Effects of Rain Intensity on Splash Dispersal of *Colletotrichum acutatum*

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ABSTRACT

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A rain simulator was used to determine the effects of intensity (millimeters per hour or milliliters per square centimeter per hour) on splash dispersal of *Colletotrichum acutatum* conidia from a point source consisting of infected strawberry fruit. Seven wide-angle spray nozzles reproduced the cumulative volume distribution of natural rains with intensities of 2 to 60 mm h^{-1}. Dispersal over a uniform soil surface was assessed in separate studies by either (i) collecting splash droplets with conidia in sheltered gravity samplers, consisting of petri plates with a selective medium for *Colletotrichum* spp. and counting colonies formed or (ii) exposing healthy immature strawberry fruit to incident and splashed water and determining the proportion of infected fruit after a 7-day incubation period. Colony density declined with distance from the source, increased over time to a maximum, and then declined. Estimated total colonies over time and space increased in a linear fashion with rain intensity. Proportion of infected fruit increased with time and decreased with distance. However, the proportion generally increased with intensity to a maximum, corresponding to about 15 to 30 mm h^{-1}, and then declined. Transport of splash droplets across the soil surface was assessed by the intensity of water splashed into gravity samplers; from 0.6 to 6.3% of incident rain was splashed, the percent increasing with intensity and time during generated rain episodes. In other studies, the proportion of conidia removed from source fruit and the rate of removal both increased with rain intensity. Wash-off of conidia from healthy fruit also was estimated by placing a fixed number of conidia on fruit, exposing the fruit to rain, and determining the proportion of fruit infected. The multiple-infection transformation (*MIT*) of disease incidence, a measure of the number of infections per fruit, declined with intensity, and the rate of change in *MIT* increased with intensity, although this rate of wash-off was less than the rate of spore removal from the source fruit. By considering essential components of splash dispersal—removal of spores from the source, transport of spores in splash droplets, and wash-off of spores from potential infection sites—it was possible to relate dispersal to the physical properties of rain and explain the previously noted variable or surprising effects of rain intensity on pathogen dissemination.

Additional keywords: anthracnose, disease spread, *Fragaria* × *ananassa*, models, quantitative epidemiology.

Spores of many fungal plant pathogens are disseminated to new infection sites by rain splash (19). Based on several studies published over the last decade, a detailed understanding has emerged of the events that occur when a water drop impacts on a lesion with spores (reviewed by Fitt et al. [21] and Madden [30]). Splash droplets are formed, some of which contain spores, that move in ballistic trajectories very short distances from the impact point (29,52). Models and descriptions of spore entrainment in droplets and droplet trajectories have been published, based partly on the physical mechanisms involved (8,18,21,27,48,53). Because of the short flight distances of splash droplets, generally less than 10 cm (30), successful deposition of spores at an infection site involves continual resplashing of droplets with spores during rain episodes. Thus, properties of ground cover (e.g., surface topography) and plant canopy (e.g., density) have a large effect on dispersal (5,17, 30,32,33,49,54). Although a detailed view of the population process of splash dispersal over a given area is emerging (19,21,30), there is still no integrated theory of spore transport similar to that developed for wind dissemination (2,3,35).

Critical information still is needed before a satisfactory theory of splash dispersal can be achieved. In particular, the role of rain intensity (millimeters per hour) remains elusive (16,32,42), even though intensity is perhaps the fundamental characteristic of rainfall ([37]; appendix). Rain intensity is an indication not only of the volume of water incident on a unit area per unit time, but also the number and size distribution (spectrum) of drops, as well as the kinetic energy of drop impactions (37). Intensity may have a positive, negative, or no significant effect on dispersal (5,6,10,36,39, 41–43). It is known that mean raindrop size increases and the size spectrum shifts to larger diameters as intensity increases (46), and correspondingly, more splash droplets are formed, and more spores are initially dispersed by the impact of large compared to small water drops (20,21,52). Attempts to relate intensity of natural rains to dispersal (or disease increase resulting from dispersal) have been hindered by short-term variation in intensity, which cannot easily be measured, inability to control initiation, and end of rain episodes, as well as variation in crop and surface properties over time and space in the field (30). However, because of the short-range nature of splash, it is possible to study dispersal with a rain simulator (21,30). Although many studies have been based on the use of a rain simulator (5,16,20,32,55,56), they cannot be used to characterize the functional relationship between intensity and dispersal because they have not considered a wide range of intensities, have considered only high intensities, or have not represented the natural raindrop size distributions.

Colletotrichum acutatum J.H. Simmonds is a splash-dispersed fungal pathogen (55) that causes anthracnose fruit rot of strawberry (24,26). Conidia are produced in acervuli and are surrounded by a mucilage matrix. We previously have characterized the spore entrainment in splash droplets (53), determined the physical relations between properties of impacting single water drops and re-

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sulting trajectories of splash droplets (52), demonstrated the pronounced effects of surface topography and plant canopy on droplet transport and dispersal (5,50,55), showed that dispersal can be approximated by a diffusion-type process (51), and found that very short-duration rains (<2 min) are sufficient for dispersal (34). To date, all controlled studies have been based on simulated rain intensities of 15 and 30 mm h^{-1}. Although dispersed spores increased with increasing intensity (31,54), there was no overall relationship between intensity and infected fruit resulting from splash dispersal (55).

The objective of this study was to characterize, using a rain simulator, the relationship between rain intensity and splash dispersal of *C. acutatum*. A wide range of intensities was chosen, and dispersal was assessed, in separate experiments, by both deposited spores in gravity samplers and disease incidence of exposed strawberry fruit. Spore removal from source fruit, water splashing over the experimental area, and wash-off of spores from the potential infection sites were determined to evaluate the components of spore dispersal at selected intensities. Results are discussed in terms of the physical properties of natural rains and the integrated effects of individual raindrop impactions on splash dispersal.

MATERIALS AND METHODS

Rain-generation system. The rain simulator developed by Reynolds et al. (38), similar to those used in soil erosion experiments (45), was used in all tests. An upward-pointing, rotating wide-angle spray nozzle (Spraying Systems, Wheaton, IL) was used to produce a spray pattern of approximately uniform rain over a 1.2 × 1.2-m area. Height of drop fall was 4 m with a chosen water pressure of 69 kPa at the nozzle orifice. Water pressure was kept constant with a precision flow valve. Drops up to 4 mm in diameter theoretically reach at least 90% of terminal velocity with this fall height (40), with the percent increasing as drop size decreases.

Seven different spray nozzles were used (Table 1). The volume of water per hour of incident rain on a unit area (i.e., intensity) was determined for each nozzle as described by Reynolds et al. (38). Briefly, 12 9-cm-diameter funnels were placed in flasks, approximately evenly, over the 1.2 × 1.2-m experimental area. Rain was generated for each nozzle for 20 to 40 min, depending on the nozzle, and the volume of water collected through each funnel was determined. Results were converted to milliliters per square centimeter per hour, and the mean of rain intensity (R) for the rain test was calculated (Table 2 contains a summary of the symbols used). The evaluation was done two to three times for each nozzle. Volume of water splashed into samplers was determined with spore gravity samplers (described below).

The cumulative volume distribution (proportion of total water volume accounted for by drops less than or equal to a certain diameter) for each nozzle, obtained from the manufacturer, was compared to the theoretical cumulative volume distribution for natural rains (37), following Reynolds et al. (38). The theoretical distributions were determined for intensities of 1 to 100 mm h^{-1} based on a modified Marshall-Palmer equation ([28,46]; equation A1 in the appendix). Agreement between a theoretical and actual distribution was determined with the Kolmogorov-Smirnov test (23), which is based on the greatest difference in cumulative value of the distributions at any drop diameter.

Inoculum production. Strawberry fruit were inoculated with *C. acutatum* and incubated, following the methods of Madden et al. (34). In brief, detached immature fruit from greenhouse-grown plants were rinsed with deionized water, surface-disinfected with 70% ethanol for 60 s, rinsed with deionized water, and placed on a horizontal 6-mm-mesh screen within 5-liter containers. Approximately 700 ml of deionized water was added to the bottom of each container, so the screen was immediately above the water; the pedicel of each fruit was placed through the screen and into the water to minimize fruit desiccation. Using an atomizer, fruit were sprayed to run-off with a conidial suspension (2.5×10^4 spores per ml), lids were placed on the containers, and the containers were incubated at 26°C for 24 h. Then, the lids were removed, and the fruit were incubated another 6 days for lesion development and conidial production. Fruit were covered by lesions with this temperature and incubation regime.

