Aerial Concentrations of Pathogens Causing Early Blight and Brown Spot Within Susceptible Potato Fields

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ABSTRACT

Early blight caused by *Alternaria solani* and brown spot caused by *A. alternata* are two common foliar diseases of potato, with early blight typically predominating in incidence and severity. Renewed interest in these two diseases has arisen as a result of notable differences in fungicide resistance profiles of the pathogens and inconsistent outcomes of disease management tactics. The pathogens share similar disease cycles, but they differ in the shape and size of their conidia. *A. solani* has a host range that includes just the Solanaceae, whereas *A. alternata* has a broad host range spanning numerous plant families. Such differences may result in differences in dispersal of the pathogens and subsequently influence epidemiology and management outcomes. In the commercial potato fields investigated in this study, the aerial conidial concentrations of *A. solani* and *A. alternata* differed significantly, with those of *A. alternata* conidia being higher in number and more variable than those

of *A. solani*. The aerial conidial concentration of *A. solani* almost always significantly decreased with height (0 to 3 m above the canopy), whereas such a decrease was only observed for 4 of 12 days for *A. alternata*. The atmospheric concentrations of *A. alternata* were higher than those of *A. solani* at both upwind and downwind edges of the field ($P < 0.0001$), indicating more potential for long-distance dispersal. A higher aerial concentration of conidia at the downwind than the upwind location was observed for *A. solani* ($P < 0.05$), whereas overall no such effect was observed for *A. alternata*. This indicated that the potato fields investigated were likely the source of *A. solani* conidia, but they may not be the sole source of *A. alternata*. Results are consistent with inoculum of *A. solani* coming primarily from within the potato crop, whereas that of *A. alternata* may be generated from diverse plant sources across the landscape.

Early blight caused by *Alternaria solani* and brown spot caused by *A. alternata* are two common foliar diseases of potato (Droby et al. 1984; Shtienberg et al. 1990; Tymon and Johnson 2014). Early blight is the more prevalent of the two diseases and is recognized by its distinctive bull's-eye-shaped lesions (3 to 12 mm in diameter) (Stevenson et al. 2001). Brown spot is a relatively minor disease on potatoes, as it causes comparatively smaller lesions (pinpoint to 10 mm in diameter) compared with early blight (Stevenson et al. 2001). The two diseases often co-occur and the pathogens have been isolated from the same plant or even the same leaf. They share similar disease cycles and both are polycyclic with asexually propagated inoculum (conidia) dispersed by wind and rain splash (Bashan et al. 1991b; van der Waals et al. 2003).

The morphology of conidia of *A. solani* and *A. alternata* is efficient for wind dispersal, but the conidia differ in their shape and size (McCartney et al. 1993). *A. solani* conidia are typically $80 \times 30 \mu\text{m}$ in length by width, whereas *A. alternata* conidia are $30 \times 10 \mu\text{m}$. Fungal spore size and shape can have a major influence on their aerodynamic characteristics and subsequently can influence the proportion of spores escaping the canopy, their long-distance dispersal in the air, and the efficiency of spore deposition, which are important steps leading to disease spread (Golan and Pringle 2017; McCartney 1990; McCartney et al. 1993). However, there are no previous studies comparing the dispersal of the two pathogens, which could provide better prescriptive management of the diseases with crop rotation in space and time, as well as fungicide selections.

Despite the potentially different dissemination pattern of *A. solani* and *A. alternata*, the two pathogens are typically managed together in the field mainly with fungicide applications as no commercial cultivar is resistant to either disease. Single-site mode of action fungicides are favored by potato growers because of their high efficacy and reduced environmental risk (Tymon and Johnson 2014). Quinone outside-inhibiting (QoI) fungicides were among them, but repetitive application of QoIs caused selection of resistance in both *Alternaria* pathogen populations (Bauske et al. 2018; Fairchild et al. 2013; Karaoglanidis et al. 2011; Pasche et al. 2004; Rosenzweig et al. 2008). In the early 2000s, the majority of *A. solani* isolates from Wisconsin field populations demonstrated QoI resistance (Rosenzweig et al. 2008), and the frequency of *A. solani* isolates showing QoI resistance was >90% in Wisconsin after 2013 and as high as 100% in 2015 (Bauske et al. 2018). In a subsequent multiyear survey focusing on both pathogen species, QoI resistance was assessed in Wisconsin *Alternaria* isolates from 2015 to 2017 with collections made during early and late seasons in commercial potato fields to determine the resistance dynamics within the production season for each species (Ding 2018). The relative abundance of QoI-resistant *A. solani* isolates was consistently high (100%) both early and late seasons in each of these years, which was consistent with Rosenzweig et al. (2008) and Bauske et al. (2018). However, with the same fungicide program applied in the same field in 2015 to 2017, the relative abundance of QoI-resistant *A. alternata* isolates was low early in the season and increased during the season, but never reached a relative abundance as high as QoI-resistant *A. solani* (Ding 2018). Interestingly, such dynamic change was consistently observed each year from 2015 to 2017 in multiple commercial potato fields in Wisconsin (Ding 2018).

