Effects of Surface Topography and Rain Intensity on Splash Dispersal of Colletotrichum acutatum

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ABSTRACT


Effects of ground cover, plant canopy density, and rain intensity on the splash dispersal of Colletotrichum acutatum were studied using a rain simulator. In one experiment, three ground covers (soil, straw, and plastic) and two rain intensities (15 and 30 mm/hr) were evaluated by collecting splash droplets with conidia in sheltered gravity samplers consisting of petri plates with a selective medium for C. acutatum. Ground covers were characterized by random roughness, the standard deviation of surface elevation. Infected fruits with sporulating lesions were clustered on the ground to serve as the inoculum source. Sampling plates were positioned 20, 40, 60, 80, 100, and 120 cm from the source and were exposed to rain for 1 min periods (every 5 min) for a total duration of 46 min. Ground cover had a major effect on splash dispersal, as measured by colonies growing in selective medium. The estimated total number of colonies over time and space (N) was inversely proportional to roughness; i.e., straw had the largest random roughness but the lowest N, and plastic the opposite. Differences in N among ground covers were due to differences in steepness of the dispersal gradients (i.e., straw had the steepest gradient and plastic the shallowest), not to the release rate of spores at the source. Total number of colonies increased with rain intensity, but the effect was due to the release rate, as measured by the intercept parameter of a gradient model. Rain intensity did not influence gradient steepness. The effect of plant canopy density on splash dispersal of C. acutatum was evaluated in a second experiment using a soil ground cover. Leaf area index (LAI) was inversely related to N. Cross-row dispersal of spores was reduced by 90% or more for two rows of plants with LAI ≥2.7 compared to no plant rows. Results support the hypothesis that surface topography (including ground cover and plant canopy) is a major factor controlling splash dispersal due to its effect on splash droplet trajectories and loss of inoculum.

Additional keywords: disease spread, Fragaria X ananassa, quantitative epidemiology.

Rain splash is a major means of spore dispersal for a large number of bacterial and fungal plant pathogens (17). Although some research can be traced to a century ago (11,17), large scale and systematic studies on splash dispersal mostly began in the last decade (11-13). A great deal of effort has been placed on studying characteristics of natural rainfall, correlating rain occurrence and intensity with disease increase, describing the mechanisms of spore dispersal in splash droplets, and quantifying the effects of inoculum source, plant canopy, and weather conditions on dispersal or disease spread (11-13). Field studies have shown that dispersal or spore deposition gradients from point sources are very steep in still air (1,8,12,17,24,29,30). This made the use of laboratory facilities, such as rain towers and wind tunnels, a very efficient way for studying rain splash dispersal under a range of manipulated conditions (10,14,19,27,33).

Previously, we have demonstrated that Colletotrichum acutatum Simmonds, causal agent of anthracnose of strawberry (Fragaria X ananassa Duchesne), is effectively dispersed by rain splash (33). This disease is becoming one of the most economically important constraints to strawberry production in the southeastern United States and California (4,6). With a rain-generating (simulation) system, we have quantitatively examined the effects of ground cover (three types), rain intensity (two levels), rain duration (four times), distance from the inoculum (two levels), source strength, and horizontal airflow on rain splash dispersal from infected strawberry fruits (33). Rain intensity (mm/hr) was shown to have a minor or inconsistent effect on dispersal, but ground cover had a strong influence, as measured by the percentage of fruits that became infected by C. acutatum. With plastic mulch, all fruit became infected over 15-60 min of rain at 30 and 60 cm from the inoculum source; with soil ground cover, disease incidence generally increased over time; but with straw cover, disease incidence either declined or remained constant over time. Use of infected fruit as the measure of dispersal limited the number of distances and rain application times that could be tested because of constraints on fruit production in the greenhouse and maintenance of inoculated fruit in the laboratory. Additionally, the experimental technique did not determine the relative importance of deposition versus wash-off of spores from the fruit.

