Effect of Strawberry Density on Dispersal of *Colletotrichum acutatum* by Simulated Rain

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ABSTRACT

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Studies were done with a rain simulator to evaluate the influence of plant density, inoculum source location, rain intensity, and their interactions on splash dispersal. Greenhouse-grown strawberry plants were mounted in support structures buried in soil to create artificial two-row canopies with 2-, 3-, 4-, and 8-cm intrarow spacings. Infected fruit placed either within or between the rows provided a source for spore dispersal by simulated rain, which was generated at constant intensities of 15 or 30 mm h^{-1}. Spore deposition, evaluated in sheltered petri plates exposed every 5 min over a period of 46 min, declined with increasing plant density when integrated over time at locations near the source. Deposition also declined with density when integrated over time and space for rows, area between rows, or the whole plot ($P < 0.05$). Within-row placement of inoculum and lower rain intensity reduced deposition compared to between-row placement and high rain intensity. Deposition gradients on plates between rows were described well by a negative exponential model. Slope parameters of spore deposition gradients between rows were not affected by any of the treatments, but the *y*-intercept was lowered by increased density, within-row placement of inoculum, and less intense rain ($P < 0.05$). Results suggest plant density affects both removal of spores from fruit and subsequent transport and may influence dispersal between sources and targets not within the canopy.

Additional keywords: anthracnose, *Fragaria* × *ananassa*, rain-splash.

Plant density is thought to have an important influence on disease dynamics, but the number of studies directly evaluating these effects is limited. It is often suggested that disease incidence and plant density are positively correlated, due to the increased probability of a given propagule contacting a host (3,5,14,15). Such an increase has been observed for celery infected by *Cercospora apii* (9), cress infected by *Pythium irregulare* (12), barley infected by *Erysiphe graminis* f. sp. *hordei* (13), wheat infected by barley yellow dwarf virus (8), and others. However, less disease or no change in response to increased plant density also has been observed for fungal pathogens (10,26) and particularly for vectored viruses (1,19,23). The importance of disease as an agent of density-dependent mortality in nonagricultural systems is beginning to be studied, but causal relations and positive correlation between density and disease are not always clear (2,4,7,17,33).

The mechanism by which host density affects disease, and, therefore, the changes in disease resulting from a change in density, may be more complex than suggested above. For example, requirements for alternate hosts may interact with density (33). A number of mechanisms labeled "indirect effects" by Burdon and Chilvers (14) influence disease after dispersal, such as changes in microclimate or host nutritional status due to density changes. Waggoner (34) points out that higher density will increase the probability of pathogen propagule impact but also may decrease the velocity of spread because of the short distance between inoculum source and target. In one study, however, velocity of spread increased with density (18).

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Density may affect dispersal further by influencing the transport medium. For example, wind velocity and turbulence may be altered by density changes (suggested by English et al. [21]). In the case of splash dispersal, the quantity, size distribution, and spatial pattern of impacting water drops may be altered by changes in plant density. Inoculum on soil or plant material may be impacted by fewer drops under a dense canopy than under a sparse canopy, and drops, on average, will be larger and slower due to leaf drip (6). Studies on *Phytophthora cactorum* and *Colletotrichum acutatum* J.H. Simmonds on strawberries have shown that less intense rain (lower volume per time) reduces spore dispersal distances (24), and larger drops carry more spores and result in greater travel distances for splash droplets (32,37).

These studies were done primarily to determine the effects of uniform changes in strawberry (*Fragaria* × *ananassa* Duchesne) density on dispersal of *C. acutatum* conidia from a point inoculum source. Secondary factors of rain intensity and inoculum location were evaluated to determine how they affect dispersal and interact with density. By evaluating several response variables (deposition at various locations, deposition integrated over time and area, and dispersal gradient parameters) and incorporating them into an analysis that explicitly evaluates factor interactions, we were able to examine several possible mechanisms by which plant density influences splash dispersal.

MATERIALS AND METHODS

Experimental setup. A previously described rain simulator (30) was used to provide rainfall of consistent and uniform intensity over an artificial strawberry canopy and surrounding area. This canopy was created by arranging strawberries in two 90-cm-long rows, 76 cm apart, in a 1.2 × 1.2-m wooden frame containing a

layer of soil 8 cm deep. Each strawberry "row" consisted of mature (fruiting) greenhouse-grown plants harvested immediately before the trial and placed in a portable support structure. These support structures were positioned in troughs in the wooden frame and buried so only the aerial parts of the plants were exposed. The support structures consisted of a 90-cm-long, 7.6-cm-diameter PVC pipe resting horizontally in a galvanized steel frame. The pipe was sealed at both ends and drilled with a row of 1.7-cm-diameter holes, at intervals of 2 cm, at the top of the pipe along its entire length. Before each experiment, the pipe was filled with water, plant roots were removed approximately 2 cm below the crown, and plants were placed in the holes. A mechanism consisting of two PVC strips bolted to the pipe and cut to accommodate the plants were clamped together using C-clamps to keep plants upright.

A multifactorial randomized complete block design was used, with four levels of plant density, two levels of rain intensity, and two positions of source inoculum. The 16 possible treatments each represent a single trial ("run") in the simulator. These were done in random order to complete a block, and three such blocks were completed sequentially (48 trials total). The intent of the blocking was to remove differences due to seasonal variation in plant growth and plant cultivar ('Midway' or 'Selva') in the greenhouse.