Inoculum density per fruit was determined by placing five fruit (which were not to be used in a rain experiment) in 100-ml beakers (one per beaker) with 60 ml of sterilized deionized water. The fruit was soaked for 30 min and then gently brushed for 2 min with a fine-tipped brush to help remove conidia. The conidial suspension (consisting of spores released with or without the aid of the brushing) was agitated for 30 s, and the numbers were estimated with a hemacytometer. Mean inoculum density was 2×10^8 (standard error = 4×10^7).

Gravity sampler study. A wood frame (1.5 × 1.5 m) was placed immediately below the rain generator. For each rain simulation, the upper surface of the frame was covered with a sterilized soil mix (soil/peat/sand, 2:2:1, by volume) to a depth of ~3 cm. This soil was discarded after a test run. A point 40 cm from a randomly selected corner was identified, and gravity samplers were positioned (one per distance) at 20, 40, 60, 80, and 100 cm from this point. The sampler consisted of a metal base on which a petri plate (9 cm diameter) was placed (Fig. 1 in Yang et al. [54]). In these experiments, a selective medium for *Colletotrichum* (54) was used. A horizontal rain shield was attached 16 cm above the metal base by a thin vertical rod. This shield prevented direct impact of generated rain into the samplers. Placement of the samplers was staggered so the obstruction was minimized, i.e., there was an unobstructed straight line between the inoculum source and the petri plate.

TABLE 1. Characteristics of wide-angle spray nozzles[a] and simulated rains for the Ohio State rain generator

Spray nozzle	Generated rain intensity[b] (R; ml cm^{-2} h^{-1})	Natural rain match[c] (I; mm h^{-1})	Splashed-rain intensity[d] (R_S; ml cm^{-2} h^{-1})		% Incident	
			12 min	27 min	12 min	27 min
2.8W	1.24 (0.24)[e]	2	0.008 (0.002)[e]	0.012 (0.002)[e]	0.6	0.9
5.6W	1.45 (0.15)	4	0.013 (0.001)	0.016 (0.003)	0.9	1.1
10W	1.93 (0.19)	7	0.022 (0.002)	0.028 (0.002)	1.1	1.5
20W	2.31 (0.16)	11	0.040 (0.008)	0.061 (0.001)	1.7	2.6
27W	3.30 (0.35)	15	0.090 (0.007)	0.122 (0.028)	2.7	3.7
35W	4.03 (0.28)	30	0.183 (0.0)	0.253 (0.001)	4.5	6.3
50W	4.86 (0.52)	60	0.194 (0.083)	0.298 (0.016)	4.0	6.1

[a] Nozzles were from Spraying Systems (Wheaton, IL) and were operated at 69-kPa water pressure.
[b] Direct interception of generated rain in funnels (R; described in Table 2).
[c] Match determined by comparing water-drop volume distributions produced by the nozzle with calculated distributions for natural rainfall intensities (37,46).
[d] Intensity of splashed water into sheltered petri plates (R_S; described in Table 2) at two times during a generated rain episode. Percent determined by dividing $100R_S$ by R.
[e] Standard error in parentheses. Number of replications is three.

Petri plates containing the selective media, with the lids removed, were placed under the rain shield at 20 and 100 cm, and the entire area was exposed to 1 min of generated rain before each run of the simulator to test for contamination. Then, five infected fruit, covered with sporulating lesions (as prepared above), were placed in a tight cluster at the previously identified point near one of the corners of the frame. Starting at 0 min into a simulated rain, plates were exposed every 5 min for 1-min periods (0 to 1, 5 to 6 min, etc.) for a total duration of 46 min. New plates were used for each time period. After exposure, lids were immediately placed on the plates, and the plates were incubated for 3 days in the dark at 26°C. Then, the plates were transferred to a refrigerator at 6°C for 2 to 3 days. *Colletotrichum* spp. colonies of characteristic size, shape, and color are produced under this regime (54). Colonies corresponding to each time (rain duration) and distance from the source were counted with the aid of a magnifying plate counter. Calibration studies showed that 65% of spores produce colonies under a wide range of inoculum-density and water-volume conditions (54). Generally, >95% of the plates had colonies characteristic only of *Colletotrichum*. If there were more than a few isolated colonies of contaminates in the plates exposed to rain before the introduction of inoculum (a rare event), the simulation run was discarded. Simulation runs were done from three to six times for each rain intensity in random order.

TABLE 2. Description of selected symbols and terms used in text[a]

Variable	Description
a	Parameter of power function (aD^p) in general integral rain equation (equation A3) [units depend on p]; Ulbrich (46, 47) and the appendix contain specific values
b_0–b_3	Parameters in equation 3 for fruit disease incidence
D	Impacting water (rain) drop diameter [cm]
D_0	Mass (volume) median diameter of drops impacting on a unit area during a rain episode [cm]; 50% of the rain volume is due to drops with diameters greater than (or less than) D_0
I	Idealized rain intensity [mm h^{-1}]
MIT	Multiple infection transformation of the proportion of fruit infected ($= -1n(1 - y)$); used explicitly with spore wash-off study for fruit exposed (MIT_E) or not exposed (control) to rain (MIT_C)
n	Total number of splash droplets produced from all drop impactions over a unit area [cm^{-2} h^{-1}]
$n(D)$	Number of droplets produced from the impact of a single drop with diameter D
N	Number of drops impacting per unit area and time [m^{-2} s^{-1}]; can be calculated with equation A4
N_0	Term of Marshall-Palmer–type equations for $N(D)$ [m^{-3} cm^{-1}]; equation A1 (30,46)
$N(D)$	Distribution of drops per unit volume of air per unit drop diameter [m^{-3} cm^{-1}]; i.e., number of drops of any diameter, D, in a unit volume of air (equation A1)
p	Parameter of power function (aD^p) in general integral rain equation (equation A3); choice of p determines units for a (46)
P	Significance level
Q'	Parameter in empirical diffusion equation in Yang et al. (51), representing (initial) release of spores at the source [min^{-1}]
r^2	Coefficient of determination
R	Measured rain intensity [ml cm^{-2} h^{-1}]
R_S	Measured splashed-rain intensity [ml cm^{-2} h^{-1}] into petri plates
R_{opt}	Optimum R; value of R where y^* is a maximum
R^*	Value of R where Σ equals 0 (predicted from equation 1)
R_S^*	Value of R_S where Σ equals 0 (predicted from equation 2)
s	Distance from a spore source [cm]
S_R	Proportion of spores removed from source fruit
S_W	Proportion of deposited spores washed off healthy fruit, estimated by MIT
t	Time [min]
$V(D)$	Velocity of drop with diameter D impacting on a surface [m s^{-1}]
$V_T(D)$	Terminal velocity of drop with diameter D [m s^{-1}]; can be predicted with a power function of D (appendix)
y	Disease incidence; e.g., proportion of strawberry fruit infected by *Colletotrichum acutatum*
y^*	Transformation of y (equation 3); e.g., $y^* = 1n[-1n(1 - y)]$
Y	Colonies in petri plates [cm^{-2} min^{-1}]
α	Parameter of diffusion equation of Yang et al. (51), a measure of steepness of dispersal gradient, as well as flight distances of splash droplets [cm^2 min^{-1}]
β_R	Rate of spore removal from source fruit [min^{-1}]
β_W	Rate of spore wash-off from healthy fruit [min^{-1}]
γ	Parameter of diffusion equation of Yang et al. (51), representing rate of spore loss [min^{-1}]
$\Gamma(\bullet)$	Statistical gamma function
Λ	Term in equation A1 [cm^{-1}]
Σ	Interpolated total number of colonies over a circular area (radius = 100 cm) for 46 min of rain; numerically estimated with Simpson's 3/8 rule
Σ_f	Σ for a fixed amount of rain (not for a fixed time)
ζ	Shape parameter of the generalized equation for $N(D)$ (equation A1); when $\zeta = 1$, equation A1 is the exponential
Υ	A general integral rainfall parameter; interpretation depends on a and p (46,47)

[a] Units, if any, are given in brackets; distance, area, time, etc., can be converted to different scales (e.g., cm to mm or m^{-2} s^{-1} to cm^{-2} h^{-1}) as needed for calculations.

