Splash Dispersal of *Colletotrichum acutatum* and *Phytophthora cactorum* from Strawberry Fruit by Single Drop Impactions

Xiusheng Yang, L. V. Madden, D. L. Reichard, L. L. Wilson, and M. A. Ellis

First, second, fourth, and fifth authors: former postdoctoral researcher, professor, research assistant, and professor, respectively, Department of Plant Pathology, Ohio Agricultural Research and Development Center (OARDC), The Ohio State University (OSU); third author: agricultural engineer, Agricultural Research Service, USDA, Wooster, OH 44691. Present address of the first author: Department of Natural Resources Management and Engineering, University of Connecticut, Storrs, CT 06269.

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ABSTRACT

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Dispersal of *Colletotrichum acutatum* conidia and *Phytophthora cactorum* sporangia by single drop impactions onto strawberry fruit was studied using a drop-generating system. Uniform water drops, 0.5–4 mm in diameter, were released from heights of 25–150 cm above infected target fruit with spores labeled with fluorescent tracer. Splash droplets were collected on water-sensitive paper. Size and distance from the source of each droplet trace were determined using an image analysis system, and number of spores contained in randomly chosen traces were counted by means of fluorescent microscopy. Size and fall height of impacting drops were shown to have a significant effect on number, mass, and travel distance of splash droplets, and on percentage of droplets with no conidia of *C. acutatum*. Only size of incident drops significantly affected droplet diameter. Number of spores per droplet generally was well described by the log-normal distribution for *C. acutatum* and the negative binomial distribution for *P. cactorum*. For *C. acutatum*, both size and fall height of impacting drops significantly affected (transformed) number of spores per droplet and total number of spores per impaction, but only drop size significantly affected spore entrainment with *P. cactorum*. Mean number of spores per droplet ranged from 6 to 134 for *C. acutatum*, and from 0.2 to 0.8 for *P. cactorum*, resulting in totals of 17–11,546 conidia or 9–56 sporangia dispersed by a single impaction. Transformed total spores per impaction for both pathogens were linearly and positively related to impact velocity of incident drops on a log scale. The percentage of splash droplets with no *C. acutatum* conidia was significantly correlated with ln(kinetic energy) of incident drops at impaction. Percentage of droplets without *P. cactorum* sporangia was unaffected by impacting drop size, fall height, or kinetic energy. A weak-positive relationship between spores per splash droplet and droplet diameter also was found for both *C. acutatum* and *P. cactorum*. Differences in dispersal between the two pathogens could be partially attributed to higher inoculum density of *C. acutatum* on the fruit surface compared to *P. cactorum*.

Additional keywords: anthracnose, dissemination, *Fragaria* × *ananassa*, leather rot, quantitative epidemiology.

Many splash-dispersed bacterial and fungal pathogens can cause substantial yield losses during growing seasons with relatively high levels of precipitation (12,18,26). Studies to date have shown that rain splash dispersal is affected by numerous factors, including characteristics of impacting raindrops (16,19,34,41), surface topography (25,36,42,43), weather (5,6,32,43), and inoculum conditions (13,35). A better understanding of splash dispersal of plant pathogens is still needed to develop or refine disease management strategies. Comprehensive reviews of recent work on splash dis-

persal were presented by Fitt et al (13) and Fitt and McCartney (12).

Any splash dispersal process generally includes two important phases: detachment of pathogens by impaction of water drops and transport of spore-bearing splash droplets to new locations (1,23,41). Physical descriptions of rain splash dispersal, therefore, require quantitative knowledge of size and velocity distributions of incident raindrops, mass and kinetic attributes of splash droplets in response to impaction, and the relationships between these (mass and kinetic) attributes and spore entrainment. All of the factors are subjected to conditions of ground surface, plant structure, weather, as well as pathogen species. Only the natural rainfall characteristics (i.e., size and velocity distributions) above a canopy are well described (2,18,27). Drop impaction was not studied in depth until the late 1960s when scientists began to investigate splash on liquid layers by using energy balance equations (21,24). Studies of drop impaction on some solid surfaces also have been conducted in limited detail, mainly for application purposes such as quantification of soil erosion (44) and splash dispersal of plant pathogens (13,19). With the aim of quantifying splash dispersal of fungal pathogens of strawberry (*Fragaria* × *ananassa* Duchesne) by single drop impactions on infected fruit, we recently quantified droplet production and movement in relation to impacting water drops (41). Using a drop-generating and high speed videographic motion-analysis system, we determined mean and distribution of size, number, mass, travel distance, splash angle, take-off velocity, and kinetic energy of splash droplets.

The importance of splash dispersal in the epidemiology of strawberry anthracnose, caused by *Colletotrichum acutatum* J. H. Simmonds, and leather rot, caused by *Phytophthora cactorum* (Lebert and Cohn) J. Schröt, has been shown in our previous studies (20,25,26,34,42,43). Using a rain simulator, we demonstrated that propagules of both pathogens are effectively dispersed under a range of rain intensity and ground cover conditions (25,42,43). The rate of increase of leather rot incidence in the field also is correlated with the amount of rainfall (26). Using an earlier version of our drop-generating system (33), the dispersal of *P. cactorum* sporangia in splash droplets was assessed in relation to low velocity impacting water drops that mimicked canopy drips (34). Variables such as the number of droplets formed, average distance traveled, and number of sporangia per droplet were determined and related to size and fall height (10–40 cm) of incident drops.

Because both *C. acutatum* and *P. cactorum* produce lesions on strawberry fruit, there is an opportunity to directly compare splash dispersal of the two fungal species from the same plant structure. The objectives, therefore, of this study were to quantify the splash dispersal of *C. acutatum* conidia from impactions of single water drops traveling at a wide range of velocities and to expand on our previous study with *P. cactorum* to determine the effect of higher velocity water drop impactions on dispersal of sporangia.

MATERIALS AND METHODS

Source fruit production. The preparation of source fruit infected by *C. acutatum* followed the procedures described previously (39,43). In brief, immature (green-to-white stage) strawberry fruit were harvested from greenhouse-grown plants (cultivar Midway), cleaned, surface-sterilized in 70% ethanol for 60 s, placed on elevated screens in 5-L plastic containers with stem tips through the screen, and immersed in deionized water. Fruit were sprayed with a conidial suspension (10^5/ml) until runoff, and incubated at 25 C for 24 h with containers closed, followed by 7 days at the same temperature with containers open. Infection of the fruit was uniform after exposure to this regime in that the entire fruit surface was covered by lesions. After incubation, source fruit were soaked in a 1.0% solution of Fluorescent Brightener 28 (Sigma Chemical Co., St. Louis, MO) for 3 h and incubated for an additional 24 h at 25 C and approximately 100% relative humidity (RH). The mucilage around the conidia did not dissolve during this treatment.

