Disease Severity Gradient of Soybean Downy Mildew from a Small Focus of Infection

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ABSTRACT

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The spread of *Peronospora manshurica* from an initial infection focus was studied in a field plot of a susceptible soybean cultivar, Williams. Inoculum was introduced in the southwest corner of the plot on potted plants previously infected in the greenhouse with unidentified Illinois biotype(s) of *P. manshurica*. Disease severity was rated around arcs at five distances from the focus of infection. The regression line slope for disease severity gradients at 30 and 44 days after inoculation agreed with the slope of a theoretical line from a point source of inoculum. The slopes of gradient estimates made after 58 days were smaller than earlier ones, indicating a more uniform level of disease throughout the plot. These later gradients also were higher above the X-axis, indicating that disease was more severe. Mean of the proportion of disease in the plot was 0.21 by 58 days after inoculation. Disease increased more rapidly at the more distant areas from the infection focus, where initial severity was lower. Spores were trapped at least once a week during the period of disease ratings. The most spores were trapped during periods of no precipitation. Frequent rainfalls, low wind speeds (0–8 km/hr), and aging of soybean leaves resulted in trapping few spores despite favorable temperatures, relative humidity, and wind directions.

Additional key words: disease intensity, *Glycine max*, spore dispersal.

Downy mildew, caused by *Peronospora manshurica* (Naum.) Syd. ex Gäum., is prevalent on soybean [Glycine *max* (L.) Merr.] in Illinois. The fungus overwinters as oospores on infected leaves and on seeds (3). The oospore-encrusted seeds often produce systemically infected seedlings when planted under cool (18–20 C) conditions (2). Disease development is favored by high humidity and temperatures of 20–24 C. Sporulation occurs between 10 C and 30 C (9). Conidia are thought to be the chief source of infection in an epidemic development of the fungus in the field. The conidia are produced on the underside of the infected leaf, from which they readily disseminate to spread the pathogen throughout the growing season. Disease severity gradients of downy mildew of soybeans have not been considered in the literature, and their quantitative aspects have not been studied.

This report describes an observed disease severity gradient in downy mildew arising from an initial infection focus in a plot of susceptible soybeans.

MATERIALS AND METHODS

Williams, a high yielding cultivar grown in 28% of the total soybean acreage in Illinois (8) and very susceptible to *P. manshurica*, was planted on 13 May 1977 at Urbana, IL. The plot consisted of 40 rows, about 76 cm apart, planted at the rate of seven to eight seeds per 30.5 cm of a row. The prevailing wind was from the southwest and the rows were planted east to west. The plot was bounded by 15-m alleys planted with a resistant cultivar, Union. On the east, west, and north sides were cornfields separated from the plot by field roads about 20 m wide. A field of resistant soybeans was planted on the south side.

Peronospora manshurica was introduced on 28 June in the extreme southwest windward corner of the field plot on infected plants grown in the greenhouse. Five pots, each with two plants, were placed within 1 m of each other between two soybean rows, and the rows were covered with a white vinyl chamber (1.5 m^3) for 2 days. The plants in the chamber were sprayed with a *P. manshurica* spore suspension to provide additional inoculum. The spores were obtained from systemically infected seedlings growing in the greenhouse and placed in a water suspension as previously described (2). The suspension was sprayed through a 20-cm opening around the lower edge of the chamber. With this method there was little or no risk of accidental spread of inoculum from the chamber. The soybean plants in the plot were at growth stage N7–8 (4) (ie, 7 or 8 internodes) when the chamber was removed. Disease ratings were made at weekly intervals thereafter at points around arcs at five distances (3.0, 6.1, 12.2, 18.3, and 27.3 m) from the initial infection focus (Fig. 1). Three diagonals originating at the focus and crossing the field were marked with plastic flagpoles

