Differences in spore size and atmospheric survival shape stark contrasts in the dispersal dynamics of two closely related fungal pathogens

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Abstract

A frequently ignored but critical aspect of microbial dispersal is survival in the atmosphere. We exposed spores of two closely related, morphologically dissimilar, and economically important fungal pathogens to typical atmospheric environments and modeled their movement in the troposphere. *Alternaria solani* conidia are nearly 10 times larger than *A. alternata* conidia, but in our experiments, most died within 24 hours, while over half of *A. alternata* conidia remained viable on day 12. Next, we modelled the movement of spores across North America. We predict 99% of the larger *A. solani* conidia settle within 24 hours, with a maximum dispersal distance of 100 km. By contrast, most *A. alternata* conidia remain airborne for more than 12 days, and dispersal over long distances(2,000 km) is likely. Counterintuitively, the larger *A. solani* conidia survive poorly, as compared to smaller *A. alternata* conidia, but also land sooner and move over shorter distances.

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1. Introduction

A frequently ignored but critical aspect of microbial dispersal is survival during travel. Fungal dispersal is mediated by spores, and in some species, spores are reported to cross continents or oceans in air currents (Bowden et al., 1971; Purdy et al., 1985; Brown and Hovmøller, 2002). But whether spores remain viable after continental or oceanic crossings is unclear (Golan and Pringle, 2017). As a result, an understanding of effective dispersal (defined as the fraction of spores returning to ground alive) remains elusive (Golan and Pringle, 2017; Lagomarsino Oneto et al., 2020). Measuring not only how far spores travel (i.e., their dispersal kernel) but also how long spores remain viable in the atmosphere (i.e., their "survival kernel") is crucial. Tracking spores and measuring germination in nature is difficult (Golan and Pringle, 2017; Malloch and Blackwell, 1992; Peay and Bruns, 2014) but measuring survival in the laboratory and connecting survival data to realistic models of movement offers one path to estimate effective dispersal.

In general, larger spores appear to germinate more readily (i.e., faster, more frequently) and to tolerate greater environmental stresses, as compared

to smaller spores (Ijadpanahsaravi et al., 2022; Altre et al., 1999; Halbwachs et al., 2017; van den Brule et al., 2020; Norros et al., 2015). The result holds in both intraspecific and interspecific comparisons, and it suggests larger spores are more fit than smaller spores. Larger biological aerosols of any sort are assumed to be more durable during travel, e.g., "The survivability of a biological particle in the atmosphere will also be affected by its size ... smaller particles being more susceptible to environmental damage" (Jones and Harrison, 2004).

Spore survival is often measured in terms of "germinability',' defined as the proportion of spores germinating after exposure to the environment or experimental manipulations (Aylor, 2017). Studies measuring germinability in contexts relevant to the atmosphere suggest survival is most impacted by water loss and damage from solar radiation (Aylor, 2017; Maddison and Manners, 1972; Rotem et al., 1985; Aylor and Sanogo, 1997; Norros et al., 2015). Desiccation sensitivity varies among species (Hawker and Madelin, 1976; Hoekstra, 2002) and appears to be determined by spore wall thickness, spore surface area, and relative water content within a spore (Norros et al., 2015; Ayerst, 1969; Gervais et al., 1988; Magan, 1988). High humidity is generally associated with increased germinability (Aylor, 2017) but some

species' spores – including smut teliospores, and *Aspergillus fumigatus* and *Penicillium spp.* conidia – are released when environments are dry (Rotem et al., 1985; Piepenbring et al., 1998; Pasanen et al., 1991), perhaps to postpone germination until after deposition.

Temperature also influences germination, but temperature's influence is not the same for every species: while colder temperatures (between 12.5 and 15.8°C) appear to maintain the germinability of *Pseudogymnoascus destructans* conidia (Verant et al., 2012), between 90-99% of *Phakopsora pachyrhizi* urediniospores fail to germinate after exposure to similarly cold temperatures (Park et al., 2008; Bonde et al., 2007; Isard et al., 2006). Temperature appears to be a minor influence for other species; *A. fumigatus* ascospores survive a broad range of temperatures, including heating at 70°C for 30 minutes (Kwon-Chung and Sugui, 2013). Some species can withstand extreme temperatures, e.g., 15% of *Cladosporium cladosporioides* conidia germinate after transient exposure to 300°C (Jung et al., 2009).

High-frequency solar radiation also influences spores' survival (Koller, 1965; Robinson, 1963). Light in the ultraviolet (UV) spectrum (400-100nm) damages the DNA of many organisms, including fungi (Maddison and Manners, 1972; Rotem et al., 1985; Diffey, 1991). Spores traveling in the tropo-

sphere are exposed exclusively to UVA (400-315nm) and UVB (315-280nm) because ozone filters shorter wavelengths (below 280nm; Iqbal, 1983; Zerefos and Bais, 1997). UV radiation varies significantly by latitude and altitude, and exposure changes according to cloud cover, time of day, season, and the integrity of the ozone layer at any given location (McKenzie et al., 2011). A spore in the atmosphere encounters variability in terms of both wavelength and dosage rate (or irradiance: W/m^2). Some species are less resilient to UV damage (e.g., *Cladosporium herbarum*; Sarantopoulou et al., 2014) than others (e.g., Mycosphaerella fijiensis; Parnell et al., 1998), and other species have adapted to avoid damage, e.g., through spore melanization (*Aspergillus niger*; Singaravelan et al., 2008) or spore clumping (*Phakopsora pachyrhizi*; Li et al., 2006, 2008).

A spore's exposure to adverse humidity, temperature and solar radiation during aerial dispersal is shaped primarily by the interplay between air turbulence and gravity; these forces keep spores aloft for different times as a function of spore shape, size and density (Lagomarsino Oneto et al., 2020; Norros et al., 2014; Rotem and Aust, 1991; Isard et al., 2011). Natural selection can affect potential flight times, e.g., by altering spore aerodynamics or the timing of spore release (Lagomarsino Oneto et al., 2020; Jongejans et al.,

2015). Fungi have also evolved traits to minimize damage from water loss or UV exposure and to navigate myriad other constraints related to movement (Golan and Pringle, 2017; Norros et al., 2015; Isard et al., 2006; Hussein et al., 2013; Jongejans et al., 2015; Calhim et al., 2018).