Three plant density levels within rows were achieved by using spacings of 2, 4, and 8 cm between plants in the support structure, corresponding to 100, 50, and 25% of the maximum possible within-row plant density. A fourth density, corresponding to 75% of the maximum, resulted from randomly eliminating a single plant from every group of four plants along a row. Rain intensities of 15 or 30 mm h^{-1} were generated with different spray nozzles in the simulator (30). A source of *C. acutatum* inoculum, which consisted of five heavily-sporulating strawberry fruit prepared as previously described (37), was placed either near the crown of the end plant in a row (protected by the canopy) or 20 cm from this point between the rows (outside the canopy and exposed to the rain). Each of the 48 trials used fresh plant material, inoculum, and sterilized soil. The assignment of the row containing (or nearest to) the inoculum and of the end of the row at which inoculum was placed were random for each trial.

Data collection. Spore deposition was assessed by exposing sheltered 6-cm-diameter petri plates of dextrose peptone yeast extract agar (DPYA), a selective medium for *C. acutatum* (20), at locations 20, 40, 60, 80, and 100 cm from the inoculum source along three transects: i) within the canopy of the row with or near the inoculum; ii) within the row without the inoculum (or farther from the inoculum); and iii) outside the canopy between rows. For trials in which the inoculum was placed within the canopy of one row, placement locations at 20 and 40 cm from the inoculum within the canopy of the other row were not possible because of the 76-cm distance between row centers. Likewise, for trials in which the inoculum was placed between rows, the 20-cm location within the row farthest from the inoculum was not possible. Each petri plate was placed under a 80-cm^2 roof of galvanized steel, supported 11 cm above the soil, to shelter the agar from the direct impact of raindrops (39). Plates along each transect were staggered to minimize interference with direct (straight-line) spore movement to plates farther away. All within-canopy locations of plates were on the side of the strawberry crowns nearest the inoculum source, except for treatments in which inoculum was located within the canopy. In this case, at the 60- and 80-cm locations only, plates were positioned on the side of the crown opposite the source to minimize interference with direct spore movement by intervening plates.