Movement of solid particles and/or liquid droplets on or across a ground surface is largely controlled by the surface topography or roughness (21). Quantification of the effect of surface topography on dispersal processes may provide additional insight into the mechanisms of splash dispersal. Therefore, as a second stage of our research on the dispersal of C. acutatum, this study was conducted to better understand the effect of surface topography (ground cover and plant canopy) and rain intensity on splash dispersal, especially the dynamic nature of spore deposition over time and distance from the source. Ground cover was characterized here by random roughness (21), and dispersal was assessed by the number of colonies resulting from conidia collected in gravity plates.

MATERIALS AND METHODS

Rain generation. The rain simulator previously described (27,33) was used to generate rain of two intensities. The simulator used wide angle spray nozzles (Spraying Systems, Inc, Wheaton, IL) attached to a 4-m-high horizontal boom to produce uniform spray patterns for a 1.2-x-1.2-m target area. Two spray nozzles, 27W and 35W, were used, resulting in cumulative distributions of raindrop volume and kinetic energy in agreement with those for natural rainfall intensities of 15 and 30 mm/hr, respectively (27).

Inoculum source. The isolate of C. acutatum was obtained from a naturally infected strawberry fruit collected near Mt. Vernon, OH, in 1985. Preparation of inoculum source fruits followed the
procedure described in our previous studies (31,33). In brief, immature (whitish stage) fruits were detached, rinsed with deionized water, sprayed with a conidial suspension (5 x 10^6 conidia per milliliter) until runoff, and incubated 6–8 days, by which time lesion development and sporulation occurred. The mean number of conidia per fruit was estimated to be 4 x 10^6.

**Ground cover and rain intensity**. Effects of ground cover and rain intensity on splash dispersal of *C. acutatum* were studied by trapping splashing droplets with gravity samplers (1) that consisted of petri plates containing a selective medium. The medium was a modified dextrose peptone yeast extract agar containing benomyl (5 mg a.i./L) (4). As in the previous study (33), three ground covers (sterilized soil mix [soil-peat-sand, 2:2:1, by volume], sterilized [autoclaved] straw, and new plastic mulch) and two rain intensities (15 and 30 mm/hr) were examined. Cover materials were supported by a square wood frame (1.5 x 1.5 m). Depth of soil mix was 6–8 cm, and that of straw was 8–10 cm. For each run of the rain simulator, five inoculated fruits with sporulating lesions were clustered on the ground 40 cm from a randomly chosen corner of the frame, to serve as inoculum source. Plates were positioned (one per distance) 20, 40, 60, 80, 100, and 120 cm from the source (Fig. 1). Each plate was placed in a metal shield (rain shield) to prevent direct rain impact. The shelter roof (12 x 12 cm) was supported 16 cm above the plate by a thin vertical rod, thus allowing mostly unobstructed trajectories of splash droplets. Shelters were staggered so that the plates were not obstructed by other shelters. To test for contamination of the ground cover, two plates were placed under shelters at 20 and 120 cm distances and exposed to 1 min of rain before each run. Then, infected fruits were introduced. Starting at 0 min into a simulated rain, plates were exposed every 5 min for 1-min periods (e.g., 0–1 min, 5–6 min, etc.) for a total duration of 46 min. New plates were used for each time period. Exposed plates were covered with lids, incubated at 25 C for 3 days in the dark, and then transferred to a refrigerator (6 C) for 3 days to arrest growth. Colonies of *Colletotrichum* spp. produce a characteristic shape and color under these conditions. Colonies per plate were counted with a magnifying plate counter (New Brunswick Scientific Co., New Brunswick, NJ). Periodically, representative colonies were transferred to potato dextrose agar to obtain greater sporulation for examination of morphological characteristics and positive identification. In general, >95% of the plates had only colonies of *C. acutatum*; >99% of all colonies were characteristic of *C. acutatum*.