Based on these results, inoculum source and pathogen dissemination patterns were considered in explaining the different dynamics in the relative abundance of QoI-resistant isolates of *A. solani* and *A. alternata* during early and late-season samplings. *A. solani* is a specific pathogen on potato and tomato, whereas

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A. alternata has a wide host range including legumes, carrots, beets, and several wild plant species (Bashan et al. 1991a; Oviedo et al. 2011; Solfrizzo et al. 2005). Hosts of *A. alternata* that do not receive QoI applications may potentially serve as a reservoir of QoI-sensitive populations. Differences in production and movement of inoculum to and from a given potato field may have led to the different degree of exposure of *A. solani* and *A. alternata* populations to QoI fungicides. Based on these differences between the two pathogen species, we hypothesized that *A. solani* inoculum originated from and persisted within or near proximity of a potato field, with limited long-distance dispersal. Furthermore, we hypothesized that *A. alternata* inoculum originated from more numerous and diverse sites, in addition to the potato field and near proximity, and would not be limited in longer-distance dispersal owing to the smaller conidial size.

A comparative study of the dissemination of the two pathogens was designed to enhance our understanding of the role pathogen dispersal plays in these pathosystems. Such work may better inform preventive disease control strategies, such as disease forecasting, in the agricultural system. The objective of this study was to compare the dissemination of *A. solani* and *A. alternata* conidia and to explore possible factors influencing their dissemination.

MATERIALS AND METHODS

Field site and disease assessment. The study was conducted in two naturally infected potato (cultivar Russet Burbank) fields in Grand Marsh, Wisconsin (located in a concentrated potato production region) in 2016 and 2017 (Supplementary Fig. S1). Each year, data were collected on 6 days spread over a period of 3 weeks when the potatoes were at growth stage 5 (maturity) (Table 1). The field sampled in 2016 was 65 ha with a maize field to the North and a soybean field on the southern edges. The field sampled in 2017 was also 65 ha with a potato field to the North and a soybean field on the southern edges. The field sampled in 2017 was directly east, across a road, from the field sampled in 2016. The fields were managed by the same farm operator in both years using standard commercial practices for potato production in the region (Jordan et al. 2017, 2018). These included regular irrigation approximately twice weekly and fungicide applications made on a weekly basis from mid-June through August. Chemically induced vine kill was conducted by the grower collaborator when early

blight severity was ~40% and brown spot severity was ~20%. Final aerial conidial samples were collected on 1 September 2016 and 28 August 2017, 2 days after vine kill when only sparse patches of green, viable plants remained.

Aerial concentration of conidia. On each assessment day, the concentrations of *A. solani* and *A. alternata* conidia in the air, *C* (conidia m⁻³), were monitored above the potato canopy using Rotorod spore samplers, whereby the conidia were collected on two plastic rods (2 × 2 × 32 mm) attached to the rotating arm 8 cm in diameter (Edmonds 1972). The Rotorod samplers were made with a motor (Electro Sales Co., Somerville, MA) that spun at 2,600 min⁻¹ when powered by a 12-V marine battery (confirmed for each sampler using a strobe light). The collection efficiency of the Rotorod samplers was calculated according to Edmonds (1972). The Rotorod samplers were mounted on a 10-m tower (positioned near the center of the field) with the center of the collection rod positioned 0, 1, 2, and 3 m above the canopy. In 2016, two additional poles were placed arbitrarily within 30 m of the tower with spore samples collected at 0 and 2 m above the canopy on each pole to provide replications; only one such additional pole was used in 2017 with samples collected at 0 and 2 m above the canopy. Samples were taken from 10:00 to 15:00 on each assessment day. Rotorods were changed each hour, resulting in five sequential samples each day. *A. solani* and *A. alternata* conidia captured on the rods were identified based on morphological features and counted with the aid of a microscope at 100× magnification. Relatively long conidia (70 to 90 μm) with long beaks (30 to 40 μm) were identified as *A. solani*. Relatively small conidia (25 to 35 μm) with short beaks (4 to 6 μm) were identified as *A. alternata*. The hourly concentration (*C_h*) of conidia was calculated using the conidial counts, duration of sampling period, and the efficiency of the Rotorod samplers. Hourly concentrations of conidia were averaged across the 5 measurement hours to generate a mean spore concentration (*C_m*) per day (5-h period).

Estimation of disease severity and total conidial production. Disease severity was assessed based on the percentage of early blight and brown spot symptoms on the leaves. Five locations within 30 m of the spore sampling tower were assessed each day of sampling by visually estimating disease severity as percentage of blighted foliage in a square-meter area.