To elucidate how patterns of spore survival define the distances reached by living spores, we tested how laboratory environments relevant to atmospheric travel impact germinability. Experiments were conducted using conidia of two economically important plant pathogens: *Alternaria alternata* and *A. solani*, whose conidia and natural histories are strikingly different. While *A. alternata* is a ubiquitous, cosmopolitan species with small spores (forming chains of obovate-obtuse conidia, 10-15µm in length), *A. solani* spores are large (forming solitary, obovate-oblong conidia 75-100µm in length) and the species is primarily associated with solanaceous (especially potato and tomato) crops (Rotem, 1994; Woudenberg et al., 2014; Barberán et al., 2015; Ding et al., 2019a). Both species pose serious threats to solanaceous crops and conidia often co-infect the same plant (Ding et al., 2019a; NARR, 2019).

In a first experiment (Experiment 1), we exposed conidia of *A. alternata* and *A. solani* to a range of relative humidities (RH), temperatures (T), and UV wavelengths and intensities (UV) for 96 hours. Data were used to identify

combinations of RH, T and UV favorable to the retention of germinability. In a second experiment (Experiment 2), we exposed approximately 1×10^6 and 1.05×10^5 spores of *A. alternata* and *A. solani*, respectively, to a favorable environment for over 12 days (288 hours), a timescale relevant to continental or oceanic dispersal (Bowden et al., 1971; Purdy et al., 1985; Singh et al., 2011; Prussin et al., 2013). We next used simulations of particle transport in atmospheres to model the dispersal of spores (Bashan et al., 1991; Mccartney et al., 1993). Ultimately, patterns of effective dispersal emerge as strikingly different between these two closely related species. Unexpectedly, we find that after exposure to atmospheric environments, the smaller spores of *A. alternata* remain viable much longer than the larger spores of *A. solani*. At the same time, *A. alternata* spores dwell much longer than *A. solani* in the atmosphere, suggesting survival to atmospheric conditions may be a trait under selective pressure.

2. Materials & Methods

2.1. Overview

In Experiment 1 we exposed conidia of *A. alternata* and *A. solani* to open air with different combinations of ultraviolet wavelengths and irradi-

ance (UV), relative humidities (RH), and temperatures (T). We chose RH and T ranges relevant to spores dispersing in the troposphere and tested ten combinations (1-10, Table S1) typical of central Wisconsin (U.S.A.) in summer (Psheidt, 1985; Ding et al., 2019b). In the troposphere, UV, RH and T naturally vary, but to compare the effects of these variables within altitudes of 6-10 km (i.e., the lower to middle troposphere [Crutcher, 1969; Blumthaler et al., 1992, 1997; Dvorkin and Steinberger, 1999]), RH and T were held steady (though adjusted for each of the ten combinations) while simultaneously testing multiple levels of UV irradiance and wavelength. We conducted experiments in a single controlled environmental chamber at the University of Wisconsin Biotron (Madison, WI, USA) and the ten combinations of RH-T were tested sequentially in this single chamber, sterilizing the chamber after each condition. For each RH-T combination, we tested 21 UV strengths, including both realistic and unrealistic irradiances (Blumthaler et al., 1992, 1997; Dvorkin and Steinberger, 1999; Table S2) for a total of 10 RH-T conditions $\times$ 21 UV strengths = 210 treatments per species. We ran each iteration of Experiment 1 for 96 hours. We measured germinability at 24, 48, 72, and 96 hours. Next, we sought to understand how long conidia could live in a nearly ideal environment, an experiment designed to

test the maximum potential reach of each species. In Experiment 2 we used a combination of UV-RH-T favorable to the retention of germinability as a single environment in two experimental runs, one for *A. alternata* (2A) and a second for *A. solani* (2S). We conducted Experiment 2 for 288 hours (12 days) and measured germinability at 0, 24, 48, 72, 144, 216, and 288 hours (or days 0, 1, 3, 6, 9, and 12). Methods used to collect and generate *A. alternata* and *A. solani* conidia are found in Supporting Information 1.

2.2. Exposing spores to different combinations of UV-RH-T (Experiments 1 and 2)

Physical setup: For Experiment 1, a series of plexiglass platforms were cut and fit as steps into a frame made of PVC pipes (Figure S1). Because irradiance is inversely proportional to the squared distance between a light source and a surface, each step could be exposed to a different intensity of UV (Figure S1 and Table S2). Each plexiglass step measured 20.32 cm wide by 66.04 cm long; six steps were placed under 40 W_{UVA}, six under 40 W_{UVB}, four under 15 W_{UVA}, four under 15 W_{UVB}, and one in 0 W (i.e., complete darkness); a total of 21 steps or surfaces.

For Experiment 2, a single 121.92 cm (48 in) long by 66.04 cm (26 in) wide plexiglass step or platform was placed under a light source at a strength

consistent with the single treatment chosen from Experiment 1.

Conidial manipulation: Experiment 1 conidia were first placed on microscope coverslips. Coverslips were prepared by spreading 50 µL of a gently mixed, concentrated conidial suspension onto the upper surface of a sterile 19x19 mm ultra-thin (0.25 mm) quartz cover slip (Chemglass Life Sciences, Vineland, New Jersey, USA). Coverslips were left to dry in darkness for a few minutes before being placed in the environmental chamber. For each of Experiment 1's 10 conditions, coverslips were placed as two rows of 16 on each step (32 coverslips per UV-RH-T treatment; 16 for each species, Figure S1); coverslips were arranged according to a randomized block design. As each of the 10 conditions included a total of 32 coverslips for each of 21 treatments the total number of coverslips for each experimental run was 672 (336 coverslips per species). In total, the 10 conditions involved 6,720 coverslips.

Experiment 2 conidia were spread onto glass slides instead of coverslips. A total of 238 25x75 mm glass microscope slides (Globe Scientific, Mahwah, New Jersey, USA) per species were coated in 200 μL of conidia suspensions and left to dry in darkness for a few minutes before being placed in the environmental chamber. For each of the two runs (2A and 2S), a total of 217 slides were randomly placed as a grid across the single plexiglass platform.

