Rain Dispersal of *Colletotrichum gloeosporioides* in Simulated Rice Field Conditions

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ABSTRACT

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Rain splash dispersal of *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, a pathogen used as a commercial mycoherbicide (a type of herbicide having fungal spores as the active ingredient) to control northern jointvetch in rice, was examined in a wind tunnel (2.3 m/sec) with simulated rain (40 mm/h). The effects of different surface conditions including water, soil, and concrete and rice density were examined. Dispersal of the pathogen was significantly reduced when weeds were present within a rice canopy. The high rice density not only reduced the steepness of the dispersal gradient but also reduced the intercept of the dispersal curve. The half-distances of the disease dispersal curves (values of distance when lesions per plant decrease by half) varied from 42 cm for weeds on a concrete surface to 99 cm for weeds on a water surface. The half-distances are much greater than the spore dispersal curves reported previously for pathogen of *Colletotrichum*. Total lesions determined by integrating power law model in the range 30–180 cm from the inoculum source were 24.7 and 38.2 for high and low rice densities and 47.1 and 67.1 for soil and water surfaces, respectively. Vertical distributions of disease lesions on plant stems were also studied. The average height of lesions on stems was significantly higher for concrete than for soil and water surfaces. The vertical distribution of lesions on plants suggested that the energy reflection of water droplets from the surface determined the height and distance of dispersal. The relationship between pathogen dispersal and disease epidemic of this pathosystem is discussed.

Additional keywords: biological control, epidemiology.

Colletotrichum gloeosporioides (Penz.) Sacc f. sp. *aeschynomene* causes an anthracnose of northern jointvetch, *Aeschynomeae viginica* (L.) BSP., a leguminous weed in rice fields. Anthracnose lesions are oval shaped with orange conidia in acervuli on stems. The pathogen has been studied since 1969 (1) and is now utilized commercially as a mycoherbicide to control northern jointvetch in rice. Many formae speciales of *C. gloeosporioides* have been studied as potential mycoherbicides (9).

Despite the broad interest and research on mycoherbicides, little knowledge is available on the dispersal of these pathogens. Anthracnose in northern jointvetch has been considered to be endemic rather than epidemic because of poor dispersal mechanisms of the pathogen and the barrier effect of rice (10). In a wind tunnel, dispersal of *C. acutatum* by simulated rain has been reported to be approximately 2 m per rain episode (12). Because *C. g. aeschynomene* has been used as a model system to study efficacy of mycoherbicides, quantitative information regarding dispersal may provide answers and ecological principles as to the endemic nature of anthracnose diseases. Such information may also aid in the economical selection of potential mycoherbicides. Data on dispersal of this pathogen will be required for future field tests of genetically engineered strains.

Recent developments in the use of simulated rain to quantify the dispersal of fungal pathogens (2–6,11,12) has provided an approach to understanding the dispersal of plant pathogens used as mycoherbicides. Simple and efficient rain towers have been developed (6,12) for this type of work to determine the effects of rainfall intensity, inoculum source, and ground cover on splash dispersal of pathogens. The objective of our study was to demonstrate and quantify the rain dispersal of *C. g. aeschynomene* under simulated rice field conditions, using a simulated rain and wind system modified from Yang et al (12).

MATERIALS AND METHODS

Rain tower and wind tunnel. A rain tower with an attached wind tunnel was constructed with slight modifications of the structure reported previously (6,12). The equipment was built on a concrete platform with a slight slope surrounded by permanent buildings on three sides and consisted of a rain tower, a wind tunnel (12), and a test chamber. The chamber had a test area of 3.5 × 1.6 m, which could be split into two test chambers of equal area (3.5 × 0.8 m) by inserting a board at the center line to facilitate comparison experiments. The surface of each test chamber could be modified according to experimental needs.