Total canopy conidial production was evaluated at 10:00 each sampling day by sampling one 0.25 × 0.25 m sampling grid from each of the five locations where disease severity had been estimated

TABLE 1. Disease severity, total number of conidia produced from the canopy, conidial escape fraction of *Alternaria solani* and *A. alternata*, and meteorological variables for assessment periods (10:00 to 15:00) at the field sites in Wisconsin in 2016 and 2017

Year	Date	<i>A. solani</i>			
		Disease severity (%) ^a	Total number of conidia (spores m ⁻²) ^b	Daily total flux (spores m ⁻²)	Escape fraction ^c
2016	15 August	6	24,000	650	0.027
	18 August	7	42,500	4,273	0.101
	22 August	16	99,000	10,237	0.103
	25 August	16	185,600	20,072	0.108
	29 August	27	105,000	9,607	0.091
	1 September	42	6,500	732	0.113
	Mean ± SD	19 ± 14	77,100 ± 66,379	7,595 ± 7,390	0.091 ± 0.032
2017	10 August	0.2	187	6	0.03
	14 August	3	45,000	793	0.018
	18 August	10	56,000	1,808	0.032
	21 August	15	68,400	4,677	0.068
	24 August	30	15,180	1,861	0.123
	28 August	40	11,600	1,010	0.087
	Mean ± SD	16 ± 16	32,728 ± 27,487	1,692 ± 1,616	0.06 ± 0.040

(Continued on next page)

^a Disease severity was assessed based on visually estimating the percentage of early blight and brown spot symptoms on the leaves in five 1-m² areas within 30 m of the 10-m spore sampling tower.
^b Total number of conidia denotes the total conidia production in the canopy by each pathogen in 1 m² as determined by washing conidia off from symptomatic leaves.
^c Escape fraction denotes the proportion of daily total flux in relation to the total number of conidia produced by each pathogen.
^d Aerial concentration of conidia moderately decreased with height.
^e Aerial concentration of conidia did not decrease with height.

in the field. From each sampling grid, six leaves were sampled (30 leaves total) and rated for disease severity as the percentage of symptomatic leaf area, and then leaves collected from one grid were placed in a 50-ml sterile plastic tube in the field and maintained in a cooler containing ice packs during transit back to the laboratory within ~7 h. Once back in the laboratory, 30 ml of sterile water was added to each tube and vortexed vigorously with an Eppendorf MixMate vortex mixer for 5 min to dislodge the conidia. After vortexing, the leaves were removed and the tube was centrifuged at 5,000 min⁻¹ for 5 min. The upper 27 ml of water was discarded and the number of conidia in the remaining 3 ml was counted. The number of conidia per percent symptomatic area (n) was determined for each leaf, averaged across the six leaves, and the total number of conidia produced per square meter in the field was estimated by multiplying n by the number of leaves in the grid and the disease severity per grid and then dividing by the area of the sampling grid.

Meteorological measurements. Wind speed, wind direction, air temperature, and relative humidity were monitored using a WatchDog 2550 Weather Station (Spectrum Technologies, Aurora, IL) mounted at a height of 10 m on the tower and data were collected every 5 min. A summary of the meteorological data is included in Supplementary Table S1. Wind speed at each sampler height was calculated using the wind speed at 10 m height and the standard logarithmic wind profile equation:

$$u = (u^*/k) \times \ln[(z - D)/z_0] \quad (1)$$

where u^* is the friction velocity (in cm s⁻¹), k is von Kármán's constant (0.4), z is the height above the ground (in cm), D is the zero-plan displacement height (in cm), and z_0 is the roughness length (in cm). D and z_0 were set to 0.2 cm and 0.105 h_0 , respectively, where h_0 is the height of the potato canopy (0.3 m during the study) (Evans 1975). Wind speed was also measured at 1 m and compared with calculated wind speed at that height to validate the wind profile model.

Escape of conidia from the canopy. Calculations of the number of conidia that escaped from the canopy were based on the assumption that the vertical diffusion of conidia is determined by the vertical diffusivity of the air (Neufeld et al. 2013; Smith and Hay 1961). This way, the diffusivity of the conidia (K_c) can be equated to the diffusivity of the momentum (K_m) and can be calculated using

the friction velocity and the vertical rate of change of the average wind speed following equation 2 (Neufeld et al. 2013; Sutton 1953):

$$K_c = K_m = u^{*2} / (du/dz) \quad (2)$$

where u^* , u , and z are as defined in equation 1, and du/dz is the vertical wind speed gradient.

For each sampling period on each sampling day, the vertical flux of conidia F_c (conidia m⁻² s⁻¹) was calculated following equation 3 (Aylor and Taylor 1983; Neufeld et al. 2013):

$$F_c = -k_c \times (\delta C / \delta z) \quad (3)$$

where $\delta C / \delta z$ is the vertical gradient of the conidium concentration as determined from the Rotorod measurements at each sampling height. Values of F_c obtained for each measurement period from 10:00 to 15:00 were integrated to generate the total daily flux (F_D , conidia m⁻²) for the day. The escape fraction of each *Alternaria* species was calculated as the fraction of conidia leaving the canopy as measured by F_D divided by the total number of conidia produced per square meter (Neufeld et al. 2013).