The remaining 21 slides were kept in complete darkness.

Light treatments: In Experiment 1, UVP XX-Series UV Bench Lamps (Analytikjena, Jena, Germany) were suspended above the plexiglass steps (Figure S1) to generate different intensities of UV (Table S2). Irradiances were measured for each step with a UV Light Meter (Sper Scientific Direct, Scottsdale, Arizona, USA) at the start of each experimental run (Table S2). To prevent leakage of UV light from one module to another, black plastic fabric was placed between modules, and the UV Light Meter was used to confirm both that no light was leaking between modules and that the step kept in darkness was dark. In Experiment 2, we had to choose either UVA or UVB, and we chose to focus on UVA because 95% of UV light in the lower atmosphere is UVA (Iqbal, 1983). The fixtures emitting only UVA (6.29 ± 0.17 W/m^2 for both species) were placed above the single treatment surface. This light treatment was determined from Experiment 1 to most favor germinability (see Results). In both experiments, day-night cycles were approximated by alternating 12 hours of continuous UV irradiation with 12 hours of darkness.

Relative humidity and temperature: In Experiment 1, the environmental chamber was calibrated to one of the 10 RH-T conditions (Table S1). These

RH and T values are typical of central Wisconsin during the peak seasonal concentrations of airborne conidia of *A. alternata* and *A. solani* (Ding et al., 2019b; Crutcher, 1969; Table S1). In Experiment 2, a single RH and T found to favor the retention of germinability for *A. alternata* (RH=90%, T= 15°C) and *A. solani* (RH=90%, T= 20°C) was held for 288 hours. In both Experiments 1 and 2, RH and T were monitored every five minutes to ensure conidia were consistently exposed to a given treatment.

2.3. Measuring germinability

Imaging: Conidia were germinated according to methods provided in Supporting Information 2. After 24 hours conidia were counted (N_{total}). The slide holder on an Olympus CX31 compound microscope (Olympus, Tokyo, Japan) was removed so that conidia could be observed directly from agar plates (Figure 1). All conidia were visualized using an Olympus PlanApo N 2x objective lens (Olympus, Tokyo, Japan). To increase light penetration through agar, the microscope light condenser was removed. Digital images were captured using a Canon EOS Rebel II (Canon, Tokyo, Japan) with a Martin Widefield 1.38x DSLR adapter for Olympus BX and SZX with 51 mm dovetail photoport (Easley, South Carolina, USA), resulting in a total magnification of 2.76x. In Experiment 1, ten non-overlapping images of

conidia were randomly captured from each plate at each condition and time, and the number of germinated spores was counted ($N_{germinate}$).

In Experiment 2 the same protocols were followed but five images were captured per plate for *A. alternata* and 20 images were captured per plate for *A solani*. Image numbers differ to account for differences in the density of conidia observed between species.

In both Experiment 1 and Experiment 2, counting took place over the course of approximately four hours each day. Spores were harvested, spread onto water agar plates, and incubated for six hours at 20°C to induce germination/a germ tube of a length sufficient for imaging. Germination was then halted by placing plates in our cold room at 4°C until the following day, or for approximately 24 hours, while other components of the experiment were conducted (in order to prevent excessive overgrowth of individual germ tubes onto each other). After 24 hours of little to no growth in the cold room, plates were imaged. Only one 'sleeve' of Petri dishes was removed from the cold room at a time to mitigate, e.g., exposure of the first plate to room temperature for zero hours versus exposure of the last plate for four hours. There was between a 20 minute to one hour difference between when the first plate per sleeve was imaged versus the last. Besides being a practical

constraint, we consider the time involved as unlikely to have introduced any bias in counting: any spore that was still alive would have germinated during the six hours of initial incubation at 20°C; even if there was some additional germination during the time used to count plates per sleeve, it is unlikely that a germ tube of sufficient size for imaging would have developed. Personal observations support our assumption that spores that had not germinated during the initial incubation also failed to germinate days after germination was induced.

Image processing: Custom algorithms developed by MIPAR v3.2 (Worthington, Ohio, USA) were used to count germinated and ungerminated conidia. Conidia size and germ tube development are different for the two *Alternaria* species, and as a result, species-tailored counting algorithms were used. A full description of image processing protocols is found in Supporting Information 2. In brief: out-of-focus features of each image were removed, as were features outside of the size range of conidia. Thresholding substantially reduced noise caused by debris and uncountable clusters of conidia (Figure 1; Figure S2). Remaining features were then classified as either germinated or ungerminated conidia. To ground truth the counting algorithms, 50 images of *A. alternata* and *A. solani* were randomly selected and germinated

and ungerminated conidia counted by eye. Manual counts of germinated and ungerminated conidia were compared to results generated from our custom software (Supporting Information 1; Figure S3).

2.4. Statistical analyses

Mixed effect models: We used the R package *glmmTMB* (Hardin and Hilbe, 2018; Bolker, 2020) to test for significant differences among the numbers of germinated conidia across treatments in Experiments 1 and 2. The number of germinated and ungerminated conidia was calculated per coverslip and modeled using a log link function and log-transformed mean total number of conidia as an offset to account for differences in the number of spores deposited on each coverslip/slide (Hardin and Hilbe, 2018).

Experiment 1 data: variables included days of exposure, UV wavelength (including darkness), distance from a UV light source, RH and T; each species was analyzed separately. Random effects were included to account for any deviations in environmental chamber performance or for fluctuations in UV intensity across a step (Figure S1). We computed full models and then simplified models by removing uninformative variables (i.e., variables not included in best-fit models) using the corrected Akaike Information Criterion (AICc) (Table 1). In addition, we performed Tukey's post-hoc tests to correct

for multiple comparisons of means on best fit models (Table S3B; Bolker, 2020).

