Splash Dispersal of *Phytophthora cactorum* from Infected Strawberry Fruit by Simulated Canopy Drip

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ABSTRACT

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Characteristics of splash dispersal of *Phytophthora cactorum* from infected strawberry fruit were studied with an integrated drop-generating and photographic system that was developed previously. Drops of 1- to 4-mm diameter were released from heights of 10, 20, and 40 cm above fruit bearing sporangia that had been labeled with a fluorescent tracer. Splash droplets produced by the impact of the incident drops were collected on sheets of water-sensitive paper. Drops with diameters of 1 and 2 mm failed to produce any splash droplets when released from heights of 20 and 10 cm, respectively. Multivariate regression analysis was used to examine the relationship between a set of intercorrelated responses and attributes of the incident drop. Responses evaluated were 1) the total number of droplets collected, 2) the average droplet diameter, 3) the total droplet volume, 4) the mean distance of droplet travel, 5) the number of droplets bearing at least one sporangium, and 6) the total number of sporangia among droplets with at least one sporangium. Incident drop attributes were drop mass or weight (W) and the velocity (V), momentum (M), and kinetic energy (E) of the drop at impact with the fruit surface. The best multivariate regression model included W, M, and V as predictors of responses 1, 2, and 4. M alone was the best predictor of response 3; in contrast, V was significant alone for responses 5 and 6. Except for droplets produced by the impact of 1-mm-diameter drops, the average diameter of droplets carrying sporangia was always significantly greater than the diameter of droplets not carrying sporangia ($P \leqslant 0.05$). The distribution of the number of sporangia per droplet was generally well described by the logarithmic distribution with zeros for each combination of incident drop diameter and height of release. The λ parameter, representing the proportion of droplets with sporangia, declined in relation to momentum at impact. The θ parameter, a measure of the mean number of sporangia per droplet (of droplets bearing sporangia), declined with increasing mass of the incident drop. There was no relation between droplet size and the number of sporangia per droplet. For all splash droplets and those with sporangia, the distribution over distance was accurately described by the Weibull distribution whose scale parameter (representing the distance within which 63% of the droplets landed) varied linearly and positively with incident drop velocity. The Weibull shape parameter (representing curve skewness) was best described as a function of both drop velocity and kinetic energy or momentum. Droplets with sporangia did not travel as far, on the average, as droplets without sporangia.

Rainfall has been recognized as an important agent for the dispersal of numerous bacterial and fungal plant pathogens since the early work of Faulwetter (4,5). An early comprehensive review of the role that rain plays in pathogen dispersal was given by Gregory (13). Fitt and McCartney (10) also reviewed more recent developments in rain splash dispersal.

Most experimental studies to date dealt exclusively with relatively large-diameter drops (4–5 mm) striking an inoculum-bearing surface at terminal velocity (13,14). However, a theoretical treatment by Saville and Hayhoe (27) brought attention to the potential for inoculum dispersal by relatively low-velocity drops originating as drips within a plant canopy. Numerical analysis of drop microphysics also indicates that large drops falling from rest attain a significant fraction of their terminal-velocity kinetic energy after relatively short fall distances (26).

Research in soil erosion also indicates the possible importance of canopy drip as a mechanism for inoculum movement. Raindrop spectra have been found to be altered in three ways by the passage of rain through a plant canopy: median drop size increases, the drop distribution becomes more uniform, and maximum drop size increases (2,25). Furthermore, Noble and Morgan (23) found that although drip from a brussels sprout canopy amounted to less than 1% of the total volume of a simulated rainfall, the effect of drip on soil erosion was disproportionately large. Similar canopy effects on increased soil erosion have been observed for cereals (19), corn, and soybean (20).

Earlier work by our group examined the dispersal of *Phytophthora cactorum* (Leb. & Cohn) Schroet. from infected strawberry fruit (*Fragaria* × *ananassa* Duch.) when incident drops were released from heights sufficient to attain approximate terminal velocity at impact (15). In the present study, we used a previously described drop-generating system (26) to study effects of drop velocity and size on pathogen dispersal in the same host-pathogen system. The objective of this work was to quantify aspects of the dispersal process under experimental conditions that simulated drip from a strawberry canopy.

MATERIALS AND METHODS

Production of infected fruit. Strawberry plants (cultivar Midway) were grown in 15-cm plastic pots in the greenhouse for 5–7 wk (until reaching the green fruit stage). Green fruit were harvested and inoculated with *P. cactorum* as previously described (15) and then incubated at 20 C and approximately 100% RH in sealed plastic containers for 5 days. Sporangia formed abundantly on fruit surfaces by the end of the incubation period. After incubation, the fruit was soaked in a 0.2% solution of Fluorescent Brightener 28 (Sigma Chemical Co., St. Louis, MO) for 3 hr and then incubated for an additional 24 hr at 20 C and approximately 100% RH.

Drop production and control of impact. A piezoelectric drop generator was used to produce 1- and 2-mm drops, and a solenoid pump drop generator was used to produce 3- and 4-mm drops (26). Infected strawberry fruit was cut in half lengthwise, and a single half was mounted with the cut side down on a stage directly below the drop generator aperture (Fig. 1). The stage was mounted on an *XY* positioning device to position the fruit under the drop generator aperture. Cards of 50- × 75-mm water-sensitive paper (Spraying Systems, Wheaton, IL) were arranged end-to-end, with the long axis of each being parallel to the long axis of the target fruit, and with the fruit oriented so that the calyx end of the berry was distal to the line of cards (Fig. 1). The *XY* positioner was used to position the fruit under the drop generator so that an incident drop would strike the long axis of the fruit at a point approximately half the distance between the calyx and the end of the berry distal to the calyx. Drops produced by the drop generators were released from heights of 10, 20, and 40 cm to simulate canopy drip in strawberry foliage. Velocities at impact were determined from multiple-exposure photographs in order to verify theoretical models for drop velocity (26).

