitating pieces of leaves with pustules in double distilled water. The suspensions were strained through cheesecloth and adjusted to 1×10^5 sporangia per ml using a hemacytometer. Suspensions were sprayed to run-off onto the adaxial surfaces of leaves using a hand-held spray bottle. Plants were spray-inoculated every 2 weeks for the duration of the study and placed in a dew chamber (Model I-60DL, Percival, Boone, IA) at 15°C with a 12-h wet cycle and 12 h of light each day for 2 to 3 days. Plants were removed from the dew chamber and incubated in the greenhouse at 20 to 30°C until symptom development, approximately 14 days after inoculation.

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Cultivar screening, inoculation, and disease assessment. The cvs. Avon, Kent, Melody, and Bloomsdale were inoculated as described above. There was no difference among cultivars ($P > 0.1$) for disease severity, which ranged from 79% for Bloomsdale to 83% for Avon on average. Kent was readily available and was used in all further experiments.

A detached-leaf method (25) was used to determine the minimum concentration of sporangia that would yield maximum severity of disease. For the detached-leaf method, two fully expanded, detached leaves were placed in a 100 × 15 mm petri dish containing 1.5% water agar amended with benzyladenine (0.5 µg/ml). The adaxial leaf surface was inoculated with 10 drops of suspension at concentrations of 0, 10, 10², 10³, 10⁴, 10⁵, and 10⁶ sporangia per ml using a sterile pipette. The plates were placed in a dew chamber at 15°C for 3 days, removed, and incubated at room temperature. Disease severity (the percentage of abaxial leaf surface with pustules) was estimated on each detached-leaf 14 days after inoculation and the experiment was repeated. Leaves were sorted into five classes from 1 to 50% using the previously published (6) area diagrams of diseased spinach leaves as a guide in making disease assessments. Two additional classes, 51 to 75% and 76 to 100%, were added to assess higher disease severities. The average percent leaf area with pustules was

then calculated using class frequencies and the midpoint value between classes (6). A minimum concentration of 10² sporangia per ml was required for disease development. Disease severity increased with inoculum concentration up to 10⁵ sporangia per ml, where it leveled off and achieved the maximum level of 83% disease on average. Therefore, 10⁵ sporangia per ml inoculum concentration was used in all further experiments.

Temperature and wetness period effects. Plants were exposed to various temperatures and periods of relative humidity (≥95%), hereafter referred to as wetness period, using dew chambers. Due to the limited availability of dew chambers, experiments were conducted over time by temperature. The order of temperature treatments was assigned at random and was repeated. Plants were spray-inoculated as described above, and placed in a dew chamber set at 6 to 28°C at 2°C increments following inoculation. Within each temperature treatment, plants were exposed to cumulative wetness periods of 0, 3, 6, 12, 24, 36, 48, 60, 72, and 84 h in increments of 12 h per 24-h period. Each dew chamber was set for a 12-h dark period of relative humidity (≥95%), which supported infection, and a 12-h light period of 70 to 75% relative humidity. The regime was used to simulate the cyclic nature of wetness periods in the field. Temperature and relative humidity in each chamber were monitored

with a seven-day recording hygrothermograph (Model 5020-A, Weathertronics, Sacramento, CA). After exposure to a specified wetness period, three plants from each chamber, a total of six plants per temperature and wetness combination, were removed at random, transferred to a greenhouse at 20 to 30°C, and maintained until symptoms developed. Inoculated plants were placed in the chamber at the beginning of a 12-h dark period. The 0-h plants were immediately transferred to the greenhouse, while the 3- and 6-h plants were removed before the completion of the first dark period. Disease severity was visually estimated on 8 to 10 leaves per plant as above.

The experimental design was a split-plot with dew chamber temperature as the whole-plot treatment and wetness period as the split-plot treatment. The whole-plot was arranged in a randomized complete block design with chamber as the blocking factor. An observation used for statistical analysis was the mean value of the 8 to 10 leaves evaluated per plant, for a total of six observations per temperature and wetness period combination. Because variances were not homogenous, the data were transformed using arcsine square root calculations. Analysis of variance (ANOVA) was performed with the PROC MIXED procedure of SAS (version 6.11, SAS Institute, Cary, NC), which considers random effects. This procedure is appropriate for a split-plot experimental design when the

Fig. 1. The observed severity ranging from 0 to 100% of white rust of spinach plotted against temperature for various wetness periods. Data points represent the mean values at 14 days after inoculation over two experiments and six plants per wetness and temperature combination ($N = 12$).