Volume of water splashed was determined with the gravity samplers in separate runs of the rain simulator. The soil mix was placed over the wood frame (as described above), and five sheltered gravity samplers were placed approximately randomly over the experimental area, with the constraint that there was at least 40 cm between samplers. Petri plates with selective media were labeled and weighed immediately before use. At 12 and 27 min into a simulated rain (with no inoculum), plates were placed under the rain shields for 2 to 4 min of exposure, depending on the rain intensity. Plates were collected and immediately weighed again to determine weight gain due to water splashed into each plate. Controls consisted of three plates with media that were weighed at the same time but not exposed to rain. Average weight loss in the control plates, due to evaporation of water in the media, was used to correct the weight changes in the rain-exposed plates. Means for splashed-water intensity (R_S) were determined for each run of the rain simulator after converting to volumetric units of milliliters per square centimeter per hour based on the density of water. Splash for each rain intensity was tested twice (with a new soil each time) in random order.

Fruit infection study. Strawberry plants (*Fragaria* × *ananassa* Duchesne 'Midway') were raised from transplants in 15-cm-diameter pots (soil/peat/sand, 2:2:1, by volume) in a greenhouse. Twenty plants with immature fruit were used for each run of the simulator.

The methods were the same as those used by Madden et al. (34) for a single rain intensity. Briefly, potted plants were held in two concentric circles (30 and 60 cm radii) by a wood frame that was placed directly below the rain generator. (Circles were used for plants, rather than straight rows [as in Boudreau and Madden (5)], so all fruits were at only two distances from the inoculum source). The frame was covered with a sterilized soil mix, as described for the gravity sampler study, so the crowns of the plants were at soil level. Five infected fruit were placed in a tight cluster on the soil surface in the center of the frame immediately before a run. Forty healthy fruit were detached from plants and placed on the soil immediately in front of the plant crowns in each of the circles. The seven rain intensities (Table 1) were tested in random order, and the experiment was done three times.

For each intensity, rain durations of 4 and 16 min were tested. These durations produced >75% infected fruit at a rain intensity of 15 mm h^{-1} (34). At the end of the each duration, 25 to 30 fruit were collected from each circle, handling only the pedicels, and placed on wire-mesh screens in 5-liter containers. The pedicels were inserted through the screen into deionized water as described for inoculum preparation. All fruit were lightly misted with sterile distilled water, covers were placed on the containers, and the containers were placed in an incubator (26°C) for 24 h. Then, lids were removed, and the containers were placed back in the incubator for another 6 to 7 days. Fruit infection was assessed by

visual examination of the fruit. As shown by previous work (55), this regime resulted in fruit infected by *C. acutatum* being covered with easily identified lesions. The proportion of infected fruit was recorded for each duration and distance (plant circle).

Spore removal. The effect of rain intensity on the removal of conidia from the source fruit was evaluated with the rain simulator. Soil mix was placed over the wood frame, and no plants were used. Five infected fruit with sporulating lesions were individually placed on the soil surface, with at least 40 cm between fruit. These fruit were inoculated 7 days earlier (described above) and incubated at 26°C. The entire area received a simulated rain of 16 min, infected fruit were collected, and spore density was determined (as described above for the routine evaluation of source fruit).

Spore density for each rain-exposed fruit (D_E) was divided by the mean spore density for five fruit not exposed to rain (i.e., control fruit; D_C); this proportion was subtracted from 1 to obtain the proportion of conidia removed by rain (i.e., $S_R = 1 - (D_E/D_C)$). The rate of spore removal was calculated by assuming an exponential decline in spore density per fruit, as found previously for exposure to 15 mm h^{-1} rains (34). Rate of removal, β_R (per minute), is given by: $\beta_R = [\ln(D_C) - \ln(D_E)]/16$. Tests were done for four rain intensities, corresponding to the 5.6W, 20W, 35W, and 50W nozzles (Table 1). Intensities were tested in random order, and the experiment was done twice.

Wash-off. The removal of deposited spores from healthy strawberry fruit (i.e., wash-off) was estimated with the rain simulator. Sterilized soil mix was placed on the wood frame without the presence of plants (as described above). Twenty healthy, immature fruit were placed individually on the soil surface, with an approximately uniform arrangement. A spore suspension (4×10^3 conidia per ml) was prepared with sterile distilled water from infected fruit incubated in the laboratory. Immediately before a simulated rain, 50 µl of the spore suspension was placed on top of each fruit with a micropipette (Eppendorf Comforpette 4700, Netheler-Hinz GmbH, Hamburg, Germany; inaccuracy = 0.6%). This resulted in an average of 200 conidia per fruit. Surface tension initially kept the water on the fruit. The entire area received a simulated rain of 16 min, and the fruit were collected and placed in 5-liter containers as was done for the gravity sampler study (described above). Twenty control fruit were inoculated with 50 µl of the spore suspension immediately before each rain but were not exposed to rain; these fruit were incubated as described for the treated fruit.

After 7 days of incubation at 26°C (1 day with the lid on the container; 6 days with no lid), infected fruit were identified by visual symptoms. The proportion of fruit infected for the exposed (y_E) and control (y_C) fruit was determined; the multiple-infection transformation ($MIT_E = -\ln(1 - y_E)$; $MIT_C = -\ln(1 - y_C)$) was calculated to give the hypothetical number of infections per fruit (9). With this regime, the overall average y_C was 0.78 (MIT_C = 1.51). Calibration studies with a range of spore densities on nonexposed fruit showed there was a linear relationship between MIT_C and spore density in the range of 10^3 to 10^4 conidia per ml (L. V. Madden, *unpublished data*).

The number of spores removed by rain wash-off for any run of the rain simulator was determined by $S_W = 1 - (MIT_E/MIT_C)$. Rate of wash-off (β_W) was calculated as: $\beta_W = [\ln(MIT_C) - \ln(MIT_E)]/16$. Tests were done for four rain intensities, corresponding to the same four nozzles used for the spore-removal experiment. Intensities were evaluated in random order, and the experiment was done four times.

Data analysis. The number of colonies in a petri plate, converted to unit area and time (Y(number per square centimeter per minute)), was determined for each distance, time (rain duration), rain intensity, and repetition. Y is proportional to the deposition flux density of conidia (51). The interpolated total number of colonies (Σ) during each 46-min period over a circular area with a radius of 100 cm was estimated with numerical integration, using the technique described in Yang et al. (54). The variable Σ is proportional to the temporal integration of total deposited conidia over a circular area.

The effects of simulated rain on Σ from the gravity sampler study, S_R and β_R from the spore-removal study, and S_W and β_W from the spore wash-off study were evaluated by generalized linear models (GLMs) (11,25). (Analysis of variance [ANOVA] is a special case of GLM used when the errors are normally distributed). Standard errors of the mean responses (prior to transformation) at each rain intensity were calculated. The experimental design of these studies was a randomized complete block, with the block being the repetition of the experiment. Based on prior work characterizing the heterogeneity of colonies in petri plates (31), Σ was transformed to $\Sigma^{1/5}$ to stabilize variances and produce an approximately normally distributed variable. Thus, ANOVA was used to analyze $\Sigma^{1/5}$. Regression analysis was used to develop simple empirical models for mean Σ as a function of mean incident rain intensity (R) and splashed-rain intensity (R_S). MINITAB (MINITAB, State College, PA) was used for the analyses.

The diffusion-type model of Yang et al. (51 [equation 4]) was fitted to the colony data to relate mean Y as a function of rain duration (t; minutes) and distance from the source (s; centimeters). Parameters of the model are: Q' (per minute), representing the initial release of spores from the inoculum source, related to inoculum density on the fruit and rain intensity (51); α (square centimeters per minute), inversely related to the steepness of the spore deposition gradient and a measure of the average flight distance of splash droplets; and γ (β in the original) (per minute), representing the rate of spore loss from the system (e.g., through the soil). Parameters were estimated by nonlinear least squares by the 3R program of BMDP (BMDP Statistical Software, Los Angeles), following the methodology in Yang et al. (51).

Because of increasing standard deviation with increasing mean of S_R, S_W, β_R, and β_W, with an approximately fixed coefficient of variation (described below), a gamma-error distribution was assumed for these four variables (11). Gamma-error GLMs were conducted by the program GLIM (25).