An additional analysis: In a separate analysis of Experiment 1 data, we tested for significant effects of UV, RH and T on conidia germination using Kruskal–Wallis tests followed by post-hoc assessments of significance using Dunn's multiple comparisons with a Benjamini-Hochberg adjustment (Table S4; R Core Team, 2022). Germination at hour 96 only was compared across (a) UV wavelengths (UVA, UVB, and darkness), (b) RHs per UV wavelength (e.g., UVA-50% RH vs. UVA-90% RH), (c) Ts per UV wavelength (e.g., UVB-20^{o}C vs. UVB-15^{o}C), and (d) conditions (e.g., Experiment 1 condition 1 vs. Experiment 1 condition 2, etc.).

2.5. Using models of atmospheric transport to simulate dispersal across space over time

To understand how patterns of germinability affect the movement of both *Alternaria* species across North America, we modelled the transport of *A. alternata* and *A. solani* conidia in the atmosphere. A full description of model parameters and methods is found in (Lagomarsino Oneto et al., 2020). Briefly: numerical simulations tracked many representative trajectories of spores in the atmosphere using meteorological data available from the Na-

tional Oceanic and Atmospheric Administration (NOAA) and the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model (Stein et al., 2015). Specifically, we used the North American Regional Reanalysis (NARR) described in (Mesinger et al., 2006), as it combines numerical simulations with observational data.

The movement of conidia through the atmosphere was modeled vertically and horizontally, with gravitational settling velocities proportionate to conidial dimensions: $a \times b = 20\mu m \times 7.5\mu m$ for *A. alternata* and $a \times b = 100\,\mu m \times 10\,\mu m$ for *A. solani*, where a and b correspond to the long and short axis of the spore, respectively. The settling velocity is calculated by HYSPLIT (Stein et al. (2015) as $v = \rho g d^2/18\mu$ where d is the equivalent diameter of the spore $d = 2\sqrt[3]{3V/4\pi}$), yielding $d = 20.5\mu m$ and $d = 43.0\mu m$ for *A. alternata* and *A. solani* respectively. Assuming spores are approximately prolate spheroids, their volume is $V = 4\pi ab^{2/3}$); where $\sigma = 1g/cm^3$ is spore density (Savage et al., 2010); $\mu = 1.8 \times 10^{-5}\ kg/(m \times s)$ is the dynamic viscosity of air and we neglect air density which is negligible with respect to spore density. Only dry deposition was included in the simulations, as we focused on understanding how patterns of germinability affect the movement of both *Alternaria* species; thus wet deposition was excluded since it can cause

highly concentrated and localized deposition (Savage et al., 2010). Models simulate dry deposition by randomly removing spores that travel close to the ground using a constant rate proportional to the deposition velocity. Turbulent eddy diffusivity was estimated following Beljaars & Holtslag ("BH"; Beljaars and Holtslag, 1991).

In each simulation, a total of 500,000 conidia of each species were released from central Wisconsin (44.119N, -89.536W). We used the North American Regional Reanalysis (NARR) dataset in simulations, and it provides weather data starting from the first 10 m layer of the atmosphere closest to the ground (it does not provide data for phenomena within the 10 m layer; Mesinger et al., 2006). We chose to use the NARR dataset because it extends over large geographic and temporal scales (it includes all of North America and provides data from 1978 to present), because it offers excellent temporal and spatial resolution, and because it provides empirical measures of meteorological phenomena. Because we use the NARR dataset, our simulations represent a spore's journey in the open atmosphere, after spores have escaped the canopy. Simulations were run per species with the following initial conditions: July 15, August 1, August 15, and September 1 at 0:00, 10:00, and 14:00 hours for the years 2009-2018 (a total of 240 combinations were tested).

Dates and times were chosen based on historical data of peak conidial concentrations (Ding et al., 2019b). Each of the 240 simulations followed 500,000 spores released simultaneously from the same location. For each spore that deposited within 288 hours, the following data were recorded: latitude at deposition, longitude at deposition, maximum height across the trajectory and time of deposition.

The output of each simulation was imported into R v3.6.2 (R Core Team, 2022). The distance travelled by each spore from take-off to deposition was calculated using the WGS84 terrestrial reference system with *geosphere v. 1.5-10* (Hijmans, 2011). To visualize the geographic spread of conidia, data were aggregated by date of release and year. The Landing times were grouped into six-hour intervals from zero to 288 hours. For each interval, the geographic distribution of spores deposited was approximated as an ellipse whose centroid is the average landing position of all conidia that had landed within the six-hour interval and whose major axis is oriented in the direction of maximum spread from the centroid. The major axis radius is equal to the standard deviation of the spatial distribution of landed spores and is oriented in the direction of maximum spread. Similarly, the minor axis represents one standard deviation of the distance travelled in the di-

rection perpendicular to the major axis by the same spores. The area of ellipses become smaller with time, especially for *A. solani*. Ellipses were calculated using *aspace v. 3.2* (Buliung and Remmel, 2008) and custom in-house scripts: https://github.com/jacobgolan/alternaria_dispersal. To minimize two dimensional distortions of spore trajectories across Earth's curved surface, the R package *sp v. 1.4-0* was used to correct the latitude and longitude of each spore from an EPSG:2288 coordinate system to EPGS:4326 (Pebesma and Bivand, 2005).

3. Results

3.1. Counting germinated spores

Germinability was successfully quantified for *A. alternata* and *A. solani* conidia using automated counting algorithms: automated and manual counts are strongly correlated (Figure S3).

3.2. Identifying parameters most likely to maximize spore germination (Experiment 1)

Fitting models: Experiment 1 data enabled identification of the combinations of UV, RH and T resulting in greatest numbers of germinated conidia (Table 1; Figure 2). Full models were computed using time, wavelength

(UVA, UVB or darkness), RH, T, and irradiance (W/m^2; Figure 2) and simplified final models were chosen by comparing models' corrected Akaike Information Criterion (AICc; Table 1).