The rain tower was 2.56 m high with a cross section of 2.56 × 1.70 m. A nozzle (Nelson Brass Nozzle, N-29C, Peoria, IL) was located 3.4 m above the test surface and sprayed upward with water pressure at 44 psi. The highest point of fall of water drops was about 6 m from the surface. The rainfall covered an area with a diameter of about 4.5 m. The uniformity of rain density inside the test chamber was tested by placing five beakers at different positions on the testing surface. Five replicate tests showed that rainfall was distributed evenly in the testing area with an intensity of 40 mm/h. A simple wind tunnel similar to that in Yang et al (12) was built to provide a horizontal air flow with three 51- × 51-cm circulators (model F0882148, Toastmaster, Inc., Booneville, MO) placed at the head of the tunnel. The speed of the airflow was uniform at 2.3 m/sec within a distance of 1.0–3.1 m from the source at a height of 30 cm.

Plant and pathogen. Seeds of northern jointvetch were germinated on moistened filter paper in petri dishes at 28 C for 24 h. Uniformly germinated seeds were then planted in vermiculite in 7- × 7- × 6-cm plastic pots, four seeds per pot, and then placed in growth chambers (model E7 Conviron, Control Environments, Pembina, ND) at 28 C with a 12-h photoperiod for growth. After 2 wk, plants were transferred to a greenhouse for another week. Plants with a diameter of 3.5 mm and 35 cm tall were used for tests.

An isolate of *C. g. aeschynomene*, RBES-3B was cultured in torula agar medium at 28 C. The cultures were used when mycelium reached the edge of the 10-cm diameter plates, approximately 6 days after inoculation. Each plate produced approximately 1.6 × 10^9 spores. In the field, conidia of *C. gloeosporioides* remain within acervuli and are released during rainfall (10). To have a reproducible amount of inoculum for inoculum sources, conidial matrices were made to simulate the lesions by placing the spores from cultures onto plant stems at heights of 10–20 cm. Each inoculum source had about 5.5 × 10^8 spores. Before testing, the simulated lesions were air dried to better represent field conditions.

Treatments. Four treatments simulating rice field conditions were established in the testing chambers. The treatments were as follows: A water surface, a soil surface, a high rice canopy density, and a low rice canopy density. An additional treatment of a concrete surface was also established. The water surface was used to simulate the early stage of a flooded rice field. A water pool 3-cm deep was established by mounting a piece of plastic sheeting to the supporting wood frame on the test surface. The soil surface simulated the early growth stages of dry rice fields and was established by placing a 3-cm layer of sandy-loam soil on the plastic sheet. The surface had a slight slope to facilitate drainage. At the lower side, a 3-cm-wide channel was made so that the water could quickly run out of the testing area through a space at the lower side of the chamber. The soil was firmed in place with a 3-min rainfall before the tests. After each test, an additional 1 cm of soil was added to minimize effects of any residual inoculum. To establish the rice canopy treatments, rice plants at the heading growth stage were transplanted from fields into 10- × 10- × 9-cm plastic pots with one plant (13 tillers per plant) per pot. The plants were then placed in one subchamber forming a canopy at a density of 80 pots per square meter (1,040 tillers per square meter) to form the high density rice canopy treatment. Residual inoculum after each test for the rice canopy was eliminated by washing the rice plants for at least 20 min with rainfall and waiting for at least 2 days before another test (8). The low-density treatment (650 rice tillers per square meter) was constructed to simulate field condition before the rice canopy closes. Experiments were conducted on still, windless days.

For all treatments, pots of northern jointvetch plants were placed on the surface parallel to the wind source at 30-cm intervals with the first row 30 cm away from the source plants. There were six rows, two pots per row, for the treatments with rice. All other treatments had eight to nine rows. Source plants were placed 1 m from circulators. Ten minutes of 40-mm/h simulated rainfall was applied to each treatment to mimic summer rainstorms in southern Arkansas. After treatment, potted plants were removed from the test chambers and transferred to a dew chamber at 28 C. For the rice canopy treatments, the canopies were disassembled when pots with northern jointvetch plants were removed. After 24 h of incubation, northern jointvetch plants were moved into a greenhouse, and the number of lesions per plant was counted 4 days after inoculation. To determine the height of spore rebounding on different surfaces, the vertical distribution of lesions on stems was quantified by dividing the vertical distance of plant stems into intervals of 1.3 cm and counting the number of lesions in each interval. The numbers of plants used were 28, 35, and 31 for concrete, soil, and water surfaces, respectively.

