Factors Influencing Airborne Conidial Concentrations of *Alternaria panax* in Cultivated American Ginseng Gardens

S. N. Hill, Graduate Research Assistant, and M. K. Hausbeck, Professor, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824

ABSTRACT

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Leaf blight, caused by *Alternaria panax*, is the most common disease of cultivated ginseng and is an annual threat. To determine the influence of weather parameters on airborne conidial concentrations (ACCs), 3- and 4-year-old commercial American ginseng (*Panax quinquefolium*) gardens were monitored from mid-May to September for two growing seasons. Hourly concentrations of airborne *A. panax* conidia were enumerated using a Burkard volumetric spore sampler. The hourly averages of air temperature, rainfall, leaf wetness, and relative humidity were also collected. Fungicides were not applied. The incidence of leaf blight was assessed in predetermined areas of the monitored gardens. Disease pressure from *A. panax* was high in 2005 and 2006 and resulted in crop defoliation. Each year, ACCs were detected beginning in late May and continued through the growing season. Daily conidial concentrations followed a diurnal pattern and were greatest during periods of rapidly decreasing relative humidity. Relative humidity was negatively correlated to hourly ACCs. Each year, hourly ACCs were negatively correlated to leaf wetness and rainfall. A positive correlation was detected for hourly ACCs following 16 h or more of rainfall. A significant correlation was observed between hourly ACCs and temperature.

American ginseng (*Panax quinquefolium*) is a perennial herb grown primarily for the medicinal properties of its root (22). Currently, more than 95% of the cultivated commercial ginseng in the United States is grown in Wisconsin (1). Cultivated ginseng is grown on a raised plant bed under a tree or black woven polypropylene canopy (22). The shade required by the crop and dense plant spacing create a microclimate with limited air movement and extended leaf wetness periods. In Michigan and Wisconsin, the annual growing season for ginseng occurs over 5 months, with plant emergence beginning in May.

Alternaria blight, incited by *Alternaria panax* Whetzel, is the most common disease of ginseng throughout the world including Alberta, Canada (3,4), Asia (31), West Virginia (29), North Carolina (7), Michigan (11,12), Oregon, and Washington (24). This disease is a yearly problem for ginseng growers in Wisconsin and can be especially destructive, causing rapid defoliation and plant death (11–13,21).

Typical *Alternaria* foliar blight symptoms include circular to irregular necrotic lesions, 2 to 20 mm in diameter. The le-

sions have a dark brown margin and a yellow-green halo (21). Elongated lesions (15 to 45 mm in length) may also develop on the stem and cause girdling (2). Infected drupes may develop a water-soaked appearance followed by the development of gray mycelium and pathogen sporulation (11,12). Infection of the root by *A. panax* is rare (21); however, root weight can be reduced when blighting of the leaves and stem causes premature defoliation (2).

A. panax conidia may be produced in short chains of two to three spores, but spores more commonly are borne singly (2,31). Although the morphology of *A. panax* conidia is variable (5), conidia are typically 150 to 160 by 12 to 20 μm (2) with vertical (one or two) and transverse (nine to 11) septa (23) and are obclavate. Conidia are disseminated via wind, rain, or splashing water (27). *A. panax* can survive as conidia or as mycelium in infected plant residue, or in and on seeds (16). Lesion development and conidial production can occur in 5 to 7 days under favorable conditions (31). Optimal conidial production and mycelial growth occur with temperatures ranging from 18 to 25°C and 24 and 27°C, respectively (2,5,6,31). Specific leaf wetness parameters required for *A. panax* to infect and produce conidia and mycelia are unknown.