The number of germinated conidia on each coverslip was modeled as a random variable distributed according to a negative binomial distribution. The expected value of the distribution, conditioned on each treatment, took the form:

$$E(N_{germinate}|t, RH, UV, T, surface, N_{total}) = N_{total} e^{\beta_0 + \beta t + \gamma_{RH} + \tau_T + \lambda_{UV} + \epsilon_{surface}}$$

where N_{total} is the total number of conidia on a coverslip (alive and dead); β is a parameter quantifying how quickly germination decreases and t is time of exposure to a specific condition (in days); γ_{RH}, τ_T and λ_{UV} are parameters quantifying the effects of RH, T and exposure to UV light. $\epsilon_{surface}$ represents the random effects on each surface (a random variable distributed according to a Gaussian centered at zero and with a standard deviation σ). The fit produces estimates for our nine coefficients of interest (β, $\gamma_{60\%}$, $\gamma_{75\%}$, $\gamma_{90\%}$, $\tau_{15°C}$, $\tau_{20°C}$, $\tau_{25°C}$, λ_{UVA} and λ_{UVB}) and we choose RH = 50%, T =

10°C and no UV exposure as a reference condition, hence $\gamma_{50\%} = \tau_{10°C} = \lambda_{dark} = 0$. Exponentiated coefficients greater than 1 translate to an increase in germinability with respect to the reference condition, and exponentiated coefficients less than one translate to a decrease in germinability with respect to the reference condition (Figure 3). β_0 is the intercept accounting for dead spores in the reference condition at $t = 0$.

We next compared models' AICc to identify the minimum number of parameters needed to explain experimental data without overfitting. The best-fitting model of *A. solani* conidia germination did not include T, but to enable comparisons between *A. solani* and *A. alternata*, we selected the second-best *A. solani* model, which included T and was identical to the best fit *A. alternata* model (Table 1).

Models identify both UV wavelengths as detrimental to germination ($e^{\lambda_{UVA}}$ = 0.89 and 0.82, and $e^{\lambda_{UVB}}$ = 0.16 and 0.37, for *A. alternata* and *A. solani* respectively, Figure 3). Conidia kept in darkness germinated most readily and UVB exposure resulted in the smallest numbers of germinated conidia (Figure 2). While we observed differences in conidial germinability among different wavelengths (Figure 2), selected models did not include irradiance (W/m^2) as a parameter (Table 1). Kruskal-Wallis followed by post-hoc Dunn

tests confirm this result (Table S4).

Relative humidities of 90% maximized germination at all temperatures and UV wavelengths ($e^{\gamma_{90\%}} = 1.25$ and 1.75 for *A. alternata* and *A. solani* respectively, Figure 3). Kruskal-Wallis followed by post-hoc Dunn tests confirm this result (Kruskal-Wallis $\chi^2 = 225.05$, 77.664, 28.624, for each species, respectively, both with df = 3, p-value < 0.0001 for each; Table S4).

Results for T were less consistent than results for RH or UV. Models suggest 15°C maximized germination for both species (Figure 3), but *A. alternata* conidia kept at 90% RH appear to germinate equally well at both 15°C and 20°C (p-value < 0.05; Table S3, Figure 2). Kruskal-Wallis followed by post-hoc Dunn tests were also inconclusive ($\chi^2 = 11.28$-55.14, df = 3, p-value < 0.01; Table S4A). Because 90% RH clearly maximized the germination of both species' conidia, temperature was reinvestigated using only the four conditions (7-10) involving 90% RH (Table 1, Figure 3):

$$E(N_{germinate}|t, RH = 90\%, UV, T, surface, N_{total}) = N_{total}e^{\beta_0+\beta t+\tau_T+\lambda_{UV}+\epsilon_{surface}}$$

Results were more consistent; according to both model effect sizes (Figure

3, Table S3), and Kruskal-Wallis and post-hoc Dunn tests (Figure 3; Table S4), 15°C is the most favorable temperature for *A. alternata* germinability, and 20°C is the most favorable for *A. solani* germinability.

Based on these results, parameters chosen for Experiment 2 included an RH of 90% and T of 15°C for *A. alternata* (2A), and 90% RH and 20°C for *A. solani* (2S). We exposed conidia to alternating periods of 12 hours UVA light and 12 hours darkness at an irradiance of 6.29±0.17 W/m^2, equivalent to the lowest UVA-40W dosage administered in Experiment 1 and a UV environment typical of the troposphere (Table S2; Iqbal, 1983).

3.3. Measuring spore germination over timescales consistent with long distance dispersal (Experiment 2):

The two *Alternaria* species demonstrated markedly different germination patterns over 288 hours. A greater total number of conidia and proportion (i.e., fraction of total conidia) of *A. alternata* conidia germinated at all sampling points (hours 0, 24, 72, 144, 214 and 288), compared to *A. solani* conidia (Figure 4D & H; Figure 5C). Germinability of *A. alternata* conidia decreased approximately linearly over time, but germinability of *A. solani* conidia fell sharply within 24 hours and subsequently plateaued (Figure 4D & H). Germinability remained at approximately 12-20% after 24 hours and a

visual inspection of *A. solani* conidia suggests most conidia germinating after 24 hours develop atypical germ tubes, compared to conidia germinating at 0 hours (Figure S6). These abnormally growing conidia could not be measured by custom MIPAR algorithms because they were designed to provide a binary classification (germinated/ungerminated). Atypical conidia grew germ tubes reaching a length of approximately 100-150 µm (compared to 200 µm or more at 0 hours) and germ tube growth was delayed (Golan pers. obs.). Differences between *A. alternata* and *A. solani* germination are corroborated by Experiment 1 data: the germinability of *A. alternata* conidia decreases linearly over time, but germinability of *A. solani* conidia falls sharply within 24 hours of the start of the experiment (Figure S5). In Experiment 2, the half-life of germinability for *A. alternata* is approximately 35 hours (i.e., 2% loss in germinability per hour under UVA). In stark contrast, the half-life of germinability for *A. solani* is approximately 1.5 hours (i.e., 47% loss in germinability within the first 24 hours).

The HYSPLIT simulations of conidia dispersing from central Wisconsin show the smaller conidia of *A. alternata* as travelling over greater ranges than the larger conidia of *A. solani* (Figure 4; Figure 5A & D). Range is dictated by spore size (Lagomarsino Oneto et al., 2020): while horizontal movement

for most spores is dominated by horizontal components of atmospheric mixing (independent of spores' specific features, e.g., size and shape), spore size plays a key role in vertical motion. Spore size dictates gravitational settling, which can be faster than vertical winds (Lagomarsino Oneto et al., 2020). A complex interplay between a spore's settling velocity and the stability of the lower atmosphere controls how long spores will remain aloft (Figure 5B) and thus how far they will travel (Figure 5D; Lagomarsino Oneto et al., 2020).