The source fruit infected by *P. cactorum* were prepared similarly (20,34). Cleaned green-to-white strawberry fruit, detached from greenhouse plants, were inoculated with a sporangial suspension (10^4/ml) and incubated at 20 C in closed plastic containers for 5 days. The entire fruit surface was covered by lesions after this 5-day incubation. The source fruit were then soaked in a 0.2% solution of Fluorescent Brightener 28 for 3 h and incubated for an additional 24 h at 20 C and approximately 100% RH. After that, the fruit were transferred to a mist chamber (Herrmidifer Co., Lancaster, PA) at the same temperature for 18–24 h with the container lids removed.

The consistency of inoculum production was regularly verified for both pathogens using the technique described in our previous studies (20,43). Mean inoculum density for *C. acutatum* was 2.5×10^8 conidia per fruit, and for *P. cactorum* mean density was 2.9×10^5 sporangia per fruit. The fluorescent stain did not affect inoculum density (L. V. Madden, *unpublished*).

Drop impaction and droplet production. A drop-generating system (31,33,34,41) was used to produce impacting water drops of uniform sizes. The system used a piezoelectric crystal (Morgan Matroc Inc., Cleveland, OH), operated by a pulse generator (Hewlett Packard Corp., Palo Alto, CA), to generate drops up to 2 mm, and a solenoid-operated miniature metering pump (Valcor Engineering Corp., Springfield, NJ) to produce drops larger than 2 mm in diameter. Size and uniformity of drop production were periodically calibrated either microscopically with drops captured in silicone gel (drops $\leq$ 2 mm) or using a weighing method (drops $>$ 2 mm) described by Reynolds et al (33). Kinetic characteristics of the incident drops (impacting velocity and kinetic energy) were determined using a newly developed videographic system that has been presented in a separate report (41). Whereas impact velocity was directly measured using the videographic system, kinetic energy was calculated from mass and impact velocity of incident drops.

The assessment of splash dispersal of *C. acutatum* conidia was made by collecting droplets with water-sensitive paper (WSP) (Spraying Systems, Co., Wheaton, IL). Half of a source fruit, cut lengthwise, was mounted with the cut side down on a target positioner, which was horizontally adjusted so that an incident drop would strike the long axis of the fruit at approximately the middle point (41). Six to nine 52- × 76-mm WSP cards were placed end-to-end, radiating from the impact point in the direction defined by the long axis of the target fruit, on a supporting platform at the same elevation as the impact point. Drops of four sizes, 0.5, 1, 2, and 4 mm in diameter, were released from up to six heights, 25, 50, 75, 100, 125, and 150 cm above the target fruit. Terminal velocities for these four drop sizes are 197, 378, 645, and 845 cm/s, respectively. At the highest fall height, between 70 and 99% of terminal velocity was obtained (41). An additional test with 3-mm drops from the 150-cm height also was performed. Each treatment combination of drop size and fall height was repeated four to six times. Twenty to 200 impactions were conducted for each treatment combination of drop size, fall height, and replicate. The number of impactions was based on the number of droplet traces on the WSP; sufficient droplets were needed to obtain precise measurements, but excessive droplets would result in overlapping traces. Data were converted for a single impaction by dividing by the number of impactions. The WSP cards and source fruit were replaced after each replication. The number of drop impactions assured that the fruit surface was wet for the splash events.

After each test, the WSP cards were coated with a layer of clear acrylic (Borden, Inc., Columbus, OH), dried, and stored in the dark. Size and horizontal travel distance of each trace on the WSP were determined using an image analysis system (Dapple Systems, Inc., Sunnyvale, CA). Droplets smaller than 0.15 mm in diameter, however, were not detected and processed in this study due to the resolution limitations of the image analysis system. (It has been shown that droplets smaller than 0.2 mm in diameter contain very few spores [5,13,34].) The diameter of droplets was calculated first from the area of traces, and then corrected using the spread factors determined by Reynolds et al

(33) for the same type of WSP. In preliminary tests, no significant difference could be found in splashes from fruit that had been or had not been soaked in the fluorescent stain (X. Yang, *unpublished*). Spores in droplet traces were counted by means of incident fluorescent microscopy. Forty to 80 droplets were randomly sampled from the WSP for the purpose of spore counting for each treatment combination of drop size, fall height, and replicate. Total number of spores dispersed per impaction was estimated from spores per droplet and total splash droplets. The latter was obtained by a spatial integration of the corresponding density function generated from the WSP data.

The equipment and operational procedures for the study with *P. cactorum* were basically the same as with *C. acutatum*. However, only three drop sizes, 2, 3, and 4 mm in diameter, and three fall heights, 100, 125, and 150 cm, were tested in this study. Seven to eight replications, 20–50 impactions each, were performed for each treatment combination of drop size and fall height.

Data analysis. Measured and calculated droplet responses for each replication included mean values of diameter (d), travel distance (x_n), travel distance weighted by mass (x_m), total number (n), and total mass (Σm) of splash droplets. Yang et al (41) gives details on these responses for the situation in which fruit were not infected. Also calculated were mean number (N_{Ca} and N_{Pc}, for *C. acutatum* and *P. cactorum*, respectively) and frequency distribution of spores per droplet, total number of spores for each impaction (ΣCa and ΣPc), and percentage of droplets without spores (p_{Ca} and p_{Pc}). The effects of drop diameter (D) and fall height (H) on the above variables were assessed with analysis of variance (ANOVA). Because all heights were not tested for each drop diameter (see Results), the interaction of D and H could not be unambiguously tested (29). To approximately evaluate the interaction, a balanced subset of the data was chosen and analyzed. Also, the velocity (V) and kinetic energy (KE) of incident drops at impaction (41) were used in regression analysis to assess the combined effect of D and H on the droplet responses.