A. panax conidia may overwinter in mulch and crop debris providing initial inoculum for emerging crops in the spring (12,25). A layer of straw mulch (15 cm in thickness) generally is applied to ginseng beds immediately following seeding and reapplied throughout the crop production

cycle as needed. In winter, straw mulch can prevent temperatures from dropping below the freezing point of the roots (around -10°C), and in summer it keeps soil temperatures 5 to 10°C below that of an unshaded, grassy area (28). Mulch also eliminates the need for irrigation, as it prevents excessive moisture loss from the soil throughout the season (28). Despite these benefits, the use of straw mulch can also be problematic, as *A. panax* mycelium can spread from plant to plant through the mulch; the pathogen may also spread through the soil as a saprophyte until it contacts the ginseng host (2,11,16). Ginseng growers practice a no-till cultivation system; mulch and plant debris are not removed during the life of the crop (≥ 3 years). Over time, infected ginseng plant tissue can become integrated into the plant beds. Currently, there are no practical methods to reduce *A. panax* inoculum overwintering on the mulch or on infected crop residue (16).

In the absence of effective treatments for *Alternaria* blight, growers could potentially lose \$13,930/ha in root revenue due to repeated crop defoliation (12). Since *A. panax* also infects drupes, seed may be threatened. With an average seed yield of 337 kg/ha, growers could lose an additional \$18,537/ha (current market value \$55/kg) due to *Alternaria* blight (12).

Cultural strategies to manage *Alternaria* blight include increasing plant spacing to enhance air circulation, removing infected foliage, and replacing straw mulch in affected areas (7). Unfortunately, these strategies are not practical and would not preclude the reintroduction of inoculum via air currents. The use of biocontrols as alternatives to traditional fungicides has also been considered for *Alternaria* blight management. *Burkholderia cepacia* AMMD (*Pseudomonas cepacia* strain AMMD) was found to effectively inhibit *Alternaria* blight under growth chamber conditions. However, the biocontrol agent did not adequately reduce disease under field conditions due to poor survival on leaf surfaces (15,19,20).

Currently, growers rely on fungicides to protect their crop from *Alternaria* blight (11). Growers must apply fungicide sprays judiciously during the relatively long growing season of May through September to protect the crop and comply with product labels. Currently, azoxystrobin and pyraclostrobin are relied upon in disease

Corresponding author: Mary Hausbeck
E-mail: hausbeck1@msu.edu

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management programs (11,12). Strobilurin fungicides must be used in alternation with protectant fungicides (i.e., mancozeb, copper, or chlorothalonil) to delay the development of pathogen resistance (11).

To support the development of new management strategies that are less dependent on fungicides, additional epidemiological information for *A. panax* is needed. Ginseng growers would like to know when fungicide applications should be initiated in the spring and which weather parameters increase airborne conidial concentrations (ACCs). The objectives of this study were to determine (i) when airborne inoculum is available each season, and (ii) which weather factors influence ACCs.

MATERIALS AND METHODS

Plot establishment. Field studies were conducted in commercial cultivated gin-

seng gardens located in Marathon County, WI. Gardens were established from stratified seed which were broadcast planted into 1.5-m-wide raised and sloped plant beds with a peak height of 30 cm. Ginseng seeds were not planted on the lower portion (20 cm) of the beds. After seeding, the entire plant bed was covered with 15 cm of straw mulch. Each year, woven polypropylene panels providing 80% shade were suspended via 2- to 3-m posts over the plant beds to mimic woodland conditions. Weeds were pulled by hand.

Two gardens were monitored for *Alternaria* blight symptoms and airborne *A. panax* conidia. Garden A (3-year-old planting) was monitored from 24 May to 22 August in 2005. Garden B was monitored as a 3-year-old garden (2005) from 26 May to 9 September and as a 4-year-old garden (2006) from 25 May to 30 August. Gardens were divided in half, resulting in

approximately 1.6 ha for disease monitoring and spore trapping. Fungicides were applied to the western half of the ginseng gardens according to commercial standards. A 2.2-m nonsprayed buffer was maintained between the eastern and western garden halves. Fungicides were not applied to the eastern half of gardens where monitoring occurred.