The number of *A. solani* conidia in the air decreases two to three times faster than the number of *A. alternata* conidia in the air (Figure 5B). By 144 hours (day 6) no *A. solani* conidia remain aloft (in any simulation). By contrast, at 288 hours (day 12) significant numbers of *A. alternata* conidia are still found in the atmosphere (in all simulations). Before all *A. solani* conidia settle, they can travel (more than 1,000 km, Figure 4, Figure 5D), but the number of conidia reaching these long distances is two orders of magnitude smaller (less than 1% of the total released), as compared to *A. alternata* (upwards of 25% of the total released; Figure 4D). At a release time of 0:00 CST, *A. solani* conidia settle to the ground before day one (Figure 4E, Figure 5B), while *A. alternaria* conidia are reaching, e.g., Greenland on day nine (∼4,000 km, Figure 4A). Even at release times 10:00 CST and 14:00 CST,

where both species disperse longer distances due to increased turbulent convection (Lagomarsino Oneto et al., 2020), their dispersal dynamics are very different (compare maps in Figure 4B-F and Figure 4C-G, and corresponding flight time and landing range statistics in Figure 5B,D).

In addition to showing differences in the geographical scale of dispersal, maps in Figure 4 also reveal different shapes of these patterns, with ranges for *A. solani* elongate and narrow, compared to the more circular, broader ranges of *A. alternata*. The probability of reaching ranges showed by ellipses in Figure 4 can be estimated from the fraction of conidia deposited in the corresponding six-hour interval (Figure 5B). Accordingly, the ranges of *A. solani*'s depict much fewer conidia, as compared to the ranges of *A. alternata*.

Next, the results of simulations and experiments are combined to obtain spores' effective dispersal, i.e., the landing distance of viable spores (Figure 5C). To this end, the trajectories of simulated spores are divided into six groups, according to their landing time. Within each group, the average landing distance (obtained from simulations) is plotted against the average viable fraction of spores at the same time (obtained from experiments). Figure 5C demonstrates that among spores that sediment at a given distance, *A. solani* has a dramatically lower germinability with respect to *A. alternata*. Figure

5C further demonstrates that most *A. solani* land at distances at which they have a higher chance to survive, i.e., closer to their source. In the aggregate, these results support the hypothesis that the species with smaller spores is under considerable selective pressure to withstand atmospheric transport.

4. Discussion

We systematically tested how temperature, relative humidity, UV light exposure, and their combinations affect the germinability of *A. alternata* and *A. solani* (Aylor, 2017; Norros et al., 2015). Next, we measured survival in a favorable environment over a timescale consistent with continental dispersal. We combined the survival data with models of spore movement to offer a realistic bound on the effective (as opposed to potential) dispersal of spores in the atmosphere (Golan and Pringle, 2017; Lagomarsino Oneto et al., 2020). We specifically chose to measure longer-timescale survival in a favorable and realistic, but unnaturally static, tropospheric environment to probe the edges of the potential reach of spores, asking, "how far would the 'luckiest' spores of either species travel"? Spores may die faster if they encounter conditions harsher than the most favorable condition. To extend our findings and explore how spore survival is shaped by the diverse environments along a spore's

trajectory, more viability experiments, as well as complex simulations, are needed; both are exciting directions for future research.

The effective dispersal of viable spores of *A. alternata* and *A. solani* emerges as very different. As an illustration, consider the ability of *A. solani* and *A. alternata* to reach Maine (a potato growing state on the east coast of North America, approximately 1,500 km from Wisconsin) when both are released at 10:00 CST: less than 1% of *A. solani* reach such a distance and most are inviable; by contrast, upwards of 25% of *A. alternata* reach Maine and 75% of them are still viable (Figure 4; Figure 5). The combination of more time aloft and greater longevity results in a larger number of *A. alternata* conidia travelling hundreds to thousands of kilometers and landing still able to cause infection (Figure 4). Less than 1% of *A. solani* conidia are still in the atmosphere after 24 hours and because these spores either cannot germinate or germinate abnormally, they are unlikely to cause disease. The conidia of *A. alternata* are both small enough to travel over 1,500 kilometers and physiologically equipped to survive the journey (Brown and Hovmøller, 2002; Magan et al., 1984; Pringle, 2013; Bush and Prochnau, 2004). We can find no data addressing the global population biology of *A. alternata*, but based on our experiments we hypothesize it may function as a single, global

population, similar to *Aspergillus fumigatus* (Pringle et al., 2005).

The larger conidia of *A. solani* are more vulnerable to atmospheric hazards than the smaller spores of *A. alternata*. A shorter lifespan of larger spores is unintuitive, as larger spores are often assumed to be more resilient than smaller spores (Norros et al., 2015; Calhim et al., 2018; Kauserud et al., 2008; Jones and Harrison, 2004). Other species of *Alternaria* with large conidia also experience rapid declines in germinability when exposed to atmospheric conditions: in one experiment, 95% of *A. macrocarpa* conidia were unable to germinate after four days (Rotem et al., 1985; Rotem and Aust, 1991). *Alternaria* fungi with large conidia are clustered in the monophyletic section *Porri* (see Figure 19 in Woudenberg et al., 2013). Perhaps species with large conidia are not under selective pressures to endure long-haul atmospheric travel because they settle out of the atmosphere quickly. From our controlled experiments with two closely related pathogens, we hypothesize a negative correlation between spore size and survival time in the atmosphere among other fungi with airborne spores.

The timing of spore liberation will also influence effective dispersal. Lagomarsino Oneto et al. (2020) established the timing of spore ejection as playing a major role in determining how long spores dwell in the atmosphere before

returning to the ground. For example, solar heat transfer causes atmospheric mixing, and consequently, all else being equal, spores released during the day settle less readily and will undergo longer journeys than spores released at night (Lagomarsino Oneto et al., 2020). Thus, we hypothesize that spore size, longevity, and the timing of spore release evolve and influence each other dynamically: spores undergoing long journeys facilitated by their small size and/or release patterns (e.g., at noon) are selected for increased atmospheric survival, whereas spores traveling short distances resulting from their large size and/or release times in calm atmospheric conditions (e.g., at night), are under less selective pressure for longer-term atmospheric survival.