The basic units of observation were the splash droplet traces left on the water-sensitive paper. For each such trace, its distance from the point of incident drop impact on the fruit was recorded with an image analysis system (Dapple Systems, Inc., Sunnyvale, CA). Trace areas were converted to droplet diameters (Fig. 6 in ref. 26). Sporangia contained in each droplet trace were counted by means of fluorescent microscopy. For each treatment combination of incident drop diameter and height of release, 50 drop impacts were performed per replicate. Treatment combinations that failed to produce any splash droplets were only replicated twice. Otherwise, the number of replications per treatment combination varied from three to six.

Statistical analyses. The Weibull cumulative probability density function (CDF) was fit, by nonlinear regression, to the cumulative proportion of splash droplets observed at each distance, for each treatment combination of drop diameter and release height. The Weibull CDF was also fit to the cumulative proportion of droplets with sporangia. The distribution, $F(x)$, can be written as

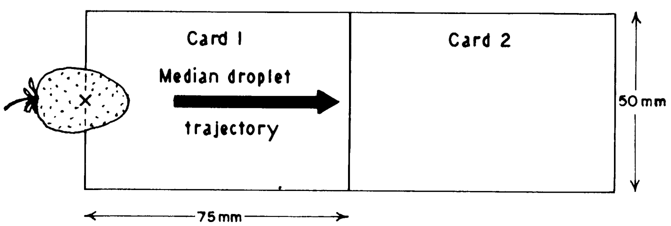

Fig. 1. Schematic diagram of the single-drop impact system showing the location of the first two water-sensitive cards.

$$F(x) = 1 - \exp[-(x/b)^c]$$

in which x represents distance, b is a scale parameter, and c is a shape parameter (24). $F(x)$ indicates the probability of a droplet's traveling a distance less than or equal to x (measured in centimeters). The scale parameter has units of distance and equals the distance within which 63% of all droplets landed. The shape parameter is dimensionless and indicates the skewness of the distribution. Fits were obtained for both the combined results of all replicates of a treatment and for replicates individually, or subsets of replicates in cases where a single replicate provided too few observations on the splash distance distribution. Linear regression was subsequently used to describe the relationships between, on the one hand, the estimated Weibull shape and scale parameters and, on the other hand, the incident drop mass or weight (W, in grams) and the velocity (V, in centimeters per second), momentum (M, in gram centimeters per second), and kinetic energy (E, in gram square centimeters per second squared) at impact (Table 1).

The distribution-fitting program of Gates and Etheridge (12) was used to identify an appropriate discrete probability distribution for the number of sporangia per splash droplet. Because of the relative scarcity of splash droplets that carried sporangia, fits were only obtained for the combined results of all replicates of a given treatment combination of drop diameter and release height.

Regression analysis (univariate and multivariate) was performed to evaluate the relation between measured or calculated variables from each replicate over all splash distances and the incident drop characteristics, W, V, M, and E. The variables for a replicate were the total number of droplets collected, the average diameter of droplets (in millimeters), the total volume of droplets (in cubic millimeters), the average horizontal distance of splash droplet travel (in millimeters), the number of droplets bearing at least one sporangium, and the total number of sporangia among droplets carrying one or more sporangia. Because of increasing variance with increasing means, all responses involving counts were transformed to the natural logarithm of the sum of the observed count plus 1, prior to regression analysis, in order to stabilize variances.

Means and standard errors for the variables listed above were calculated for each combination of drop diameter and release height. The variables were not transformed for these statistics. Pairs of means were compared with t-tests, using the formula for unequal variances.

RESULTS

Splash droplets. A total of 2,800 drop impacts were performed, producing a total of 12,467 droplets that were collected on water-sensitive paper, digitized for size, and observed for the presence of sporangia. Drops with diameters of 1 and 2 mm failed to produce droplets when released from heights less than 40 and 20 cm, respectively. Including only treatment combinations that resulted

TABLE 1. Theoretical attributes of drops released from various heights

Diameter (mm)	Height of fall (cm)	Mass (mg)	Velocity (cm/sec)	Percentage of terminal velocity (%)	Momentum (g·cm/sec)	Kinetic energy (g·cm^2/sec^2)
1	10	0.52	143	37.8	0.07	5.3
	20	0.52	189	50.0	0.10	9.3
	40	0.52	243	64.3	0.13	15.3
2	10	4.19	173	26.8	0.73	62.7
	20	4.19	234	36.3	0.98	114.7
	40	4.19	311	48.2	1.31	202.6
3	10	14.14	182	23.2	2.57	234.2
	20	14.14	248	31.6	3.50	434.8
	40	14.14	332	42.3	4.68	779.3
4	10	33.51	182	21.5	6.10	555.0
	20	33.51	249	29.5	8.34	1,038.8
	40	33.51	335	39.6	11.22	1,880.3

TABLE 2. Summary of droplet responses by incident drop diameter and height of release

