Vortex-induced dispersal of a plant pathogen by raindrop impact

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Raindrop impact on infected plants can disperse micron-sized propagules of plant pathogens (e.g., spores of fungi). Little is known about the mechanism of how plant pathogens are liberated and transported due to raindrop impact. We used high-speed photography to observe thousands of dry-dispersed spores of the rust fungus 	extit{Puccinia triticina} being liberated from infected wheat plants following the impact of a single raindrop. We revealed that an air vortex ring was formed during the raindrop impact and carried the dry-dispersed spores away from the surface of the host plant. The maximum height and travel distance of the airborne spores increased with the aid of the air vortex. This unique mechanism of vortex-induced dispersal dynamics was characterized to predict trajectories of spores. Finally, we found that the spores transported by the air vortex can reach beyond the laminar boundary layer of leaves, which would enable the long-distance transport of plant pathogens through the atmosphere.

Results

Types of Spore Dispersal. We observed and analyzed the dispersal motion of urediniospores of 	extit{P. triticina} when a raindrop hits an infected leaf containing uredinia (Fig. 1A). Fig. 1B shows that a falling drop is powerful enough to liberate spores. Here, we identified two types of spore dispersal. The first is a wet splash dispersal of spores that has been studied previously. When a droplet impacts a surface, the contact line of the spreading edge of the drop becomes unstable and forms several daughter splash droplets due to the high inertia of the drop and the hydrophobic property of a leaf substrate (20). As shown on the right side of Fig. 1B, splashing droplets fly out along with spores inside. Spores inside a splash droplet are shown in Fig. 1C and SI Appendix, section A. Although the number of spores inside a splash droplet is more than that of dry-dispersed spores (5), the splash droplets are too heavy to follow the surrounding air current, limiting the range of dispersal.

The second is a dry-spore dispersal, that is, not wetted by a raindrop. The dry-spore dispersal is attributed to either the vibration of a wheat leaf (Fig. 1B) or the direct impact of the drop (Fig. 1C, Inset). Most spores are liberated from the upper surface, especially due to drop impact (top right side of Fig. 1B). However, spores on the lower surface can be thrown by a rapidly decelerating leaf, but the number of ejected spores from the lower surface is less than that from the upper surface.

Measurement of the Number of Ejected Spores. We measured the number of dry-dispersed spores, \( N_s \), after a drop impact. We first attached a rust-infected wheat leaf on a Petri dish. Then, a drop was released onto the wheat leaf at different heights to vary the...
impact speed. We measured the number of dry-dispersed spores using a Nikon D500 camera with a pixel resolution of 6,016 × 4,000; the area of most spores was measured to be about five square pixels (SI Appendix, section A). Fig. 1C shows both wet-dispersed spores and thousands of dry-dispersed spores. Fig. 1D shows that the number of dry-dispersed spores, \( N_\text{disp} \), is on the order of \( 10^4 \) and increases with the impact velocity, \( U_d \). Such an increase of \( N_\text{disp} \) with respect to \( U_d \) can be attributed not only to higher impulse momentum (5) but also to an increase in the drop-contact area as the drop spreads (21).

**Ejection Mechanism.** Spores are detached by the spreading motion of a drop as it exerts dynamic force on the spores. The dynamic force to drag and scavenge spores, \( C_D \rho_a \bar{U}^2 r_s^2 \) (\( \sim 10 \) nN), exceeds the interparticle force of *P. triticina* spores, 0.7 nN (SI Appendix, section B). Here, \( C_D \), \( \rho_a \), \( \bar{U} \), and \( r_s \) are the drag coefficient, the liquid density, the speed of the liquid contact line (on the order of 1 m/s), and the radius of a spore (\( \approx 10 \) μm). The detached spores stay on the advancing meniscus and then collide with dry spores on the way. By assuming elastic collision between spores on the meniscus and dry spores, the ejection velocity of dry spores, \( V_e \), would be proportional to the impact velocity of a falling drop, \( U_d \). A detailed theoretical model and experimental validation of spore ejection are presented in *SI Appendix*, section C.