Disease incidence. Within the eastern half of the monitored ginseng gardens, four plant bed sections (3 m in length) were randomly selected prior to the start of spore trapping for disease monitoring. Each bed section contained approximately 250 plants. The number of plants with one or more lesions was determined in each plant bed section. Disease incidence was assessed three times during the 2005 growing season and every other week in 2006.

Weather monitoring. Hourly measurements of air temperature and relative

Fig. 1. Hourly averages for *Alternaria panax* airborne conidial concentrations (ACCs), leaf wetness (LW), relative humidity (RH), and temperature in commercial Wisconsin ginseng gardens A-2005, B-2005, and B-2006.

humidity were recorded using a data recorder (Watchdog series 450, Spectrum Technologies, Inc., Plainfield, IL). A Watchdog leaf wetness sensor (Spectrum Technologies) was placed at a 45° angle facing north within the upper 25% of the ginseng canopy. Moisture occurring for a period greater than 1 min/h was recorded. Rainfall was measured hourly using a tipping-bucket rain collector (series 3554WD, Spectrum Technologies). In each garden, environmental monitoring equipment was placed within 1.5 m of the spore trap. Sensor positions were adjusted periodically as needed. Weather data were downloaded weekly to a laptop computer using commercially available software (Specware 6.01 and 7.01, Spectrum Technologies).

Inoculum availability and airborne concentrations. Concentrations of airborne *A. panax* conidia were monitored each growing season from 2005 to 2006 using a 7-day volumetric spore sampler (Burkard Scientific, Uxbridge, UK). Monitoring began in May (prior to the development of Alternaria blight symptoms) and concluded in August or September. One spore trap per garden was placed within the plant sections monitored for disease incidence. Spore traps were operated at a flow rate of 10 liter/min, and the orifice was free to move with changing wind direction. The orifice height was maintained 10 to 15 cm above the canopy. Conidia were collected on melinex tape (Burkard Scientific) coated with an adhesive mixture of petroleum jelly and paraffin (9:1, wt/wt) after heating to 60°C and cooling overnight before being dissolved with sufficient toluene to give a viscous consistency. Prior to petroleum jelly and paraffin coating, the melinex tapes were coated with a polyvinyl alcohol (Airvol/Gelvatol, grade 603, Burkard Scientific) and phenol mixture (35 g polyvinyl alcohol, 25 ml glycerol, 50 ml distilled water, heated to 40°C to combine, then cooled to 23°C before adding 2 g of phenol) and allowed to dry overnight at 23°C. Prepared tapes were maintained at 23°C until use.

Tapes were removed from the spore trap weekly and cut into 48-mm lengths and scored using a razor blade at 2-mm intervals corresponding to a 24-h day. Tape sections were mounted onto 3 × 2 cm glass slides and stained with aniline blue (0.14 mg aniline blue, 20 ml distilled water, 15 ml glycerol, 10 ml of 85% lactic acid). Slides were covered with 22 × 50 mm coverslips and sealed using Cytoseal (Richard-Allan Scientific, Kalamazoo, MI). Mounted slides were allowed to dry at 23°C for at least 24 h before microscopic observation at ×200.

Conidia were counted using a compound light microscope. *A. panax* was identified based on morphological characters (2,23). Occasionally, conidia of *A. alternata* (20 to 63 µm) and *A. solani* (150 to 300 µm) (26) were observed but were

not included in hourly conidial counts. Hourly conidial concentrations were converted to numbers of conidia per cubic meter of air sampled per hour. To eliminate zeros within the data set, 1 was added to each hourly count.

Statistical analysis. Data were first inspected visually by plotting ACC values against temperature (°C), relative humidity

(%), rainfall, and leaf wetness (min/h) within each 24-h period corresponding to 0000 to 2300 h for each garden location. This allowed us to identify positive or negative associations among variables. Visual correlations were verified using PROC CORR (SAS Institute Inc., Cary, NC) to determine correlation coefficients among hourly ACC and weather parameters (10).