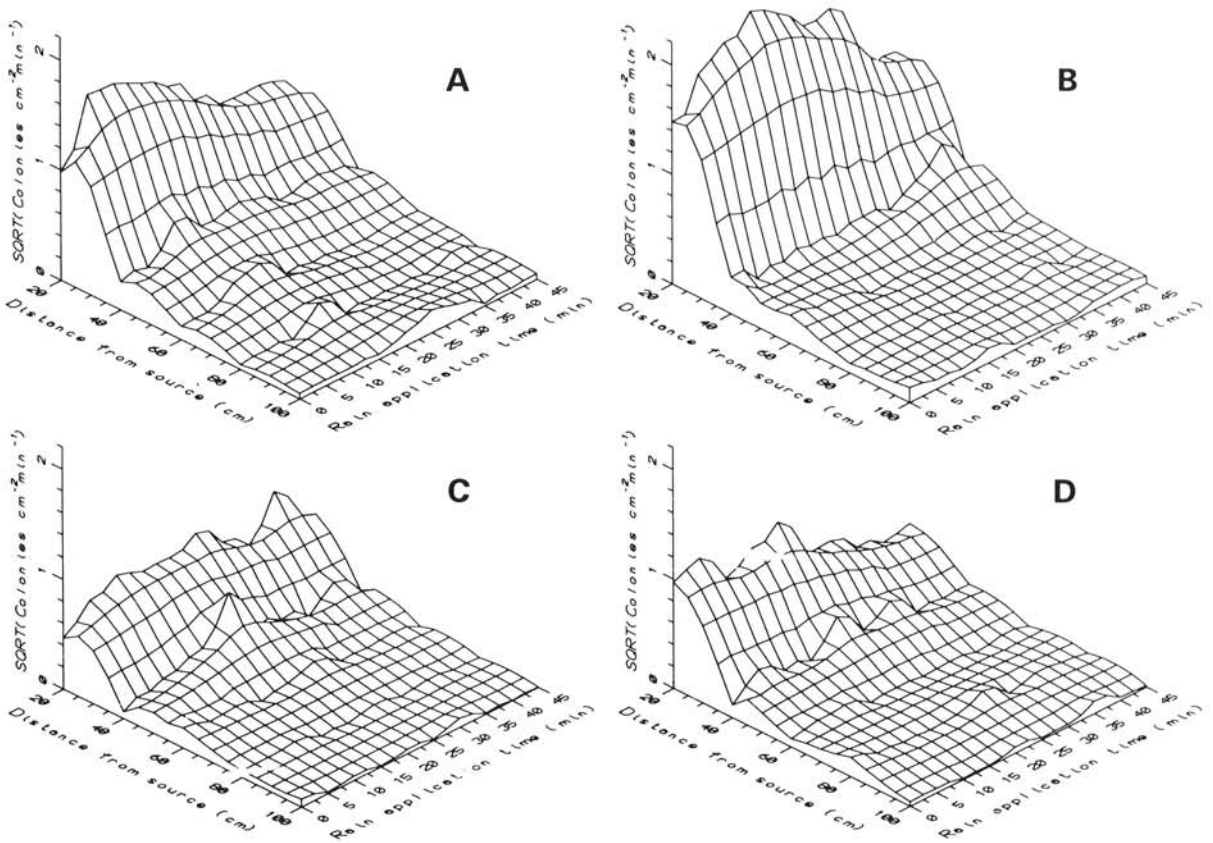

Fig. 1. Deposition of *Colletotrichum acutatum* conidia in an artificial strawberry canopy within the row nearest the inoculum source during a 46-min simulated rain, as influenced by plant density. All points are derived through interpolation from mean values of 12 trials performed at a given density (combined data from three blocks for each of two rain intensities and two inoculum locations). For each trial, petri dishes were exposed for 1 to 2 min at 5-min intervals at 20, 40, 60, 80, and 100 cm from an inoculum source. Maximum density was 2 cm between plants within rows. **A,** 25%; **B,** 50%; **C,** 75%; and **D,** 100% of maximum density. Square-root scale used to better visualize high and low deposition rates on the same graph.

Each trial involved first wetting the plants and soil for 2 min with no inoculum present. In the first block of trials (i.e., the first 16 runs), control plates were exposed to 1 min of rain in each transect at the locations most proximal and most distal to the location at which the source would be placed. Inoculum was added, and plates were exposed for 1-min periods every 5 min after rainfall had been initiated, i.e., 0 to 1, 5 to 6, 10 to 11 min, and so on up to 45 to 46 min. The exposure times for all plates, including controls, were increased to 2 min during the second and third trial blocks. Exposed plates were incubated at 26°C for 72 h, then placed at 4°C for 2 to 10 days, when colonies with morphologies characteristic of *C. acutatum* were counted (39). For all blocks, deposition rate was determined as conidia per square centimeter per minute. Ten plants were randomly sampled at the end of each trial from each row. They were washed to remove soil, and the area of each leaf on each plant was determined with an electronic meter (Li-Cor, Lincoln, NE).

Analysis. The leaf area index (LAI) for each trial was determined by multiplying the leaf area per plant for that trial by the total number of plants, then dividing by the area of the simulated canopy. Mean LAI was determined for each of the four treatments, and analysis of variance (ANOVA) was used to verify that the four treatments corresponded to distinct LAI classes.

Total deposition at each sample location for each trial was estimated by integrating the rates of spore deposition (spores per square centimeter per minute) for each location over the entire time of the trial. This was accomplished using a midpoint-mean method, which is analogous to calculating the area under the disease progress curve (16). Total spore deposition for the entire area between canopies and for the area of the canopy in the rows nearest and farthest from the inoculum were estimated by using time-integrated deposition values for a given location, as calculated above, and integrating over area. The midpoint-mean method was again employed, this time by multiplying mean spore deposition-per-square-centimeter values for two adjacent sample locations by the area of canopy or soil between those locations, i.e., of circular arcs truncated by the boundary between the canopy and bare soil. Estimates of total deposition for both rows and the area between them were summed to obtain an estimate of total deposition in the entire plot.

The effect of distance from source on deposition within and between rows also was assessed by fitting a negative exponential dispersal model to the calculated total deposition at each sample location. This was done by regressing the log(1 + total deposition per square centimeter) on distance (centimeters). The slope (b) is a measure of gradient steepness, and the intercept (log[a]) is an estimate of logarithm of deposition at 0 cm. Gradients were determined separately for the transect between canopies and for the transect within the canopy of the row containing the inoculum. The third transect, i.e., the row farthest from the inoculum source, was not included in this analysis due to the absence of the 20-cm sample location.