Fig. 2. The observed severity ranging from 0 to 100% of white rust of spinach plotted against wetness period for various temperatures. Data points represent the mean values at 14 days after inoculation over two experiments and six plants per temperature and wetness combination ($N = 12$).

whole-plot error term and the blocking factor are random (20). Multiple regression analysis, performed with the PROC REG procedure of SAS, was used to determine the functional relationship of wetness period and temperature on disease severity. A regression equation was computed using the PROC REG procedure. An F test, the coefficient of determination (R^2), and the adjusted R^2 for degree of freedom evaluated the goodness of fit of the regression model.

RESULTS

Results for the two experiments were similar. ANOVA indicated that there was

no effect ($P \geq 0.5$) of experiment, chamber, or the interaction between experiment and chamber on disease severity. There also were no significant interactions among experiment, chamber, and temperature and among experiment, chamber, and wetness period ($P \geq 0.5$). Therefore, data by experiment and chamber were combined. In the combined analysis, the interaction between temperature and wetness period was significant ($P \leq 0.0001$).

Disease was observed at all temperatures evaluated, and generally, there was a sigmoidal increase in disease severity with increase in wetness period (Fig. 1). The

optimum temperature for disease development ranged from 12 to 18°C. The maximum disease severity occurred at 12°C at the 84-h wetness period, where disease severity approached 90%. Disease severity also was high at 14 to 18°C and ranged from 70 to 80% at the 84-h wetness period. Higher ($>18^\circ\text{C}$) or lower temperatures ($<12^\circ\text{C}$) resulted in lower levels of disease severity. Disease severity was $<1\%$ at 6 and 28°C for all wetness periods. Disease severity increased from 6 to 10°C, peaked at 12°C, leveled off from 14 to 18°C, and declined from 20 to 28°C (Fig. 2).

White rust was observed at all wetness periods (Fig. 2). Disease did not develop at the 0-h wetness period for any of the temperatures evaluated. Although maximum disease severity occurred at the 84-h wetness period for all temperatures, the minimum wetness duration required for disease development was 3 h at 12 to 22°C. As temperatures increased or decreased from the 12 to 22°C range, a longer wetness period was required for disease development. A minimum wetness period of 6 to 12 h was required for disease development at the suboptimal temperatures of 8, 10, 24, and 26°C. A minimum of 72 and 60 h of wetness were required for disease development at 6 and 28°C, respectively (Fig. 2).

A regression analysis was performed to better assess the relationship of temperature and wetness period to the arcsine square root transformed disease severity.

Table 1. Estimated parameter values from the polynomial regression equation describing the relationship of postinoculation wetness period (W) and temperature (T) on severity of white rust of spinach (Y)^a

Parameter ^b	Parameter estimate	Standard Error
b_0 (intercept)	-0.912896	0.09920274
b_1	-0.065962	0.00669859
b_2	0.000593	0.00008146
b_3	0.199248	0.02106211
b_4	-0.010643	0.00133817
b_5	0.000168	0.00002607
b_6	0.014963	0.00142220
b_7	-0.000131	0.00001730
b_8	-0.000717	0.00009036
b_9	6.363×10^{-5}	0.00000110
b_{10}	9.642×10^{-5}	0.00000176
b_{11}	-8.739×10^{-8}	0.00000002

^a Data from two experiments did not differ significantly ($P \leq 0.5$) and were combined for analysis. Regression analysis was performed on arcsine square root transformed values of disease severity.

^b All parameters were significant at $P \leq 0.0001$.

Fig. 3. Observed and predicted values of severity (Y) of white rust of spinach plotted against wetness period (W) by temperature. Lines represent predicted values generated by the polynomial model (see text) and the parameter estimates in Table 1. Data points represent the observed values (arcsine square root transformed) 14 days after inoculation averaged over two experiments and six plants per wetness and temperature combination ($N = 12$).

Fig. 4. Observed and predicted values of severity (Y) of white rust of spinach plotted against temperature (T) by wetness period. Lines represent predicted values generated by the polynomial model (see text) and the parameter estimates in Table 1. Data points represent the observed values (arcsine square root transformed) 14 days after inoculation averaged over two experiments and six plants per temperature and wetness combination ($N = 12$).

Regression analysis showed significant linear, quadratic, and cubic relationships between disease severity and temperature (T) averaged across wetness period ($P \leq 0.0001$). There also were significant linear and quadratic relationships between disease severity and wetness period (W) averaged across temperature ($P \leq 0.0001$). The W -linear $\times$ T -linear, W -quadratic $\times$ T -linear, W -linear $\times$ T -quadratic, W -quadratic $\times$ T -quadratic, W -linear $\times$ T -cubic, and W -quadratic $\times$ T -cubic interactions also were significant ($P \leq 0.0001$).