Fig. 2. Weekly (wk) totals for rainfall and *Alternaria panax* airborne conidial concentrations (ACCs) as observed in commercial Wisconsin ginseng gardens A-2005, B-2005, and B-2006.

Regression analysis was performed using the PROC MIXED (SAS) to investigate the effect of single and two-factor interactions between weather parameters on hourly ACC values for all gardens. ACC values were regressed on temperature, relative humidity, leaf wetness, temperature $\times$ leaf wetness, and relative humidity $\times$ leaf wetness. Residuals from the regression model were evaluated for normality. Time series analysis procedures (PROC ARIMA, SAS) were used when residuals were dependent. Hourly average ACC data were differenced at lag 1 so that the data series was stationary. For each garden, ARMA models were chosen based on minimum Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (SBC) values.

RESULTS

Conidial availability, diurnal pattern, and airborne concentrations. Each year, ACCs were detected at the onset of spore trapping and occurred daily throughout the monitoring period. In 2005, ACCs during the first week of monitoring were $<1,000/\text{m}^3/\text{h}$, whereas in 2006 the ACCs were $>1,000/\text{m}^3/\text{h}$. When hourly ACCs were averaged over the monitoring periods, peaks occurred at 1100 h for garden B-2006 (257 conidia/ m^3/h), 1200 h for garden A-2005 (87 conidia/ m^3/h), and 1300 h for garden B-2005 (23 conidia/ m^3/h). Relatively few conidia were trapped between 0000 h and 0500 h, with an average of 26, 8, and 44 conidia/ m^3/h in garden A-2005, B-2005, and B-2006, respectively (Fig. 1).

ACCs and weather effects. Weekly rainfall averaged 12.9, 9.9, and 111.0 mm for gardens A-2005, B-2005, and B-2006, respectively (Fig. 2). Average hourly rainfall in gardens A-2005 and B-2006 was negatively correlated with hourly ACC values (Table 1). A positive correlation was observed for ACCs occurring 16 h or more after rainfall was detected. Across all gardens monitored, the highest weekly ACCs were observed during the latter portion of each growing season (e.g., July, garden B-2005; August, gardens A-2005 and B-2006) and typically were preceded by rainfall events (Fig. 2). Leaf wetness was detected and occurred with a diurnal periodicity in gardens A-2005 and B-2005 (Fig. 1). Within the 4-year-old ginseng garden (B-2006), leaf wetness did not fluctuate as observed in the 3-year-old gardens (Fig. 1). Hourly ACC values were negatively

correlated to leaf wetness in 2005 and 2006 (Table 1).

Within each 24-h period, ACCs peaked during periods of decreasing (20 to 80%) relative humidity for 2005 and 2006. Relative humidity was negatively correlated to hourly ACCs; each year the correlation was significant (Table 1). Temperature was positively correlated to ACCs and was significant each year (Table 1). Temperatures between 18 and 25°C were most common during all of the monitoring periods. Temperatures rarely fell below 10°C, with fewer than 10 h/week on average recorded. Temperatures exceeded 25°C each year, with an average of 19 to 24 h/week observed. The highest hourly temperature recorded in each garden was 34.5°C (A-2005), 36.6°C (B-2005), and 44.4°C (B-2006).

Regression analysis of *A. panax* conidial concentrations against temperature, leaf wetness, and relative humidity was described by the model ($-633.24 + \text{garden effect} + 5.0365 (\text{relative humidity}) + 23.7799 (\text{temperature}) - 45.2915 (\text{leaf wetness}) + 0.3027 (\text{relative humidity} \times \text{leaf wetness}) + 1.1772 (\text{temperature} \times \text{leaf}$

wetness). Standard errors and *P* values for terms in the regression model are presented in Table 2. Residues for garden A-2005, B-2005, and B-2006 were analyzed using ARIMA models (0,1,0), (2,1,1), and (0,1,1), respectively (Table 3). The effect of temperature, leaf wetness, and the cross interactions of temperature $\times$ leaf wetness and relative humidity $\times$ leaf wetness were significant to the model (Table 2).