To determine the percentage germination of conidia and colony formation, spore suspensions (~0.25 ml) were added to the plates with the selective medium. Concentrations of 75–3,000 spores per milliliter were evaluated, using five plates per concentration. Over this range of spore concentrations, which produced no colonies typically found in the rain simulation experiments, 66% of conidia produced colonies.

Random roughness, defined as the standard deviation (σ) of elevation from baseline (3,20), was chosen as a basic index to quantify surface characteristics of the ground covers. Random roughness for the three ground covers was determined using a portable laser scanner (20) developed at the National Soil Erosion Research Laboratory, Purdue University, West Lafayette, IN. The scanner consisted of an optical transducer for measuring elevation, a motor-driven transversing frame for sequentially positioning the transducer, and a personal computer for system controlling and data recording. An area of 1 x 1-m of each ground cover was scanned. The position resolution along the scanning lines was 1 mm for all three covers, and the advance increment in the transverse direction was 3 mm for straw and 5 mm for plastic and soil.

**Plant barrier**. The effect of the plant canopy on dispersal was assessed using the same rain-generating system. The frame was altered to contain two troughs, 76 cm apart, corresponding to the typical field condition for row spacing. Greenhouse or field-grown plants were placed tightly in the troughs, which were then filled with soil to cover roots, and then the entire frame was covered with soil (6–8 cm). Field and greenhouse plants were used to form two different densities. The leaf area index (LAI) for each plant “row” was determined following Yang et al. (32) with an image analyzing software (5). Source fruits were placed on the outside of one row, and plates in rain shelters were used to collect droplets at 40 and 80 cm (both separated from the source by one plant row) and 120 cm (separated by two rows), all within the uniform zone of rain. These distances were perpendicular to the row direction. Other methods were the same as those described for the tests without plants.

**Data analysis**. Number of colonies in each petri plate (n, cm^-2 min^-1) was the measure of deposition flux density of conidia, which is proportional to the conidia being splashed per unit area per unit time. All treatments were repeated at least three times, excluding those discarded because of contamination found in the control plates.

Assuming equal dispersal in all directions because of the lack of air flow, the interpolated total number of colonies during each 46-min period over a circular area with radius of 120 cm (the maximum distance measured) (N) was estimated by first converting the measurements to colonies per minute per centimeter of a circular ring, and then integrating over both the distance and time domains. The integration was approximated by Simpson’s 3/8 rule with the aid of the SURFER 3-D graphical software (4V) (15). The variable N is proportional to a temporal integration of total conidia over the circular area. Both N and n, however, are scaled measures of conidia because all spores intercepted by plates did not cause colonies. Data of N were analyzed with analysis of variance (ANOVA), using MINITAB (25, procedure GLM) to determine the effects of ground cover and rain intensity. Ground cover then was partitioned into linear and quadratic effects, using orthogonal polynomials in which the coefficients were based on the levels of random roughness. Total number of colonies was also calculated for the plant canopy experiment. The effect of plant density and rain intensity on N was evaluated with ANOVA (25).

In the first experiment, regression analysis also was used to assess the effect of distance from the source, x, on n at each time for each replicate, cover, and rain intensity. A simple model was initially chosen as:

\[ \ln(n) = a + b \times x^2, \]

where a and b were parameters estimated from data, related to source strength or spore release rate (a) and steepness of the dispersal gradient, i.e., change in n over x (b) (12). Release rate refers not to total spores on the infected fruit but to the number that are splashed from the source by water drops. The equation is consistent with an analytical solution of a diffusion model with impulse initial and boundary conditions in cylindrical coordinates (2). Calculated values of parameters a and b for selected times were analyzed with ANOVA using MINITAB (25).

