Effects of Temperature and Wetness Duration on the Sporulation of *Phomopsis viticola* on Infected Grape Canes

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Abstract

In 2008, research was initiated to examine effects of temperature and wetness duration on the sporulation of *Phomopsis viticola* on infected grape canes and to determine effects of interrupted wetness duration on sporulation. To determine effects of temperature (T) and wetness duration (WD) on sporulation, a split-plot experimental design was used, with T (5, 12, 15, 18, 20, 22, 25, 28, and 35°C) assigned to whole-plots and WD (11, 23, 35, 47, and 71 h) assigned to sub-plots. Linear and nonlinear mixed models were fitted to the data. Lower and upper limits of sporulation were found to be at 5 and 35°C, respectively. Optimum sporulation was near 22°C, and sporulation increased with increasing WD. Of the examined models, a generalized Analytis Beta model fit the data best. To determine effects of wetness interruption (IWD), a split-plot was used, with T (12, 15, and 20°C) assigned to whole-plots and IWD (0, 2, 4, 8, 12, and 24 h) assigned to sub-plots. Generally, sporulation declined with increasing IWD. An IWD of 12 h or more resulted in significantly and substantially less sporulation compared to the control (0 h IWD). Using a repeated-measures design, spore density and environmental data were measured in the vineyard during and following individual rain events; a preliminary model predicted the temporal trend in spore density within the vineyard fairly well ($R^2 = 0.719$), although absolute magnitude of sporulation could not be predicted.

Introduction

Despite fairly extensive fungicide programs, the incidence of Phomopsis cane and leaf spot (PCLS) appears to be increasing in many Ohio vineyards. This disease, caused by *Phomopsis viticola*, occurs worldwide wherever grapes are grown but is most economically important in the Midwest and Northeast temperate regions of the USA (6). In the presence of favorable environmental conditions, PCLS is capable of reducing yields by 30% (3,5). All above ground tissues of the grapevine are susceptible to infection by *P. viticola* during some stage in their development; however, PCLS has its greatest negative impact on yield when affecting fruit and rachises, which remain susceptible throughout the growing season (4). Berries infected by *P. viticola* develop a rot near harvest and become unmarketable (6). Rachises with high levels of PCLS become weakened, which can result in yield loss due to premature fruit drop (6). Leaves with PCLS lesions have a reduced photosynthetic capacity, and when heavily infected, they become wrinkled and abscise prematurely. Severely infected canes become weakened, which predisposes them to winter injury (6).

Phomopsis cane and leaf spot is a monocyclic disease (1). During wet conditions in the spring (from bud break until shortly after the end of bloom), pycnidia on canes and rachises infected during the previous growing season extrude cirrhi. This gelatinous matrix dissolves in the presence of water, allowing conidia to be splash-dispersed to susceptible grape tissues, resulting in new infections. Towards the end of the growing season, when canes and
rachises begin to produce a periderm layer, *P. viticola* forms pycnidia within these tissues. These pycnidia, as well as hyphae within dormant buds (9), act as overwintering structures for the fungus, but pycnidiospores (e.g., α-conidia) typically are not produced until the following growing season (1).

The effect of temperature and wetness duration on infection of grape canes and leaves by *P. viticola* α-conidia has been published (5,7); however, the effect of temperature and plant-surface wetness duration on the sporulation of *P. viticola* in the field has not been reported. This is an extremely important component of the disease cycle. Under environmental conditions that are not favorable for sporulation, fungicide applications for disease control would not be required, even if conditions for infection were favorable. Understanding the environmental factors that favor sporulation may be economically beneficial in controlling the disease, either by improving the timing of fungicide applications or eliminating unneeded applications. Improving chemical control of this disease is important because PCLS-resistant cultivars are not currently available (3,6,8).

In 2008, research was initiated to determine the temperature and wetness duration conditions required for sporulation of *P. viticola*. These studies should be helpful in predicting the timing and amount of sporulation (disease risk) of *P. viticola* in grape vineyards. The objectives of this research were to: (i) examine the effects of temperature and wetness duration on the sporulation of *P. viticola* on infected grape canes and to develop a predictive model for sporulation within vineyards; (ii) determine the effects of interrupted wetness duration (split wetness periods) on sporulation of *P. viticola*; and (iii) measure spore density and environmental variables in the vineyard in order to evaluate the predictive model developed under Objective 1.