The effects of simulated rain on the proportion of infected fruit (y) from the fruit study were first evaluated with ANOVA. Experimental design was a repeated measures factorial, with rain intensity (nozzle type) and block (experiment repetition) as the randomized factors, distance from the source as a spatially repeated measure, and rain duration as a temporally repeated measure. To help stabilize variances and produce an approximately linear scale,

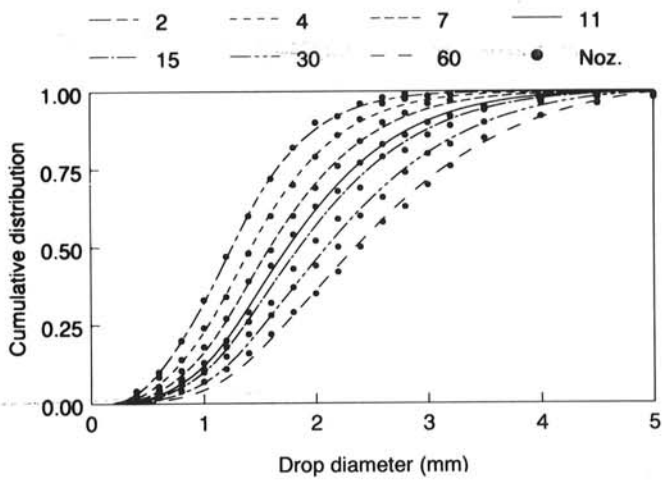

Fig. 1. Theoretical cumulative volume distributions for the indicated rain intensities (I = millimeters per hour), based on the Marshall-Palmer equation (46,47) (curves), and cumulative volume distributions for seven wide-angle spray nozzles (Spraying Systems) (points). Nozzle descriptions are given in Table 1. Left side: 2 mm h^{-1} and 2.8W nozzle; right side: 60 mm h^{-1} and 50W nozzle.

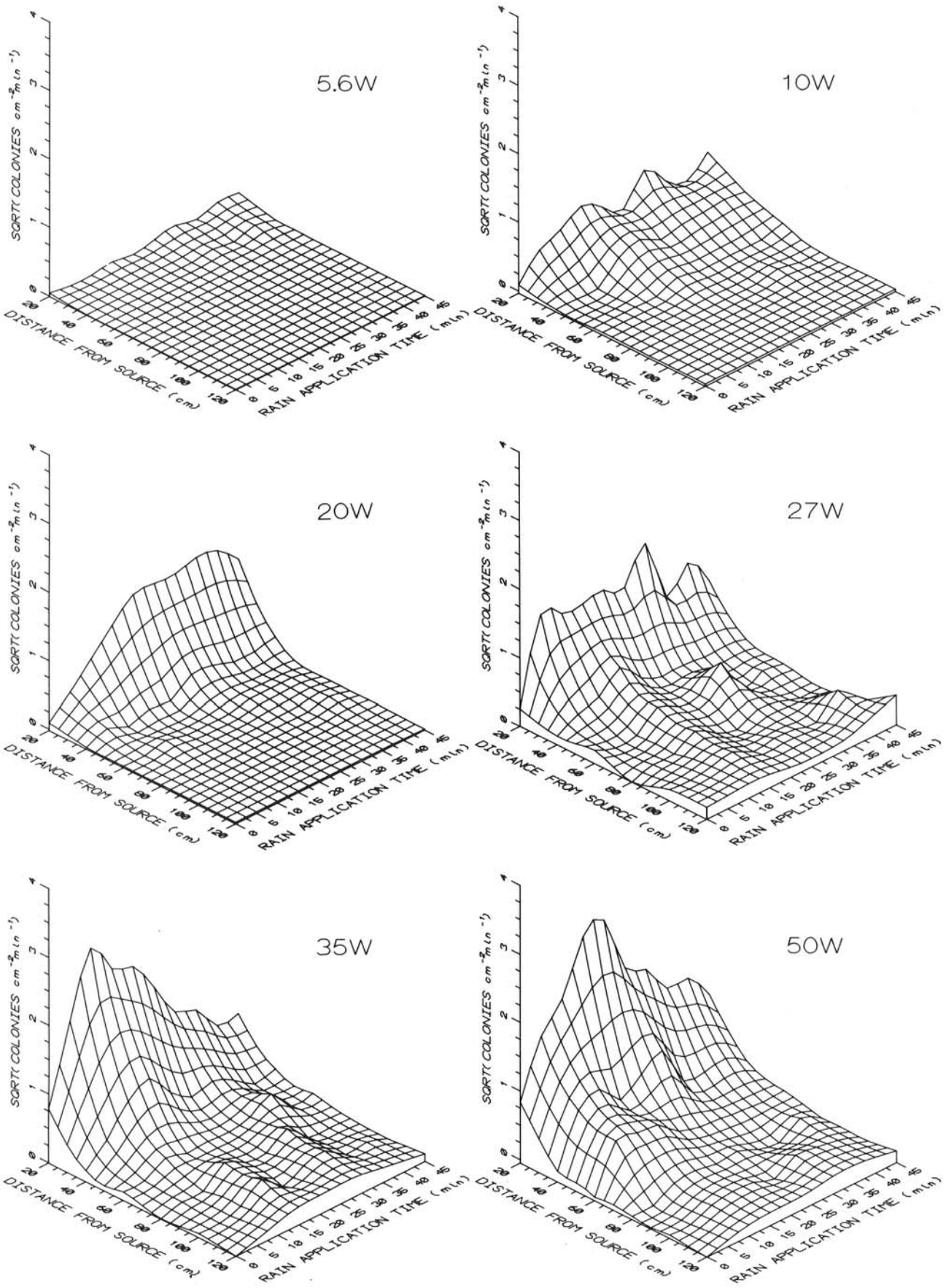

Fig. 2. Mean number of *Colletotrichum acutatum* colonies (Y; per square centimeter per minute) on a selective medium (under a rain shield) in relation to rain duration (t; minutes) and distance (s; centimeters) from the inoculum source for rains generated by six of seven wide-angle nozzles (Spraying Systems; Table 1). Results for the smallest nozzle (2.8W) are not shown because of low numbers (Table 3). A square-root scale was used for Y to better illustrate the data at 60 cm and beyond, where colony density was low.

the "complementary log-log transformation" was used (11): $y^* = 1n[-1n(1 - y)]$. In essence, this is a log transformation of the *MIT* of infected fruit. The repeated measures ANOVA was performed with the 2V program of BMDP. Based on the significant terms of the ANOVA, y^* was modeled as a function of incident (R) and splashed-rain intensity (R_S) by GLMs, with an assumed binomial distribution for y. GLIM (25) was used for model development.

RESULTS

Simulated rain. The seven nozzles, operated at a pressure of 69 kPa, produced mean rain intensities (R) ranging from 1.2 to 4.9 ml cm^{-2} h^{-1} (Table 1). Variation in incident rain over the experimental surface was low, as indicated by the low standard errors. The largest coefficient of variation of the mean was 19% (=(0.24/1.24) × 100), corresponding to the lowest rain intensity (2.8W nozzle).

The cumulative volume distribution of simulated rains could be paired with the theoretical distributions for natural rains between 2 and 60 mm h^{-1} (Fig. 1; appendix). The Kolmogorov-Smirnov goodness-of-fit test statistic was not significant for any nozzle ($P > 0.10$). Although the match between theoretical natural rains and the simulated rains was not exact, there was sufficient agreement to treat the simulated rains as representative of natural ones (38). For convenience, I is used to represent the intensity of the theoretical (idealized) natural rain approximated by the rain simulator (millimeters per hour); R is used for measured rain intensity (milliliters per square centimeter per hour) (Table 2 describes the symbols). Because 1 ml = 1 cm^3, milliliters per square centimeter per hour can be expressed as cubic centimeters per square centimeter per hour or simply centimeters per hour. Thus, $10R$ gives measured rain intensity in units of millimeters per hour. As can be seen in Table 1, $10R$ was greater than I for all nozzles, except the largest (50W), demonstrating that although the nozzles (operated at 69 kPa) generally reproduced the distribution of drop sizes in natural rains of the listed intensities (Fig. 1), they did not give the same intensity of water. Therefore, I is used as a label for the rain generated by a nozzle, and R is used in all analyses to relate dispersal to intensity (described below).