**RESULTS**

**Surface and canopy quantification**. The three ground covers possessed distinct surface characteristics, as expressed by random
Fig. 2. Spatial and temporal patterns of colonies (n, cm⁻² min⁻¹) of Colletotrichum acutatum due to rain splashing with intensities of 15 and 30 mm/hr for plastic, soil, and straw ground covers. Points represent the means of four replications. The square-root scale was chosen to better illustrate the data beyond 60 cm from the source, where few colonies were observed.
roughness. Values of random roughness ($\sigma$) were 2.7, 6.5, and 13.4 mm for plastic, soil, and straw, respectively. Greenhouse and field plant cover was quantified by LAI, with means of 2.72 and 4.90 over the canopy crown and 1.00 and 2.32 over the whole sample area, respectively.

**General dispersal characteristics.** General patterns of colonies (n, cm$^{-2}$ min$^{-1}$) for each ground cover and rain intensity, with respect to both time and distance, are illustrated in Fig. 2. The vertical scale was the square-root transformation of $n$ to better illustrate the data beyond 60 cm from the source, where $n$ was equal or close to zero in many cases. The deposition flux density of conidia (as measured by $n$) rapidly decreased with distance, but it increased with time at the beginning of rain application and then decreased. Unlike soil and plastic, straw had very few colonies beyond 40 cm. The maxima of measurements with time were reached earlier for rains of 30 mm/hr than of 15 mm/hr. The rate of change of $n$ over time was higher with higher rain intensity also, especially near the source. Overall magnitude of $n$ was strongly related to rain intensity, with many more colonies at the high compared to the low-intensity rain.

**Effects of ground cover and rain intensity.** Effects of rain intensity and ground cover on the total number of colonies ($N$) were both significant (P$\leq$0.01) and P$\leq$0.05, respectively, whereas the interaction of intensity and cover was not (P$>0.1$). Mean values of $N$ for each ground cover and rain intensity are shown in Fig. 3. Lack of a significant interaction indicated that differences in ground cover did not depend on rain intensity, and vice versa. The relationship between mean $N$ and surface roughness is shown in the nested graph of Fig. 3. Orthogonal polynomials indicated that a linear relationship existed between surface roughness and $N$ (P$\leq$0.01); the quadratic effect was not significant (P $>0.2$).

Equation 1 provided a good fit to the data ($n$ vs $x$) for all time tested. The coefficient of determination, $R^2$, averaged 0.81 (standard deviation = 0.17) for a total of 144 fittings. Figure 4 shows a comparison of the predictions with observations for the three ground covers with 30-mm/hr rain intensity at $t$ = 10 min. The scale was normalized at $x$ = 20 cm (i.e., $n=1$ at $x=20$) to accentuate the dispersal. Fig. 3. Ground gradient of $n$ at any given time was the steepest for straw and shallowest for plastic. Regression parameters varied among times but were fairly consistent among replicates. The dependence of parameter $b$ in equation 1 on roughness is also illustrated in Fig. 4 (nested graph, $t$ = 10 min). The effect of ground cover on the gradient steepness, indicated by parameter $b$, was significant (P $\leq$ 0.01), whereas the release rate at $x = 0$ ($a$, source strength) was not (P $>0.1$), as tested with ANOVA. In contrast, rain intensity had a significant effect on parameter $a$ (P $\leq$ 0.05) but not on $b$ (P $>0.1$). The interaction of cover and intensity was not significant (P $>0.1$) for both estimated parameters.

**Effect of plant canopy.** Calculated $N$ during the 46-min period over the whole sample area was significantly affected (P $\leq$ 0.05) by density of plant canopy (Table 1). With plants present, the effect of rain intensity and the interaction of rain intensity and ground cover were insignificant (P $>0.1$). Even the relatively low-density canopy resulted in a drastic reduction in $N$, compared to results with no canopy.