Comparison of upwind and downwind conidial concentration. In 2017, two poles were set up at the northern and southern ends of the field, 1.5 m outside the field. Rotorod samplers were attached to each pole at 0.3 and 2.3 m above the ground (0 and 2 m above the height of the potato canopy, respectively). A sampling period of 2 h was used. The conidia captured on the rods were counted and the hourly concentration was calculated the same way as that from the 10-m tower. Wind direction was recorded each assessment day for the identification of upwind and downwind locations.

Settling velocity. Settling velocity was measured with conidia of each pathogen using a procedure described by Ferrandino and Aylor (1984). Conidia were dislodged from 2-week-old cultures grown on clarified V8 medium by flooding the dishes and gently rubbing the surface with a glass rod. The conidium suspension was collected in a 1.5-ml tube and dried in a laminar flow hood for 1 week. Conidia were separated from each other by beating with sand granules.

A 1-m glass tube with a diameter of 5 cm was used to measure the settling velocity. The top of the glass tube was covered with aluminum foil with a small slit. In a room with an LED light

TABLE 1. (Continued from previous page)

<i>A. alternata</i>						
Disease severity (%)	Total number of conidia (spores m ⁻²)	Daily total flux (spores m ⁻²)	Escape fraction	Wind speed (m s ⁻¹)	Temperature (°C)	Relative humidity (%)
6.6	67,320	6,520	0.097	1.4	25.7	56.1
11	82,500	18,749	0.227	2.3	28.1	61.2
12	227,250	35,575	0.157	4.5	22.8	54.3
15	244,000	97,540	0.4	2.3	23.6	57.6
16	190,000	43,164 ^d	0.227	1.6	26.3	71.1
18	26,500	744 ^e	0.028	3.2	19.5	57.3
13.1 ± 4.1	139,595 ± 92,085	33,715 ± 35,261	0.189 ± 0.129	2.6 ± 1.1	24.3 ± 3.0	59.6 ± 6.1
1	10,693	4,031 ^c	0.377	5.4	25.1	55
2	21,120	1,021 ^c	0.048	2.1	20.9	67.3
3	46,800	1,342 ^c	0.029	4.3	20.7	68.2
10	88,000	6,012 ^c	0.068	2	23.3	80.1
20	14,720	1,916 ^c	0.13	2.1	17.5	46.7
25	12,500	1,261 ^c	0.101	0.7	18.2	87.3
10.2 ± 10.2	32,305 ± 30,350	2,597 ± 2,001	0.126 ± 0.128	2.8 ± 1.7	21.0 ± 2.9	67.4 ± 15.1

positioned under the tube as the only light source, conidia were allowed to drop through the slit, and the dropping time over a defined distance was recorded for calculating settling velocity. The experiment was conducted three times for each pathogen.

Statistical analysis. The mean total number of conidia produced per square meter by each pathogen, daily flux of conidia produced by each pathogen per square meter, and the escape fraction of each pathogen were compared throughout the 6 days each year with pairwise *t* tests using GraphPad Prism version 6.02 (GraphPad Software, San Diego, CA). Aerial conidial concentrations of each species collected from canopy level on each sampling day were regressed against the total number of conidia produced by each corresponding pathogen on that day to test their correlations. The escape fraction of each pathogen was also regressed against the mean wind speed of that day to test how wind speed influenced the escape fractions. The regression analyses were conducted with GraphPad Prism version 6.02.

The C_m of conidia at each height was transformed based on the natural logarithm and fitted to a linear regression equation that was adapted from the negative exponential equation (Cooke et al. 2006):

$$\ln(C_m) = \ln(C_{m0}) - \alpha h \quad (4)$$

where C_{m0} is C_m at the canopy level, α is a coefficient, and h is the height above the canopy.

An *F* test was used to examine whether α , the slope in the regression, was significant for each pathogen from each assessment day. An analysis of covariance was conducted to compare α of the two pathogens obtained from each assessment day.

The decreasing rates of the daily conidial concentration at each height above the potato canopy C_m of *A. solani* and *A. alternata* from both years were compared by linear regression analysis using PROC GLM in SAS version 9.4 (SAS Institute, Cary, NC):

$$\ln(C_m) = h + \text{species} + \text{species} \times h + d + y \quad (5)$$

where h is the height above the canopy, species is *A. solani* (as 0) and *A. alternata* (as 1), d is the assessment day, and y is the sampling year.

t tests were used to compare the aerial conidial concentration at upwind and downwind locations at each height for each pathogen species.

RESULTS

Disease severity and spore production. In both 2016 and 2017, early blight and brown spot were first observed, at low severity, starting in July. Disease developed rapidly during mid-August (Table 1). Early blight progressed faster than brown spot in both years. The total number of conidia produced per square meter was as low as 187 conidia m^{-2} (10 August 2017) to as high as 185,600 conidia m^{-2} (25 August 2016) for *A. solani*, and 10,693 conidia m^{-2} (10 August 2017) to 244,000 conidia m^{-2} (25 August 2016) for *A. alternata* (Table 1). There was a significantly higher number of *A. alternata* conidia produced from the canopy than *A. solani* in 2016 ($t = 3.946$, $df = 5$, $P = 0.0054$), but the numbers were similar in 2017 ($t = 0.068$, $df = 5$, $P = 0.4741$). The total number of conidia produced per square meter peaked when disease severity was $\sim 15\%$ for *A. solani* and 10% for *A. alternata* both years, and then declined as the disease continued to progress.