Splashed-water intensity (R_S; milliliters per square centimeter per hour) increased with increasing incident rain intensity and with duration of rain (Table 1). Splashed-water volume at 27 min into a generated rain episode was from 1.2 to 1.5 times greater than that at 12 min into a rain episode, likely due to the increasing saturation of the soil mix (7,54). The percentage of incident rain that was splashed increased in a fairly linear manner with incident rain intensity until $R \approx 4$ ml cm^{-2} h^{-1} (i.e., $I = 30$ mm h^{-1}, 35W nozzle); there was a slight decrease in the splash percent at the highest intensity (Table 1).

Gravity sampler study. The number of colonies (Y) generally decreased with distance at any rain duration for all generated rain intensities (Fig. 2). (Results for the 2.8W nozzle are not shown in the figure because means were very close to 0). There were few colonies beyond 40 cm. Y increased with duration to a maximum and then decreased. This temporal relation was most obvious for distances close to the inoculum source (e.g., 20 cm) because of the higher values at these distances.

Estimated total colonies for 46 min, over a circle with a radius of 100 cm (Σ), increased in a linear fashion with R (or R_S) (Table 3), with means ranging from ~4×10^2 for $I = 2$ mm h^{-1} (2.8W nozzle) to ~9×10^5 for $I = 60$ mm h^{-1} (50W nozzle). Standard errors of the means also increased with rain intensity, but the fifth-root transformation stabilized variances. There was a highly significant effect ($P < 0.001$) of rain intensity on $\Sigma^{1/5}$, but there was no significant effect of experiment repetition (block) on $\Sigma^{1/5}$ ($P > 0.20$). Thus, means across experiment repetitions can be presented (Table 3). A simple linear relation best described Σ as a function of R:

$$\Sigma = \underset{(6.1 \times 10^4)}{-3.6 \times 10^5} + \underset{(2.0 \times 10^4)}{2.5 \times 10^5 R} \quad (1)$$

in which numbers in parentheses under the coefficients are standard errors of the estimated parameters. The coefficient of determination (r^2) equaled 0.97. The predicted value of R at which $\Sigma = 0$ (R^*) is given by 3.6/2.5 = 1.44 ml cm^{-2} h^{-1}, an intensity intermediate between the lowest two rain intensities tested (Table 1).

For splashed-rain intensity, a linear relation also was found:

$$\Sigma = \underset{(2.6 \times 10^4)}{-1.1 \times 10^4} + \underset{(2.0 \times 10^5)}{3.5 \times 10^6 R_S} \quad (2)$$

This equation had an r^2 of 0.98. Σ equaled 0 at a predicted splashed-rain intensity (R_S^*) of 1.1/350 = 0.003 ml cm^{-2} h^{-1}, which was less than for the 2.8W nozzle (Table 1).

With a fixed time duration (46 min), rain intensity (milliliters per square centimeter per hour) is confounded with total amount (milliliters per square centimeter), because amount equals (46/60)R. To help distinguish intensity from total amount of rain, Σ was recalculated for durations that resulted in a fixed amount of 1.11 ml cm^{-2} (Σ_f), corresponding to the total rain amount over 46 min with the 5.6W nozzle (Table 1). The data from the 2.8W nozzle was not considered, because there was some indication that this intensity was below an intensity threshold of 1.4 ml cm^{-2} h^{-1} (R^*) (described above). Times to reach 1.11 ml were 46, 34.6, 28.9, 20.2, 16.6, and 13.7 min for the last six nozzles. Σ_f was not

TABLE 3. Effects of spray nozzle (=rain intensity) on number of colonies of *Colletotrichum acutatum* in petri plates, removal of spores from infected strawberry fruit, and wash-off of spores from healthy fruit

Spray nozzle	No. of reps.[a]	Colonies[b]	Transformed colonies[c]	Spore removal[d] (proportion)	Rate of spore removal[e] (min^{-1})	Spore wash-off[f] (proportion)	Rate of spore wash-off[g] (min^{-1})
2.8W	5	3.8×10^2 (2.4×10^2)[h]	1.58 (0.97)[h]	...[i]	...	...	...
5.6W	4	1.1×10^3 (5.4×10^2)	3.75 (0.46)	0.46 (0.05)[h]	0.04 (0.01)[h]	0.22 (0.03)[h]	0.016 (0.003)[h]
10W	4	7.6×10^4 (3.6×10^4)	8.64 (1.19)	...	...	...	...
20W	3	2.5×10^5 (1.3×10^5)	11.07 (1.84)	0.59 (0.10)	0.06 (0.01)	0.33 (0.04)	0.025 (0.006)
27W	3	3.5×10^5 (6.4×10^4)	12.82 (0.45)	...	...	...	...
35W	6	7.2×10^5 (1.1×10^5)	14.67 (0.48)	0.80 (0.11)	0.10 (0.02)	0.57 (0.03)	0.053 (0.010)
50W	6	8.8×10^5 (1.9×10^5)	15.16 (0.66)	0.83 (0.14)	0.12 (0.03)	0.59 (0.08)	0.056 (0.011)

[a] Number of repetitions for calculating colonies in plates.
[b] Total number of colonies over a circular area with a radius of 100 cm and rain duration of 46 min (Σ). Mean over replications is shown.
[c] Σ for each replication transformed to $\Sigma^{1/5}$ prior to calculating mean and other statistics.
[d] Number of spores removed from strawberry fruit after 16 min of rain divided by the number before rain. Mean of two repetitions.
[e] Change in 1n(spores/fruit) for fruit before and after 16 min of rain divided by 16.
[f] One minus (multiple-infection transformation [*MIT*] of the proportion of diseased fruit after 16 min of rain divided by *MIT* of nonexposed proportion of diseased fruit). Mean of four repetitions.
[g] Change in 1n(*MIT*) for fruit before and after 16 min of rain divided by 16.
[h] Standard error given in parentheses.
[i] Not determined.

determined for the 50W nozzle, because only data for three times (0, 5, and 10 min) would have been used, which was considered insufficient for the numerical integration. Mean values of Σ_f were 1.1×10^3 (5.6W nozzle), 5.7×10^4 (10W), 1.4×10^5 (20W), 1.6×10^5 (27W), and 3.3×10^5 (35W), direct evidence of increasing number of colonies with increasing rain intensity when rain amount was fixed.

The diffusion-type model of Yang et al. (51) provided a satisfactory fit to the Y data in relation to rain duration and distance (Table 4). Values of r^2 were relatively high for intensities of 11 to 60 mm h^{-1} (nozzles 20W to 50W); the lower r^2 values at intensities below 11 mm h^{-1} reflected the small change in Y with change in s or t (Fig. 2). The estimated parameter for initial release of spores from the inoculum source (Q') generally increased with increasing intensity. Rate of spore loss (γ) also increased with increasing intensity. However, for the lowest three intensities, the estimate of γ was too close to 0 to be estimated precisely. Estimates of α, the parameter for trajectory length, was erratic and not related to rain intensity, as was found previously (30,51).

Fruit infection study. Rain intensity had a strong effect on fruit disease incidence (y), but the magnitude of the effect depended on rain duration and distance from the inoculum source (Fig. 3). At 30 cm and 4 min of rain, mean y increased from near 0 at the lowest intensity to about 0.8 at $R = 3.3$ ml cm^{-2} h^{-1} (corresponding to $I =$ 15 mm h^{-1} or the 27W nozzle). Then, y decreased at higher R. The same general relation occurred at 16 min and 30 cm, although all means were higher (Fig. 3). The highest mean was 1.0 (i.e., all fruit were infected in all repetitions [$I = 15$ mm h^{-1}]); y's for the highest two intensities ($I = 30$ and 60 mm h^{-1}) were about 0.97. At 60 cm from the source and 4 min of rain, few fruit became infected at any intensity, although there was a slight increase in y with R. With 16 min of rain at this distance, higher mean y was found than at 4 min, with the maximum observed incidence at $R =$ 3.3 ml cm^{-2} h^{-1} (Fig. 3).

Analysis of the complementary log-log transformation of incidence (y^*) indicated a significant effect of rain intensity ($P < 0.001$), distance from source ($P < 0.001$), rain duration ($P < 0.001$), and the interaction of rain intensity and distance from source ($P =$ 0.02). Experiment repetition (block) and all other interactions were not significant ($P > 0.20$). The one significant interaction indicated distance affects how y^* changes with R (Fig. 3).