The distribution of spores per droplet was quantified by fitting statistical distributions to the spore data for each drop size and fall height combination. For *P. cactorum*, the Poisson, negative binomial, and logarithmic-with-zeros distributions were fitted

TABLE 1. Summary of the droplet responses and spore entrainment in relation to diameter of incident drops and fall height[a]

Incident drops		Replicates *Colletotrichum acutatum*/ *Phytopthora cactorum*	Mean droplet responses[b]					Spore entrainment[c]					
								C. acutatum			*P. cactorum*		
D (mm)	H (cm)		d (mm)	n	Σm (mg)	x_n (mm)	x_m (mm)	N_{ca}	ΣCa	p_{ca} (%)	N_{Pc}	ΣPc	p_{Pc} (%)
1	25	6/–	0.34	0.6	0.02	30.5	26.8	75	45	0	...	...	...
			(0.05)	(0.5)	(0.01)	(11.3)	(10.7)	(25)	(15)	(0)			
	50	6/–	0.32	2.8	0.08	38.8	37.1	6	17	49	...	...	...
			(0.02)	(1.5)	(0.04)	(10.6)	(10.7)	(6)	(16)	(18)			
	75	6/–	0.34	2.6	0.12	30.1	22.9	12	31	44	...	...	...
			(0.03)	(1.3)	(0.10)	(5.8)	(5.1)	(17)	(44)	(23)			
	100	6/–	0.33	5.9	0.20	44.2	30.6	39	232	10	...	...	...
			(0.01)	(1.6)	(0.07)	(7.4)	(8.7)	(53)	(310)	(7)			
2	25	6/–	0.41	13.3	1.1	41.9	29.8	43	565	11	...	...	...
			(0.01)	(3.9)	(0.5)	(3.0)	(3.6)	(49)	(646)	(21)			
	50	6/–	0.45	16.1	1.5	42.3	30.0	47	761	8	...	...	...
			(0.03)	(1.1)	(0.4)	(7.4)	(2.7)	(30)	(478)	(7)			
	75	6/–	0.39	24.3	2.1	62.4	39.8	84	2,049	8	...	...	...
			(0.05)	(7.1)	(1.0)	(18.6)	(13.6)	(61)	(1,484)	(5)			
	100	6/8	0.47	24.0	3.3	56.9	32.3	105	2,512	36	0.58	14	68
			(0.04)	(5.1)	(1.4)	(18.5)	(5.5)	(125)	(2,989)	(18)	(0.50)	(12)	(23)
	125	4/7	0.38	31.5	2.6	63.2	35.3	98	3,089	42	0.27	9	74
			(0.07)	(15.6)	(1.8)	(20.9)	(7.2)	(54)	(1,703)	(13)	(0.25)	(8)	(14)
	150	4/7	0.42	34.0	3.1	72.5	39.5	134	4,559	7	0.48	16	74
			(0.03)	(12.9)	(1.1)	(26.0)	(1.6)	(29)	(979)	(11)	(0.36)	(11)	(14)
3	100	–/8	0.46	45.0	6.2	60.5	35.4	...	...	...	0.74	33	58
			(0.02)	(8.1)	(2.0)	(6.2)	(5.4)				(0.75)	(34)	(30)
	125	–/7	0.52	55.2	10.4	55.7	46.6	...	...	...	0.33	18	79
			(0.03)	(19.4)	(5.0)	(10.3)	(15.3)				(0.40)	(22)	(22)
	150	4/8	0.51	64.7	11.8	77.0	58.4	119	7,694	13	0.73	47	65
			(0.05)	(14.9)	(3.1)	(11.8)	(8.8)	(58)	(3,757)	(10)	(0.87)	(56)	(32)
4	25	6/–	0.43	27.9	3.5	46.8	25.9	72	2,022	2	...	...	...
			(0.03)	(9.4)	(1.6)	(3.4)	(10.2)	(96)	(2,680)	(3)			
	50	6/–	0.42	38.6	11.8	47.6	20.7	29	1,105	7	...	...	...
			(0.02)	(1.4)	(7.4)	(11.8)	(5.2)	(23)	(896)	(6)			
	75	6/–	0.46	46.0	19.9	55.3	20.5	29	1,338	8	...	...	...
			(0.02)	(3.3)	(5.9)	(19.9)	(3.2)	(15)	(711)	(11)			
	100	6/8	0.51	47.3	19.4	60.0	21.2	57	2,718	7	0.82	39	57
			(0.07)	(16.4)	(9.0)	(19.4)	(4.2)	(39)	(1,853)	(6)	(0.56)	(26)	(25)
	125	5/7	0.54	81.9	21.6	74.2	61.1	37	3,016	3	0.58	47	66
			(0.04)	(17.3)	(6.3)	(10.1)	(10.6)	(18)	(1,470)	(5)	(0.43)	(36)	(24)
	150	4/8	0.49	108.9	21.3	91.1	79.5	106	11,546	5	0.51	56	69
			(0.03)	(11.0)	(3.3)	(15.1)	(24.0)	(45)	(4,934)	(5)	(0.48)	(53)	(27)

[a] D = diameter of incident drops; H = fall height; d = diameter of splash droplets; n = total number of droplets per impaction; X_m = total mass of droplets per impaction; x_n = travel distance of droplets from impact point; x_m = travel distance of droplets weighted by mass; N_{ca}, ΣCa, and p_{ca} = mean spores per droplet, total spores per impaction, and percentage of droplets with no spores, respectively, for *C. acutatum*, and N_{Pc}, ΣPc, and p_{Pc} = mean spores per droplet, total spores per impaction, and percentage of droplets with no spores, respectively, for *P. cactorum*. Variables n and Σm were obtained by a spatial integration of the density functions generated for the water-sensitive paper data.

[b] Means and standard errors (in parenthesis) over the indicated replicates of 20–200 impactions each, estimated for a single impaction by a water drop with a given diameter released from a given height.

[c] Means and standard errors (in parenthesis) for a single splash droplet, over the indicated replicates.

using the program of Gates and Etheridge (15), as suggested by Reynolds et al (34). The goodness-of-fit was assessed with a chi-square (χ^2) test.

Because of the high number of *C. acutatum* conidia per droplet (see Results), the normal and log-normal distributions were fitted to the data for this pathogen. Discrete distributions are approximated by continuous distributions when numbers are large (38). The MINITAB (30) distribution fitting procedure was used to fit the normal distribution, and the goodness-of-fit was assessed with the Kolmogorov-Smirnov statistic (δ), which is the maximum difference between the observed and theoretical cumulative distributions (17). (The test statistic usually is represented by D but was labeled δ here to avoid confusion with drop diameter.) The log-normal distribution was fitted by first taking logarithms of spores per droplet. Droplets with no spores were omitted from this analysis. A normal distribution of log-transformed data is equivalent to a log-normal distribution of the original data. Skewness (β) of the distributions for *C. acutatum* and *P. cactorum* also was calculated using BMDP (4).

ANOVA, regression, and correlation analyses were performed using MINITAB (30). Analyses for each pathogen were done separately because of differences in spore type and inoculum density. Before analysis, spores per droplet and total spores per impaction were transformed based on the most appropriate statistical distribution describing the data. Relationships between spores per droplet and droplet size for both pathogens also were evaluated by regression analysis by first partitioning droplets into groups based on size (i.e., 0–0.25, 0.25–0.5, ... mm in diameter) and calculating means for each droplet size. Separate means were calculated for each D, H, and replicate combination. Weighted regression was used in this case in which weights were the number of observations for each mean.