**Discussion.**

In this study we have confirmed our previous results (33) showing that C. acutatum is dispersed by rain splash and also demonstrated the strong effects of ground cover and plant barrier on the dispersal process. In prior studies we used infected fruits as the measure of dispersal (23,27,33) to determine the significant effect of rain splash on the spore dispersal of C. acutatum and Phytophthora cactorum (Lebert & Cohn) Schr., the cause of strawberry leaf blight. Although percentage of infected fruits was a useful variable in those studies, it did not permit the testing of many rain durations and distances on spore dispersal. Also, infected fruits associated with a given duration of rain resulted from the cumulative deposition of spore-carrying droplets onto the fruits and the wash-off of the spores (i.e., from $t = 0$ to fruit removal time). Collecting droplets in gravity plates with selective medium and counting fungal colonies gave a more direct indication of deposition of spore-carrying droplets and a better spatial and temporal resolution to the collected data. Data collected could also be used in developing and validating dispersal models based on physical principles.

Previously, ground cover was reported (23,27,33) to be a very important factor affecting spore dispersal. Total number of colonies during the 46-min period over the sample area was shown here to be significantly different among plastic, soil, and straw covers. With plastic, the mean value of $N$ was about 1.5 times that of soil and twice that of straw (Fig. 3). Mean total colonies were inversely proportional to, and highly correlated with, random roughness of the covers, leading to the hypothesis that roughness is a key parameter controlling rain splash. This is consistent with studies showing that roughness controls many transport processes on and across the ground surface boundary, such as water infiltration, runoff, and soil detachment (21). The hypothesis is also supported by our recent study with P. cactorum (23), which showed that plastic had the highest fruit disease incidence, straw the lowest, and soil and sand intermediate. Soil and sand used

![Fig. 3. Effect of ground cover and rain intensity on the mean total number of colonies estimated for a 46-min period over a circular area with a radius of 120 cm ($N$). The vertical line indicates the value of standard error of difference (SED) for the means across intensities. The nested graph shows the relation between surface random roughness and mean $N$. Vertical segments indicate the standard deviations of data.](image1)

![Fig. 4. Effect of ground cover on gradient of colonies ($n$, cm$^{-2}$ min$^{-1}$) over distance at 10 min into a 30-mm/hr rain. The vertical scale was normalized at 20 cm from the source so that the gradients can be compared. Data points are means of $n$, and smooth curves are the corresponding predicted values. The nested graph shows the relationship between the regression parameter $b$ in equation 1 and surface random roughness, where vertical segments indicate standard deviations of data.](image2)
TABLE 1. Effect of plant canopy on the dispersal of *Colletotrichum acutatum* with soil ground cover

<table>
<thead>
<tr>
<th>Rain intensity (mm/hr)</th>
<th>Average leaf area index (LAI)</th>
<th>Flux density ((v_i), colonies cm(^{-2}) min(^{-1}))</th>
<th>Total colonies ((N))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 cm</td>
<td>80 cm</td>
<td>120 cm</td>
</tr>
<tr>
<td>15</td>
<td>0.00(^a)</td>
<td>0.08(^b)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>2.72</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>4.90</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.00</td>
<td>0.38</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>2.72</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>4.90</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\(^a\) Data for LAI = 0 was from the first experiment and not included in the analysis for plant canopy.

\(^b\) Means of four replicates.

\(^c\) Integrated means over 46-min period and the whole sample area (radius = 120 cm), using Simpson's 3/8 rule with the aid of the SURFER 3-D graphical software.

in that study had the same random roughness (X. Yang, unpublished).

With the same rain intensity, the difference in colonies among cover types was mainly caused by the difference in the steepness of the dispersal gradients, as shown in Fig. 4. The gradient was the steepest for straw and shallowest for plastic, thus positively proportional to surface random roughness. Since most splash-dispersed spores are below the 20 cm (7.1-13.28), conidial dispersal and hence the gradient was formed only by pneumophores. Specifically, the gradient depends on the rates of horizontal dispersal and loss of spores through the ground cover. According to diffusion theory, the parameter \(b\) (equation 1) only includes the effect of the horizontal dispersal, not the loss of spores, indicating that a negative relation exists between droplet trajectories and surface random roughness (X. Yang, unpublished). The insignificant effect of the three ground covers on \(a\) (equation 1) indicated that, as expected, cover did not influence the release rate of spores from the source or source strength (Fig. 2).