Incident drop			Droplet responses[a]					
Drop diameter (mm)	Height of fall (cm)	Number of replicates	TN[b]	AD (mm)	TV (mm^3)	$\bar{X}$ (mm)	NBS	TS
1	40	6	15.17	0.30	0.04	16.85	0.83	0.83
			4.53	0.05	0.04	5.71	0.31	0.31
2	20	6	21.00	0.31	0.04	44.90	6.50	25.33
			5.10	0.08	0.03	10.76	1.23	6.83
2	40	6	58.17	0.35	0.07	46.47	8.17	17.00
			6.01	0.09	0.06	13.21	2.27	5.52
3	10	6	71.17	1.05	1.19	30.43	2.00	4.67
			6.76	0.23	0.73	7.43	0.63	2.22
3	20	6	176.67	0.76	0.53	43.74	19.67	97.00
			19.65	0.19	0.42	11.79	4.07	33.62
3	40	6	380.17	0.53	0.18	47.11	54.67	165.00
			32.02	0.13	0.15	13.43	8.25	44.53
4	10	3	56.33	1.43	3.47	26.91	3.67	5.00
			7.69	0.50	3.37	10.81	0.67	2.00
4	20	5	440.80	0.93	1.16	35.38	24.20	27.80
			33.46	0.29	1.04	10.84	1.62	2.48
4	40	6	875.67	0.74	0.90	54.81	50.83	63.67
			52.73	0.26	1.33	16.17	13.20	16.37

[a]The numbers for each table entry are the mean and the standard error of the estimate. Each estimate is based on 50 drop impacts per replicate. TN = total number of droplets per replicate; AD = average droplet diameter; TV = total volume of droplets per replicate; $\bar{X}$ = average splash droplet travel per replicate; NBS = number of droplets bearing sporangia per replicate; TS = total number of sporangia per replicate.

[b]Droplets were collected on 50- × 75-mm cards of water-sensitive paper, arranged linearly along the median trajectory of droplet travel (Fig. 1).

TABLE 3. Correlations between droplet responses when responses are summarized by replicate within each combination of incident drop diameter and height of release[a]

	TN[b]	AD	TV	$\bar{X}$	NBS[b]
AD	0.62***				
TV	0.56***	0.45**			
$\bar{X}$	0.75***	0.28	0.32*		
NBS[b]	0.83***	0.29*	0.45**	0.70***	
TS[b]	0.76***	0.23	0.31*	0.73***	0.96***

[a]TN = number of droplets; AD = average droplet diameter; TV = total volume of droplets; $\bar{X}$ = average splash droplet travel; NBS = number of droplets bearing sporangia; TS = total number of sporangia. *, **, and *** indicate a significant correlation at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively. Degrees of freedom = 55.

[b]Transformed to the natural logarithm of the sum of the observed count plus 1.

in splash droplet formation, average responses per replicate exhibited strong trends with respect to the diameter of the impacting drop and the height of drop release (Table 2). Within a drop diameter class, the total number of droplets, the number with sporangia, and the average distance traveled always increased with increasing height of drop release. The total number of sporangia also generally increased with drop height for a given diameter. The exception was that the numbers of sporangia were not significantly different ($P > 0.05$) for 2-mm drops released from heights of 20 and 40 cm (Table 2). In contrast, values for average droplet diameter and total droplet volume for 3- and 4-mm drops declined with increasing height of release. No differences in these two variables were apparent for 2-mm drops released from heights of 20 and 40 cm. In fact, impacts of 1-mm drops did not appear to differ from impacts of 2-mm drops with respect to average droplet diameter and total volume.

Responses over all splash distances can also be viewed in terms of changes in drop diameter for a fixed height of release. When comparisons are made on this basis, it can be seen that the total number of droplets, their average diameter, and their total volume generally increased with increasing impact drop diameter for a given height of release (Table 2). In contrast, the average distance traveled and the number of droplets with sporangia appeared to increase initially but then stabilized at large drop diameters (3 mm), and the total number of sporangia showed evidence of a quadratic trend (Table 2).

The variables summarized in Table 2 were, in general, highly correlated (Table 3). This correlation indicated the need for a multivariate regression approach (21) to quantify responses as a function of *W, M, V*, and *E*. The selection of the best set of independent variables describing the joint response was based on the Hotelling T^2 (21). A multivariate linear model with *W, M*, and *V* provided the best fit to the data ($P(T^2 = 1{,}665) < 0.00001$). An examination of the univariate statistics for the response variables indicated that average droplet diameter and the logarithm of the total number of droplets were well described by models involving *W, M*, and *V*; these terms also were significant in the model for average travel distance, but the total variation explained in this case was lower (Table 4). *M* alone was sufficient for predicting total volume. In contrast, variation in the logarithm of the number of droplets with sporangia and the logarithm of the number of sporangia was explained by *V* alone.