RESULTS

Mean droplet responses. More than 65,000 splash droplets were captured on WSP cards and digitized for size and distance from the impacting point. Of the digitized droplets, 3,222 and 3,286 were observed for the presence of spores of *C. acutatum* and *P. cactorum*, respectively. Listed in Table 1 are means and standard errors (in parentheses) of the main droplet responses summarized by incident drop diameter (D) and fall height (H). Impacting drops with 0.5-mm diameter did not produce splash droplets detectable by the image analysis system and are not included in this report. Because it was very difficult or impossible to keep small drops released from large heights from falling on the target, due to air turbulence, 1-mm drops released from heights of 125 and 150 cm also were excluded from this study. (Drops of 1 mm in diameter, however, reached 83% of terminal velocity by a fall height of 100 cm.) Generally, means of droplet number (n), total mass (Σm), and travel distance (x_n) all increased with H within each class of D. For a given fall height, droplet responses n, Σm, and x_n also increased with D. The mean droplet diameter (d) and travel distance weighted by mass (x_m), however, were shown to significantly increase only with D or H, respectively, as indicated by the ANOVA results in Table 2. For all cases, x_m was smaller than x_n (Table 1), an indication of strong clustering of large droplets near the impact point. There was a significant interaction of D and H only for Σm and x_m.

Spores per droplet and total spores dispersed. The distributions of *C. acutatum* conidia per droplet were highly and positively skewed, as indicated by the calculated skewness values of the original data (β_0, Table 3). This was because more than half of the droplets had less than 20 spores, but some droplets had as many as 1,000 or more (maximum = 1,685). Fifteen of the 17 β_0 values were greater than 2.0. As expected from the skewness values, the normal distribution did not provide an acceptable fit to any of the observed distributions as determined by the δ statistic ($P < 0.01$, data not shown). Eliminating droplets with no conidia did not improve the fit by the normal distribution (X. Yang and L. V. Madden, *unpublished*). Logarithmic transformation of the data generally resulted in symmetrical distributions, as represented by their skewness (β_L, Table 3). Sixteen out of 17 β_L values were between −1 and 1. The normal distribution provided an acceptable fit to most of the log-transformed data sets, which is equivalent to the log-normal distribution fitting the untransformed data for all droplets with one or more conidia. The parameters μ and σ in Table 3 represent the mean and standard deviation of the log-transformed values. Among the 17 fittings of log-normal distribution for each combination of D and H, only four had significant values of the δ statistic, with three of them significant only at $P = 0.05$. An example of such fittings with the log-normal distribution is shown in Figure 1. Plots of μ versus D and H did not reveal any relationships, probably because of removing the data points with zero spores. Therefore, a pooled (or common) estimate of σ was obtained by subtracting the estimated mean for each D and H combination from all the observations for that treatment. The data were pooled, and then the normal distribution was fitted to the log-transformed data. This estimate of σ equaled 1.4.

Because of the positive skewness of the original data of spores per droplet and the generally good fit of the log-normal distribution, N_{Ca} and ΣCa were log-transformed before ANOVA and regression analyses. The (transformed) N_{Ca} and ΣCa were both significantly affected by D and H ($P < 0.01$), but not by their interaction ($P > 0.05$) (Table 2). In general, ΣCa increased with both D and H, mostly caused by the increase in droplet number (Table 1). Despite the significance, N_{Ca} did not show a strong increase or decrease with change in either D or H. The percentage of droplets with no spores (p_{Ca}) generally decreased

TABLE 2. Effects of incident drop diameter and fall height on mean droplet responses as determined by analysis of variance[a]

	F statistic[b]										
						C. acutatum			*P. cactorum*		
Factor	d	n	Σm	x_n	x_m	N_{ca}	ΣCa	p_{ca}	N_{pc}	ΣPc	p_{pc}
D	25.8	43.2	48.1	7.3	0.4	5.7	51.5	7.4	8.6	5.8	1.5
	***[c]	***	***	***	NS	***	***	***	***	**	NS
H	1.5	16.9	5.3	11.1	10.1	3.5	8.9	2.4	3.9	0.3	2.9
	NS	***	***	***	***	**	***	*	NS	NS	NS
$D \times H$	2.3	1.5	4.7	1.5	2.5	2.9	2.1	11.4	2.4	1.0	0.8
	NS	NS	***	NS	*	NS	NS	***	NS	NS	NS

[a] The major effect of D (diameter of incident drops) and H (fall height) were determined with all treatment combinations as presented in Table 1. Spore entrainment was transformed based on the log-normal and negative bionomial distributions, for *C. acutatum* and *P. cactorum*, respectively, before analysis. The effect of interaction between D and H on droplet responses and dispersal of *C. acutatum*, however, were based on a balanced subset of data only.

[b] d = Diameter of splash droplets; n = total number of droplets per impaction; Σm = total mass of droplets per impaction; x_n = travel distance of droplets from impact point; x_m = travel distance of droplets weighted by mass; N_{ca} and ΣCa = mean spores per droplet and total spores per impaction of *C. acutatum*, respectively; N_{pc} and ΣPc = mean spores per droplet and total spores per impaction of *P. cactorum*, respectively; p_{ca} and p_{pc} = percentage of droplets with no spores of *C. acutatum* and *P. cactorum*, respectively.

[c] Asterisks *, **, and *** indicate significance at $P = 0.05$, 0.01, and 0.001, respectively. NS = not significant ($P > 0.05$).

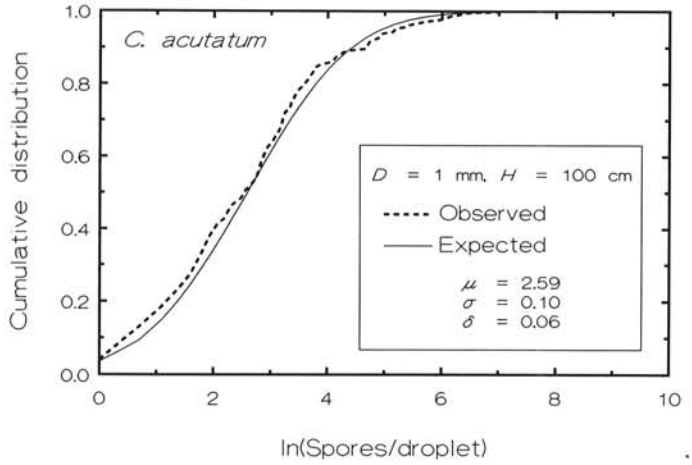

Fig. 1. Cumulative proportion of the logarithmic transforms of *Colletotrichum acutatum* conidia per droplet and the corresponding predictions by the log-normal distribution function, in which D = impacting drop diameter; H = fall height; μ and σ = parameters of the log-normal distribution; and δ = Kolmogorov-Smirnov statistic of goodness-of-fit test. The number of observations = 214.

as H and (especially) D increased (<10% for 4-mm drops), and was strongly affected by the interaction of D and H ($P < 0.001$, Table 2).