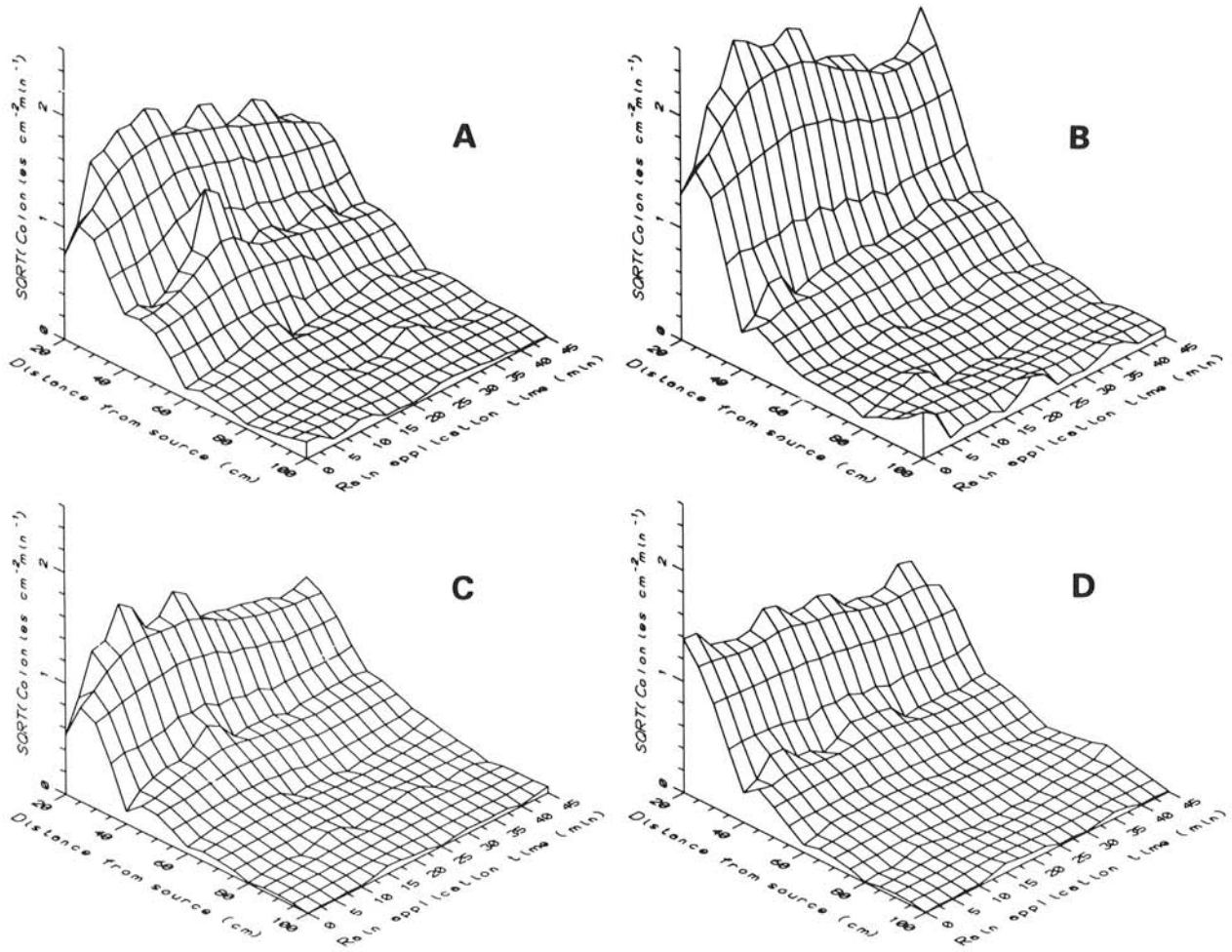

Fig. 2. Deposition of *Colletotrichum acutatum* conidia between rows of an artificial strawberry canopy during a 46-min simulated rain, as influenced by plant density. All points are derived through interpolation from mean values of 12 trials performed at a given density (combined data from three blocks for each of two rain intensities and two inoculum locations). For each trial, petri dishes were exposed for 1 to 2 min at 5-min intervals at 20, 40, 60, 80, and 100 cm from an inoculum source. Maximum density was 2 cm between plants within rows. **A,** 25%; **B,** 50%; **C,** 75%; and **D,** 100% of maximum density. Square-root scale used to better visualize high and low deposition rates on the same graph.

The effects of rain intensity, plant density, inoculum exposure, and their interactions on the response variables (total deposition at each sample location, total deposition for various areas, b, and $\log[a]$) were determined with ANOVA. Separate analyses were done for each row and between rows because of the unequal number of sampling plates in each area. Orthogonal polynomials were calculated to determine if there were linear, quadratic, or cubic changes in response variables with plant density. Tukey's test (41) was used to separate means when a factor or interaction was significant. Log transformations of the deposition data were necessary to satisfy the assumptions of ANOVA because of the relationship between the variance and mean of deposition data (25). Mean values for treatments were presented in the original units by back-transforming the means of the transformed values.

RESULTS

There was no significant effect of block on spore deposition for any location or area evaluated by ANOVA, nor on gradient slope or intercept ($P > 0.10$). Although all trials were conducted at different times using different plants, this did not have a systematic effect on experimental outcome.

LAI. Mean LAI was 3.76 (SE = 0.32), 3.17 (SE = 0.21), 1.79 (SE = 0.15), and 0.93 (SE = 0.06) for plant densities of 100, 75, 50, and 25% of maximum density, respectively (2, 3, 4, and 8 cm between plants within rows). Expressed as percentage of maximum LAI, values are 100, 84, 48, and 25% of 3.76, corresponding approximately to the range of densities used. As expected, LAI values were significantly affected by plant density ($P < 0.001$). LAI varied significantly with blocks, which corresponded to the time of year in which trials were conducted.

Gradient analysis. Decline in spore deposition with distance from the source occurred in all trials, similar in general pattern to the surface mean plots in Figures 1 through 3. Between-row data generally were described well by the negative exponential model (75% of the coefficients of determination [r^2] were greater than 0.79) and significant ($P < 0.05$).

Between rows, slopes of the fits of the linearized negative exponential model to spore deposition data were not affected by density ($P = 0.187$, based on ANOVA), but intercept values were affected by density ($P = 0.013$) (Table 1). Intercepts for the 25 and 50% plant densities were significantly higher than those for 75 and 100% densities ($P < 0.05$) by Tukey's test. Partitioning the density effect into orthoganol polynomials indicated that linear ($P = 0.007$) and cubic ($P = 0.067$) components both contributed to these changes but that the quadratic term did not ($P = 0.985$).