TABLE 1. *F* tests and significance levels ($P > F$) from analysis of variance of the effects of replicate (block), treatment (plot), distance (split plot), and pot on the number of lesions per northern jointvetch plant, *Aeschynomeae viginica*, infected with *Colletotrichum gloeosporioides* f. sp. *aeschynomene*

Variable[a]	*F*	$P < F$
Replication	0.53	0.312
Treatment	53.2	0.0001
Distance	121.67	0.0001
Treatment × distance	8.20	0.0001
Pot	3.29	0.108
Plant	1.53	0.200

[a] Error terms were pot × plant interaction for pot and plant and treatment × distance × replication for other variables.

Data analysis. The experiment was a two-factor design; treatment and distance were considered as the two factors. Each experiment was repeated five times and the five experiments were considered five replicates in data analysis. The number of lesions per plant with respect to distance was the dependent variable. SAS (7) procedure of variance analysis (ANOVA) was used to evaluate the effect of the factors. To compare the results of treatments with rice to results of other treatments, only data of the first six rows (30–180 cm from the source inoculum) were used in modeling and variance analysis.

The relationship between lesions per plant (Y) and the distance (X) was determined for each treatment by fitting data with two commonly used empirical dispersal models (3,12). The first is the power law model:

$$\mathrm{Ln}(Y) = B_0 + B_1 \mathrm{Ln}(X) \quad (1)$$

and the second is the exponential model:

$$\ln(Y) = B_0 + B_1 X \quad (2)$$

where B_0 and B_1 are the regression coefficients. In both equations, the parameter B_1 describes the rate of decrease of Y with distance. Regression results were compared between the two models. Regression coefficients were also compared among the treatments to determine the effects of surface and rice density on disease dispersal.

Surface effects were also compared among the treatments by calculating the following dispersal parameters for each treatment. Half-distance ($X_{0.5}$, cm), defined as a value when Y decreases by half as value of X increases by a constant, was calculated using $X_{0.5} = 0.693/d$ (d equal to constant B_1 in eq. 2). The number of theoretical total lesions was calculated by integrating the power law model in a range of 30–180 cm from inoculum source with 10-cm intervals, assuming a 10-cm plant-to-plant distance. Average lesions per plant at distance 30 cm from source (Y_{max}) was also compared among the concrete, soil, and water treatments.

To compare the vertical distributions of lesions on stems among the surfaces, frequency of the distributions was examined by plotting number of lesions against the height. Then, statistical distribution models Poisson and negative binomial were tested for goodness-of-fit to the data of each surface. Average lesion height on stems (H_{avg}, cm) was calculated for each surface.

RESULTS

The effects of treatment and distance as well as their interactions for lesions per plant were all significant (Table 1). Effects of

TABLE 2. Regression analysis between disease lesions per plant (Y) and dispersal distance (X) by using power law model [$\ln(Y) = B_0 + B_1\ln(X)$] and exponential model [$\ln(Y) = B_0 + B_1X$][a]

Treatment	B_0 ± Standard[b]	B_1 ± Standard	r^2[c]	$P > F$
$\ln(Y) = B_0 + B_1\ln(X)$				
Concrete	7.70 ± 0.60	−1.38 ± 0.14	0.77	0.001
Soil	5.15 ± 0.68	−0.92 ± 0.14	0.55	0.001
Water	4.75 ± 0.56	−0.75 ± 0.20	0.32	0.001
Low-density rice	5.95 ± 0.80	−1.18 ± 0.18	0.61	0.001
High-density rice	3.71 ± 0.69	−0.74 ± 0.18	0.42	0.001
$\ln(Y) = B_0 + B_1X$				
Concrete	3.21 ± 0.19	−0.016 ± 0.001	0.78	0.001
Soil	1.99 ± 0.29	−0.010 ± 0.002	0.44	0.001
Water	2.07 ± 0.30	−0.007 ± 0.002	0.28	0.001
Low-density rice	1.97 ± 0.28	−0.013 ± 0.002	0.59	0.001
High-density rice	1.16 ± 0.26	−0.008 ± 0.002	0.29	0.001