The distribution of *P. cactorum* sporangia per droplet also had a high positive skewness. Six of nine β_0 values (Table 4) exceeded 2.0 and about two-thirds of all droplets contained no spores (Table 1). The Poisson distribution did not fit any of the observed distributions as indicated by χ^2 statistic ($P < 0.05$, data not shown). The logarithmic-with-zeros distribution provided an acceptable fit to only four of the distributions. However, the two-parameter negative binomial distribution fit most of the observed distributions ($P > 0.05$). The parameter k of the negative binomial is an index of dispersion and is inversely related to the variance of the observations (7). The second parameter, p, is equal to mean/k. Table 4 lists the estimated parameters, and Figure 2 gives an example of the fit-for-one data set. Only one of the nine treatment combinations had a significant lack of fit.

Plots of $1/k$ versus D, H, and mean number of sporangia per droplet (3) did not reveal any relationships between the dispersion parameter and the experimental factors or measured variables. Therefore, a common k was calculated using the method of Bliss and Owen (3). The common k was estimated as 0.80. As suggested by Bliss and Owen (3), N_{Pc} and ΣPc were transformed to $\ln(N_{Pc}$

TABLE 3. Fit of the log-normal distribution to the spore numbers of *Colletotrichum acutatum* per droplet[a]

Incident drops		Observations for fitting with normal/log-normal distribution	Skewness		Log-normal distribution parameters		
D	H		β_O	β_L	μ	σ	δ Statistic
1	25	40/ 40	0.82	−0.11	4.06	0.14	0.12
	50	213/110	2.19	−0.06	1.85	0.12	0.11
	75	216/120	3.25	0.51	1.82	0.14	0.14*[b]
	100	240/214	6.64	0.46	2.59	0.10	0.06
2	25	119/111	3.82	0.21	2.84	0.12	0.08
	50	189/175	2.44	0.24	2.69	0.12	0.11*
	75	214/198	2.81	−0.47	3.59	0.12	0.08
	100	212/132	3.51	0.02	3.22	0.19	0.10
	125	108/ 62	2.24	−0.23	3.39	0.32	0.17
	150	192/178	2.09	−0.89	4.63	0.07	0.07
3	150	187/165	1.65	−1.16	4.36	0.10	0.11*
4	25	227/222	2.87	−0.28	2.96	0.10	0.12**
	50	240/224	3.14	0.18	2.56	0.09	0.06
	75	235/216	5.18	−0.20	2.64	0.09	0.07
	100	235/218	3.27	0.09	2.99	0.11	0.07
	125	163/157	3.36	0.29	2.64	0.11	0.07
	150	192/181	2.78	−0.84	4.04	0.10	0.08

[a] D = diameter of incident drops; H = fall height; β_O = coefficient of skewness for the original observations; β_L = coefficient of skewness for the log-transformed data; μ and σ = model parameters of the log-normal distribution; and δ statistic = the Kolmogorov-Smirnov one-sample test statistic.

[b] Asterisks * and ** indicate significance at $P = 0.05$ and $P = 0.01$, respectively. Note that significance ($P \leq 0.05$) indicates lack of fit.

TABLE 4. Fit of the negative binomial distribution to the spore numbers of *Phytophthora cactorum* per droplet[a]

Incident drops		Number of observations		Distribution parameters			
D	H		β_O	p	k	χ^2	P^b
2	100	381	2.01	1.02	0.59	5.76	0.33
	125	253	4.35	0.35	0.98	1.82	0.40
	150	292	1.67	1.30	0.34	4.31	0.36
3	100	297	1.80	1.17	0.66	9.17	0.10
	125	350	2.62	0.69	0.44	0.83	0.66
	150	456	1.42	1.56	0.55	10.01	0.19
4	100	369	2.48	0.82	1.06	15.77	0.01
	125	387	3.55	0.54	1.10	4.88	0.18
	150	501	2.71	0.69	0.84	3.31	0.51

[a] D = diameter of incident drops; H = fall height; β_O = coefficient of skewness for the original observations; p and k = model parameters of the negative binomial probability density function.

[b] Significance level. Values of $P \leq 0.05$ indicate lack of fit.

$+ k/2$) and $\ln(\Sigma Pc + k/2)$, respectively, before ANOVA and regression analysis. This transformation stabilizes variances and produces symmetrical (normal) distributions. ANOVA indicated that transformed N_{Pc} and ΣPc were significantly affected by D, but not H or the interaction of D and H (Table 2). In general, transformed N_{Pc} and ΣPc increased with D. The percentage of droplets with no *P. cactorum* sporangia (p_{Pc}), however, was not affected by either D or H ($P > 0.05$, Table 2), with values clustered in the range of 57–74% (Table 1).

Spore dispersal in relation to kinetic attributes of incident drops. Correlation analysis was performed to determine significant relations between total dispersed spores per impaction and kinetic attributes of incident drops (i.e., impact velocity [V] and kinetic energy [KE]). Correlation coefficients were determined for original and transformed spore data and for original and various transformations of V and KE. Based on the high correlations, regression analysis was conducted to relate transformed ΣCa and ΣPc to $\ln(V)$. With R^2 values of 0.77 ($P < 0.001$) and 0.65 ($P < 0.01$), the best relationships, as shown in Figures 3 and 4, were found as:

$$\ln(\Sigma Ca) = -28.51 + 5.85\ln(V) \quad (1)$$

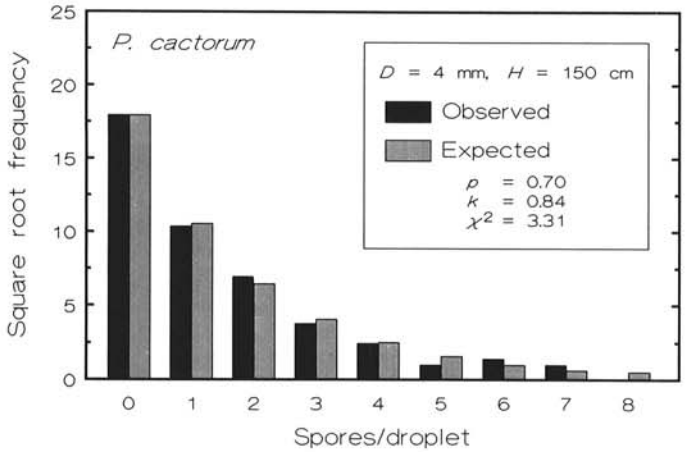

Fig. 2. Frequency distribution of *Phytophthora cactorum* sporangia per droplet and corresponding predictions of the negative binomial probability density function, in which D = impacting drop diameter; H = fall height; p and k = parameters of the negative binomial distribution; and χ^2 = chi-square statistic of goodness-of-fit test. The number of observations = 501.