The intercept of the dispersal gradient increased as rain intensity was increased ($P = 0.002$) (Table 1) between rows, and gradient slopes tended to be steeper at higher rain intensity ($P = 0.089$). Inoculum location also affected the intercept of the spore

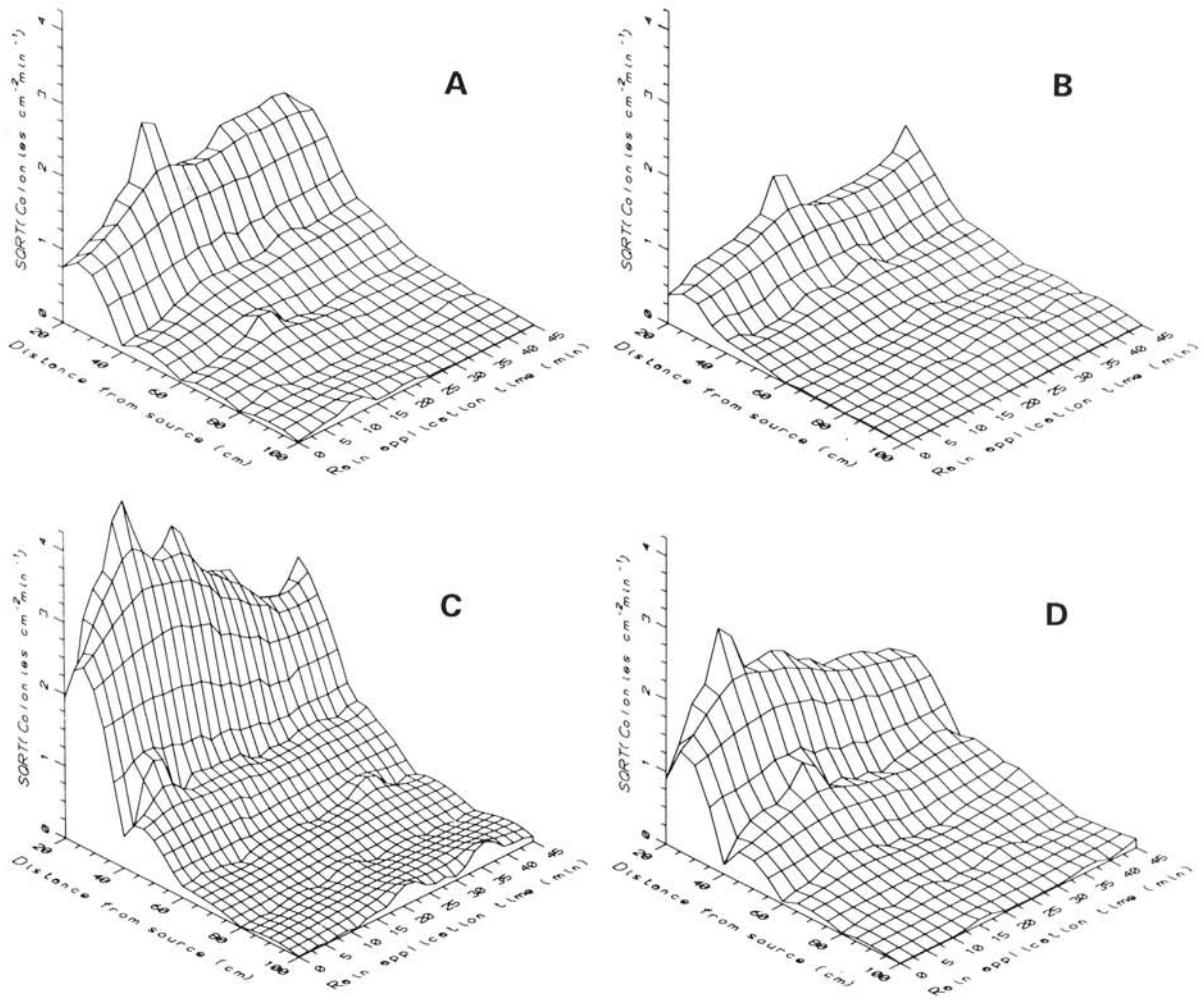

Fig. 3. Deposition of *Colletotrichum acutatum* conidia between rows of an artificial strawberry canopy during a 46-min simulated rain, as influenced by plant density and inoculum position. All points are derived through interpolation from mean values of six trials performed at a given density and inoculum location (combined data from two rain intensities × three blocks). For each trial, petri dishes were exposed for 1 to 2 min at 5-min intervals at 20, 40, 60, 80, and 100 cm from inoculum source. Maximum density was 2 cm between plants within rows. **A and B,** 50 and 75%, respectively, of maximum density, inoculum source within canopy; **C and D,** 50 and 75%, respectively, of maximum density, inoculum source outside canopy. Square-root scale used to better visualize high and low deposition rates on the same graph.

deposition gradient, which increased when inoculum was located outside the canopy ($P = 0.016$). The slope was not affected. No interactions for any combination of plant density, rain intensity, and inoculum location were significant for either slope or intercept of between-row dispersal gradients ($P > 0.10$).

Within the strawberry row nearest the inoculum, the decline in spore deposition with distance from the source appeared less rapid than that between rows (Fig. 1 versus Fig. 2). In fact, dispersal gradients of the negative exponential model based on total deposition over 45 min were not significant for within-row data in 31 (65%) of the trials (failure to reject H_0:$b = 0$ at $P = 0.05$). The coefficients of determination [r^2] for all 31 trials were less than 0.75. Because the model did not account for the data well, comparisons of slope and intercept using ANOVA were not made among treatments for within-row data. However, it is clear that the presence of plants affects dispersal gradients, in that a clear gradient could be observed on bare soil between rows but not within rows. This was true even though altering plant density had no effect on gradient slopes measured between rows, as indicated above.