The distribution of distances traveled by droplets was positively skewed. Figure 2 contains an example for one replicate. The maximum number of droplets generally occurred at a distance of 7 to 32 mm from the target. The Weibull cumulative distribution function provided a consistently accurate description of the cumulative proportion of droplets over distance (Table 5). Only three R^2 values were less than 99%. Although the significance test results were only approximate for these nonlinear models (18), the extremely high values of the *F* statistics are clearly indicative of good model fits. Furthermore, residuals were typically less than 1% of the observed values. There was little difference between the results for the combined data from all replicates and the results from individual replicates or groups of replicates. A plot of the fitted cumulative distributions (Fig. 3) for all replications combined shows a shift to the right as diameter and fall height increased. Moreover, a stepwise regression analysis of the relation between the Weibull scale parameter (b) and *W, V, M*, and *E*, resulted in the following model (Fig. 4, top):

$$b = 3.64 + 0.15V \quad (1)$$

Equation 1 accounted for 43% of experimental variation

TABLE 4. Univariate regressions of droplet responses on incident drop momentum, mass, and velocity

Droplet response[a]	Regression coefficients[b] Intercept	Momentum (g·cm/sec)	Mass (g)	Velocity (cm/sec)	R^2 [c]	F statistic[d]
TN[e]	−3.46 (0.74***)	−0.53 (0.15***)	237.90 (39.34***)	0.029 (0.003***)	0.92	96.61***
AD	−0.08 (0.18 NS)	−0.21 (0.04***)	77.91 (9.47***)	0.001 (0.0005*)	0.87	55.43***
TV	200.34 (174.00 NS)	113.81 (35.43**)	−10,339.62 (9,272.11 NS)	−1.19 (0.71 NS)	0.66	33.88***
$\bar{X}$	−34.89 (10.30***)	−4.19 (2.10*)	1,374.44 (550.42**)	0.26 (0.04**)	0.61	26.72***
NBS[e]	−2.73 (0.76***)	−0.09 (0.15 NS)	72.11 (40.36 NS)	0.016 (0.003***)	0.69	38.90***
TS[e]	−3.39 (1.16**)	−0.23 (0.24 NS)	101.30 (61.87 NS)	0.021 (0.005***)	0.72	19.39***

[a] TN = total number of droplets; AD = average droplet diameter; TV = total volume of droplets; $\bar{X}$ = average splash droplet travel; NBS = number of droplets bearing sporangia; TS = total number of sporangia.
[b] The standard error of the estimate is shown in parentheses underneath each regression parameter. The significance of each parameter, given that the others are in the model, is indicated by asterisks. *, **, and *** indicate significance at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively. NS = not significant.
[c] Proportion of response variation that is accounted for.
[d] F statistic for significance of the regression. Significance levels are as given in footnote b. Degrees of freedom are 3 and 52.
[e] Response was transformed to the natural logarithm of the sum of the observed count plus 1.

($P[F(1,34) = 25.51] < 0.0001$). Plots of the standardized deleted residuals (22) failed to reveal either a lack of fit or errors of unusually large standard deviation, and no additional terms (e.g., M or E) contributed significantly to the regression. The Weibull shape parameter (c) had a more complex relationship to the

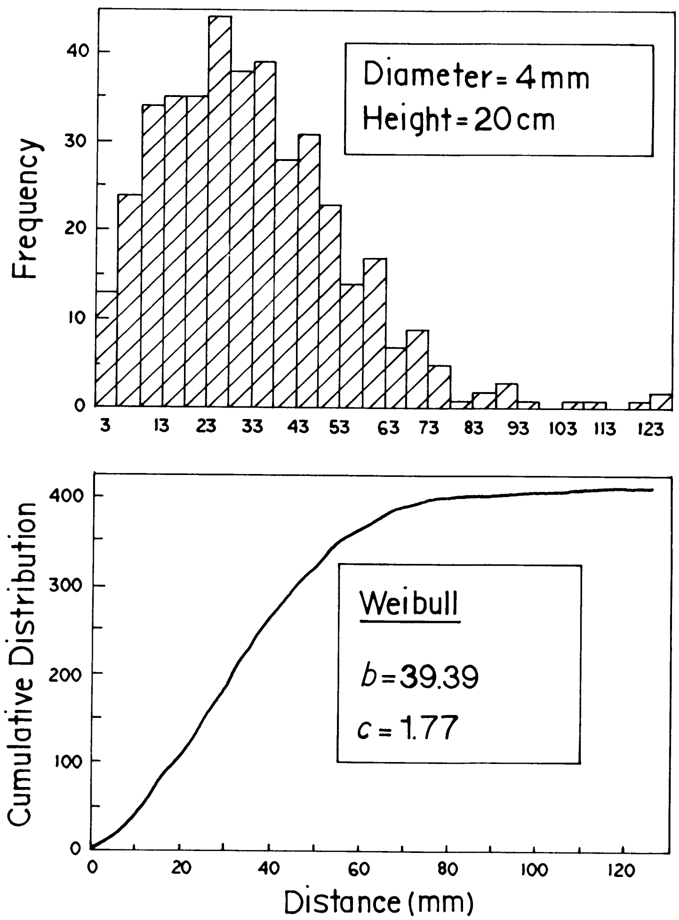

Fig. 2. Frequency distribution (above) and cumulative distribution (below) of splash droplets over distance from the strawberry target. In the frequency distribution graph, droplets were grouped in 5-mm-wide intervals for presentation purposes. The data are from replicate 3.

independent variables (Fig. 4, bottom). The best regression model was found to be

$$c = 2.25 - 0.0026V + 0.00016E \quad (2a)$$

which can also be written as

$$c = 2.25 - V(0.0026 - 0.00008M) \quad (2b)$$

Equation 2b accounted for only 21% of experimental variation ($P[F(2,33) = 4.49] < 0.05$), but, again, an examination of the standardized deleted residuals revealed no deficiencies in the model.