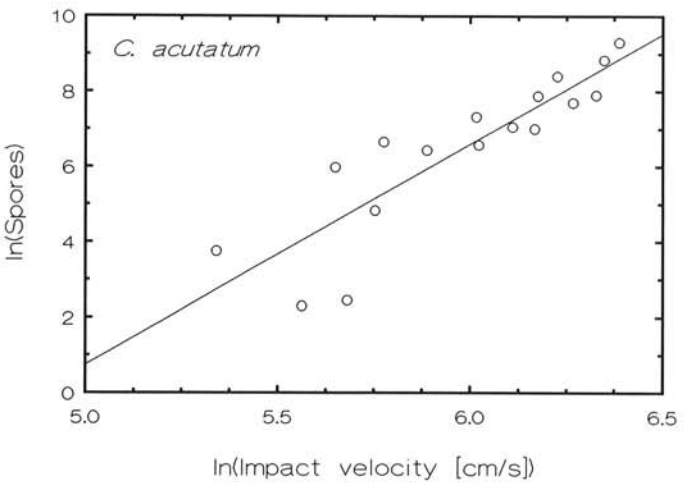

Fig. 3. Relationship between total spores of *Colletotrichum acutatum* dispersed per impaction and velocity of the impacting water drops. Data are means of replicates, and the solid line represents predictions from the regression equation (Eq. 1).

for *C. acutatum*, and

$$\ln(\Sigma Pc + k/2) = -33.23 + 5.83\ln(V) \quad (2)$$

for *P. cactorum*. Plots of residuals indicated that the transformations resulted in acceptable regression models (7).

The relationships between percentages of droplets with no spores (p_{Ca} and p_{Pc}) and kinetic attributes of incident drops (V and KE) were evaluated similarly. Highest correlations were found between the logarithmic transformation of KE and both p_{Ca} and p_{Pc}. Excluding an exceptional value of p_{Ca} for 1-mm drops released from 25-cm height, a significant ($R^2 = 0.40$, $P < 0.01$) regression equation was found for p_{Ca} as:

$$p_{Ca} = 37.28 - 5.19\ln(KE) \quad (3)$$

There was, however, no significant regression found between p_{Pc} and V, KE, or their transformations. The grand mean and standard error of p_{Pc} were 67.3 and 23.8, respectively. Figure 5 shows

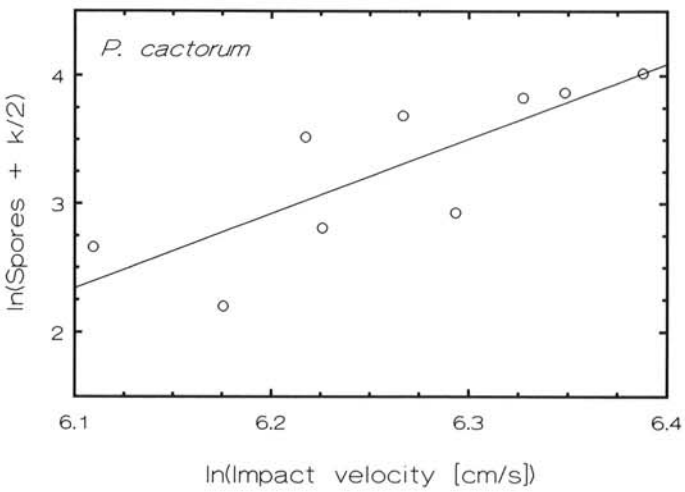

Fig. 4. Relationship between total spores of *Phytophthora cactorum* dispersed per impaction and velocity of impacting water drops. Data are means of replicates, and the solid line represents predictions from the regression equation (Eq. 2). The value of $k/2$ equals 0.4, in which k is the common index of dispersion from the negative binomial distribution.

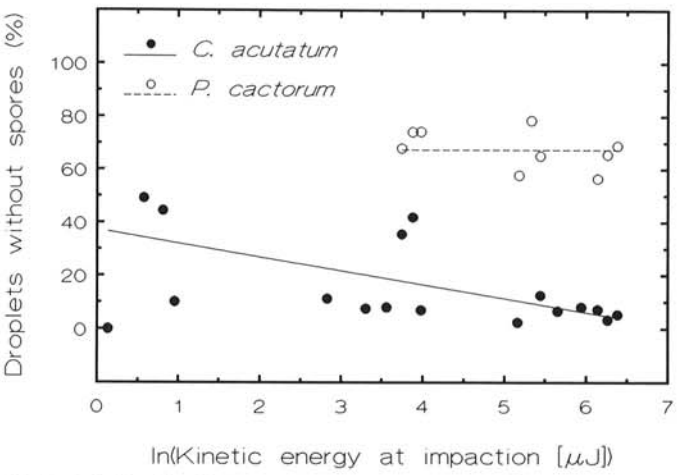

Fig. 5. Relationships between percentage of droplets with no spores and kinetic energy of impacting water drops for *Colletotrichum acutatum* conidia (solid circle and line) and *Phytophthora cactorum* sporangia (hollow circle and broken line), respectively. Lines represent predictions from the regression equation (Eq. 3, for *C. acutatum*) or the mean (for *P. cactorum*).

a plot of observed p_{Ca} versus $\ln(KE)$, together with predicted values from Equation 3. Means of p_{Pc} for each combination of D and H also were plotted for purposes of comparison.

Relationships between droplet size and spores per droplet. There was no significant relationship between travel distance of splash droplets and number of spores per droplet based on a correlation analysis ($P > 0.05$ for both pathogens; data not shown). However, a weak correlation (0.33–0.61) between droplet size and transformed number of spores per droplet was found for both *C. acutatum* and *P. cactorum*. Linear relationships were found between transformed spores per droplet and droplet diameter (Figs. 6,7), based on weighted regression analysis:

$$\ln(N_{Ca}) = 2.99 + 1.56d \quad (4)$$

and

$$\ln(N_{Pc} + k/2) = -0.82 + 1.22d \quad (5)$$

for *C. acutatum* and *P. cactorum*, respectively. The R^2 values for Equations 4 and 5 were only 0.11 ($P < 0.001$) and 0.38 ($P < 0.001$), respectively. Residual plots indicated that the chosen regression models were acceptable.