Dynamics of conidial concentration. The concentration of conidia of both *Alternaria* species fluctuated during the sampling time (Figs. 1 and 2; Supplementary Fig. S2; Supplementary Table S2). No consistent daily patterns of aerial concentration of *Alternaria* conidia in relation to temperature or relative humidity were observed. The aerial conidial concentration of *A. alternata* was higher than that of *A. solani* ($P < 0.0001$). The aerial conidial concentration of each species followed that of their total number of conidia produced per square meter in the canopy in 2016 ($P = 0.0199$ for *A. solani* and $P = 0.0705$ for *A. alternata*), but such correlation was absent for *A. solani* ($P = 0.4608$) and *A. alternata* ($P = 0.8370$) in 2017.

The conidial concentration decreased with height more frequently for *A. solani* than for *A. alternata* (Fig. 3). The *F* test showed that linear regression of conidial concentration with height was significant on 11 of 12 sampling days for *A. solani*, whereas the conidial concentration for *A. alternata* decreased significantly with height on only 4 of the 12 sampling days over the 2 years (Table 2). According to the multiple linear regression model (Table 3), the C_m of *A. solani* decreased faster with height than that of *A. alternata* ($t = 2.35$, $df = 82$, $P < 0.05$).

Fig. 1. Hourly aerial conidial concentrations of *Alternaria solani* and *A. alternata* from representative assessment days in 2016. Disease severity (DS) was assessed based on visually estimating the percentage of early blight and brown spot symptoms on the plants. Hourly aerial conidial concentrations were obtained using the number of conidia captured with Rotorod samplers.

Daily flux and escape fraction. Daily total conidium flux of both *Alternaria* species increased with increasing disease severity before declining with reduction of the plant tissue corresponding to the increasing amount of disease and subsequent vine kill. *A. solani* had a smaller daily total flux ($t = 2.285$, $df = 5$, $P = 0.0355$) and a smaller escape fraction ($t = 1.963$, $df = 5$, $P = 0.0535$) than *A. alternata* in 2016 but not in 2017 ($P > 0.05$). Higher wind speed most often resulted in a larger escape fraction (Table 1), although in general there were no significant correlations between escape fraction and mean wind speed for either pathogen ($P > 0.05$). Temperature and relative humidity were not correlated with conidium escape (Table 1).

Conidium concentration in upwind and downwind directions. Wind directions were considered as North wind and South wind because the additional two poles were located at the northern and southern ends of the field in 2017. This way, the source of the *Alternaria* conidia was simplified as either from within the field or from outside. Aerial conidial concentrations of *A. alternata* were significantly higher than those of *A. solani* ($P < 0.0001$) at both upwind and downwind locations (Fig. 4). The aerial conidial concentration of *A. solani* was higher at the downwind edge of the field than the upwind edge ($P = 0.0329$ at 0.3 m and $P = 0.0101$ at 2.3 m above the ground) and again was higher in the middle of the field than at the downwind edge ($P = 0.0235$ at 0.3 m and $P = 0.0562$ at 2.3 m above the ground). However, no significant difference in aerial conidial concentration of *A. alternata* was observed between the downwind and upwind edges of the field or between the edges of the field and the middle of the field (Supplementary Tables S3 and S4).

Settling velocity. The settling velocity of *A. solani* was $2.04 \pm 0.21 \text{ cm s}^{-1}$, which was significantly larger ($t = 3.061$, $df = 4$, $P = 0.0188$) than that of *A. alternata* ($1.26 \pm 0.14 \text{ cm s}^{-1}$).

DISCUSSION

Although *A. solani* and *A. alternata* are closely related and have many similar biological and epidemiological characteristics, their dispersal behavior differed significantly. Conidia of both species were previously considered as effectively dispersed by wind (McCartney et al. 1993). Nonetheless, we identified significant differences between *A. solani* and *A. alternata* conidia in atmospheric concentration, horizontal movement, vertical gradient, and

Fig. 3. Vertical profiles of *Alternaria solani* and *A. alternata* conidial concentration (transformed with the natural logarithm) above the infected potato field, averaged from 10:00 to 15:00 on each assessment day during **A**, 2016 and **B**, 2017. Solid lines represent *A. solani* and dashed lines represent *A. alternata*. C_m = mean spore concentration and Ln = natural logarithm.