The potential trade-offs driving spore size and survival during dispersal remain largely unexplored, as do the evolutionary forces shaping spore morphology in general (Pringle et al., 2015; but see Aguilar-Trigueros et al., 2023). Why don't all fungi evolve small, hardy spores capable of long distance dispersal? For the kingdom as a whole, one answer involves phylogenetic constraints. For example, the biomechanics of ascospore and basidiospore launching are fundamentally different, and so ascospores take different sizes, as compared to basidiospores (Aguilar-Trigueros et al., 2023). Ecological niche may also shape spore size, for example, symbiotic species appear to

have consistently larger spores than asymbiotic species, at least for some guilds of fungi (Aguilar-Trigueros et al., 2023).

Spores are exposed to myriad physical and biological variables as they leave sporocarps and disperse. Different variables likely impose different selective pressures for spores to be either larger or smaller (Lagomarsino Oneto et al., 2020). Spore liberation itself involves a fascinating sequence of physical processes whose efficiency depends on a spore's shape, as well as on the biomechanics of the release process, reviewed by Ingold (1965) and in Pringle et al. (2017). Once liberated, spores must escape the boundary layer of still air surrounding the parent fungus. Larger spores may escape more easily because their size correlates with the thickness of the boundary layer (Pringle et al., 2017) or because their increased mass propels them several centimeters away from the parent upon ejection (Money, 1998). Smaller spores may cross the boundary layer by creating their own collectively generated wind, or "puffing," a phenomenon characteristic of cup fungi, e.g., *Sclerotinia sclerotiorum* (Roper et al., 2010). After escaping the boundary layer, spores also interact with the canopy in a highly size-dependent manner; e.g., smaller spores are more likely to evade obstacles including overhanging leaves or stems (Norros et al., 2014). Once spores reach the atmosphere, the duration

of a spore's flight will depend on both their size as well as the timing and location of release (Lagomarsino Oneto et al., 2020). All else being equal, small spores will travel further than large spores.

Once a spore lands on a substrate, other variables become relevant. For example, parental investment (Zimmerman et al., 2016) increases with a spore's biomass, and greater investment may be an advantage as a spore grows into an independent mycelium because larger spores are more likely to establish, as compared to smaller spores (see e.g., Altre et al., 1999; Norros et al., 2015; Halbwachs et al., 2017; van den Brule et al., 2020; Ijadpanah-saravi et al., 2022). However, smaller spores may be favoured because they can be produced in larger numbers, increasing the probability of successful establishment by at least one spore in uncertain downstream environments (Nagarajan and Singh, 1990, 1990).

However, whether a species and its spores optimize on travelling short versus long distances will depend on the life history of the species, and general statements for all "fungi" are not possible (Golan and Pringle, 2017). In the case of the two *Alternaria* species used in this study, natural history offers clues. *Alternaria solani* is one of the most harmful solanaceous plant pathogens affecting potato and tomato crops in temperate agricultural re-

gions globally. Unmanaged, *A. solani* can reduce potato and tomato crops by 30% or more (Chaudhary et al., 2021). The pathogen is necrotrophic and crop debris provides a ready source of viable inoculum for subsequent susceptible crop plantings in relatively close proximity. The fungus does not need to travel long distances in order to reinfect crops, and we hypothesize the pathogen's relatively larger conidial size and more limited dispersal is caused by its relatively smaller host range and selective pressures to stay within potato and tomato fields planted in a tight geographical region. By contrast, *A. alternata* is a common, opportunistic, and cosmopolitan pathogen of animals (including humans) as well as crops. Over 380 host species are recognized as susceptible. In potato and tomato crops, *A. alternata* is considered a minor pathogen. It infects weakened or stressed plants and takes advantage of plants previously infected by other pathogens (including *A. solani*), by doing so, it acts saprophytically. The relatively smaller conidial size and capacity for traveling longer distances may be tied to its broader host range and distinct ecology and pathology, as compared to *A. solani*.

We have focused on specific release times and dates, motivated by the need to carefully build a case study, experimentally manipulating and modelling two pathogens that do release spores from the same locations at specific

seasons and times of the day. Our results are robust to changes in model parameters: first, the comparative results hold at all three release times, times chosen to represent extremely different atmospheric environments (e.g. weak vs. intense turbulence). Further, results will generalize to other locations, as long as they have regular diurnal patterns in turbulence. Rhythmic diurnal patterns are quite common (Lagomarsino Oneto et al., 2020.

We find factors affecting spore survival that are in broad agreement with data generated by other studies (Aylor and Sanogo, 1997; Leach, 1967; Fourtouni et al., 1998; Braga et al., 2015; Garcia-Cela et al., 2016). For example, Magan et al. (1984) also found RH as crucial to determining *A. alternata* germinability. In another experiment, the germinability of *A. solani* conidia decreased by 20% after eight hours' exposure to sunlight Rotem et al. (1985).

While UVB is clearly detrimental to germinability (Figure 2; Figure 3; Figure S4; Figure S5), in our experiments a small number of conidia exposed to UVB still germinated. We hypothesize these spores were shielded within clusters of spores. The clumping of dispersing spores is a rarely investigated phenomenon but it may be an important strategy used by fungi to survive harsh environments (Golan and Pringle, 2017; Li et al., 2006; Dias, 2008; Furukawa et al., 2005; Schwinghamer, 1958).

Tests of our hypotheses should include multiple large sets of closely related species with distinct spore morphologies and dispersal strategies. Because the genus *Alternaria* encompasses a diversity of spore shapes and sizes, it emerges as a model for studying the biophysical constraints and evolutionary tradeoffs of fungal dispersal within a phylogenetic framework. Our results also suggest that measures of spore survival rates can be incorporated within dispersion models (e.g., HYSPLIT [Stein et al., 2015], NAME [Jones et al., 2007], SRAPS [Isard et al., 2011], or the Ethiopian Wheat Rust Early Warning System [Allen-Sader et al., 2019]) to generate in-depth quantitative assessments of spatio-temporal dispersal patterns and pathways.