**Spore-Traveling Distance and Trajectory.** For spores moving through quiescent air, the Reynolds number, \( R_e = \rho_s \bar{V}_s (2r_s)/\mu_a \lesssim 1 \), is small, where \( \rho_s \) and \( \mu_a \) are the density and dynamic viscosity of air (\( \rho_a = 1.2 \) kg/m\(^3\), \( \mu_a = 1.82 \times 10^{-5} \) Pa·s), and \( \bar{V}_s \) is the instantaneous speed of a spore. Here, the instantaneous speed decreases over time due to the air drag after reaching its maximum speed at the moment of ejection (\( \bar{V}_s \sim 1 \) m/s). Thus, the trajectory of spores can be predicted by balancing inertial force \( \rho_s (4/3)\pi r_s^3 (d \bar{V}_s/dt) \), Stokes drag \( 6\pi \mu_a \bar{V}_s r_s \), and gravity, where \( \rho_s \) is the spore density. By integrating these forces with the initial condition as \( V_e(t = 0) = \bar{V}_s \), a spore-travel distance \( d_{\text{trav}} \) can be written as follows:

\[
d_{\text{trav}} = \frac{2\rho_s r_s^2 V_e \cos \alpha_e}{9\mu_a} \left[ 1 - e^{-9\rho_s r_s^2/(2\rho_a r_s^2)} \right].
\]  

where \( \alpha_e \) and \( t_e \) correspond to the ejection angle and residence time of a spore in air, respectively. For ejected spores with \( V_e = 1 \) m/s, the relaxation timescale, \( \tau_r = (2/9)\rho_s r_s^2 /\mu_a \), is about \( 10^{-3} \) s, whereas the residence timescale, \( t_e \), becomes only \( 10^{-2} \) s. Therefore, the travel distance, \( d_{\text{trav}} \), is close to \( V_e \tau_r \cos \alpha_e \) since the exponential term, \( e^{-t_e/\tau_r} \), in Eq. 1 is quite small, on the order of \( 10^{-5} \).

Fig. 1C shows horizontal distances of dispersed spores measured from the drop-impact point, \( D_{\text{disp}} \). To avoid any confusion, it is worth mentioning that the travel distance, \( d_{\text{trav}} \), is measured from spore’s resting position, not from the drop-impact point. Hence, \( d_{\text{trav}} \) is always smaller than \( D_{\text{disp}} \). Interestingly, we found that the dispersal distance of spores exceeds theoretical values from the above force balance. Fig. 1E shows the consistent discrepancy over different drop speeds.

To rectify the discrepancy on dispersal distance, we visualized the side-view trajectory of rust spores as in Fig. 2A, which shows that spores ballistically move shortly after the ejection (see the upper panel inset) and then swirl around (see the lower panel inset). The overall trajectory of one spore is shown as the black line in the lower panel. Fig. 2B illustrates a detailed trajectory from an experiment and a simulated trajectory using the ballistic model above. The ballistic model shows a right-triangle path (closed symbols) known as the Tartaglia’s trajectory (22), whereas the experimental trajectory presents a swirling motion with a longer travel distance (open symbols). In addition, the maximum height reached by the spore is a bit higher than the predicted height from the ballistic model. To explain this...
discrepancy, we need to understand airflow around liberated spores upon drop impact.

**Trajectory Comparison Using Artificial Spores.** To visualize the airflow, we used glass beads as surrogates for spores (glass sphere type I in Table 1). The density and radius of particles are close to those of actual wheat spores. We observed similar dispersal behaviors of actual wheat spores. In the very beginning, ballistic trajectories were observed as shown in the first panel of Fig. 3. Then, the glass beads were swirling around and slowly move to the right (the second, third, and fourth panels of Fig. 3). Since the glass beads are light, this swirling motion can represent the pathlines of air current. To confirm this, we calculated the Stokes number, \(St\), defined as a ratio of the relaxation time of a particle \(\tau_s = (2/9) \rho U_c D^2/\mu\) to the characteristic flow time \(\tau_f = \bar{r}_w/U_w\). Here \(\bar{r}_w \sim 1 \text{ mm}\) and \(U_w \sim 0.1 \text{ m/s}\) are the mean radius and velocity of the swirling flows. The Stokes number is very small for artificial spore I \((St = 0.001 - 0.13)\). Also, for the wheat spore, \(St\) is also 0.15, which indicates that such swirling motion is induced by surrounding airflows.