Splash droplets with sporangia. For each treatment combination of incident drop diameter and height of release, we compared the average diameter of droplets bearing sporangia with that of droplets without sporangia (Table 6). The average diameter of droplets bearing sporangia was always significantly larger ($P \leq 0.05$) than that of droplets without sporangia, with the exception of droplets from 1-mm-diameter incident drops, for which there was no difference. A regression analysis was then done to relate the number of sporangia per droplet to droplet diameter, as done by others (10). For each release height and incident drop diameter, there was no significant relationship ($P > 0.20$) between the two variables. Square root and logarithmic transformations of the number of sporangia did not produce any better fits.

Most droplets had no sporangia, and there was a sharp decline in the frequency of droplets with one sporangium, two sporangia, and so on, per droplet (Fig. 5). Because of the large number of droplets with no sporangia (see also Table 6) and the highly skewed frequency distribution (Fig. 5), the number of sporangia in splash droplets was generally best described by the logarithmic distribution with zeros (16) (Table 7). Data were combined for all replicates to obtain enough observations for fitting of the discrete distribution. The logarithmic-with-zeros distribution is defined as

$$P(x) = 1 - \lambda, \qquad x = 0 \quad (3a)$$

$$P(x) = \lambda\theta^x / -x\ln(1-\theta), \qquad x > 0 \quad (3b)$$

in which λ specifies the proportion of nonzero observations, and θ is related to the expectation of the nonzero observations. $P(0)$ indicates the probability that a droplet has no sporangia; $P(1)$, one sporangium; and so on. As specified by λ, $P(0)$ (i.e., $1 - \lambda$) was very large. Fits of the negative binomial to the data were similar to those obtained with the logarithmic distribution with zeros, but the latter distribution produced a better fit. In contrast, the Poisson and Poisson-with-zeros distributions consistently fit the data very poorly. In both instances in which the logarithmic distribution with zeros failed to fit the observed distribution, a similar lack of fit was obtained with the negative binomial. Moreover, the lack of fit in both instances appeared to be the result of an unusually high frequency in a single cell. The clearest relationship between λ and θ on the one hand and the independent variables W, V, M, and E on the other hand was that involving M or W. Both λ and θ showed an overall decline in response to increasing M. The best model for λ was

$$\lambda = 0.20 - 0.16 \log(M) \quad (4)$$

which explained 51.9% of the variability ($P[F(1,6) = 6.48] < 0.05$).

However, the best model for θ was

$$\theta = 0.87 - 0.06W \quad (5)$$

which explained 75.9% of the variability ($P[F(1,6) = 44.40] < 0.01$). No other terms could be added in a stepwise regression analysis.

As with all droplets, the Weibull CDF provided an excellent fit to the cumulative proportion of droplets with sporangia over distance from the target (Table 8). Because of the small number of droplets with sporangia, data from all replicates were combined. The estimated scale and shape parameters were less than those for all droplets (cf. Table 6). The shape parameter was often 1 or close to 1, indicating an approximately exponential decline in sporangia-bearing droplets over distance (16).

The scale parameter was most highly correlated with velocity at

TABLE 5. Fit of the Weibull cumulative distribution to the number of splash droplets over distance

Drop diameter (mm)	Height of fall (cm)	Replicate[a]	Parameters[b] b	c	MSE[c] ($\times 10^{-4}$)	F statistic[d]	R^2 [e]
1	40	1–3	20.19	1.26	4.2	119	99.6
		4–6	15.21	1.62	27.0	338	97.2
		All	16.79	1.43	5.0	2,566	99.5
2	20	1–3	51.48	1.77	6.4	3,619	99.2
		4–6	44.58	1.55	5.1	2,813	99.5
		All	49.68	1.72	2.0	17,842	99.8
2	40	1–2	49.11	1.31	7.0	4,205	99.1
		3–4	40.20	1.13	9.0	3,092	99.0
		5–6	66.38	1.91	3.0	10.733	99.7
		All	51.70	1.32	3.0	16,347	99.6
3	10	1–2	37.60	1.95	1.0	27.955	99.9
		3–4	35.02	1.92	6.0	5,023	99.4
		5–6	36.03	1.98	4.0	8,420	99.6
		All	34.00	1.90	0.8	55,712	99.9
3	20	1	43.73	1.53	3.0	11,210	99.6
		2	51.81	1.32	2.0	15,930	99.7
		3	44.71	1.53	3.0	7,698	99.7
		4	52.24	1.67	3.0	10,595	99.7
		5	52.56	1.98	3.0	10,568	99.7
		6	51.93	1.63	6.0	6,795	99.4
		All	56.75	1.63	6.0	119,783	99.9
3	40	1	53.74	1.61	2.0	28.207	99.8
		2	54.23	1.23	2.0	26.195	99.8
		3	51.37	1.70	2.0	29.142	99.8
		4	67.91	1.81	2.0	37,820	99.8
		5	35.97	1.34	9.0	4,627	99.0
		6	42.40	1.81	1.0	42,550	99.9
		All	51.18	1.48	0.5	162,550	99.9
4	10	1	31.04	2.40	18.0	575	98.3
		2	30.48	1.80	5.5	3,489	99.4
		3	24.58	1.55	17.4	740	98.2
		All	28.68	1.83	5.4	5,179	99.5
4	20	1	41.20	1.47	3.1	18,475	99.7
		2	37.11	1.76	1.2	37,699	99.9
		3	39.39	1.77	0.6	80,167	99.9
		4	34.95	1.56	3.9	9,711	99.6
		5	36.76	1.84	1.4	33,994	99.9
		All	38.39	1.64	1.1	58,403	99.9
4	40	1	70.66	1.88	2.0	45,862	99.8
		2	62.45	1.44	0.2	420,090	99.9
		3	51.64	1.55	3.2	23,189	99.7
		4	62.84	1.57	1.2	70,299	99.9
		5	49.03	1.96	2.4	29,723	99.8
		6	52.39	1.64	3.5	41,473	99.7
		All	58.64	1.56	1.4	76,502	99.9