TABLE 1. Parameters resulting from fits of a linearized negative exponential dispersal model to data of *Colletotrichum acutatum* conidia deposition between rows of an artificial strawberry canopy during a 46-min simulated rain, as influenced by plant density, inoculum source location, and rain intensity

Treatment	Model parameters[a]	
	Slope	Intercept
Density[b]		
25	−0.040	4.00
50	−0.038	3.85
75	−0.031	2.97
100	−0.035	3.04
Significance level (P)[c]	0.187	0.013
SED[d]	0.0032	0.290
Location of inoculum source		
Within canopy	−0.035	3.08
Outside canopy	−0.036	3.76
Significance level (P)	0.925	0.016
SED	0.0022	0.205
Rain intensity		
15 mm h^{-1}	−0.033	2.98
30 mm h^{-1}	−0.038	3.78
Significance level (P)	0.089	0.002
SED	0.0022	0.205

[a] Mean values derived from three replicate trials in which petri dishes were exposed for 1 to 2 min at 5-min intervals at 20, 40, 60, 80, and 100 cm from inoculum source. Slope and intercept calculated by least-squares regression of log(CFU) on distance from source for each trial.
[b] Percentage of a maximum density of 2 cm between each plant.
[c] Probability of falsely rejecting H_0: Main effect (density, source location, or rain intensity) does not influence parameter, determined by analysis of variance.
[d] Standard error of the difference. Error df = 26 for slope and 28 for intercept.

Density effects on spore deposition. Deposition rates for *C. acutatum* conidia within (Fig. 1) and between strawberry rows (Fig. 2) increased during approximately the first 15 min of rain, then declined for positions near the source and at low plant density. Graphs of mean spore deposition plotted as a function on time and distance (Figs. 1 through 3) revealed that fewer spores were deposited at high plant densities but that the effect was not linear. A large reduction occurred between 50 and 75% density, with little discernible difference in deposition between 75 and 100%. Further, there tended to be an increase in mean deposition between 25 and 50% density between rows. This pattern was more clearly apparent in area data (Table 2), in which total deposition was integrated over space and time. ANOVA indicated a highly significant effect of plant density on logarithm of deposition ($P < 0.01$). Based on Tukey's test, no significant difference in deposition occurred between 75 and 100% density, but a significant (more than fivefold in magnitude) increase in deposition occurred (in absolute units) as density was reduced from 75 to 50% ($P < 0.05$). From 50 to 25% density, deposition did not significantly increase further.

The nonlinear increase in deposition as density declined was evident when the density factor was partitioned into orthogonal polynomials. The linear component contributed to changes in deposition ($P < 0.05$ for all areas sampled), and the quadratic term did not ($P > 0.25$ for all areas sampled), but nonlinearity was apparent in the significant contribution of the cubic term, in which $P = 0.060$, 0.015, 0.104, and 0.020 for the near-row, between-

TABLE 2. Estimated logarithms of total deposition of *Colletotrichum acutatum* conidia (back-transformed means in parentheses) in an artificial strawberry canopy during a 46-min simulated rain, as influenced by plant density, inoculum source location, and rain intensity

Treatment	Area of deposition[a]			
	Near row	Between rows	Far row	Whole plot
Plant density[b]				
25	4.3 (20,456[c])	4.7 (47,303)	3.3 (2,227)	4.9 (84,918)
50	4.5 (31,707)	5.0 (103,495)	3.7 (5,089)	5.2 (151,747)
75	3.8 (6,163)	4.3 (19,699)	2.8 (695)	4.5 (28,849)
100	3.6 (4,088)	4.3 (20,402)	2.7 (460)	4.5 (28,443)
Significance level (P)[d]	0.002	0.007	0.027	0.004
SED[e]	0.23	0.22	0.36	0.22
Location of inoculum source				
Within canopy	3.5 (3,137)	4.2 (15,974)	2.5 (346)	4.3 (22,092)
Outside canopy	4.6 (40,747)	4.9 (87,813)	3.7 (5,496)	5.2 (147,191)
Significance level (P)	<0.001	<0.001	<0.001	<0.001
SED	0.16	0.16	0.26	0.15
Rain intensity				
15 mm h^{-1}	3.9 (7,966)	4.2 (16,488)	2.9 (875)	4.5 (29,198)
30 mm h^{-1}	4.2 (16,046)	4.9 (85,073)	3.3 (2,175)	5.0 (111,368)
Significance level (P)	0.074	<0.001	0.131	0.001
SED	0.16	0.16	0.26	0.15

[a] Areas relative to inoculum source; near row = row containing or nearest inoculum, far row = row farthest from inoculum. Areas, averaged over all trials, were 3,510 cm^2 for both rows, 3,240 cm^2 between rows, and 10,260 cm^2 for the entire plot.
[b] Percentage of a maximum within-row spacing of 2 cm between each plant.
[c] Antilog$_{10}$ of mean of three replicate trials. Each replicate is $\log_{10}(x + 1)$, where x is calculated by integrating, over total time of rainfall (46 min) and area of deposition, CFU in petri dishes.
[d] Probability of falsely rejecting H_0: Main effect (density, source location, or rain intensity) does not influence deposition, determined by analysis of variance performed on log-transformed data.
[e] Standard error of the difference. Error df = 30 in all cases.

row, far-row, and whole-plot areas, respectively. These results were consistent for either row, between the rows, and, therefore, over the whole plot. In addition, more spores were deposited between rows than within rows and in the row nearest the source than in the far row. Separate ANOVA performed on deposition at 20, 40, and 60 cm distances, integrated over time, showed the same pattern (M. A. Boudreau, *unpublished data*); at 80 or 100 cm, few spores were sampled, and no differences among treatments could be detected.