**Temperature, Wetness Duration, and Sporulation of *P. viticola***

To investigate the effects of temperature (T) and wetness duration (WD) on the sporulation of *P. viticola* on infected grape canes, a growth chamber experiment was conducted utilizing a split-plot experimental design. T treatments (5, 12, 15, 18, 20, 22, 25, 28, and 35°C) were assigned to the whole-plot and WD treatments (11, 23, 35, 47, and 71 h) were assigned to the sub-plot. All cane samples (~10 cm long) used in this experiment were collected on one sampling date near the end of the dormant overwintering period (mid March). After sampling, canes were immediately placed in four layers of 2-mil freezer bags, sealed with a twist tie, and stored in a -20°C freezer. Canes were randomly removed from the freezer in groups of 75 (5 WD per T, 15 canes per treatment). Canes were allowed to thaw and were then surface disinfested by soaking canes for 5 sec in 70% ethanol, then 1 min in 0.5% sodium hypochlorite, and then rinsing under deionized water for 2 min. After surface disinfection, canes were placed in moist chambers (22 × 22 × 10 cm, 4.5-liter plastic containers) with enough deionized water in the bottom to achieve the dew point across all T examined. This resulted in relative humidity at or near 100%, and, thus, maintained a layer of free water on the canes. Metal screens were bent to hold canes above the deionized water and below the transparent plastic top of the moist chamber. Moist chambers were randomly arranged in the center of a growth chamber (Model 57, Controlled Environments Inc., Pembina, ND). The growth chamber was lit with fluorescent lights on a 16:8 h photoperiod. Moist chambers were arbitrarily removed from the growth chamber at 11, 23, 35, 47, and 71 h. Samples were vortexed (speed = 5) in 20 ml deionized water for 10 sec (Vortex Genie 2, Fisher Scientific, Pittsburgh, PA). Sporulation on each cane was quantified as the average of six hemacytometer readings multiplied by 20 ml/mm². Surface area was calculated assuming a surface area given by a cylinder. Because disease severity did not significantly affect the amount of sporulation of *P. viticola* within infected canes (unpublished), sporulation per sample was not standardized by disease severity.
Data were obtained from two experimental repetitions, each with two blocks. A generalization of Analytis’ Beta model for T and WD was used to quantify the results (NLMIXED procedure in SAS 9.2, SAS Institute Inc., Cary, NC).

Initial results indicate that sporulation of *P. viticola* on infected canes can occur between the cardinal points of 5 and 35°C, with an optimum of 22°C (see Fig. 1). Little to no sporulation was observed at 11 h WD from 5 to 35°C. At WD above 11 h, sporulation increased with increasing WD to the maximum observed WD of 71 h.

**Interrupted Wetness Duration and Sporulation of *P. viticola***

In order to examine the effects of interrupted wetness duration (IWD) on the sporulation of *P. viticola*, as well as to assist in the analysis of environmental data collected in the vineyard (see Objective 3), a growth chamber experiment was conducted utilizing a split-plot experimental design. T treatments (12, 15, and 20°C) were assigned to the whole-plots, and the length of IWD treatments (0, 2, 4, 8, 12, and 24 h) was assigned to the sub-plots. Cane collection, storage, surface disinfestation, and moist chamber protocols were the same as described in Objective 1. After an initial WD of 24 h, metal screens that supported the canes were removed from chambers and placed on top of the resealed moist chambers. This allowed the canes to dry in less than 5 min. During periods of IWD, moist chambers remained in the controlled environmental chamber, which resulted in consistent T. The positive control consisted of 0 h IWD, during which the canes remained in the moist chamber without interruption of the WD. After each IWD treatment, canes and metal screens were rewetted with deionized water using an atomizer (pressure: ~55 kPa, from a distance of ~19 cm) (Model 5KH33DN16AX, General Electric, Fairfield, CT) to the point just before runoff and returned to their respective moist chambers for a final WD of 24 h. Sporulation was quantified as previously described. The experiment contained two blocks and was conducted once.

A generalized linear mixed model was fitted to the sporulation data using the GLIMMIX procedure of SAS to determine the effects of IWD and T, and their interactions (i.e., whether the effect of IWD is dependent upon T), on sporulation. One-sided contrasts of the least squares means were used to determine which IWD reduced sporulation compared to the control.