The relationship between y^* and R could be described by a cubic function:

$$y^* = b_0 + b_1R + b_3R^3 \quad (3)$$

in which b_0 to b_3 are parameters estimated with generalized linear modeling (25) (Table 5). The models for combinations of distance and time explained a high percentage of the deviance (analogous to the residual sum of squares of normal-error models). The b_0 parameter is an indication of the height of the curve (in the transformed scale), and the other parameters show the change in y^* with changing R. The large value of b_0 for 30 cm and 16 min (–5.51) reflects the very high overall incidence with this combination (Fig. 3). The b_1 and b_3 terms were remarkably similar for three of the four combinations of distance and time (Table 5). The major difference was for the short rain duration at the greater distance from the source (4 min and 60 cm), where the magnitude of the parameters was lower than the others, reflecting the minor change in y^* with change in R.

TABLE 4. Estimated parameters (and standard errors) of the diffusion-type model of Yang et al. (51) fitted to mean number of colonies of *Colletotrichum acutatum* (Y; cm^{-2} min^{-1}) in relation to rain duration (t; min) and distance from the inoculum source (s; cm)[a]

Spray nozzle	Estimated parameters (SE)			r^{2b} (%)
	Q'	α	γ	
2.8W	8.3 (0.8)	5.9 (1.0)	$<10^{-5c}$ …[d]	62
5.6W	1.2×10^2 (37)	0.8 (0.1)	$<10^{-5}$ …	76
10W	1.5×10^3 (1.0×10^2)	6.7 (0.8)	$<10^{-5}$ …	78
20W	1.7×10^4 (1.5×10^3)	2.0 (0.1)	0.027 (0.005)	98
27W	8.7×10^3 (7.7×10^2)	8.1 (1.3)	0.010 (0.008)	80
35W	7.3×10^4 (2.7×10^3)	5.1 (0.3)	0.076 (0.006)	97
50W	2.2×10^5 (2.4×10^4)	2.4 (0.3)	0.093 (0.014)	91

[a] Model: $Y = [Q'/(4\pi\alpha t)] \cdot \exp\{-[s^2/(4\alpha t)]\} \cdot \exp(-\gamma t)$. The text and Table 2 describe the parameters and units.
[b] Coefficient of determination.
[c] Estimate close to 0; cannot estimate precisely.
[d] Not determined.

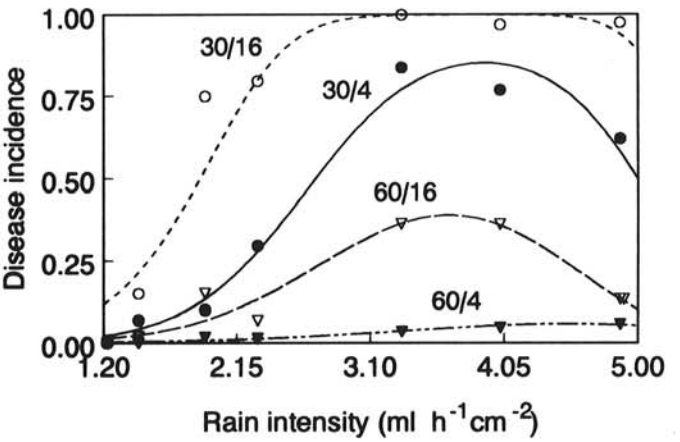

Fig. 3. Mean proportion of strawberry fruit infected by *Colletotrichum acutatum* (y) in relation to rain intensity (R; milliliters per square centimeter per hour) for rain durations (t; minutes) of 4 and 16 min and distances (s; centimeters) from the inoculum source of 30 and 60 cm. Labels on curves given as s/t. At the lowest R value, all y values are 0, even though only one of the four points can be seen. Curves represent the predicted proportion of infected fruit by equation 3 and the parameter estimates in Table 5.

TABLE 5. Estimated parameters of the model (equation 3)[a] for the relationship between strawberry fruit infected by *Colletotrichum acutatum* and rain intensity (R; ml cm^{-2} h^{-1}) and splashed-rain intensity (R_S) for two distances from the source and two times from the initiation of rain episodes

Distance (cm)	Time (min)	Estimated parameters (SE)			Optimum[b]	Explained deviance(%)
		b_0	b_1	b_3		
R:						
30	4	–7.39 (0.55)	3.80 (0.24)	–0.067 (0.004)	3.9	95.7
30	16	–5.51 (0.47)	2.96 (0.26)	–0.068 (0.007)	3.8	87.0
60	4	–7.91 (1.95)	1.70 (0.87)	–0.028 (0.022)	4.5	85.9
60	16	–7.50 (0.77)	2.78 (0.35)	–0.069 (0.010)	3.7	86.3
R_S:						
30	4	–3.14 (0.21)	38.84 (2.75)	–449.4 (37.4)	0.17	93.3
30	16	–1.61 (0.15)	39.38 (4.08)	–479.0 (60.1)	0.16	73.7
60	4	–5.37 (0.76)	19.66 (10.22)	–165.5 (141.8)	0.20	76.5
60	16	–3.72 (0.30)	32.31 (3.91)	–406.3 (54.9)	0.16	80.0

[a] $\ln[-\ln(1 - y)] = b_0 + b_1X + b_3X^3$, in which y is the proportion of infected fruit, b_0, b_1, and b_3 are parameters, and X represents R or R_S (Table 2).
[b] Predicted optimum rain intensity or splashed-rain intensity (ml cm^{-2} h^{-1}), based on estimated parameters: $\{[-b_1/(3b_3)]^{1/2}\}$.

Optimum rain intensity (R_{opt}), i.e., the value of R where y^* is a maximum, can be predicted by $[-b_1/(3b_3)]^{1/2}$. This value was about 3.8 ml cm^{-2} h^{-1} for three of the combinations (Table 5), intermediate between the rains generated by the 27W (I = 15 mm h^{-1}) and 35W (I = 30 mm h^{-1}) nozzles (Table 1). At 60 cm from the source and only 4 min of rain, where little deposition of conidia was expected, R_{opt} was predicted to be 4.5 ml cm^{-2} h^{-1}, which was greater than the highest simulated rain.

The relation between y^* and splash intensity (R_S) was not as strong as between y^* and R (Table 5), i.e., variability was higher, and the cubic model (with R_S substituted for R in equation 3) generally explained a lower percentage of the deviance. However, optimum R_S corresponded to an incident rain intensity intermediate between I = 15 and 30 mm h^{-1} (Table 1), which agrees with the model for R.

Rain amount (milliliters per square centimeter) was not a useful predictor of y^* compared to intensity (milliliters per square centimeter per hour) . Total amount of rain was lower for all intensities at 4 min compared to 16 min of rain, yet there was considerably higher disease incidence (especially at 30 cm) at the high intensities with 4 min of rain compared to low intensities at 16 min of rain. The most similar rain amounts were for the 2.8W nozzle at 16 min (0.33 ml cm^{-2}) and the 50W nozzle at 4 min (0.32 ml cm^{-2}). However, mean incidence was about 62% with 4 min of the high rain intensity (50W nozzle) and 0% for 16 min of the low rain intensity (2.8W nozzle) (Fig. 3).

Spore removal. Rain intensity had a significant effect on the proportion of spores removed from infected source fruit (S_R) and the rate of spore removal (β_R) ($P < 0.05$). Experiment repetition (block) was not significant. Simulated rain removed from 46% (I = 4 mm h^{-1}) to 83% (I = 60 mm h^{-1}) of the *C. acutatum* conidia over the first 16 min of rain exposure (Table 3); β_R increased from 0.04 to 0.12 min^{-1} over the same range of rain intensities. The increase was nearly linear with respect to R (or I) over most of the rain intensity range, although the increase between the last two intensities was somewhat less.

Wash-off. The proportion of conidia removed from healthy fruit, as measured by infected fruit, significantly increased with rain intensity ($P < 0.05$). Repetition was not significant. With the highest rain intensity (I = 60 mm h^{-1}), there was an approximately 60% reduction in the *MIT* of the proportion infected (MIT_E) compared to a non–rain-exposed control (MIT_C) (Table 3), i.e., S_W = 0.59 implies that 59% of the conidia that hypothetically could infect the fruit were washed off the fruit by rain. The reduction was about 20% for the lowest tested rain intensity (I = 4 mm h^{-1}). As with spore removal from source fruit, rate of wash-off (β_W) increased with R (or I).