DISCUSSION

Dispersal of *C. acutatum* and *P. cactorum* spores by single drop impactions was characterized in this study by a range of droplet responses, mean and distribution of number of spores per droplet, and total pathogen propagules dispersed per impaction. Except for drops less than 0.5 mm in diameter, incident drops had sizes that encompassed the spectrum of natural rainfalls (8) and reached a high percentage of their terminal (and natural rainfall) velocities (33). Small drops (≤0.5 mm) do not result in splash droplets and therefore do not contribute to splash dispersal. Due to the asymptotic behavior of mass and kinetic energy reflections (i.e., the proportion of mass and kinetic energy of the incident drops transferred to [reflected in] splash droplets approached constants above relatively low impact velocities [41]), the results reported here can be generalized to natural raindrop impactions. Splash droplet formation and spore dispersal results should be applicable to impactions throughout rain episodes except when spore depletion becomes a factor, or the fruit are submersed in puddles of water.

In our preceding study of single drop impaction on healthy strawberry fruit (41), droplet responses were described by their size, number, mass, travel distance, initial velocity, splash angle, kinetic energy, and reflective factors of mass and kinetic energy. The characteristics of mass reflection in this study (Tables 1,2) were agreeable with those for healthy fruit, except that, in general, the travel distances of droplets from infected fruit were smaller. One important factor affecting splash is the characteristic of the impact surface, normally indexed by surface tension (24). With infected fruit and sporulating lesions, more energy was believed to be dissipated for deformation of fruit surface during impaction (37). The ratio of the travel distance of droplets with spores given here to (divided by) our previous results with healthy fruit (41) was calculated as 0.85 (standard error = 0.13).

Gregory et al (19) reported that a single impaction by a 5-mm water drop at terminal velocity on a 0.1-mm-deep suspension of *Fusarium solani* (10^5 spores cm^{-2}) could produce over 5,000 droplets with about 50% containing one or more fungal spores. Similar numbers of droplets and spore-carrying droplets for impactions by 4- or 5-mm water drops also were found in later studies with spore suspensions of *Septoria nodorum* (5), *Pyrenopeziza brassicae* (9), and *Pseudocercosporella herpotrichoides* (11). Our studies with infected fruit, however, observed fewer splash droplets per impaction (also see 34,41). The difference in splash characteristics of impaction surfaces, as indicated by many studies (19,24,37,41), should account for the major part of the discrepancy. In general, water drop impactions on plant surfaces produce fewer droplets than on a liquid suspension (13). Another factor is the resolution of our image analysis system, which did not allow detection of droplets smaller than 0.15 mm in diameter. Because very few spores are carried in tiny droplets (≤0.2 mm) (5,11,13,34), our estimated total number of dispersed spores is believed to be close to but slightly less than that present in all splash droplets.

Mean number of spores per splash droplet with *C. acutatum* (N_{Ca}) was quite large, varying from 6 to 134 over the range of incident drop conditions studied. By considering the number of splash droplets (n) per incident drop, 17–11,546 conidia per drop impaction (ΣCa) were dispersed mean distances between 30 and 90 mm, indicating that the pathogen could be effectively dispersed by raindrops. Compared to *C. acutatum*, mean number of spores per droplet and total spores per impaction with *P. cactorum* were much smaller, ranging from 0.27 to 0.82 for N_{Pc} and from 9 to 56 for ΣPc, respectively, with about two-thirds of the droplets with no spores. A major reason for the difference between the two pathogens was the different magnitude in inoculum density. Fruit infected by *C. acutatum* had about 10^3 times more spores than fruit infected by *P. cactorum*. No attempt was made to standardize densities because inoculum levels are typical for these pathogens (25,34,39,43), and an infected fruit is the significant

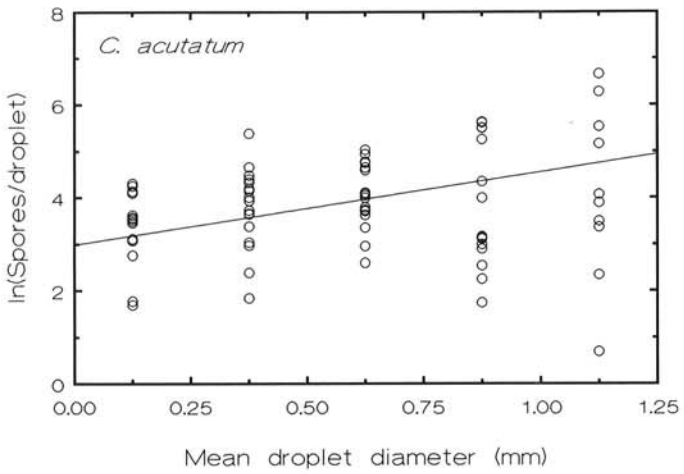

Fig. 6. Transformed number of *Colletotrichum acutatum* conidia per droplet in relation to mean splash droplet diameter. Data are means of replicates within each division of droplet diameter, and the solid line represents predictions from the regression equation (Eq. 4).

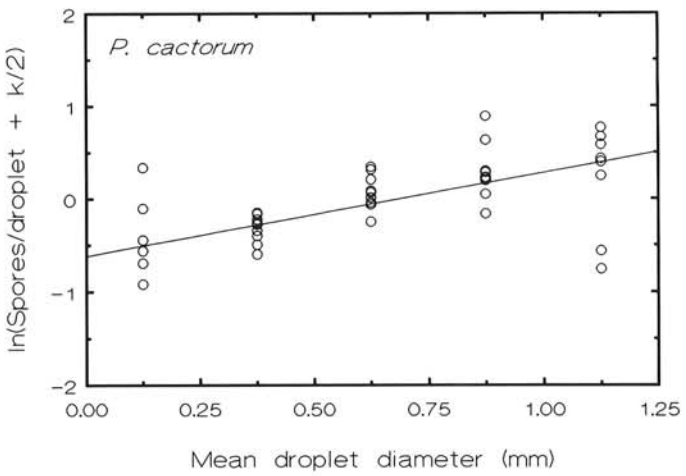

Fig. 7. Transformed number of *Phytophthora cactorum* sporangia per droplet in relation to mean splash droplet diameter. Data are means of replicates within each division of droplet diameter, and the solid line represents predictions from the regression equation (Eq. 5). The value of $k/2$ equals 0.4, in which k is the common index of dispersion from the negative binomial distribution.

epidemiological unit in the field. A higher percentage of *P. cactorum* sporangia produce infections compared to *C. acutatum* conidia with the same environmental conditions (L. V. Madden, *unpublished*). Conidia of *C. acutatum* also are produced in mucilage in acervuli. When sufficiently wetted, the mucilage dissolves, and a thin film of conidia in suspension on the fruit surface is produced. Surface tension and other properties of this suspension may increase spore entrainment compared to spores suspended in water (13). However, on average (based on Table 1), there were about 188 times more *C. acutatum* conidia dispersed compared to *P. cactorum* sporangia, a ratio less than for inoculum density (10^3) for the two pathogens. Presumably, other factors, as well, contribute to spore entrainment.