Analysis of data from the first repetition indicated that sporulation generally declined with increasing IWD (Fig. 2). Both IWD and T affected sporulation (*P* < 0.01). However, there was no interaction of T and IWD (*P* = 0.828); thus, one-sided contrasts of the least squares means were conducted on the sporulation data across all three T. An IWD of 12 h or more resulted in less sporulation compared to the control (*P* < 0.01) (Fig. 2, Table 1). These results suggest that predictions of sporulation in the vineyard should be determined by summing sporulation predicted to occur during each wetness period separated by a threshold IWD (12 h), rather than predicting sporulation based on cumulative WD from all wetness events between the end of each rain event.
Fig. 1. Predicted effects of temperature and wetness duration on sporulation of *P. viticola* on infected grape canes, based on fit of a generalized Beta model to the data of experiments from 2009 and 2010.
Fig. 2. Effect of interrupted wetness duration on sporulation of *P. viticola* on infected grape canes, 2010. Error bars are standard errors about the mean of 2 replicates.

Table 1. Differences of least squares means of *P. viticola* sporulation on grapevine canes under different interrupted wetness durations.

<table>
<thead>
<tr>
<th>Contrast (h)</th>
<th>Estimated difference&lt;sup&gt;x&lt;/sup&gt;</th>
<th>&lt;sup&gt;y&lt;/sup&gt;p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 vs. 2</td>
<td>0.265 (0.259)</td>
<td>0.1601</td>
</tr>
<tr>
<td>0 vs. 4</td>
<td>0.419 (0.294)</td>
<td>0.0863</td>
</tr>
<tr>
<td>0 vs. 8</td>
<td>0.321 (0.275)</td>
<td>0.1299</td>
</tr>
<tr>
<td>0 vs. 12</td>
<td>1.234 (0.382)</td>
<td>0.0025</td>
</tr>
<tr>
<td>0 vs. 24</td>
<td>2.556 (0.625)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

<sup>x</sup> Values are estimates (standard errors) of differences of treatment least squares means with the control.

<sup>y</sup> P < 0.01 for a significant difference between the control (no wetness interruption) and the indicated IWD (one-sided test, PROC GLIMMIX).

**Sporulation in the Field**

To evaluate the model developed under Objective 1 in the vineyard, a repeated-measures experiment was conducted. Fifteen spore traps were placed next to the main trunks of randomly selected grapevines, three traps per row, in the five most easterly rows of a ‘Catawba’ vineyard. Spore traps consisted of 2-liter plastic bottles connected to 196-mm Urbanti funnels (CP Lab Safety, Novato, CA) via Nalgene premium tubing. Traps were collected after every rain event from near grape bud break through bloom until no spores of *P. viticola* were observed for three consecutive rain events. A rain event was defined as any period of precipitation after which any quantifiable amount of water was collected in the traps (0.1 mm or more). After being collected, spore traps were immediately refrigerated, and their spores were counted. Spore density per trap was measured as the average of six hemacytometer readings multiplied by the amount of water collected in each trap. Average spore density per trap was calculated from all fifteen traps for each rain event. Rain events separated by less than 12 h were pooled for collection and quantification purposes, based on the IWD threshold concluded from Objective 2. *Phomopsis viticola* spores (α-conidia) were identified by morphology and verified by isolating and culturing on streptomycin-amended potato dextrose agar. Environmental variables (e.g., T, leaf WD, and precipitation) were collected every 5 min with a data logger.
Thermisters and leaf wetness sensors (two each) were positioned in the canopy ~1.65 m above ground, and a rain gauge was positioned outside the canopy 1.8 m above ground. Data were collected in 2009 and 2010. The generalized Beta model developed under Objective 1 was evaluated for its appropriateness in characterizing spore density after each rain event based on corresponding environmental data and spore density observed with spore traps. The nonlinear model was used to predict spore density based on average $T$ during leaf wetness and total hours of WD for each leaf wetness period within each rain event; predicted spore density for each wetness event were summed for each rain event. However, this model was developed based on sporulation occurring on canes with a known unit surface area, and since the spore traps in the field would be sampling from an unknown total surface area of infected tissue, the developed Beta model would only be able to predict relative amounts of spore density. Furthermore, since the sporulation potential of infected tissues in the vineyard would be changing over the course of the growing season, predicted spore density would ultimately need to be scaled by some unit of time to account for this variable.