[a] Five different dispersal treatments using northern jointvetch (*Aeschynomene viginica*) plants and *Colletotrichum gloeosporioides* f. sp. *aeschynomene* were conducted in a rain-wind tunnel.
[b] Standard deviation of regression coefficients.
[c] Coefficient of determination.

replicate, pot, and plant were not significant. Coefficients of determination were significant for all treatments for both the power law model and the exponential model with r^2 values of power law models slightly greater than those of the exponential models for all treatments except concrete (Table 2). For the two models, both intercepts (B_0) and regression coefficients (B_1) were significantly different between treatments for low and high rice densities (Fig. 1 and Table 2). On concrete, there was an average of 17 lesions per plant 30 cm from inoculum source, and this surface had the steepest disease gradient. Treatments on a water surface and high rice density had the flattest disease gradients. The soil surface resulted in the same intercept as that of the water surface. Splash dispersal for both water and concrete surfaces produced at least one lesion per plant as far as 270 cm from the inoculum source. In treatment with a high density of rice, disease lesions were observed on some plants 180 cm from the inoculum source. The high rice density reduced both the steepness of the dispersal gradient (slope) and the intercept of dispersal curve.

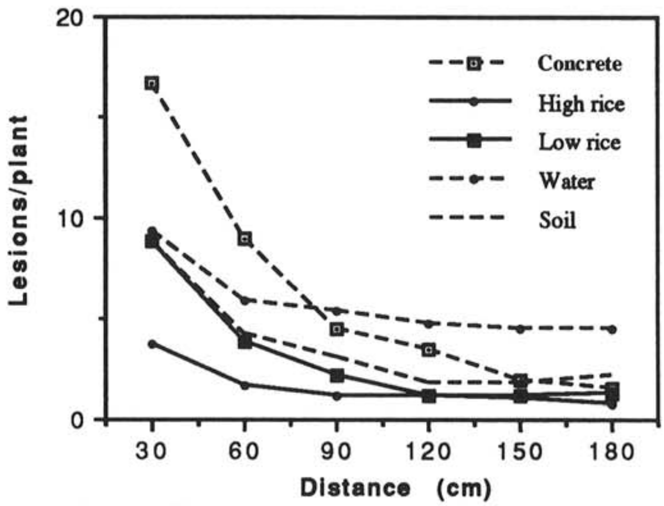

Fig. 1. Effects of different surfaces and rice densities on spore dispersal of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* observed as lesions on stems of northern jointvetch (*Aeschynomeae viginica*) at different distances from a source of inoculum. All experiments were treated with a 10-min 40-mm/h simulated rainfall with 2.3 m/sec wind.

TABLE 3. Parameters of rain-splash dispersal for five different surface conditions obtained from experiments in a wind tunnel with simulated rain using northern jointvetch (*Aeschynomene viginica*) plants and *Colletotrichum gloeosporioides* f. sp. *aeschynomene*

Treatment	$X_{0.5}$ [a]	Total lesions[b]	Y_{max} [c]	H_{avg} [d]	Poisson distribution[e] h_{mean} [f]	R^2 [g]
Concrete	42	100	16.7	8.14	8.01	0.82
Soil	69	47.1	8.5	5.62	5.56	0.96
Water	99	67.0	8.5	5.73	5.26	0.91
Low rice density	53	38.2	8.3	...[h]	...	...
High rice density	92	24.7	4.1	...	...	...

[a] $X_{0.5}$ = half-distance (cm), $0.693/d$, defined as a value of distance when lesions per plant decreases by a half as X increase by a constant. d equal to constant B_1 in exponential models (Table 2).
[b] Total lesions were determined by integrating in the range of 30–180 cm (10-cm interval) using the power law model.
[c] Y_{max} = average lesions per plant at distance 30 cm from source.
[d] H_{avg} = observed average lesion height on stems (cm).
[e] Nonlinear regression between number of lesions and lesion height on plant stems using the Poisson distribution model.
[f] h_{mean} = estimated average lesion height on plant stems (cm).
[g] R^2 = coefficients of determination of the nonlinear regression, which were significant at level $P > 0.01$.
[h] Not measured.