Fig. 2. Hourly aerial conidial concentrations of *Alternaria solani* and *A. alternata* from representative assessment days in 2017. Disease severity (DS) was assessed based on visually estimating the percentage of early blight and brown spot symptoms on the plants. Hourly aerial conidial concentrations were obtained using the number of conidia captured with Rotorod samplers.

escape from the canopy of a susceptible potato crop. The concentration of conidia in the atmosphere above the potato canopy in our study was in line with concentrations from other similar studies with similarly shaped propagules (Maya-Manzano et al. 2016; van der Waals et al. 2003), and the aerial conidial concentration of *A. alternata* in general was several times higher than that of *A. solani*. Although on occasion there were significant gradients of *A. alternata* escaping from the potato canopy, in more than half of the sampling days the major source of *A. alternata* conidia was most likely from surrounding sources, outside of the potato field investigated. This was distinctly different from the pattern of conidial dispersal for *A. solani*, where the potato canopy in and around our sampling location was likely the major source of *A. solani* conidia.

Our findings may explain the difference in QoI resistance between the two species described earlier (Ding 2018). Given the vertical flux and horizontal dispersal data, we hypothesize that conidia of *A. alternata* over the potato canopy are produced over a wide area, including natural vegetation such as woodlots, tree lines, and scrub areas as well as agricultural vegetation such as corn and soybeans. Most of these potential sources of *A. alternata* conidia would likely not have received routine applications of QoI fungicides. The influx of wild-type (or unexposed to QoI fungicides) potato-pathogenic *Alternaria* inoculum into the potato field may reduce the potential for widespread QoI resistance by providing a continual source of fungicide-sensitive genotypes to the population in the potato canopy. As the field season progresses, these outside sources appear to decline in importance as greater numbers of conidia are produced in the canopy and are exposed to QoI fungicides. On the other hand, primary inoculum of *A. solani* originates on potato residue from fields near the current potato crop. Our data suggest that subsequent to initial infections of the current-season crop, most *A. solani* inoculum originates in the potato field from those early infections, and thus, the population is under selection for QoI resistance every season.

The concentration of fungal spores, or other fungus-like propagules, decreases exponentially from finite sources with increasing altitude (Eversmeyer et al. 1973). The magnitude of decrease in concentration with height is an indicator of the concentration of spores originating

from the source (Eversmeyer and Kramer 1987). The net movement or flux of spores in an upward vertical direction from plant canopies has been measured in a relatively small number of studies (Aylor and Ferrandino 1985; Aylor and Taylor 1983; Aylor et al. 2001; Neufeld et al. 2013). In our study, a decreasing gradient of aerial conidial concentration with height was not frequently observed with *A. alternata*. Even on those 4 of 12 days when such a decreasing gradient was observed, it did not decrease as rapidly as did the conidial concentration of *A. solani* with increasing height (Fig. 3; Table 2). The rapid decrease of conidial concentration of *A. solani* suggested that the majority of aerial conidia originated from within the assessed potato fields.

Many spores produced in foliar lesions never leave the plant canopy (Aylor 2017). The proportion of spores that are lofted into the atmosphere above the canopy is referred to as the escape fraction. In this study, aerial conidia of *Alternaria* spp. were sampled between 10:00 and 15:00 on each assessment day at different altitudes to measure the escape of conidia from the canopy and the vertical flux above the canopy. There was a significant difference in the escape of conidia between the two species, with escape being greater for *A. alternata*. We measured the proportion of conidia of *A. solani* escaping from the potato canopy to be between 0.03 and 0.12, while escape fractions for *A. alternata* were between 0.03 and 0.40. In 11 of 12 days of sampling over the 2 years of this study, the escape fraction of *A. alternata* was

TABLE 3. Parameters in the linear regression model of aerial conidial concentrations of *Alternaria solani* and *A. alternata* against height above the potato canopy, *Alternaria* species, and sampling year

Parameter	Estimate	SE	P value
Intercept	3.91	0.3310	<0.0001
h^a	-0.12	0.1055	0.2445
Species ^b	-2.30	0.2792	<0.0001
h species	-0.35	0.1493	0.0214
Year ^c	2.24	0.2890	<0.0001

^a h denotes the height above the potato canopy.

^b Species denotes the *Alternaria* species, where 0 = *A. solani* and 1 = *A. alternata*.

^c 0 = 2016 and 1 = 2017.

TABLE 2. Gradient models for aerial conidial concentrations of *Alternaria solani* and *A. alternata* measured above potato fields in central Wisconsin after natural logarithm transformation and fitting to modified negative exponential equation 4