Rain intensity effects on spore deposition. Increasing rain intensity from 15 to 30 mm h^{-1} doubled the deposition within rows (Table 2). There also was a large and significant ($P < 0.001$) increase in deposition between rows, and, therefore, for the whole plot, as intensity increased. Rain intensity did not significantly ($P > 0.05$) interact with other factors or factor combinations, based on ANOVA.

Inoculum location effects on spore deposition. Inoculum source location strongly influenced deposition (Table 2). More than six times the number of spores (absolute units) were deposited in the plot when inoculum was located outside rather than within the canopy, and this increase was highly significant for all areas of the plot ($P < 0.001$).

For sources of inoculum located both within and outside the canopy, the graphs of mean spore deposition over time and distance (Fig. 3) indicated that deposition tended to decrease as plant densities increased. ANOVA indicated that the interaction of plant density and inoculum source location was marginally significant ($P = 0.083$) for spores sampled between rows and for the entire plot (P = 0.097) (Table 3). Deposition was only reduced at high plant density (75 or 100% compared to 25 and 50%) when inoculum was located within the canopy. When spores were sampled between rows, plant density only had an effect when the inoculum source was placed within the canopy row.

DISCUSSION

The main factor under consideration in this study, within-row plant density, had consistent effects on dispersal. Yang et al. (39) observed large reductions in *C. acutatum* deposition with increased strawberry LAI using the same rain simulator system, but dispersal only across one or two rows was considered, and only two canopy densities were compared. No consideration of spore transport within versus between canopies was made in their study. The design of the current experiments allowed some determination of the mechanisms responsible for the observed reduction in deposition with increasing density. The mechanisms relate to rain intensity and inoculum source location and will be discussed after these factors are considered.

Effects of rain intensity on disease incidence have been ambiguous, particularly in field studies, for several splash-dispersed pathogens, including *C. acutatum*, *Rhynchosporium secalis*, *Phytophthora cactorum*, and *P. capsici* (11,22,31,40). For example, rain intensity did not significantly affect incidence of strawberry anthracnose in simulated rain studies (40) nor was mean rain intensity a predictor of strawberry leather rot incidence in regression studies using field plots (31). In the study reported here, spore deposition increased with higher rain intensity, which corroborates earlier work on *C. acutatum* (39). These results may be due to the increased number and size of drops in more intense rains (24) and the reproduced accuracy in the rain simulator (30). Larger drop impactions have greater kinetic energy, entrain more *C. acutatum* spores in resulting splash droplets, and transport spores longer distances in larger droplets compared to impaction by smaller drops (35,37,38). These factors affect removal of spores from the source fruit, supported here by the effects of intensity on dispersal gradient height (log[*a*]).

We observed that inoculum placed under the canopy resulted in much less deposition than inoculum placed outside the canopy. This probably was due to the reduced removal of spores from source fruit, since rain intensity decreases under a canopy due to interception by leaves and stems (6,29). Some of the incident drops coalesce and drip from leaves, however, and the large size spectrum of these drops relative to unimpeded rain has been studied in terms of their high soil-erosion and spore-dispersal potential (28,32). If rain interception is responsible for the inoculum location effects in our study, then leaf drip does not play a large role in splash dispersal. This may be related to the short stature of strawberry plants, which prevents drops from leaves from reaching a significant fraction of terminal velocity. Therefore, a different pattern of splash dispersal than we observed for strawberries might result for plants with different physiognomic characteristics.

Possible mechanisms of reduction in spore deposition include reduction in spore removal from the inoculum source (mechanism 1); reduction in resplash of spore-carrying droplets on the surface (mechanism 2); and interception of spores by the plant canopy (mechanism 3). All three mechanisms may operate in nature.

In the first case, increasing plant density may diminish rain in-

TABLE 3. Estimated logarithms of total deposition of *Colletotrichum acutatum* conidia (back-transformed means in parentheses) in an artificial strawberry canopy during a 46-min simulated rain, as influenced by plant density × inoculum source location interaction

Treatment		Area of deposition[a]			
Inoculum source location	Plant density[b]	Near row	Between rows	Far row	Whole plot
Within row	25	3.9 (8,876[c])	4.6 (40,986a)	3.0 (1,020)	4.8 (62,305)
	50	4.1 (11,471)	4.7 (53,253)	3.3 (1,863)	4.9 (75,012)
	75	3.2 (1,584)	3.7 (5,404)	2.0 (98)	3.9 (7,739)
	100	2.8 (601)	3.7 (5,519)	1.9 (77)	3.8 (6,587)
Outside row	25	4.7 (47,145)	4.7 (54,592)	3.7 (4,862)	5.1 (115,742)
	50	4.9 (87,640)	5.3 (201,136)	4.1 (13,904)	5.5 (306,987)
	75	4.4 (23,779)	4.9 (71,808)	3.7 (4,935)	5.0 (107,545)
	100	4.4 (27,823)	4.9 (75,417)	3.4 (2,736)	5.1 (122,826)
Significance level (P)[d]		0.213	0.083	0.421	0.097
SED[e]		0.33	0.31	0.51	0.31

[a] Areas relative to inoculum source; near row = row containing or nearest inoculum, far row = row furthest from inoculum. Areas, averaged over all trials, are 3,510 cm^2 for both rows, 3,240 cm^2 between rows, and 10,260 cm^2 for entire plot.