The shortest half-distance was 42 cm for concrete, whereas the longest ones were 99 and 92 cm for water and high rice density, respectively (Table 3). Among the five treatments, the dispersal on concrete had the greatest calculated total lesions for the range of 30–180 cm from inoculum source. Although the intercept of water was not significantly different from those of soil and low rice density (Table 2), the calculated total lesions for water were 40% greater than total lesions calculated for the latter two. In comparison with water, there was a 63% reduction in total lesions for the high rice density (Table 3). The highest and the lowest average numbers of lesions per plant at first row (Y_{max}) were for concrete and high rice density, respectively. Y_{max} was the same for water, soil, and low density (Table 3).

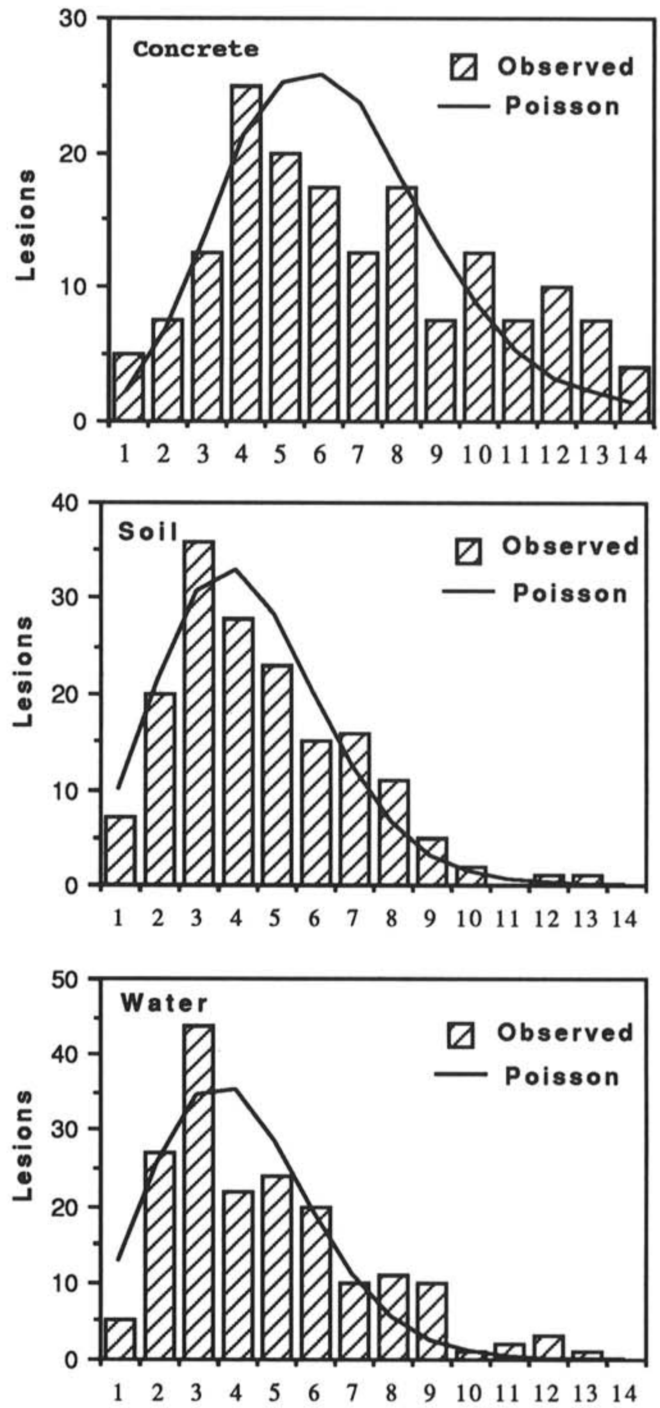

Fig. 2. Vertical distribution of anthracnose lesions on stems of northern jointvetch, *Aeschynomeae viginica*, infected with *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. The observed distribution was fit with the Poisson distribution model.