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at their intersections with the arcs. Plants in approximately 1.5 m of two rows around the flagpoles and at both ends of each arc were rated for disease severity. This plot arrangement permitted five ratings on each arc. A modified Horsfall and Barratt scale (7) was used to rate disease severity. The percentage severity (as percentage of leaf area infected) was calculated by conversion of disease ratings with the Elanco conversion tables (Elanco Products Co., Indianapolis, IN 46140). Disease severity was then expressed as proportion of disease (x); $0 < x < 1$ (5). The growth stage of soybean plants (4) was recorded on each disease rating date. The regression of disease severity on distance from the initial infection focus for each rating date was estimated with log-transformed data as follows: $\log_{10}y = \log_{10}a + b\log_{10}x$ with y = percentage disease severity and x = distance in meters (5). The regression of disease progress over time at each arc was estimated with logistic transformation as follows: $\log_e y = \log_e a + bx$ with $y = \log_e x(1-x)^{-1}$ for amount of disease and x = time in days (11).

Microclimatological data were obtained from an electronic weather station (Climatronics Corp., Hauppauge, NY 11787) located on the west side of the plot (Fig. 1). This weather station measured wind speed, wind direction, temperature, relative humidity, and precipitation. All measurements were recorded continuously by a single DC recorder with a 1-mo storage capacity.

Rotorod samplers with U-shaped brass rods (Metronics Associates, Inc., Palo Alto, CA 94304) were used to sample conidia above the soybeans; both halves of the entire forward-facing rod surface were covered with 3M double-stick tape to provide a spore-trapping surface. The rods were kept about 30 cm above the plants. Samplers were operated for 30 min between 0900 and 1100 hours at the intersections of the outer two diagonal lines with each of the three arcs (Fig. 1). The entire tape strip on the rod surface was removed after exposure and placed on microscope slides for examination. Downy mildew spores on the tape were counted at a magnification of 100. The number of spores per liter of air was calculated from the total number of spores trapped per sample and the volume of air that the sample represents (10). Spores were trapped at least once each week from 12 July, when the disease first appeared to be spreading, until 25 August, when the field had reached a uniform level of infection.

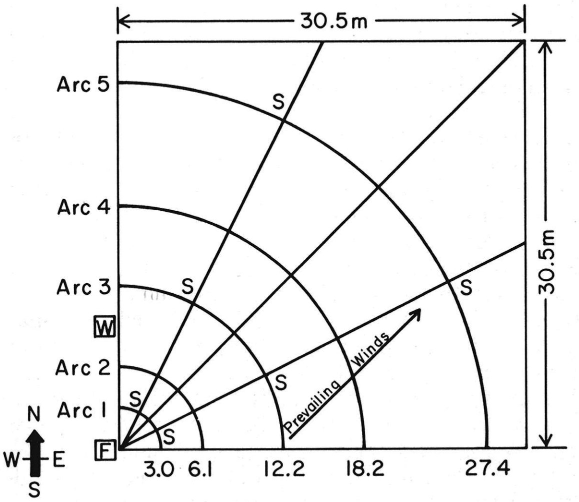

Fig. 1. Diagram of the field plot, showing the focus of infection (F), arcs at five distances from (F); three diagonal lines and the points of intersection with the arcs, with both ends of the arcs, as replicate sites for disease severity estimates; and the locations of an electronic weather station (W), and the rotorod spore samples (S).

RESULTS

Few lesions appeared on the upper leaves of the soybeans examined on arc 1 by 14 days after inoculation, when the plants were at growth stage N9–10. Disease severity did not increase until 23 days after inoculation,