[b] Percentage of a maximum density of 2 cm between each plant.

[c] Antilog$_{10}$ of mean of three replicate trials. Each replicate is $\log_{10}(x + 1)$, where x is calculated by integrating, over total time of rainfall (46 min) and area of deposition, CFU in petri dishes.

[d] Probability of falsely rejecting H_0: Density × source location interaction does not influence deposition, determined by analysis of variance performed on log-transformed data.

[e] Standard error of the difference. Error df = 30 in all cases.

tensity at the impaction sites, as discussed above, and, thus, reduce the number of spores removed from source fruit located under the canopy. Our observation that dispersal gradient intercept, but not slope, decreased as density increased is consistent with this mechanism. Furthermore, it is likely that once an inoculum source is sufficiently sheltered from direct rainfall, the value of additional leaf area to intercept more rain will diminish. This explains, in part, the nonlinear relationship between plant density (or LAI) and spore dispersal, indicated by the significant effects of the cubic orthogonal polynomials for the density factor. In our case, a threshold for rain interception may occur between 50 and 75% density.

One difficulty with mechanism 1 (reduced spore removal from the source) being solely responsible for density effects on dispersal is that this mechanism would predict a strong density × inoculum source location interaction. Although absolute values for spore deposition (back-transformed figures in Table 3) may appear to indicate such an interaction, the log-transformed values and the statistical tests performed on them indicate only a weak interaction (Table 3). Madden (25) has shown this transformation to be appropriate theoretically (based on Taylor's power law) and empirically for this experimental system, and it adequately satisfied the assumptions for ANOVA in the experiments reported here. Our results indicate that inoculum exposed to direct rainfall (i.e., between rows; Table 2) responded to changes in density, as indicated by the significant main effect of density. One explanation is related to mechanism 2, in which rain interception by the canopy might influence dispersal by reducing resplash of spores on soil as plant density increases. Spores originating and sampled outside the plant canopy can still travel within the canopy for one or more splash events if the random walk theory of splash dispersal is tenable (27,36). Because splash of water drops away from strawberry canopies is greatly affected by plant density (35), increasing density would affect the movement of spores within and across canopies, even when the source of spores is not covered by the canopy. The weak interaction of density and inoculum source location also indicates that density effects are most pronounced for within-row inoculum.

The third mechanism by which density can affect dispersal, that of spore interception by the plants prior to deposition at the final infection sites, also reduces overall deposition. This would not necessarily be affected by source location, but would be expected to alter within-row deposition primarily. Again, the weak density × inoculum location interaction indicates that this mechanism is at work in our system but only in combination with other mechanisms.

Previous work indicated alterations in dispersal gradient steepness due to changes in surface roughness or the presence of plants (39,40). Gradient steepness relates to horizontal movement of spores (affected by mechanisms 2 and 3), rather than removal from source (mechanism 1). Yang et al. (36,39) found that increased roughness of ground cover (plastic versus soil versus straw) increased steepness of spore deposition gradients, and this could be attributed to similar changes in gradients for droplet number and mass (35). Decreases in gradient steepness were observed when plants were incorporated into rain-splash experiments, either for gradients of *C. acutatum* spores, water droplet number, or droplet mass (35,39).

In our study, plant density did not affect gradient steepness, but the results are not directly comparable with the earlier work. Although plant density can be regarded as affecting surface topography, the spatial scale is different from that addressed in the previous studies (e.g., variation in surface height due to sparse plants versus variation due to straw mulch). Moreover, in prior studies spore deposition rates were not measured between rows (39), and splash droplet gradients were measured away from plants in areas protected from direct rainfall (35). It is notable, however, that Yang and Madden (35) found that, although plants reduced gradient steepness of droplet number and mass compared to no plants, increasing LAI above zero had no influence on gradient steepness, which is consistent with our observations (this was not evaluated in Yang et al. [39]). Although our work suggests that mechanisms 2 and 3 do play a role in splash dispersal, the present and previous studies demonstrate that only gradient height, not steepness, is affected consistently by plant density.

Other unknown mechanisms may be at work in this system, but currently, our results can be fully explained only by requiring that a large fraction of spores deposited between rows travel under the canopy, even if they originate from an inoculum source between the rows. Of course, direction of spore movement should be random in still air on a horizontal surface (27,36), and it may be that sufficiently few spores move between rows exclusively to prevent plant density from having some effect on between-row deposition.

Clearly, the interactions of plant density with dispersal are both real and complex, and these must be coupled to density influence on infection processes and propagule production. A better understanding of these phenomena, and incorporation into a comprehensive model, will be necessary to employ density as an effective disease management or disease risk forecasting tool.

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