Published reports of the mean number of spores per droplet and total spores per impaction vary widely for different pathogens and crops. For a 4- or 5-mm water drop impaction, dispersed spores ranged from dozens for drops falling on infected plant materials (5,14) to hundreds of thousands for impactions falling onto spore suspensions (9–11,19). Our results are, in general, intermediate between the published cases for plant materials and spore suspensions. Differences in spore suspension concentration, depth, and other experimental conditions make it difficult to compare results from the published data. As shown here, pathogen spore type and inoculum density have a large influence on splash dispersal.

Distributions of *C. acutatum* conidia per droplet were highly skewed, and the log-normal distribution was found to fit most of the frequency distributions for droplets with one or more spores (Fig. 1 and Table 3). Both (transformed) N_{Ca} and ΣCa were strongly dependent on D and H (Table 2). Although there was no simple positive or negative linear relationship found between (transformed) N_{Ca} and D or H, a significant regression of ΣCa on impact velocity, V, was obtained on a log scale, which was agreeable with previous observations that the effectiveness of splash dispersal, in general, was partly determined by the kinetic attributes of the incident drops (13,37). The relationship between ΣCa and V, together with information on N_{Ca}, p_{Ca}, and the common log-normal σ value determined for all observed droplets with spores, provides valuable information for further modeling of splash dispersal of *C. acutatum*.

For *P. cactorum*, the highly skewed distributions of N_{Pc} were well fitted by the negative binomial function. In a study of spore dispersal by simulated canopy drips (10–40 cm fall height of drops), Reynolds et al (34) described the frequency of N_{Pc} by the logarithmic-with-zeros distribution for impacts of low velocity water drops. Because the negative binomial distribution converges to the logarithmic-with-zeros as k approaches zero (22), our results were consistent with the finding of Reynolds et al (34) in a broad sense. In fact, fits of the negative binomial distribution to the data of the low velocity impaction also were adequate in most cases (34, L. V. Madden, *unpublished*). Negative binomial transforms of N_{Pc} and total spores per impaction, ΣPc, depended strongly on D, but not H or the interactions between D and H (Table 2), which also agreed with the results of Reynolds et al (34). Similar to *C. acutatum*, a linear relationship between the transformed ΣPc and V was found, but the percentage of explained variability (R^2) was less than for *C. acutatum*.

Fitt et al (13) showed a linear relationship between number of spores per droplet and droplet size (mass) for primary splash droplets with *Rhynchosporium secalis*. This is equivalent to a linear relationship between the cube root of spore numbers and droplet diameter. In other studies, a simple relationship between square root of spore numbers and droplet diameter was observed for *P. herpotrichoides* (9,11), *S. nodorum* (5,6), and *Pseudocercosporella capsellae* (10). Working with *P. cactorum*, however, Reynolds et al (34) found no relationship between sporangia per droplet and size of splash droplet from impacting water drops released from heights up to 40 cm above inoculum targets. Instead, they only found that droplets bearing spores were significantly larger than droplets without spores. In this study, a weak relationship between the transformed spores per droplet and droplet diameter was found for both *C. acutatum* and *P. cactorum* (Eqs. 4,5 and Figs. 6,7). Whereas both the regressions were significant, values of R^2 were rather low, indicating only a marginal improvement in predictive ability over simply using a mean number per droplet. Regression of square root of spore numbers on droplet diameter also was performed to compare our results with others. The relationship was significant for both *C. acutatum* and *P. cactorum*, but the R^2 values were lower, and the residual plots showed a slight pattern (X. Yang and L. V. Madden, *unpublished*) that was not evident in residual plots for Equations 4 and 5. Compared to the same kind of relationship for impactions onto *P. brassicae* suspension given by Fatemi and Fitt (9), the slopes (0.78 for *C. acutatum* and 0.64 for *P. cactorum*, respectively) were flatter. For a droplet diameter of 1 mm, *P. brassicae* had a predicted 241 spores per droplet. This compares with the predicted of about 95 conidia per droplet for *C. acutatum* and only one sporangium for *P. cactorum*. The low velocity drip impactions on fruit infected by *P. cactorum* used by Reynolds et al (34) probably did not produce a great enough range of droplet sizes to detect the slight increase in spore numbers as droplet diameter increased. It is doubtful that researchers will be able to clearly distinguish between square root, cube root, or logarithm models with the high observed variability that may be typical. The logarithmic transformation (or $\ln[N_{Pc} + k/2]$) was used here to be consistent with the observed distributions of spores per droplet.

Theoretical mechanisms of the splash dispersal process are not yet fully understood. Pathogens dispersed by rain are carried by splash droplets that follow trajectories determined largely by initial parameters such as impact velocity or kinetic energy. Modeling splash dispersal from initial velocity and trajectory of droplets with Newtonian dynamics, therefore, has been strongly recommended (23,28,41). Approximations are possible, however, by using diffusion theory (40). By first studying single drop impaction on healthy fruit, we successfully characterized droplet movement away from strawberry surfaces (41). We found that splash droplet diameter, number, mass, and travel distance are dependent on impacting drop velocity and kinetic energy. In this study, we extended these results by relating dispersed spores of two pathogens to the splash event. Here we found that total spores dispersed for both pathogens also was dependent on drop velocity. In natural rains, drop velocity is a function of drop size (2,33), and mean drop size increases with rain intensity (27). Primary or initial dispersal from the inoculum sources would be expected to increase as rain intensity increased. However, there is no simple relationship between simulated or actual rain intensity and resulting final levels of dispersal (25,42,43), presumably because of the strong effects of ground cover, plant canopy, or multiple resplashing of spore-carrying droplets. With additional knowledge of effects of ground surface and plant canopy on individual splash droplet trajectories, a stochastic simulator of splash dispersal based on Newtonian dynamics thus can be constructed. Such a simulator will permit a rational evaluation of the factors governing splash dispersal.

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