DISCUSSION

Rain intensity had a dramatic effect on splash dispersal of conidia of *C. acutatum*. Increasing intensity of generated rain resulted in increased volume or mass of water splashed, number of colonies in gravity samplers, and removal of spores from infected source fruit, but also inferred loss of spores (through the soil) and wash-off of deposited spores from healthy strawberry fruit. Infection of rain-exposed healthy fruit increased with intensity to a maximum and then declined somewhat, depending on the distance from the source and duration of rain. Results can explain the previously inconsistent, variable, or unclear effects of rain intensity on splash dispersal (5,6,16,20,38,41,42,55).

In natural rains, increasing rain intensity is linked with an increasing number of raindrops per unit area and time (37) and also a shift in the drop size distribution to larger diameters (47) (Fig. 4A). For instance, the mass median drop diameter (D_0), i.e., the median size of the drops on a volume basis, increases from about 1 mm with a 2 mm h^{-1} (idealized) natural rain to about 2 mm with a 60 mm h^{-1} rain (46) (Fig. 4B). There is also a corresponding in-

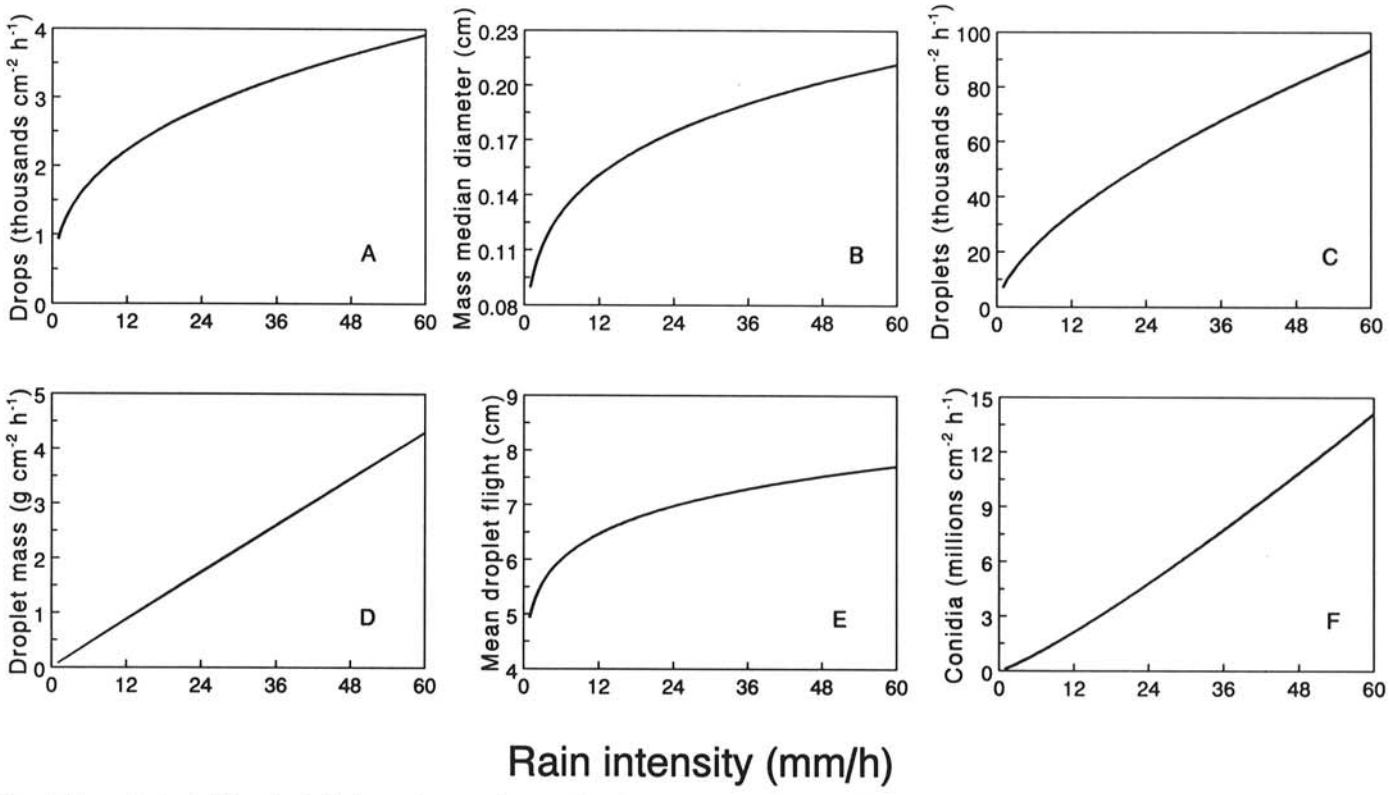

Fig. 4. Theoretical rainfall and splash-dispersal properties as a function of rain intensity, I (millimeters per hour). **A,** Number of impacting drops (of all diameters) per unit time and area (equation A4, with a = 17.67 and p = 0.67). **B,** Mass (volume) median diameter (appendix). **C,** Number of splash droplets formed from rain drops impacting on strawberry fruit (equation A4, with a = 8,717 and p = 2.01). **D,** Total mass of the splash droplets in **C** (equation A4, with a = 6.2 and p = 3.67). **E,** Average flight (travel) distance for the droplets in **C** (equation A4, with a = 17.5 × 10^5 and p = 2.53, divided by equation A4, with a = 8,717 and p = 2.01). **F,** Total number of dispersed conidia in the splash droplets in **C** (equation 4A, with a = 69.5 × 10^6 and p = 4.6).

crease in frequency of drops of any given diameter with increasing intensity. As shown by Reynolds et al. (38), there are nearly twice as many 4-mm-diameter drops in a 60 mm h^{-1} rain compared to a 15 mm h^{-1} rain. When a single 4-mm drop impacts on an infected strawberry surface at close to terminal velocity, it produces seven times the number of splash droplets, 100 times the total mass of the splash droplets, 1.2 times the average droplet flight distance, and disperses 50 times the number of *C. acutatum* conidia compared to impact by a 1-mm drop (52,53). Similar effects of drop size have been found by other researchers (8,18,20).

Although we did not directly sample individual splash droplets over the experimental area as was done with some studies with rain simulators (20,50), theoretical results can be obtained based on our previous work with single drops (52,53) by the analytical technique of Ulbrich (46,47) (appendix). Splash results shown here (Fig. 4C through F) are population totals or averages calculated from single-drop impactions on strawberry surfaces and do not represent resplashing across a surface (50) nor the depletion of spores at the source (16). Model results indicate that one can expect increases in the number of splash droplets from source fruit (Fig. 4C), total mass (or volume) of droplets splashed (Fig. 4D), mean flight (or travel) distance of droplets (Fig. 4E), and number of conidia dispersed (Fig. 4F) with increasing intensity of rain. The mass of droplets is especially relevant because the number of spores per droplet is dependent on droplet mass (8,21,27,53). In a detailed empirical study of splash over soil, straw, and plastic, but with just two generated rain intensities (15 and 30 mm h^{-1}), we found an increasing number and mass of splash droplets as well as increasing length of droplet flights (50). The latter was determined by the gradient of droplet numbers from a rain impaction area. About 30 to 38% of the mass of incident raindrops was splashed (50), as measured by droplet traces on water-sensitive paper, with the percent increasing with intensity. A higher percentage of splashed water was detected with single-drop impactions on fruit (52), equal to about two-thirds of the incident, when drops were traveling close to terminal velocity. This is reflected in the composite results of Figure 4D. Lower values for the rain simulator are due to the effects of surface topography on droplet splashing as discussed at length in Madden (30) and Yang et al. (50).

Mechanisms would include the loss of incident rain by surface infiltration of impacting drops of splash droplets, as well as altered droplet flight trajectories and transfer of kinetic energy from the drop to the droplet. Results in the current study also show increasing mass of water splashed into gravity samplers with increasing rain intensity but with lower percentages of incident (<7%) than found by Yang et al. (50). This is likely due to the lower efficiency of the gravity samplers used here compared to the water-sensitive paper used by Yang et al. (50,51). Because of the wide range of droplet sizes produced from impactions, there is no optimum sampler (19) for splash-dispersal studies.