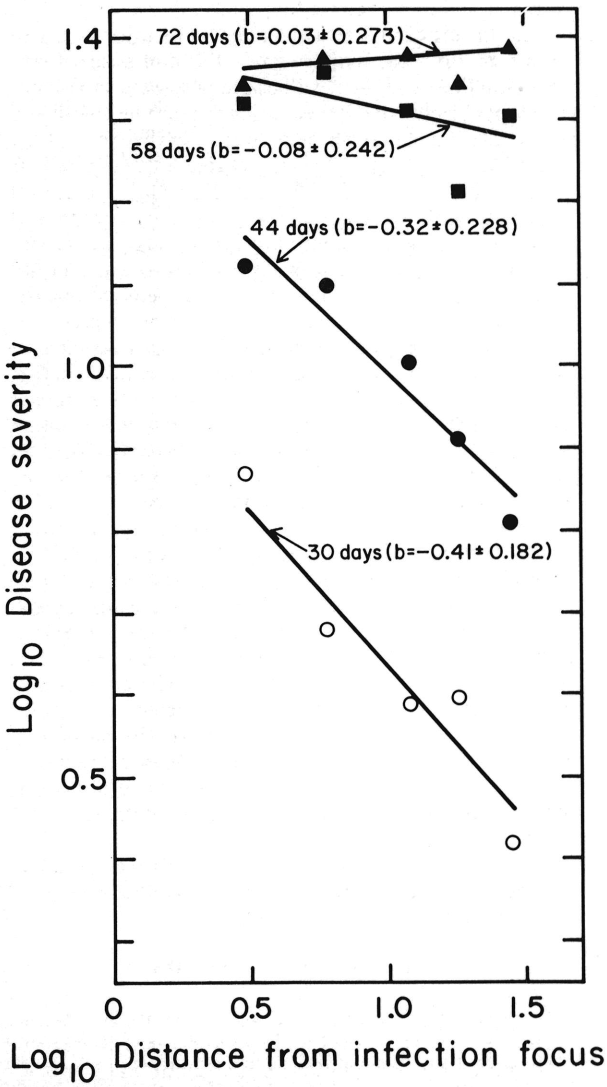

Fig. 2. Disease severity gradients with increasing distance trom the initial infection focus for downy mildew on Williams soybean at selected times after inoculation of the focus.

but then it increased rapidly in the next week, as proportion of disease reached an average 0.07 at arc 1 by 30 days after inoculation (Table 1). Plants developed from stage N7–8 at inoculation to stage R3 [pods 0.5 cm long at one of the four uppermost nodes with a completely unrolled leaf (4)] at the time of the first visual rating. Disease increased with time at each arc, reaching a uniform level of about x = 0.21 throughout the area sampled by 58 days after inoculation. At this date, the coefficient of determination (r^2) for the proportion of the variation in disease severity that can be attributed to the distance was low (0.19) and nonsignificant ($P \geqslant 0.05$). There was no apparent increase in disease severity after the 58th day; most lesions on the leaves were without conidiophores and were dark gray to brown spots resembling scars. On the 72nd day, plants had reached physiologic maturity, and most of their lower leaves were turning yellow and dropping.

The best-fitting regression lines for disease severity gradients with distance on four rating dates are given in Fig. 2. In addition, the calculated values for log a (a scaling factor that partly fixes the position of the regression line) and the r^2 values are presented in Table 1. The slopes (b) for the disease severity gradients at 30 and 44 days after inoculation were similar to the slope of the theoretical line from a point source of inoculum as discussed by Gregory (5). The slopes for the gradients at 58 and 72 days were smaller (ie, b values approached zero), indicating a more uniform level of disease throughout the plot. The regression lines for the amount of disease on these later days also were higher above the X-axis, indicating that disease severity was greater.

The regression lines for disease progress over time at each of three distances (at arcs 1, 3, and 5) are given in Fig. 3. The slope for the disease increase at arc 5 was steeper than for increases at arcs 1 and 3. At the first five rating dates, the regression line for arc 5 was lower than for arcs 1 and 3. The r^2 values generally increased with distance from the infection focus (Table 1).