[a] For some combinations of drop diameter and release height, replicates were combined to provide a sufficiently large sample.
[b] The parameters b and c are the Weibull scale and shape parameters, respectively.
[c] Mean square error.
[d] Approximate F statistic. Significance levels were not calculated, since values are only approximate.
[e] Percentage of variation explained by the Weibull model.

impact; higher velocities produced greater distances traveled. The best regression model for b based on the data in Table 8 was

$$b = -16.88 + 0.18V \quad (6)$$

which explained 86.6% of the variation ($P[F(1,6) = 38.8] < 0.01$). The best model for c was

$$c = 1.3 - 0.001V + 0.00028E \quad (7)$$

which explained 61.9% of the variability ($P[F(2,5) = 4.06] < 0.10$).

DISCUSSION

In this study, splash droplet formation on strawberry fruit, the number of droplets bearing sporangia, the probability that a droplet carried one or more sporangia, and the distances traveled could be described in terms of the physical properties of incident drops at the point of impact. Momentum at impact, except for 1- or 2-mm-diameter drops at very low impact velocities, which are rare for canopy drip (19,25), was generally sufficient to cause water splash and disperse sporangia. Six intercorrelated droplet response characteristics (Table 3) were found to be predictable in terms of the physical properties of an impacting drop, i.e., M, W, and V (Table 4). Not all of the drop properties were significant in univariate regression for the responses. In particular, the response of total volume depended significantly ($P < 0.05$) only on M, whereas both the logarithm of the number of droplets with sporangia and the logarithm of the total number of sporangia depended only on V. It is important for the reader to bear in mind that most of the data and associated models presented in this study are specific to our experimental system, since system responses were only recorded along the median splash droplet trajectory.

Regression coefficients for M, W, and V were all significant for the regression of the logarithm of the total number of droplets, average droplet diameter, and the average distance of droplet travel. Since $M = W \cdot V$, the inclusion of M in a regression model

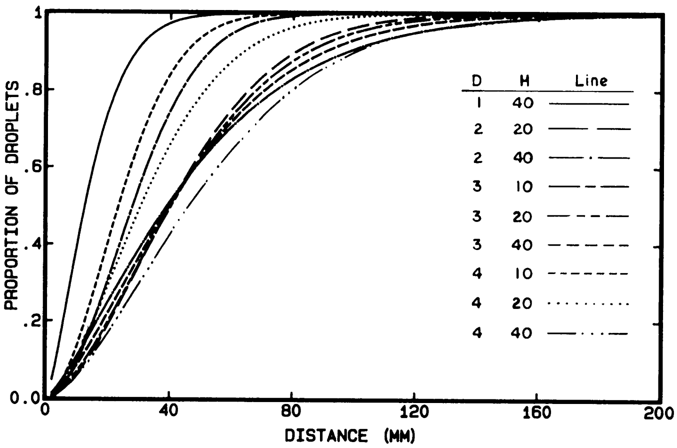

Fig. 3. Predicted cumulative distribution of splash droplets over distance from the strawberry target based on the Weibull distribution (Table 5). Curves are shown for each combination of drop diameter (D) and fall height (H), predicted from estimated parameters when all replicates were combined.

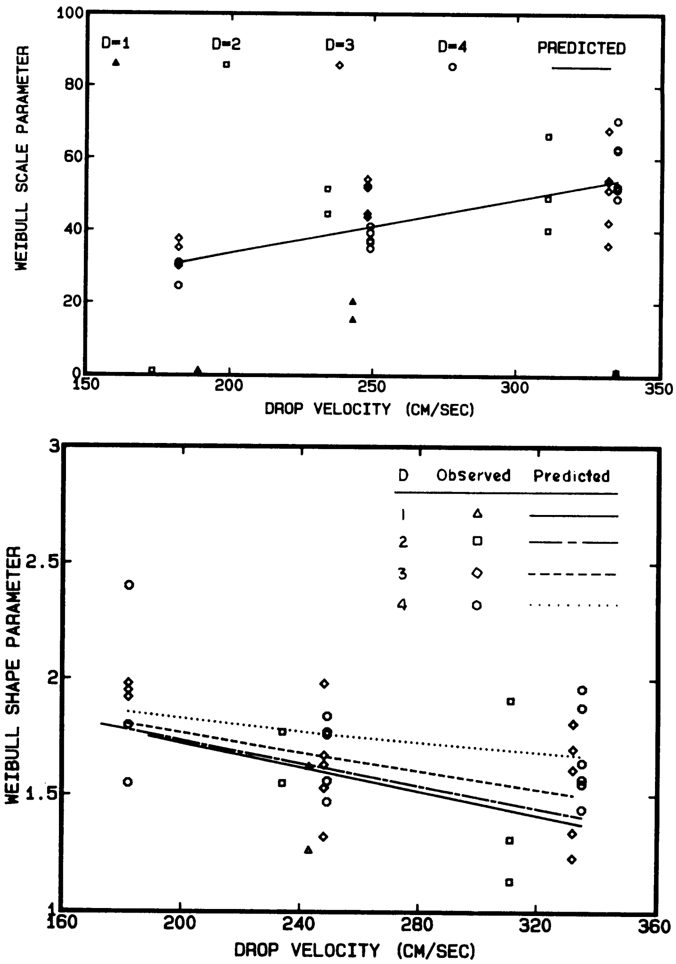

Fig. 4. Relationship between estimated Weibull scale parameter, b (above), and shape parameter, c (below), for the cumulative distribution of splash droplets over distance and the velocity of an impacting drop. For the shape parameter, separate curves are drawn for the four drop diameters, which were multiplied by velocity (from eq. 2) to obtain momentum.