Disease incidence. Each year, disease increased throughout the growing season, resulting in significant levels of foliar blighting (Fig. 3). The first *Alternaria* leaf blight lesions were observed on the foliage on 24 and 20 June of 2005 and 2006, respectively. A significant correlation ($P = 0.0048$) between weekly ACCs and plant disease incidence was observed (Fig. 3). Commonly, high levels (85%) of crop defoliation were observed in early August. By the end of the monitoring period, many of the garden sections were completely defoliated.

DISCUSSION

Ginseng growers are interested in adopting integrated pest management to reduce

Table 2. Parameter estimates, standard errors, and *P* values for effects in regression analysis describing the relationship between airborne *Alternaria panax* conidial concentrations, temperature, relative humidity, and leaf wetness

Effect ^a	Estimate	Standard error	<i>P</i> value
Intercept	-633.24	369.13	0.0912
Garden A-2005	-84.8388	18.1173	<0.0001
Garden B-2005	-138.84	9.7343	<0.0001
Garden B-2006	0	—	—
Temperature	23.7799	9.7751	0.0178
Relative humidity	5.0365	2.5529	0.0529
Leaf wetness	-45.2915	19.2453	0.0217
Temperature $\times$ leaf wetness	1.1772	0.4871	0.0186
Relative humidity $\times$ leaf wetness	0.3027	0.1272	0.0204

^a Values were obtained using PROC MIXED (SAS, Cary, NC).

Table 3. Estimates, standard errors, and *P* values for residues following regression modeling as determined by ARIMA^a

Effect	Estimate	Standard error	<i>P</i> value
Garden A-2005			
Residue mean	0.17558	2.46271	0.9438
Garden B-2005			
Residue mean	0.31689	2.33803	0.8936
Auto regressive	0.47847	0.31797	0.1488
Moving average	1.03274	0.24537	0.0005
Garden B-2006			
Residue mean	1.14591	3.00563	0.7068
Moving average	0.54076	0.18478	0.0081

^a ARIMA models (0,1,0), (2,1,1), and (0,1,1) were for residue analysis in gardens A-2005, B-2005, and B-2006, respectively. Significant effects occur with $P < 0.005$.

Table 1. Correlation coefficients between hourly airborne *Alternaria panax* conidial concentrations and hourly weather parameters in commercial Wisconsin ginseng gardens monitored in 2005 and 2006

Garden-year	Weather parameters ^a							
	Temperature		Relative humidity		Rainfall		Leaf wetness	
A-2005	0.59060	(0.0024)	-0.56871	(0.0037)	-0.26154	(0.2179)	-0.42341	(0.0439)
B-2005	0.98220	(<0.0001)	-0.97809	(<0.0001)	0.51047	(0.0108)	-0.93043	(<0.0001)
B-2006	0.85860	(<0.0001)	-0.79165	(<0.0001)	-0.64149	(0.0007)	-0.66561	(0.0004)

^a Values within parentheses indicate significant correlation ($P \leq 0.05$).

fungicide use. To this end, we determined when conidia of *A. panax* are available during the growing season and the weather factors that influence ACCs. Airborne *A. panax* conidia were detected at the onset of the growing season, prior to the development of lesions, suggesting that ginseng growers should initiate fungicide sprays immediately upon plant emergence, especially in conjunction with rainfall events. Currently, many growers initiate fungicide sprays early in the season, but previously, it was not known whether this practice was justified. Also, the detection of conidia throughout the growing season into late August and early September indicates that growers should consider continuing fungicide sprays through September into mid-October for gardens that are not being currently harvested.