Daily temperatures ranged from 12 to 37 C during the period from inoculation to final disease severity rating (28 June–8 September), with usual daily means of 18–28 C. On many days, temperature of 21–24 C prevailed for more than 10 hr, providing conditions favorable for the sporulation and spread of *P. manshurica*. The numbers of *P. manshurica* spores trapped by the rod samplers at arcs

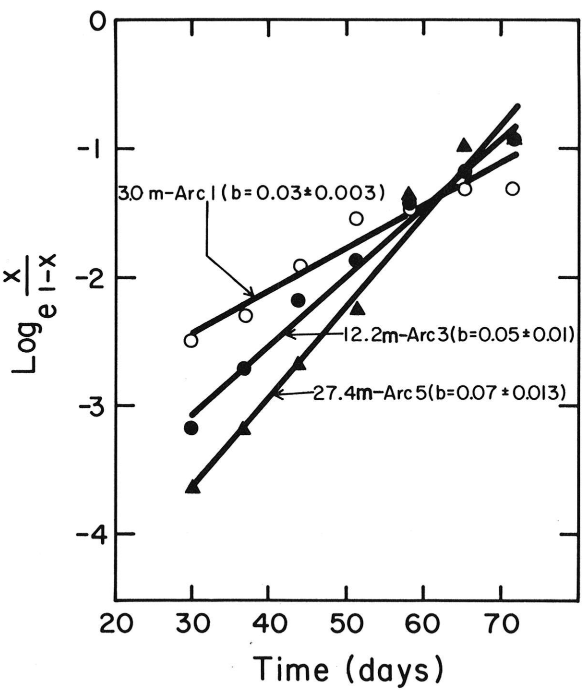

Fig. 3. Disease progress (logit scale) for downy mildew on Williams soybean at selected distances from an initial infection focus. Slope of line (b) corresponds to Van der Plank's (11) average apparent infection rate.

TABLE 1. Proportion of downy mildew on Williams soybean at five distances from an initial focus of infection with *Peronospora manshurica*

Distance from infection focus (m)	Proportion of downy mildew (x)[a] after inoculation of focus at: 30 (days)	37 (days)	44 (days)	51 (days)	58 (days)	65 (days)	72 (days)	$\log_e a$[b]	r^2 (%)[c]
3.0	0.07	0.09	0.13	0.18	0.21	0.22	0.22	−3.39	91
6.1	0.05	0.07	0.13	0.21	0.25	0.25	0.25	−4.18	86
12.2	0.04	0.06	0.10	0.13	0.21	0.23	0.26	−4.67	96
18.2	0.04	0.05	0.08	0.11	0.20	0.22	0.22	−4.62	97
27.4	0.02	0.04	0.06	0.09	0.21	0.25	0.25	−5.67	95
$\log_{10} a$[d]	1.04	1.17	1.31	1.45	1.39	1.34	1.35		
r^2(%)[e]	81	97	85	70	19	9	9		

[a]Each value is the mean of five replicate estimates.
[b]Scaling factor that partly fixes the position of the line that describes the regression of disease progress on time.
[c]Coefficient of determination for the regression of disease progress on time.
[d]Scaling factor that partly fixes the position of the line that describes the regression of disease severity on distance.
[e]Coefficient of determination for the regression of disease severity on distance.

1, 3, and 5 and the daily rainfall during the experiment are shown in Fig. 4. Only 4 spores were collected at arc 1 by 14 July (16 days after inoculation of the initial infection focus). On 28 July, many spores (190 spores/3,600 liters of air space) were trapped at arc 1. During southerly and southwesterly winds to 13 km/hr on the morning of 3 August, the largest number of spores (280 spores/3,600 liters of air space) were trapped at arc 3. Strong southwesterly winds should have been favorable for spore trapping because of the position of the traps relative to the infection focus. The trapping of large numbers of spores on 28 July and 3 August also occurred during a period of no precipitation.

DISCUSSION

Factors affecting types of disease gradients have been discussed by numerous investigators (1,5,11). Contamination by inoculum from outside sources other than the initial infection focus, variation in method of disease rating, and variation in rating dates are some of the factors that may cause the slopes of disease gradients to deviate from the slope of the theoretical line (1).