The following model best defined the relationship of temperature (T) and wetness period (W) on the arcsine square root transformed white rust severity (Y):

$$Y = b_0 + b_1W + b_2W^2 + b_3T + b_4T^2 + b_5T^3 + b_6WT + b_7W^2T + b_8WT^2 + b_9W^2T^2 + b_{10}WT^3 + b_{11}W^2T^3$$

where b_0 - b_{11} are the parameter estimates (Table 1). The R^2 and adjusted R^2 were 0.8917 and 0.8909, respectively. The values predicted by the polynomial model fit well the observed means at each temperature (Fig. 3) and wetness period (Fig. 4). There was an increase in predicted disease severity with an increase in W until 60 h at all values of T , except at 6 and 28°C (Fig. 3). Unimodal curves were produced when disease severity was plotted against temperature (Fig. 4). Predicted responses to temperature were reduced with decreasing values of wetness duration. The predicted disease severity was at maximum at 14°C and 60 h of leaf wetness. The predicted range of optimum temperatures was 12 to 18°C, and disease severity increased with an increase in wetness duration until 60 h (Fig. 5).

DISCUSSION

Temperature and wetness period are important factors for white rust development in spinach. The interaction between temperature and wetness period significantly influenced disease development. No disease developed until 72 and 60 h of wetness at 6 and 28°C, respectively. Temperatures of 12 to 18°C favored disease development when accompanied by only 3 h of wetness. Disease severity increased following 12 h of wetness at 8, 10, 20, 22, and 24°C, which contributed to the interaction.

Disease was not observed at the 0-h wetness period even in the presence of inoculum and optimal temperatures indicating that wetness is required for disease development. These results are similar to those of Raabe and Pound (17) who determined that the optimum temperature for indirect germination of sporangia was from 12 to 16°C and that free surface moisture was required for germination.

Disease was observed at all temperatures evaluated in this study (6 to 28°C), although disease severity at 6 and 28°C was <1% for all wetness periods. Disease se-

verity also decreased when temperature increased or decreased from the optimum range of 12 to 18°C. Zoospore release from sporangia of *A. occidentalis*, *A. tragopogi*, and *A. candida* can occur over a relatively wide temperature range of 2 to 25°C (9,13,17). However, germination decreased when temperature was above or below the optimal range of 10 to 16°C (9,13,17). The decrease in disease seen in this study may be due to a reduction in zoospore release at suboptimal temperatures.

Free surface moisture is a requirement for development of other diseases caused by oomycetes. For downy mildew of cantaloupe caused by *Pseudoperonospora cubensis*, 4 to 6 h of free surface moisture is required for infection and disease development (10,23). Late blight outbreaks, caused by *Phytophthora infestans*, are greatest when zoospores are formed, and when there is a film of moisture on the leaf surface (1). High relative humidity ($\geq 95\%$) favors outbreaks of downy mildew of crucifers, caused by *Peronospora parasitica*, and promotes sporulation (12).

In this study, there was a general trend for disease severity to increase with an increase in wetness period duration. This finding is not unexpected, since continuous free moisture is required for the indirect germination of sporangia, germination of zoospores, and penetration of leaf tissues. The longer the duration of leaf wetness the greater the likelihood of infection and dis-

ease development (1,10). According to Gross et al. (11), the effects of temperature are more pronounced for longer wetness duration periods than shorter periods indicating that leaf wetness is perhaps the critical environmental variable for infection, though temperature regulates the rapidity and level of disease development.

A high proportion of the variability of mean disease severity was accounted for by the components of the polynomial model. The model fit the observed data well with the following exceptions. Predicted disease severity was at a maximum at 14°C and 60 h of wetness. However, the observed maximum disease severity occurred at 12°C and at 84 h of wetness. The model also predicted that disease severity increased with wetness duration until 60 h. However, observed disease severity continued to increase after 60 h of wetness. In the observed data, there was a distinct maximum in disease severity at 12°C for all wetness periods. Although the observed and predicted disease severities did not always match, the model was adequate for describing the range of optimal temperatures and wetness periods.

This study verified that the 12-h period of continuous wetness, previously identified in the field as a threshold for fungicide application, supported disease development (7). Disease severity ranged from 0 to nearly 70% at this wetness period depending on temperature. Results of this study

indicated however, that as few as 3 h of wetness were required for disease development at optimum temperatures, while 6 to 12 h were necessary at suboptimal temperatures of 8, 10, 20, and 22°C. A wetness period of 3 h often occurs in a single dew period in the field, and thus makes verification of this wetness period in the field very important. Although interrupted wetness periods were applied, temperature was kept constant in the chamber. In the field, humidity and temperature both fluctuate. In addition, differences in plants grown in the field versus those grown in the greenhouse may be important.

This model describes the influence of temperature and wetness duration on white rust development. The defined relationship between white rust disease development and environmental conditions might be useful for timing fungicide sprays in the field. The response surface will be used as a framework for developing and testing a weather-based spray advisory for white rust in the field.

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Fig. 5. Response surface for the polynomial model (see text) describing the influence of temperature and wetness period on the severity of white rust of spinach.

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