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Data Availability Statement

All data and scripts can be found at https://github.com/jacobgolan/Alternaria_Dispersal and DOI10.17605/OSF.IO/R98HA

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981 **5. Figure Legends**

982 **Figure 1:** Example images of germinating conidia of *A. alternata* (A,
983 blue) and *A. solani* (B, red) on water agar plates. Boxes 1 and 2 show ger-
984 minated and ungerminated conidia, respectively. Boxes 3-5 illustrate debris,
985 out-of-focus conidia, and uncountable clusters of conidia, respectively.
986
987 **Figure 2:** Experiment 1 (A-F): Proportion of conidia relative to initial
988 number of conidia germinating after 96 hours for all tested relative humidi-
989 ties (RH %), temperatures (T in ^{o}C), and UV dosages. Data of *A. alternata*
990 (A, C, D) are blue and data of *A. solani* (B, E, F) are red. Tones of blue
991 and red mark different RH environments.
992
993 **Figure 3:** Summary of effect sizes for parameters included in best-fit gener-
994 alized linear mixed models. Effect sizes shown in exponentiated form. Panels
995 (A) and (B) show estimates for models including all ten Experiment 1 condi-
996 tions, and (C) and (D) show effect sizes from models fitting only conditions
997 7-10, or the conditions for which RH was held at 90%. Values for *A. alternata*
998 and *A. solani* shown in blue and red, respectively. Statistically significant
999 effects sizes are marked with an asterisk (*, $p < 0.05$; **, $p < 0.001$; ***, p
1000 < 0.0001).
1001
1002 **Figure 4:** Spatial visualization of HYSPLIT models (A-C, E-G). From top
1003 to bottom: data corresponding to release times 0:00, 10:00 and 14:00 CTS

from all 40 dates simulated across 10 years for *A. alternata* (A-C) and *A. solani* (E-G). Each ellipse corresponds to the position of spores that deposit within an interval of 6 hours (for better visualization, select intervals are highlighted with a dotted circumference). The opacity of each ellipse decreases as a function of the number of spores deposited. The centroid of each ellipse is located at the average landing position of all conidia in the same time interval; Ellipse major axes are in the direction of maximum spread from the centroid; minor axes are perpendicular to the major axis. Axis lengths are the standard deviation of the spatial area over which spores have deposited within a time interval. Panels D & H show the proportion of germinated spores averaged per block (slide) in Experiment 2. Data in blue and red for *A. alternata* and *A. solani*, respectively. Data for spores kept in darkness shown in grey inset.

Figure 5: (A) Results of HYSPLIT models of spore dispersal showing spores remaining airborne as a function of time after take-off. Small insets show the same data zoomed in for the first 12 hours. All conidia of *A. solani* settle before the end of the 288-hour simulation. Shades correspond to the number of standard errors from the mean; means represented by black trend lines. (B) Fraction of launched spores deposited in each consecutive six-hours interval. The x-axis indicates the end of each interval. Each point represents the fraction of spores used to define the geometry of ellipses in Figure 4A-C and E-G. (C) Fraction of deposited spores that germinate (i.e., are viable), as a function of landing distance. The x-axis shows distance travelled from the take-off point (from HYSPLIT simulations) averaged among all spores per each time point used to test germinability in Experiment 2. The y-axis shows the fraction of viable spores (from Experiment 2 data) that have landed (from HYSPLIT simulations). Error bars show the standard error of the fraction of viable spores that have landed. (D) Fraction of launched spores landing at a given distance from the take-off point. A spore is counted as landed at a distance x if it has landed at any location between the take-off point and up to 100 km (the maximum distance simulated in HYSPLIT models). In all panels, data for *A. alternata* and *A. solani* are shown in shades of blue and red, respectively. Trajectories were simulated for three release times: 0:00 (solid line), 10:00 (dotted line), and 14:00 (dashed line).

6. Supporting Information Figure Legends

Figure S1: Experiment 1 experimental setup. Clear plexiglass surfaces (or "steps") were arranged at different heights beneath a UVA or UVB light source. Quartz cover slips coated in spore suspensions of either *A. alternata* or *A. solani* were placed on each plexiglass step using a randomized block design. UV bulbs were suspended above the 20 steps (an additional step was kept in complete darkness, labelled "Dark"). A module encompasses all surfaces underneath one of the four UV bulbs of a given wavelength (UVA or UVB) and power (Watts). There are four modules. The experimental apparatus was placed in an environmental chamber and a single relative humidity (RH) and temperature (T) specific to one of ten environments (conditions 1-10) was maintained and monitored every five minutes by an automated system during each of the experimental runs (1-10). Experimental runs took place in series, one after the other, using the same chamber. Lights cycled through a 12-hour on, 12-hour off schedule for the 96 hours of each experimental run and four cover slips were sampled from each step at 24-hour intervals. Black plastic tarp was placed between each module to prevent leakage of UV light between modules. Conidia did not germinate on cover slips.

Figure S2: Sample of MIPAR software, here used to count conidia of *A. alternata*.

Figure S3: Comparison of automated and manual counting of germinated and ungerminated spores. A logarithmic scale was chosen to aid visualization. A simple linear regression was performed on untransformed data (grey line) and compared to a dotted black line of an ideal 1:1 relationship between both counting methods.

Figure S4: Complete results of Experiment 1 conditions 1-10 on *A. alternata* for hours 24 and 96. Dotted lines connect the median proportion of germinated spores per light source for illustrative purposes.

Figure S5: Complete results of Experiment 1 conditions 1-10 on *A. solani* for hours 24 and 96. Dotted lines connect the median proportion of germinated spores per light source for illustrative purposes.

Figure S6: Experiment 2: Images of abnormal and delayed germ tube development, *A. solani*.

Figure S7: Summary of spore germinability at sampling hours 0, 24, 48, 72 and 96 under Experiment 1 conditions most favoring germinability for each species. To aid visualization, dots are randomly placed about their corresponding sampling time on the x-axis. Data are summarized with a linear regression line per species.