Year	Date	Species	$\alpha \pm \text{SE}^a$	$C_0 \pm \text{SE}^b$	R^2	P value ($\alpha \neq 0$)	α (<i>A. solani</i>) > α (<i>A. alternata</i>)	
							t (df = 4)	P value
2016	15 August	<i>A. solani</i>	-0.4080 $\pm$ 0.07255	1.896 $\pm$ 0.1357	0.9405	0.0302	-3.01	0.0397
	15 August	<i>A. alternata</i>	-0.1643 $\pm$ 0.03614	4.908 $\pm$ 0.06761	0.9117	0.0451		
	18 August	<i>A. solani</i>	-0.4623 $\pm$ 0.03264	2.807 $\pm$ 0.06107	0.9901	0.0049	-5.98	0.0039
	18 August	<i>A. alternata</i>	-0.1700 $\pm$ 0.03641	4.995 $\pm$ 0.06811	0.916	0.0429		
	22 August	<i>A. solani</i>	-0.4082 $\pm$ 0.02588	3.063 $\pm$ 0.04842	0.992	0.004	-8.76	0.0009
	22 August	<i>A. alternata</i>	-0.1317 $\pm$ 0.01809	5.088 $\pm$ 0.03385	0.9636	0.0184		
	25 August	<i>A. solani</i>	-0.4511 $\pm$ 0.07895	4.146 $\pm$ 0.1477	0.9423	0.0293	-2.13	0.1006
	25 August	<i>A. alternata</i>	-0.2628 $\pm$ 0.04011	6.112 $\pm$ 0.07503	0.9555	0.0225		
	29 August	<i>A. solani</i>	-0.4998 $\pm$ 0.08754	4.077 $\pm$ 0.1638	0.9422	0.0293	-2	0.1164
	29 August	<i>A. alternata</i>	-0.2655 $\pm$ 0.07807	5.878 $\pm$ 0.1461	0.8525	0.0767		
	1 September	<i>A. solani</i>	-0.1299 $\pm$ 0.009537	1.559 $\pm$ 0.01784	0.9893	0.0053	-3.17	0.034
	1 September	<i>A. alternata</i>	-0.01955 $\pm$ 0.03353	4.165 $\pm$ 0.06273	0.1453	0.6188		
	10 August	<i>A. solani</i>	0.07072 $\pm$ 0.1399	-1.654 $\pm$ 0.2617	0.1134	0.6633	0.51	0.6359
	10 August	<i>A. alternata</i>	-0.001942 $\pm$ 0.02484	4.918 $\pm$ 0.04646	0.003	0.9448		
2017	14 August	<i>A. solani</i>	-0.8462 $\pm$ 0.1062	1.066 $\pm$ 0.1986	0.9695	0.0154	-7.71	0.0015
	14 August	<i>A. alternata</i>	-0.02487 $\pm$ 0.009322	3.696 $\pm$ 0.01744	0.7806	0.1165		
	18 August	<i>A. solani</i>	-0.4622 $\pm$ 0.09200	1.414 $\pm$ 0.1721	0.9266	0.0374	-4.17	0.014
	18 August	<i>A. alternata</i>	-0.06140 $\pm$ 0.02752	2.382 $\pm$ 0.05149	0.7133	0.1554		
	21 August	<i>A. solani</i>	-0.8171 $\pm$ 0.1376	2.476 $\pm$ 0.2575	0.9463	0.0272	-5.35	0.0059
	21 August	<i>A. alternata</i>	-0.06204 $\pm$ 0.03071	4.398 $\pm$ 0.05746	0.6711	0.1808		
	24 August	<i>A. solani</i>	-0.8585 $\pm$ 0.2312	1.341 $\pm$ 0.4326	0.8733	0.0655	-2.95	0.042
	24 August	<i>A. alternata</i>	-0.1510 $\pm$ 0.06397	2.582 $\pm$ 0.1197	0.7358	0.1422		
	28 August	<i>A. solani</i>	-0.4125 $\pm$ 0.1099	2.395 $\pm$ 0.2057	0.8756	0.0643	-1.81	0.1444
	28 August	<i>A. alternata</i>	-0.1696 $\pm$ 0.07683	3.091 $\pm$ 0.1437	0.7091	0.1579		

^a α is the slope of each conidial concentration gradient regression model.

^b C_0 denotes the aerial conidial concentration of each *Alternaria* species at the potato canopy level (0.3 m above the ground).

higher than that of *A. solani*. Therefore, vertical transport of *A. solani* conidia was less efficient than *A. alternata*.

The ability of particles such as spores and pollen in the range of 1 to 100 μm in diameter to be lofted into the atmosphere is a function of aerodynamic size. This size correlates with settling velocity (McCartney 1990; McCartney et al. 1993; Zanatta et al. 2016). The settling velocity of *A. solani* ($2.04 \pm 0.21 \text{ cm s}^{-1}$) was significantly larger ($P=0.0188$) than that of *A. alternata* ($1.26 \pm 0.14 \text{ cm s}^{-1}$). Our measured settling velocities were similar to those calculated from the aerodynamic diameters of *Alternaria* spp. measured by McCartney et al. (1993). Aerodynamically larger spores are more likely to either impact on plant surfaces or settle out onto plants or soil. Therefore, it is expected that *A. alternata* would have a high flux from a source compared with *A. solani* as a result of its smaller size and lower settling velocity.