The two most commonly used empirical models of the number of spores deposited with distance from a source are the power law (16) and the exponential (22) model, with the latter fitting splash dispersal data better in most cases (8,9,12). From a viewpoint of mass conservation, the exponential model with distance being the independent variable is an outcome of the analysis of a one-dimensional transfer problem (with a line source). Using distance as one of the ordinates in a cylindrical coordinate system for a point source, the solution naturally turns out to be a function of the distance squared (2), which coincides with the statistical Gaussian models employed in some studies on dispersal of airborne pathogens (1,18,26). The high \(R^2\) values of our regression analyses indicated that equation 1 was indeed a good starting model for single-source splash dispersal problems.

A gradient over distance in number of spores being splashed (as measured by colonies) existed for all ground covers and rain intensities, including plastic. This would have been so even if no spore losses occurred through the ground, because deposition area increased as distance increased from the source. In contrast, when infected fruits were used as the dispersal measure, disease incidence quickly reached 100% with plastic cover at all distances tested (33). This was because of the cumulative properties of that system, i.e., fruits were exposed for a relatively long period of time (15-60 min), which resulted in enough spores being deposited to cause 100% infection. The colony per plate system thus provided more information on the dispersal of *C. acutatum*.

For a given ground cover, change of rain intensity caused the release rate to vary with no change in gradient steepness. This can be seen from the fact that rain intensity significantly affected \(a\) (equation 1) and \(N\) but not \(b\). Generally, the heavier the rain, the more spores were released from the source and dispersed. The mean value of \(N\) for rain of 30 mm/hr was nearly double that for rain of 15 mm/hr. Since only two rain intensities were tested, a functional relationship between \(N\) (or \(a\) and intensity cannot be concluded. In our previous study with *C. acutatum* (using infected fruit as the dispersal measure), rain intensity showed a minor or inconsistent effect on disease incidence (33). The lack of a linear relation between dispersed spores and intensity was also observed by other researchers (8,30). We believe that several processes are responsible for our previous results. First, the increase in kinetic energy as rain intensity increases results in more spore dispersal from the source fruit (higher release rate) and redistribution by splashing. Second, the increased volume of water with increased rain intensity likely results in greater loss of spores through the ground cover and wash-off of spores from the fruit surface. Depletion of spores from the source is a third process involved, as found by Fitt et al. (8). The difference between our results for infected fruits and for colonics suggests that wash-off highly influenced fruit infection. This is supported by our results with *P. cactorum*, in which a 30-mm/hr rain had lower disease incidence than a 15-mm/hr rain (23).

Plant canopy is another aspect of surface topography that influences splash dispersal (12,29). Our study indicated that dispersal of *C. acutatum* in the cross-row direction was strongly affected by the presence and density of plants. With two rows of plants of LAI 2.7 and 4.9, the total number of colonies decreased by 95 and 99.5%, respectively. Plant canopy reduced dispersal so much that any effect of rain intensity could not be detected. This may also help to explain the minor or inconsistent effect of rain intensity in our previous studies (23,27,33), in which circles of plants were used. For a strawberry row canopy, spread of anthracnose thus may be mainly down the row or through gaps between plants.

In conclusion, ground cover and plant density have a major impact on the splash dispersal of *C. acutatum*, whereas rain intensity strongly influences the release rate of spores from the source. Because of the presumed importance of resplashing or redistribution of spore-carrying droplets during rain episodes, results should be applicable to a large number of splash-dispersed pathogens. Knowledge of the effects of surface topography should be of value in disease control when ground cover can be manipulated, as is the case with strawberry.

LITERATURE CITED