The vertical distribution of lesions was skewed with 90% of the lesions on stems within 10 cm height for soil and water surface and within 15 cm for concrete (Fig. 2). The distribution patterns for water and soil surfaces were similar. The distribution for concrete was skewed but not as steep as those for water and soil. The three distributions fit Poisson distribution model better than negative binomial model. The distributions for soil and water surfaces fit Poisson model better than that for concrete (Fig. 2 and Table 3). The estimated mean heights of lesions by Poisson model (h_{mean}) were close to the observed average heights (H_{avg}). The h_{mean} and H_{avg} for concrete were greater than those for soil and water (Table 3). The observed peaks of distribution were at 3.9 cm for soil and water and at 5.2 cm for concrete (Fig. 2).

DISCUSSION

Rain-splashed dispersal of spores was a function of roughness of topography (11). Previous studies (5,11) found that different surface topographies, soil, plastic, and straw affected the amount and actual travel distance of splashed conidia. Spore dispersal distance was inversely proportional to roughness (11). Our results with soil, water, and concrete support these findings. The water surface provided the smoothest surface and had a very long flat curve for inoculum spread, while the curve for concrete and soil is relatively steep (Table 2).

Results of the experiments done on concrete indicate that in addition to surface roughness, the energy reflection of droplets may be another factor determining vertical distribution of spores splashed by rain. Although concrete is not smoother than water, the intercept on concrete was greater than that of water (Fig. 1). In comparison to concrete, more energy was used for surface deformation in soil or for viscous dispatch in water. Therefore, less energy was reflected by splash droplets on soil and water, resulting in greater trajectories in concrete than in other treatments. The height of rebounding determines the number of lesions per plant because infection only occurs on stem. Thus, the higher the rebounding, the greater the number of lesions per plant. The redeposition of inoculum is a continuous event of dropping and rebounding. Higher rebounding ability may provide more inoculum at greater distances. For northern jointvetch anthracnose, most of the lesions were distributed on the lower stems, which provided a measure of the height of rebounding. For instance, the average height H_{avg} may reflect the height of rebounding, which was also associated with average number of lesions per plant at a distance of 30 cm Y_{max} (Table 3). Intercepts (B_1 in exponential model) for water, soil, and low density rice canopy were not significantly different, probably because a water film on surfaces of these treatments resulted in a similar energy reflection.

Fitt et al (3) reported that the half-distances of spore dispersal curves for different fungi were less than 10 cm even with 2–3 m/sec wind. Our half-distances for dispersal measured by lesions varied from 44 to 99 cm (Table 3), which are much greater than those for spore dispersal. This difference may result from the fact that the limited number of potential infection sites per plants fails to reflect the greater actual number of spores caught. The maximum lesions per plant (Y_{max}) may be limited by the number of potential infection sites per plant as affected by different surface conditions (Table 3). Parameters among concrete, soil, and water (Tables 2 and 3) showed that the greater the maximum lesions per plant (Y_{max}) were, the shorter the half-distance ($X_{0.5}$) and the steeper the disease gradients (B_1 in power law model) were.

There were advantages and disadvantages in our experimental system. The plant and the pathogen are very suitable for quantification of rain-splash dispersal. Young plants usually are not branched and have a slender stem, uniform in diameter. Infection by the pathogen causes a clearly defined lesion on plant stems within 4 days after inoculation. Our wind tunnel was built outdoors, so the height of the water droplet was not limited by the space of a building. However, the efficiency was reduced because of less control to the weather conditions. The experiments could be conducted only in still air and only during certain parts of the year. Turbulence was sometimes observed during tests although the weather was calm, which could contribute to the experimental errors, which were much greater than these obtained from indoor experiments (11,12). Furthermore, the residual inoculum could also result in unexplained variation in our regressions.

Anthracnose of northern jointvetch is considered to be an endemic disease, and it has been suggested that the endemicity is due to limited dispersal of the pathogen within the rice canopy (10). The significant reduction of spore dispersal by the rice canopy in our study (Fig. 1) supports this assumption. Because weeds are patchily distributed in rice, the barrier effect of the rice canopy restricts the disease, as a whole, to endemic levels even though disease levels may be high in some patches. If the distance between diseased plants and target plants is greater than the maximum dispersal distance of the pathogen in rice, the chance of increase of disease in target plants is small for each rain episode. Application of the pathogen as a mycoherbicide overcomes such a limiting factor by increasing the inoculum and aiding dispersal (10).

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