There was qualitative agreement between experimental results here and theoretical predictions for splashing of spores from source fruit exposed to idealized rain episodes (Fig. 4), i.e., the increase in Σ (or Σ_f), S_R, β_R, as well as Q' and γ from the diffusion-type model with R (or I) was expected based on theoretical increased droplet mass, droplet number, flight distance, and number of dispersed conidia from lesions on strawberries with idealized rain. Even though wash-off of spores (β_W and S_W) increased with increasing R (or I), the rate of wash-off was less than the rate of removal of spores from source fruit (Table 3). Although the calculation of β_W was based on the assumption that the proportion of infected fruit was an indication of the number of infections (9), it gives some suggestion that spores are less readily removed from an infection site than they are from a source (lesion). The loss of spores from the system, due to depletion of spores at the source (represented by β_R) and infiltration of the soil surface (γ; [30]), together with wash-off (β_W, S_W), would account for the reduced infection of fruit at the highest rain intensities (Fig. 3). This also can be seen as the reduction in colony density per unit time near the source at sufficiently long rain durations with the gravity sampler study (Fig. 2); this reduction was most obvious at the highest rain intensities, where the greatest colony densities also occurred.

In many crops, development of anthracnose caused by *Colletotrichum* spp. is strongly associated with rainfall (1,12–15,22,44). This is not surprising considering that dispersal of conidia depends on impact by raindrops (19,30,55,56). Some simple empirical models have been developed to predict disease level in the field with rain amount as one of the predictor variables (33,44). In general, past studies with *Colletotrichum* spp. have not thoroughly considered which of the interrelated properties of natural rain—intensity, total amount in an episode, or duration, for example—are the best predictors of this component of disease development. For instance, infection of strawberry fruit by *C. acutatum* in the field was correlated with rain amount that occurred one latent period earlier (33), but intensity could not be related to infection because rainfall was not collected often enough to precisely determine intensity. Where intensity was determined, in studies of diseases caused by *Colletotrichum* spp. and other splash-dispersed pathogens, often it was a poorer preenvironmental variables (6,10,39,43). This may be due to the variation in intensity over time. Calculating average intensity in natural rains as the total amount divided by duration in episodes that last many hours or even days would be very misleading regarding the distributional characteristics of impacting drops at any moment during the episode (28). Even when short-duration estimates of intensity are available (e.g., 10-min periods), it is not yet clear how to summarize intensity for the episode. One possibility is the maximum intensity recorded (39), but a weighted integration over time probably will be needed. Under controlled conditions here, rain intensity was a more useful predictor of dispersal than amount, based on the increasing Σ_f (Σ for a fixed rain amount) with increasing intensity, as well as the poor relationship between disease incidence (y) and rain amount.

Published work shows high variability in droplet splashing with natural rains (41,42). These results are not surprising considering the multiple effects found here (e.g., spore removal, transport, and wash-off) for controlled and constant rain intensity. Further complicating field studies is the variation in intensity over time in rain episodes (as discussed above), different drop size distributions (represented by the ζ parameter of equation A1) for different types of rain events (thunderstorms, frontal convective, etc.), as well as changing crop phenology and variation in the pathogen during an epidemic (43). Future research will need to focus on evaluating splash dispersal in relation to the interaction of these factors with rain intensity.

APPENDIX

Raindrop size distribution. The number of raindrops per unit volume of air per unit diameter interval of drop, $N(D)$ (per cubic meter per centimeter), is a function of rain intensity, I (millimeters per hour), and is described by a generalization of the Marshall-Palmer equation (30,46). $N(D)$ can be written as

$$N(D) = N_0 D^{\zeta} \exp(-\Lambda D) \quad \text{(A1)}$$

in which D is the drop diameter (centimeters), and N_0, Λ, and ζ are parameters. For most rain episodes, $N_0 = 8 \times 10^5$ m^{-3} cm^{-1}, and Λ is a function of I, $\Lambda = 41I^{-0.21}$. Equation A1 is a form of the gamma probability density function (28), with scale parameter $1/\Lambda$ and shape parameter $\zeta + 1$, multiplied by the total drop number density ($N^* = N_0\Gamma(\zeta + 1)\Lambda^{-1-\zeta}$). The unitless parameter, ζ, generally takes on values between –2 and 2, depending on the type of rain episode. Time-averaged values of ζ often are close to 0 (28, 46), and we assume that $\zeta = 0$ here. With this restriction, equation A1 simplifies to $N(D) = N_0 \exp(-\Lambda D)$, the exponential distribution.

Integration. The number of raindrops of unit diameter interval impacting per unit area and time can be determined by multiplying

equation A1 by the velocity of the drops, $V_T(D)$ (units in meters per second). $V_T(D)$ can be well approximated as a function of D (4,46):

$$V_T(D) = 17.67D^{0.67} \quad \text{(A2)}$$

The total number of drops impacting per unit area and time (N; per square meter per second) is determined by the integration

$$N = \int V_T(D)N(D)dD = \int (17.67D^{0.67})N(D)dD$$

where the integration is from the minimum (D_{min}; ~0.02 cm) to the maximum (D_{max}; ~0.5 cm) drop diameter in a rain episode.

The integration can be written generally as

$$Y = \int a\, D^p\, N(D)dD \quad \text{(A3)}$$

in which Y is known as the integral rainfall parameter, with units and interpretation depending on a and p. For total number of impacting drops, $Y = N$ (equation A3), $a = 17.67$, and $p = 0.67$. Under a wide range of values of a and p, the integration in equation A3 from D_{min} to D_{max} is approximately equal to the integration from 0 to ∞ (46,47). In the latter case, equation A3 can be written approximately as

$$Y = a\Gamma(p + \zeta + 1)\,(3.67 + \zeta)^{-(p+\zeta+1)}\, N_0 D_0^{(p+\zeta+1)} \quad \text{(A4)}$$

in which $\Gamma(\cdot)$ is the gamma function and D_0 is the mass median diameter of rain (centimeters), which is given by $D_0 = (3.67 + \zeta)/(41I^{-0.21})$ (46). The advantage of equations A3 and A4 is that the solution can be determined for a rain episode if the property of individual raindrops can be represented as a power function of drop diameter (e.g., aD^p) or the product of several power functions (e.g., $cD^e \times fD^g = cfD^{(e+f)}$; thus, $a = cf$ and $p = e + f$). For instance, because of unit density of water, the mass and volume of drops are given by $(\pi/6)D^3$. Thus, total volume of water per unit area and time is determined by first multiplying individual drop velocity by volume:

$$17.67D^{0.67} \times (\pi/6)D^3 = (17.67 \times 0.5236)D^{(0.67+3)} = 9.25D^{3.67}$$

So, $a = 9.25$ and $p = 3.67$ in equation A4. Conversion of results to other units can be accomplished easily, as for Figure 4B (e.g., per square centimeter per hour). In fact, one check of the accuracy of equation A4 can be done by predicting I ($a = 33.31$ and $p = 3.67$), which is the starting point of all calculations, because Λ of equation A1 is a function of I. Percent error in predicting intensity is 10% at $I = 15$ mm h^{-1} and 6% at $I = 60$ mm h^{-1}.

Splash-dispersal prediction. Many properties of splash dispersal from single-drop impactions can be described by a power function of the velocity of the drop at impact ($V(D)$) (52,53). For instance, the number of droplets produced from a single impact by a drop of diameter $D[n(D)]$ can be written as

$$n(D) = 1.58[V(D)]^2 \quad \text{(A5)}$$

which is a revised description of the data in Table 2 in Yang et al. (52). Because one can assume that drops are traveling at terminal velocity (equation A2) in natural rains, one can substitute $V_T(D)$ for $V(D)$. Using equation A2, the number of splash droplets from a single-drop impact ($n(D)$) is now predicted as a function of D:

$$n(D) = 1.58[17.67D^{0.67}]^2 = (1.58 \times 17.67^2)D^{1.34} = 493.3D^{1.34} \quad \text{(A6)}$$

For the total number of splash droplets produced per unit time and area resulting from all drop impactions (n), one multiplies $N(D)$ by $V_T(D)$ to get impacting drops (N) and then by $n(D)$ (equation A6) to get droplets from the impacting drops:

$$n = \int n(D)V_T(D)N(D)dD = \int (493.3 \times 17.67)D^{(1.34+0.67)}\, N(D)dD$$

$$= \int 8716.6D^{2.01}\, N(D)dD$$

Thus, the a and p parameters of equation A3 to obtain $Y = n$ are: $a = 8716.6$ and $p = 2.01$.

One can use equation A4 to calculate total droplets without performing a numerical integration. Equation A4 also was used (Fig. 4) to determine mean flight distances (total flight distances divided by number of droplets), mass of splash droplets, and total number of conidia dispersed, using experimental results from single-drop impactions on strawberry fruit surfaces (52,53), including additional analyses to obtain equations in the form of equation A5.

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