**Effect of Surrounding Airflows.** Bischofberger et al. (23) showed the experimental evidence of a vortex ring created by a spreading drop. Here, we also visualized the vortex ring using a smoke generator (Chauvet DJ H700), as shown in Fig. 4A. Fig. 4B shows the schematic of forming the air vortex via a spreading drop. The airflow was induced by the spreading drop, whose speed was proportional to the inertia of the impacting drop. Then, a vortex ring was shed as a boundary layer keeps propagating laterally, after the spreading drop reached its maximum radius. We experimentally measured the initial magnitude of circulation using particle tracking velocimetry (PTV) (SI Appendix, section D). Fig. 4C shows that \(\Gamma(t \approx 0)\) is proportional to \(U_d\) and \(R_d\), which indicates that circulation \(\Gamma(t \approx 0)\) was induced by the drop inertia. Also, we observed that the air vortex is dissipated over time and lasted only for a few tens of milliseconds, as shown in Fig. 4E. Therefore, airborne spores are swirled up by an air vortex and then fall back under gravity after a few tens of milliseconds, which can explain complicated trajectories in Fig. 2.

**Theoretical Model of the Magnitude of Circulation in Air.** We developed a scaling relation of \(\Gamma(t \approx 0)\) for a better understanding of the vortex motion. The shear motion of a spreading drop drives airflow and eventually creates an air vortex in the air. This spreading energy per unit length, \(E_d\), is given as \(\int_0^{t_m} \int_\Omega \phi \bar{d}\Omega dt \approx \phi \Omega t_m\), where \(\phi\), \(\Omega\), and \(t_m\) are the viscous dissipation function per unit length, the effective area of a viscous fluid, and the time duration for a spreading drop to reach its maximum radius, respectively. The dissipation function \(\phi\) scales as \(\mu_a (U_s/\delta_a)^3 \sim \mu_a U_c^2 / (R_d^2 R_a)\) using the Blasius boundary thickness of air, \(\delta_a\), where \(U_s\) and \(R_a\) are the mean speed of a liquid contact line and the Reynolds number of air, \(\rho_a U_d (2R_d)/\mu_a\). The cross-sectional area of the boundary layer scales as \(\Omega \sim R_m \delta_a\) and \(t_m \sim R_d/U_d\). Also, the maximum spreading radius of an impacting drop, \(R_m\), scales as \(R_d R_a^{1/4}\) (21), where \(R_a = \rho_a U_d (2R_d)/\mu_a\) with the dynamic viscosity of a liquid, \(\mu_a\). We finally get the spreading energy as \(E_d \sim \mu_a U_d R_a R_m^{3/4} R_d^{1/4}\) by substituting \(R_m\) and \(U_c\) as derived in SI Appendix, section E. The rotational energy of the air vortex, \(E_R\), is given as \(\rho_a \bar{r}_w^2 (t \approx 0) = \rho_a \bar{r}_w^2 (\bar{r}_w \bar{w})^2\) (24), where \(\bar{r}_w\) and \(\bar{w}\) are the mean radius and vorticity of the air vortex, respectively. By balancing spreading and rotational energies, we get the circulation as

\[
\Gamma(t \approx 0) \sim \rho_a^{-1/4} R_a^{3/8} U_d R_d.
\]

Our scaling relation shows the dependence of \(R_a\) on the initial circulation, \(\Gamma(t \approx 0)\), Fig. 4D shows good agreement of our scaling argument with experimental data. Furthermore, this association explains experimental observations previously reported in ref. 23.

**Simulation of Spore Trajectories.** We also simulated the trajectories of spores by solving the equation of motion considering inertial force, air drag force, and gravitational force:

\[
\rho_s \left( \frac{4}{3} \pi r_s^3 \right) \frac{d\bar{V}_s}{dt} = 6\pi \mu_a r_s (\bar{V}_s - \bar{U}_w) - \rho_s \left( \frac{4}{3} \pi r_s^3 \right) \bar{g}.
\]

Here, \(\bar{g}\) is the gravitational acceleration and we neglect the Basset force because of low \(\rho_s\) and \(\mu_a\). To estimate the airflow, \(\bar{U}_w\), we used potential theory and the method of images along with our estimated circulation. Thus, the corresponding