Peaks of airborne *A. panax* conidia in ginseng gardens were correlated with specific weather factors. ACCs were greatest during periods of reduced relative humidity. While conidia were detected during periods of high relative humidity, concentrations were low and a significant negative correlation was found. This suggests that conidia of *A. panax* are not actively released under high humidity conditions. The reduced ACCs during rain events could be attributed to rain scrubbing from the atmosphere (9). Despite a negative correlation between hourly rainfall and ACCs, a positive correlation was detected for ACCs following 16 h or more after a rainfall event. Rain may temporarily remove conidia from the air, but the coincident leaf wetness period provides favorable conditions for sporulation (14). Optimal conidial production of *A. panax* occurs with temperatures ranging from 18 to 25°C (2,31). In our study, significant ACCs were observed within that range and $\geq 25^{\circ}\text{C}$.

Similar correlations between ACCs and weather parameters observed in our study have been reported for other *Alternaria* spp. *A. solani* conidia in controlled environmental chambers were released with sudden increases or decreases in relative humidity, vibration, and rain events (8). Conidia of *A. tenuis* on banana are released during a shift from wet to dry (18). During surface drying, air bubbles are formed on conidial chains of *A. tenuis*, causing them to twist and resulting in the release of conidia (18). Conidia of *A. tenuis* are also released following both an increase and a decrease in relative humidity (17). Conidia of *A. alternata* on tangerine were similarly released following sudden increases or decreases in relative humidity and with simulated rainfall events (30). While moisture is important for sporulation of *Alternaria*, most *Alternaria* spp. including *A. panax* are able to survive in dry conditions (27,30,31).

The correlation between weather variables and ACCs is critical to the develop-

ment of disease models and management. Recently, the disease forecasting system TOM-CAST was evaluated as a tool for timing fungicide sprays for *Alternaria* blight in ginseng gardens (14). TOM-CAST, used for the management of *A. solani*, prompts fungicide applications at specific disease thresholds by calculating daily disease severity values (DSVs) based on cumulative hours of leaf wetness and temperature. In evaluating TOM-CAST using a 10- or 15-DSV program to prompt fungicide applications, neither was able to adequately protect the foliage and seed compared to 7-day spray programs (14). Utilizing lowered DSVs would likely result in a spray schedule with similar num-

bers of applications as a 7-day program. It is possible that other factors, such as the level of overwintering inoculum, may affect ACCs. Also, the biology of *A. panax* and its response to temperature ranges may differ from the other pathogens where TOM-CAST has been successfully used.

Temperatures considered in the TOM-CAST forecaster range from 13 to 29°C and are based on formation of *A. solani* conidia and conidiophores. In our study, temperature was positively correlated with ACCs; garden temperatures were commonly between 18 and 25°C. With the use of a black polypropylene canopy, temperatures within the ginseng gardens occasionally exceeded 29°C. Future studies with

Fig. 3. Percentage of plants with *Alternaria panax* symptoms and seasonal airborne conidial concentrations (ACCs) per week (wk) observed in ginseng gardens A-2005, B-2005, and B-2006.

TOM-CAST for use within ginseng gardens could include a broader temperature range.

Leaf wetness is a common and important component of disease models and forecasters (30). The leaf wetness sensor in our study was placed within the top 25% of the canopy, 25 cm from the bed gutter which is along the top ridge of the plant bed. The detection of leaf wetness may be compromised at this location since air flow may be greater here than at other locations along the plant bed (e.g., near the bed gutter). Future studies could incorporate an additional leaf wetness sensor along the side of the ginseng bed, where the plant canopy tends to remain wet longer due to limited air movement. Additional studies using controlled environmental chambers to analyze conidial response to weather parameters could be conducted to further refine disease models and forecasting programs. In particular, determining leaf wetness parameters for *A. panax* and confirming the temperature ranges for conidial sporulation and survival is important. Assessing the effect of canopy density among 2- to 4-year-old ginseng crops on pathogen sporulation and disease development may also be helpful.

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