The slopes for the increase of soybean downy mildew from a small focus of infection generally agreed with the slope of a theoretical gradient line described by Gregory (5). There was no evidence of contamination from outside

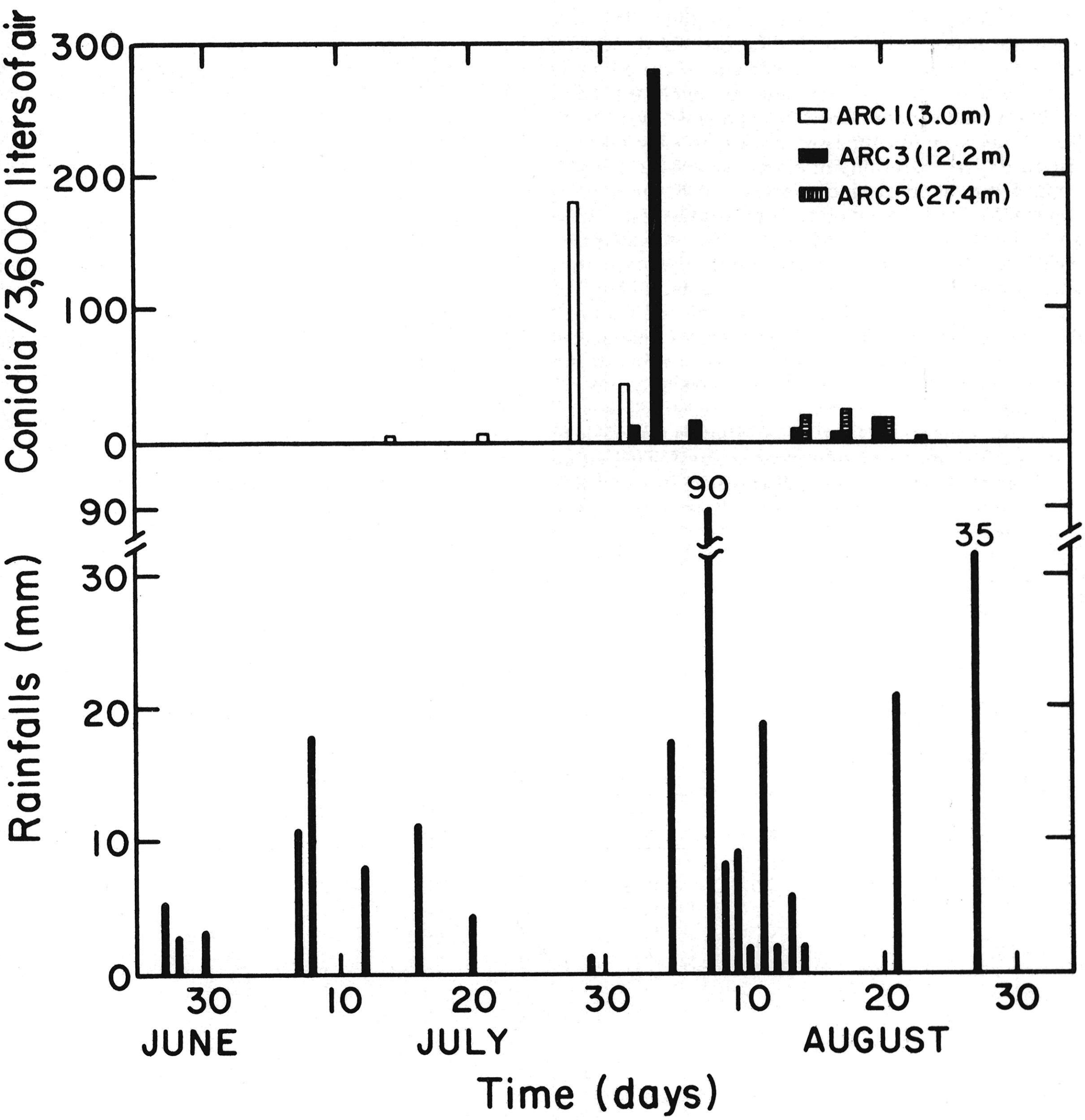

Fig. 4. Daily rainfall during the experiment and numbers of *Peronospora manshurica* conidia trapped at three distances from the initial infection focus (at arcs 1, 3, and 5) in a plot of Williams soybeans. Conidia were trapped at least once each week from 12 July to 25 August.