The vertical flux and escape fraction are related to the take-off of the conidia. Many pathogens are firmly attached to their sporophores, and therefore the detachment of conidia requires a unique threshold of wind speed to strike them directly (Waggoner and Aylor 2000). This threshold may not be necessarily the same for the two *Alternaria* species, but their conidiophore structures and the way conidia are borne may also contribute to differences in their ability to escape the canopy. *A. solani* produces individual conidia, whereas *A. alternata* produces conidia in chains with each successive conidium being smaller in size (Zheng et al. 2015). Chains of conidia were rarely observed on the sampling rods, suggesting that mature conidia detached from the chains easily by wind and the conidia were most likely dispersed individually. Therefore, in addition to the possibility of increasing the number of conidia by producing them in chains, *A. alternata* may also facilitate the take-off of the conidia that were not immediately attached to the host.

If the potato field being assessed was the major source of *Alternaria* conidia, the dispersal gradient would follow the wind direction (Bashan et al. 1991b). This was indeed the case with *A. solani*. There was a clear trend of a higher concentration of *A. solani* conidia at the downwind location than the upwind location consistent for all of the assessment days, no matter whether the neighboring potato field to the North was in the upwind or downwind direction, suggesting that the potato field assessed was the source of *A. solani* conidia. However, there was no such consistent trend for *A. alternata*. There were several days when there were high numbers of *A. alternata* conidia captured at the upwind location, indicating that there was a larger number of atmospheric conidia of that species than the number of conidia produced from the potato canopy being studied. This also indicated that the large fluctuation of aerial conidial concentration of *A. alternata* in the potato field was not attributed to the local source (specific field of investigation) of inoculum (Timmer et al. 1998). Such a large amount of external *A. alternata* conidia would also explain the lack of a vertical gradient or slow vertical decrease of *A. alternata* conidial concentration with height.

The vertical flux and escape fraction of conidia also depends both on turbulence and the rate of spore release (Aylor and Taylor 1983). With the similar wind speed on 14 August and 24 August 2017, a higher escape fraction was observed on 24 August 2017 for both pathogens when relative humidity was low. Wind speed as well as wind direction did not vary appreciably based on 5-min interval measurements over the course of any day. Low relative humidity facilitates the release of spores of other fungal pathogens (Aylor and Taylor 1983; Timmer et al. 1998). Similarly, a lower relative humidity together with a higher wind speed on 25 August 2016 than 29 August 2016 also was associated with a relatively higher escape fraction for both pathogens. This result suggested that the two *Alternaria* pathogens may have similar dispersal responses to environmental factors. However, with similar temperature and relative humidity on 14 August 2017 and 18 August 2017, a higher wind speed was associated with a higher escape fraction for *A. solani* but a lower escape fraction for *A. alternata*, indicating that in addition to these environmental factors, there may be other

factors influencing the escape of conidia, such as high background aerial conidial concentration of *A. alternata*. This also explained why the escape fraction of each species was not simply significantly correlated with wind, temperature, or relative humidity, as the sporulation and dissemination process of a pathogen relies on many other factors not examined here.

Overall, a higher production of conidia as measured by the total number of conidia produced per square meter was observed with a higher disease severity for *A. solani* and *A. alternata*. Disease severity in 2016 was slightly higher than in 2017, and subsequently more conidia were produced from within the canopy. A significant decrease in concentration with height of *A. alternata* was typically observed when the total number of conidia produced from the canopy was high, such as the first 4 days in 2016. This could be attributable to a large number of conidia being contributed by the potato field, so that the external *A. alternata* conidia failed to obscure the gradient. Although disease caused by *A. solani* was more severe than that caused by *A. alternata*, the former produced a smaller number of conidia in the canopy. This may be because *A. alternata*, producing conidia smaller in size and borne in chains, may form a higher number of conidia per unit lesion area than *A. solani* (Zheng et al. 2015).

Knowledge of differences in dissemination between the two *Alternaria* species may be useful when considering management practices. Because of the broad host range and large movement of *A. alternata* conidia, a significant proportion of the population may receive no fungicide exposure or a lower level of fungicide selection pressure and therefore the risk of fungicide resistance would be lower compared with that of the population of *A. solani*. Fungicides

Fig. 4. Comparison of aerial conidial concentration of **A.** *Alternaria solani* and **B.** *A. alternata* at upwind and downwind locations. Data were collected in 2017, and the aerial conidial concentration of each *Alternaria* pathogen was collected 0 and 2 m above 0.3 m, the height of the potato canopy. Each box represents the range of aerial conidial concentrations and the bars mark the mean of the aerial conidial concentration. *The mean aerial conidial concentration for *A. alternata* at the lower height of the upwind location on 21 August 2017 was 2,027 conidia m⁻³, and the range was 1,176 to 2,908 conidia m⁻³.

with single-site modes of action, such as the QoIs, may still be applied to control *A. alternata* during the early season, as resistance frequency in the pathogen population may be relatively low. Such differences in dispersal also imply that a longer crop rotation may aid in management of early blight caused by *A. solani* in an individual field (Weisz et al. 1994), but it may not be as beneficial in managing brown spot caused by *A. alternata* because external sources of inoculum likely play a larger role in spore introduction than for *A. solani* (Timmer et al. 1998). Both pathogens favor mature or senescent plants, so it is important to avoid early planted fields in the upwind direction of the potato field when deploying crop rotations.

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