TABLE 6. Droplet diameters with and without sporangia

Drop diameter (mm)	Height of fall (cm)	Droplets with sporangia present			Droplets with sporangia absent			
		N[a]	Mean diameter (mm)	SE	N[a]	Mean diameter (mm)	SE	t statistic[b]
1	40	5	0.32	0.0610	86	0.30	0.0143	0.319 NS
2	20	39	0.43	0.0256	87	0.25	0.0200	2.68**
	40	49	0.47	0.0359	300	0.33	0.0130	3.67***
3	10	12	1.48	0.1860	415	1.04	0.0276	2.34*
	20	118	0.88	0.0342	942	0.74	0.0156	3.72***
	40	328	0.74	0.0172	1,953	0.49	0.0072	13.41***
4	10	44	2.31	0.2543	632	1.37	0.0675	3.57***
	20	121	1.72	0.0633	2,083	0.88	0.0136	25.21***
	40	305	1.44	0.0401	4,949	0.70	0.0085	18.05***

[a] N = number of droplets.
[b] *, **, and *** indicate significance at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively. NS = not significant.

indicates a statistical interaction between W and V, so that these three responses cannot be described in terms of simple effects due to W and V alone. The models for these three responses (Table 4) can all be written in the general form

$$Y = b_0 + b_1 V + b_2 W - b_3 M \quad (8a)$$

in which Y represents one of the three responses, and the parameters are nonzero values. Equation 8a can also be written as

$$Y = b_0 + b_1 V + b_2 W - b_3 WV \quad (8b)$$

which can be rearranged as

$$Y = b_0 + b_1 V + b_2[1 - (b_3/b_2)V]W \quad (8c)$$

From equation 8c, it is clear that the effect of W on Y is positive when $V < b_2/b_3$ and negative when $V > b_2/b_3$. There is also a critical value, $V_c = b_2/b_3$, at which the slope changes sign. The values of V_c for the models for the logarithm of the total number of droplets, average diameter, and average distance were 449, 371, and 328 $cm \cdot sec^{-1}$, respectively. The change in Y with change in W is given by $b_2[1 - (b_3/b_2)V]$. As V increases, this slope becomes smaller and eventually becomes negative. However, because of the $b_1 V$ term, the overall level of Y increases with increasing V. The behavior exhibited by some responses may be a consequence of the experimental system. Responses were measured along the median droplet trajectory (Fig. 1). As V (and therefore M) was experimentally increased, we observed progressively greater droplet scattering about the median trajectory, and this is reflected in the models for the logarithm of the total number of droplets and average distance (Table 4 and eq. 8c). It is uncertain whether values of $V > V_c$ in the models represent realistic system behavior, since values of V_c for average droplet diameter and the logarithm of the total number of droplets were greater than our experimental values of V (Table 1). The value of V_c for the average distance model only slightly exceeded most experimental values of V.

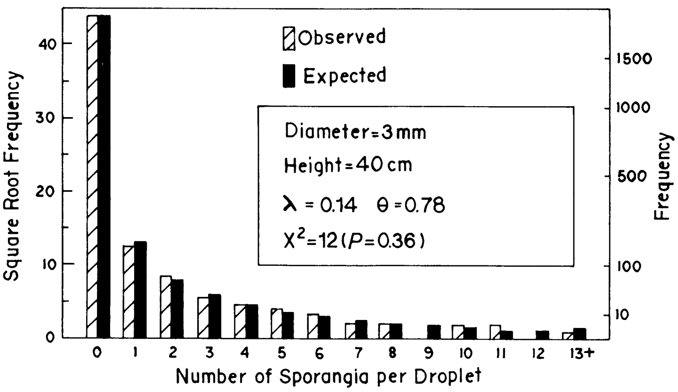

Fig. 5. Frequency distribution of the number of sporangia per splash droplet and the expected frequency based on the logarithmic-with-zeros distribution (Table 7).

TABLE 7. Fit of logarithmic-with-zeros distribution to the number of sporangia per droplet

Drop diameter (mm)	Height of fall (cm)	Parameters[a] λ	θ	Chi-square	df[b]	P
1	40	—[c]	—	—	—	—
2	20	0.29	0.85	7.70	7	0.36
	40	0.14	0.73	1.88	5	0.60
3	10	0.03	0.67	1.85	2	0.39
	20	0.10	0.82	18.12	8	0.02
	40	0.14	0.78	12.01	11	0.36
4	10	0.06	0.28	2.91	1	0.05
	20	0.05	0.24	0.16	1	0.34
	40	0.06	0.35	3.10	3	0.38

[a]See text for the definition of the probability density function.
[b]Degrees of freedom.
[c]The number of observations in the nonzero frequency classes was insufficient to estimate parameters of the distribution.