<table>
<thead>
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<th>Type</th>
<th>Particle</th>
<th>(\rho_s) kg/m³</th>
<th>(r_s) μm</th>
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<tbody>
<tr>
<td>I</td>
<td>Hollow glass sphere</td>
<td>1,100</td>
<td>1 to 10</td>
</tr>
<tr>
<td>II</td>
<td>Soda lime sphere</td>
<td>2,500</td>
<td>19.0 to 22.5</td>
</tr>
<tr>
<td>III</td>
<td>Soda lime sphere</td>
<td>2,500</td>
<td>45.0 to 53.0</td>
</tr>
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complex potential $W$ can be written as $W = -i\pi/(2\pi) \log \left([z - d]/(z + d)\right)$ (25), where $z$ is a complex variable of a position and $d$ is a distance from the wall. Here, the vortex circulation, $\Gamma$, dissipates over time after the vortex is shed from a spreading drop. The wall distance, $d$, increases over time as $d \sim \sqrt{\nu a t}$, where $\nu a$ is the kinematic viscosity of air while the vortex dissipates and diffuses away (SI Appendix, section F).

By differentiating $W$ with respect to $z$, we can determine $\bar{U}_w$. Finally, we can numerically solve Eq. 3 with given ejection velocity and angle of spores. Fig. 5 shows that computed trajectories from Eq. 3 follow experimental trajectories quite well.

The vortex generated from a spreading raindrop can blow spores farther away and also cause the swirling motion of the spores, resulting in transport over higher and longer distances. Fig. 5 also shows that the maximum height of liberated spores increases in the presence of the air vortex (red and orange circles and lines), and thus most liberated spores can escape beyond the boundary layer of a leaf ($d_1 \approx 1.72 \sqrt{\nu a L}/U \approx 0.7$–2.1 mm; the length of leaf, $L \approx 10$ cm; and a typical wind speed, $U = 1–10$ m/s) (26). Thus, spores liberated by an air vortex may cross the laminar boundary layer and be exposed to the wind, with the potential to travel over long distances in the atmosphere.

**Discussion**

Various spore-dispersal mechanisms have been reported previously. These include dispersal resulting from changes in temperature and relative humidity (27), in response to mechanical stimuli (28), from insect movement (29), along with dew and splash droplets (30), and due to cavitation bubbles (31). In this paper, we report a dry-dispersal mechanism of a plant pathogen via raindrop impact. When a raindrop hits a rust-infected plant leaf, the drop initially pushes the spores to be liberated from the leaf surface. Then, the spores follow swirling trajectories aided by the air vortex ring formed by the raindrop. As a result, the spores travel longer distances and reach greater heights, thereby reaching outside the boundary layer to be swept away by wind. Therefore, a number of pathogenic spores can escape

**Fig. 3.** Visualization of the dispersal motion of glass beads (surrogates for spores) when $[R_d, U_d] = [1.9 \text{ mm, 3.1 m/s}]$. Ballistic motion of the beads was observed only at the beginning stage of dispersal, but later spores exhibited a swirling motion (Movies S3 and S4).

**Fig. 4.** (A) Smoke visualization of the air vortex with $[R_d, U_d] = [1.4 \text{ mm, 3.3 m/s}]$. The white dashed curve represents the final shape of a drop and the solid curve indicates the intermediate shape of a spreading drop at the maximum spreading radius. (B) Schematic of the formation of an air vortex, driving spores to swirl around. (C) Magnitude of a circulation $\Gamma$ versus an impact velocity $U_d$ depending on a drop radius $R_d$. (D) $\Gamma$ is replotted according to our scaling law (Eq. 2). (E) Contour map of a vorticity obtained from particle image velocimetry (PIV) measurements using glass beads with $[R_d, U_d] = [1.9 \text{ mm, 3.1 m/s}]$, showing the dissipation of the air vortex with elapsed times (see SI Appendix, section D).
from the region of an infected plant and possibly land on other susceptible plants.

A few limitations exist to simulating the trajectory of rust spores: splash droplets and the leaf's flexibility. First, the splash droplets affect the shape and magnitude of the air vortex, which change the trajectory of spores only near the end (SI Appendix, section F). Second, a flexible leaf will vibrate due to the drop impact, which increases the number of spores. However, the response of the leaf is much longer than spreading time of the drop, which minimizes its effect on liberated spores. Nevertheless, we consistently observed that both rust spores and surrogate glass beads show the swirling trajectories (Fig. 2) and are able to escape from the leaf boundary as $d_{\text{min}}$ and maximum height are larger than the width of leaf and $d_L$, respectively. Our finding could be extended to crops impacted by rust diseases, such as coffee and corn, thereby making $d_{\text{min}}$ much longer than that without the air vortex (SI Appendix, section G).