In 2009 and 2010, sporulation of $P. viticola$ occurred in the vineyard shortly after bud break and continued through bloom (Fig. 3). This confirms the importance of controlling this disease with fungicides during the early part of the growing season (2,7,8). Variability between observed and predicted spore density was greater in 2009 than in 2010 (Fig. 3). Spore density was under-predicted from 28 May 2009 to 1 June 2009 and over-predicted from 12 June 2009 to 25 June 2009. In 2010, predicted spore density followed the general trend of observed spore density, although the model appeared to over-predict spore density both early (before 8 May) and late (after 9 June) during the course of the experiment. These errors may be explained by two factors that were unaccounted for in this analysis: (i) the sporulation potential of infected tissues in the vineyard changes as those tissues age, and (ii) not accounting for how varying amounts of rainfall might affect dispersal of conidia to varying extents. The first is likely to contribute to differences (i.e., relative over-estimation) observed early and late during the sampling period, whereas accounting for the effects of rainfall may help explain periods when the model over-predicted spore density near the middle of the experimental period (during times of high sporulation potential). Thus, incorporation of such factors into the developed Beta model might improve the relationship between observed and predicted spore density. The relationship of predicted and observed sporulation in the vineyard for the combined data from 2009 and 2010 was fairly high (Fig. 3). However, the lack of many data points for observed spore density between 4 and 16 million $\alpha$-conidia/trap reflects the importance of including results from a third experimental repetition in the final analysis.
Fig. 3. Predicted and observed spore density in a 'Catawba' grapevine vineyard in 2009 (A) and 2010 (B). Bars represent mean a-conidia per trap per sporulation event. Dates correspond to rain event ending dates. The plotted line represents sporulation/mm² infected cane tissue predicted by the model developed under Objective 1. Plot of observed and predicted spore density in spore traps for the 'Catawba' vineyard in 2009 and 2010 (C).
The temporal trend of (predicted or observed) spore density, rather than its magnitude at a given time, is emphasized here based on the preliminary analytical results, as the discrepancy between the magnitude of predicted and observed spore density will be affected by biotic (e.g., age of infected tissues) and environmental factors that can change during the season but can be measured. Nevertheless, preliminary results show that the developed model can predict the temporal trend in spore density in the vineyard fairly well ($R^2 = 0.719$, regression equation forced through the origin). Analysis based on data from one or more additional growing seasons will be carried out with the goal of improving the predictions of spore density in the vineyard.

**Significance**

The results of this research add to our knowledge of the epidemiology of Phomopsis cane and leaf spot. Increasing our knowledge of the epidemiology will hopefully allow us to develop more effective management programs for this disease. Erincik et al. developed a predictive model for infection of grapevine canes and leaves by *P. viticola* based on T and leaf WD (5). Nita et al. incorporated the model into a disease warning (forecasting) system and validated the accuracy of the model in the vineyard (7). The warning system was shown to be useful in reducing the overall number of fungicide applications while providing about the same level of control as a calendar-based protectant program (7). The use of disease forecasting to schedule fungicide applications is an attractive alternative to the standard calendar-based protectant program that schedules the application of fungicide every 7 to 10 days, regardless of whether or not high-risk periods for infection have occurred. In dry growing seasons, applying fungicides in response to predicted infection periods should result in improved timing of fungicide application and a reduction in overall fungicide use. This approach to disease management complements the current philosophy and use of integrated disease management programs.

One weakness of the warning system is that it does not take the presence, production, and dispersal of primary inoculum into account. Obviously, unless the proper conditions for sporulation (and dispersal) have been met prior to the infection event, there can be no infection. Moreover, for a given risk of infection, the number of infections will be proportional to the level of spore production (up to a saturation level). Although sporulation and infection are both influenced similarly by T and WD, these processes occur separately (and sequentially) with respect to time, and because environmental conditions in the vineyard change over time, integration of the two models would proceed in a compartmentalized manner. That is, the risk of infection at a given time would be predicted based on the current T and WD conditions (at an assumed fixed level of spore density in the vineyard); then the infection risk would be adjusted up or down based on the predicted level of spore density, with this spore prediction being a function of prior T and WD conditions. This would not necessarily result in correlated predictions of sporulation and infection because of the time sequence involved. Because a fungicide application would only be recommended if conditions favored (relatively) high sporulation followed by (relatively) high probability of infection, it is expected that the integration of the models would result in a less than additive effect with respect to the number of fungicide applications needed to provide control comparable to a protectant spray program. In particular, the integration of these models is expected to result in fewer fungicide applications than the model based just on infection. The increased generality of a warning system based on both sporulation and infection could improve our ability to control this disease, which could also result in increased profitability to United States grape producers and a potential reduction in overall fungicide use and deposition in the environment. However, future experiments will need to be conducted to provide data indicative of the economic impact of utilizing such an integrated model in order to demonstrate to growers the usefulness of incorporating the model into their pest management program.


**Literature Cited**