the experimental plot. The smaller slopes of the disease gradients measured at 58 or more days after inoculation were caused by the continuous spread of conidia from the initial infection focus and the eventual dispersal of conidia produced at secondary lesions. The flattening of curves representing the disease gradients as a pathogen spreads from a point source has been described previously (5).

Comparison of disease progress curves at each measured distance from the initial infection focus showed that disease increased more rapidly when initial disease damage levels were lower. From 29 June to 8 September, conditions were favorable for conidial germination within 12 hr, and the disease incidence should have increased rapidly. However, the level of infection was not uniform at areas sampled in the plot until the 58th day after inoculation. At this time, differences in the disease levels at all distances from the infection focus probably were due to random variations and not significant. Thus, as downy mildew approached 100% possible severity ($x \leqslant 0.25$), curves representing the disease severity gradient flattened. The uniformity of disease severity in the plot implied very short-distance dispersal of *P. manshurica* conidia (5). The frequent rainfalls during the first 22 days after inoculation may have delayed an increased incidence of disease through the plot (horizontal spread among plants). Heavy rains can cause "rain scrubbing" or "washout" of spores suspended in the air (6). On rainy days, more conidia may have been carried from infected upper leaves to uninfected lower leaves (vertical spread within plants). The most conidia were trapped after no rain had fallen for more than a week, even though wind speeds were less than 13 km/hr. No attempt was made to correlate the numbers of spores trapped with either microclimatological records or disease severity since daily spore totals and leaf-wetness periods were unknown. Apparently, both soybean growth stage at the time of infection and weather conditions can influence disease severity gradients for downy mildew of soybeans.

LITERATURE CITED

1. CAMMACK., R. H. 1958. Factors affecting infection gradients from a point source of Puccinia polysora in a plot of Zea mays. Ann. Appl. Biol. 46:186-197.
2. DUNLEAVY, J. M. 1971. Races of Peronospora manshurica in the United States. Am. J. Bot. 58:209-211.
3. DUNLEAVY, J. M., D. W. CHAMBERLAIN, and J. P. ROSS. 1966. Soybean diseases. U.S. Dep. Agric. Handb. 302. 37 p.
4. FEHR, W. R., C. E. CAVINESS, D. T. BURMOOD, and J. S. PENNINGTON. 1971. Stage of development descriptions for soybeans, Glycine max (L.) Merrill (Abstr.). Crop Sci. 11:929-931.
5. GREGORY, P. H. 1973. Microbiology of the atmosphere, 2nd ed. Leonard Hill, London. 377 p.
6. HIRST, J. M., and O. J. STEDMAN. 1963. Dry liberation of fungus spores by rain drops. J. Gen. Microbiol. 33:335-344.
7. HORSFALL, J. G., and R. W. BARRATT. 1945. An improved system for measuring plant diseases (Abstr.). Phytopathology 35:655.
8. ILLINOIS COOPERATIVE CROP REPORTING SERVICE. 1976. Illinois soybean varieties. September 9, Springfield, IL.
9. MC KENZIE, J. R., and T. D. WYLLIE. 1971. The effect of temperature and lesion size on the sporulation of Peronospora manshurica. Phytopathol. Z. 71:321-326.
10. METRONICS ASSOCIATES. 1976. Operating instructions for the rotorod sampler. Technical Manual No. 6:36-39. Metronics Associates, Inc., Palo Alto, CA. 41 p.
11. VAN DER PLANK, J. E. 1963. Plant disease: Epidemics and control. Academic Press, New York. 206 p.