Outside the boundary layer, air flows are turbulent. So, the Stokes number of spores in that region is much less than 1; $S_{Lk} = t_v / t_k$ where the particle relaxation timescale is $t_k = (2/9) \rho_d r_d^2 / \mu_d$ $\sim 10^{-3}$ s and the Kolmogorov timescale is $t_k = (\nu_k / \epsilon)^{1/2} = 2^{-1/2} t_k$ $\sim 10^{-1}$ s. The turbulent kinetic dissipation rate $\epsilon$ is expected to be $10^{-3} - 10^{-2} m^2 s^{-3}$ at the half-height of a typical canopy (32). Thus, many airborne spores with $S_{Lk} < 1$ can be immediately dispersed by the turbulent eddies, similar to ocean spray droplets (33). Furthermore, plant pathogens can travel over kilometer distances via wind currents (34). Therefore, the dry dispersal of plant pathogens via a vortex ring may be a critical component of the spread of a plant pathogen in the atmosphere over long distances in the atmosphere.

Materials and Methods

Preparation of Infected Wheat Leaves. Lines of wheat susceptible to wheat leaf rust infection were cultivated under controlled growth conditions (135 $\mu$mol m$^{-2}$ s$^{-1}$ light for 12 h/d, 25°C) for 6 wk following seed germination. Healthy leaves were inoculated using a suspension of $P. triticina$ urediniospores in water with 0.001% of detergent as an adjuvant. Inoculated plants were then incubated in an isolating–isohumidity chamber at 15°C and 99% relative humidity for 24 h then removed back to previous controlled growth conditions. After 7 to 10 d, pustules of $P. triticina$ spores had sufficiently broken through the epidermis of wheat leaf, as illustrated in Fig. 1A.

Properties of Rust Pustules and Spores. The shape of the pustules is assumed to be an elliptic cylinder, where the length of major and minor axes were measured to be 1.02 ± 0.17 mm and 0.30 ± 0.04 mm, respectively, and the thickness is measured to be 0.21 ± 0.01 mm. The radius of $P. triticina$ spores, $r_s$ was measured to be 9.7 ± 0.6 $\mu$m using a scanning electron microscope. Measuring the terminal velocity of individually falling spores, the density of $P. triticina$ spores, $\rho_s$ was calculated to be 1.294 ± 0.84 kg/m$^3$ (SI Appendix, section H), whose value is close to the density of other microspores, such as fungal spores (35) and pollen (36).

Drop Impact Conditions. A syringe needle was used to generate water drops of radius $R_d$ ranging from 1.4 to 1.9 mm, within the range of the typical size of raindrops (37). Drops were released from a certain height, falling under gravity, and then striking either an anchored leaf (cantelever) or a leaf fully supported by a rigid bottom. By varying the drop-releasing height from the substrate, the impact velocity of a water drop, $U_{wp}$, is changed to be between 1.5 and 4.6 m/s. Thus, the corresponding Weber number $We_d = \mu_d U_{wp} (2R_d / \gamma) = \rho_d R_d (U_{wp} / \gamma)$ and Reynolds number $Re_d = \rho_d U_{wp} (2R_d / \mu_d) = \rho_d (U_{wp} / \mu_d)$ of an impacting drop range from 88 to 1,117 and from 4,200 to 17,480, respectively. Here we specially focused on drop impact on a rigid infected leaf to elucidate the fundamental mechanisms of vortex-induced spore spreading. In the timescale comparison, the spreading time of water drops, $t_w \sim R_d / U_{wp}$, is on the order of 1 ms, which is much less than the vibration time of leaves (~50 ms (38)). Thus, the vibration of the leaf by drop impact is assumed to be insignificant to the initial liberation process of disease spreading. Also, we focused on the first impact of a simulated raindrop because the number of the removed spores was observed to rapidly decrease with the number of successive drop impacts (5).

Properties of Artificial Leaf and Spores. Artificial leaf and spores were used to quantify the dynamics of disease spreading. For the artificial leaf, we used a polycarbonate film. For the artificial spores, we used micron-sized glass beads to simulate rust spores in the field. By varying the drop-releasing height from the substrate, the thickness ranged between 100 and 250 $\mu$m, which is a value similar to the thickness of pustules. Here, the Stokes numbers of artificial spores I, II, and III were measured to be 0.001 to 0.13, 1.1 to 1.5, and 6.1 to 8.5, respectively.

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