Whereas the behavior of the models for droplet numbers and distance traveled can be explained in terms of a dilution effect due to increased droplet scatter, we believe the applicability of the same model form for the average diameter of droplets is related to increased drop shattering at high impact velocities. Although the absolute numbers of droplets bearing sporangia and the total number of sporangia increased with V (Table 4), the efficiency of inoculum entrainment in individual droplets, as measured by either the number of sporangia per droplet or the probability of a droplet's bearing sporangia, decreased with log(M). Efficiency also declined with V (eqs. 3a and 4). The apparent contradiction between increasing absolute numbers and decreasing efficiency of inoculum entrainment with increasing V is a result of the fact that the total number of droplets increased exponentially with an increase in V (Table 4), as evidenced by the linear relationships between the logarithm of the total number of droplets with sporangia and V, whereas efficiency (e.g., λ in eq. 4) only decreased linearly with log(M).

Responses involving average droplet diameter and the efficiency of inoculum entrainment are most likely independent of splash droplet trajectory. Furthermore, other studies indicate that marked differences in spore shape do not affect the entrainment of spores in splash droplets (3).

For all combinations of drop diameter and height of release, except for 1-mm drops released from 40 cm, droplets bearing sporangia were significantly larger than droplets without sporangia (Table 6). The same observations have also been made regarding splash dispersal of *Pseudocercosporella herpotrichoides* (Fron) Deighton (3,9) and *Septoria nodorum* Berk. (1) when large drops were allowed to strike inoculum-bearing surfaces, either petri dishes or wheat straw, at terminal velocity. However, in contrast to these other studies, we could find no relationships between droplet size and the number of sporangia. As indicated by the logarithmic distribution with zeros, the probability of a droplet's containing at least one sporangium ranged from 0.03 to 0.29, depending on momentum at impact (Table 7). Of the droplets bearing sporangia, the mean number per droplet declined with increasing weight of the impacting drop.

Studies using large-diameter drops falling at terminal velocity have consistently shown that the number of droplets, the number of inoculum-bearing droplets, and the number of propagules all decline exponentially with distance (1,3,6–9,11,14,15). Macdonald and McCartney (17) further showed that splash droplets follow

TABLE 8. Fit of the Weibull cumulative distribution to the number of splash droplets with sporangia over distance

Drop diameter (mm)	Height of fall (cm)	Parameters[a] b	c	MSE[b] ($\times 10^{-4}$)	F statistic[c]	R^2 [d]
1	40	—[e]	—	—	—	—
2	20	27.75	1.18	0.21	19,904	99.9
	40	37.50	1.00	9.60	432	99.3
3	10	15.50	1.00	29.40	96	98.5
	20	36.40	1.16	0.33	15,369	99.9
	40	38.92	1.22	5.13	1,024	99.7
4	10	10.64	1.45	7.84	341	99.6
	20	25.94	1.24	1.40	3,148	99.9
	40	44.18	1.48	0.36	14,227	99.9

[a]The parameters b and c are the Weibull scale and shape parameters, respectively.
[b]Mean square error.
[c]Approximate F statistic.
[d]Percentage of variation explained by the Weibull model.
[e]The number of observations was insufficient to estimate parameters.

trajectories predicted from Newtonian dynamics. In the present study, the number of droplets bearing sporangia and the number of all droplets changed over distance according to the Weibull distribution (Tables 6 and 8). When the shape parameter, c, of the Weibull distribution has a value of 1, the Weibull distribution is identical to the negative exponential distribution, indicating an exponential decline in the number of droplets with distance. The mode, i.e., the distance of the maximum number of droplets, is 0 when $c = 1$. The mode is related to the shape parameter (c) and the scale parameter (b) as follows:

$$\text{mode} = b[(c - 1)/c]^{1/c} \quad (9)$$

From Table 5, the mode in all replicates, for all combinations of drop diameter and release height, was greater than 0, because $c > 1$ in all cases. This indicates that the proportion of total droplets increased to a maximum and then declined to 0 with increasing distance.

Given the estimated values of b and c (Tables 5 and 8), the mode of droplet distribution over distance can be calculated for each table entry. Another approach, however, is to consider the manner in which the mode varies with W, V, M, and E. From equations 1 and 6, b was described as a linear function of V. The value of c was found to be a relatively complex function of both V and M (eqs. 2 and 7). As long as $M < 32.5$ g·cm·sec^{-1}, the expression in parentheses in equation 2b is positive; the corresponding term of equation 7 is positive when $M < 7.1$ g·cm·sec^{-1}. The values of M in this experiment were much less than 32.5 g·cm·sec^{-1} and mostly less than 7.1 g·cm·sec^{-1}, so that the term in parentheses was generally positive. Thus, with our data, increasing values of V lead to smaller values of c. For $c > 1$, the term in brackets in equation 9 gets smaller as c approaches 1; likewise, increases in b cause the mode to become larger. Thus, two competing effects on the mode of droplet distribution exist. Careful study of the Weibull CDFs (Fig. 2) shows, however, that for a given incident drop mass, increasing V shifts the mode to the right. Because of the lack of a relationship between droplet size and the number of sporangia, there was no reason to follow the trajectories of individual droplets or group droplets into size categories.

The present study demonstrates the role of canopy drip in inoculum dispersal even with a low-canopy crop. In the case of low-intensity rains, in which the mass median diameter of raindrops is less than 1 mm, canopy drip due to drop action on plant surfaces still provides an effective mechanism for splash dispersal. Additional studies are planned to describe the splash dispersal of *P. cactorum* resulting from the impact of drops at terminal velocity and to ascertain the relative importance of direct rainfall versus drip in the epidemiology of this pathogen.

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