Naturally Produced Aerosols of Pseudomonas glycinea

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ABSTRACT

Aerosols of *Pseudomonas glycinea* were detected in an infected soybean plot during rainstorms and sprinkler irrigation. Predominant particle size was 2.1-3.3μm and aerosols contained an average of 151 bacteria/m^3 during rain, and 94 bacteria/m^3 during sprinkler irrigation.

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Additional key words: Pseudomonas syringae, bacterial blight, dispersal of bacteria, epidemiology, *Glycine max,* soybean.

Bacterial blight of soybean, caused by *Pseudomonas glycinea* Coerper [*Pseudomonas syringae* van Hall (4)], is a chronic disease throughout soybean growing regions of the United States. Primary inoculum for the disease probably comes from infected seeds, and subsequent spread is thought to be via rainsplash (5). Since raindrops impacting wet bacteria-laden leaf surfaces should also generate aerosols (8) [i.e., collections of particles between 0.5μm and 20μm diameter suspended in air (6)] and these particles could be significant in epidemiology, we sampled air above a plot of soybeans and assayed for presence, numbers, and particle size distribution of airborne *P. glycinea* during rainstorms and sprinkling irrigation.

MATERIALS AND METHODS.—Samples were taken at the downwind boundary of a 28 × 40-meter plot of *Glycine max* (L.) Merr. 'Acme' grown on St. Paul campus field plots. Plants were naturally infected by *P. glycinea,* but the center one-third of the plot was also inoculated with race 2 of the pathogen 2 weeks prior to the first sampling. The plants were inoculated by spraying them at night with a knapsack sprayer containing approximately 5×10^7 bacteria/ml. The plot was periodically irrigated via sprinklers which delivered 5.5 cm of water per hour.

The Andersen viable particle sampler (Andersen 2000, Inc., P.O. Box 20769, Atlanta, GA 30320) was used to assay for bacterial aerosols (1). The sampler divided airborne particles into six size ranges (2, 7) and collected the particles on agar-filled petri plates placed beneath each stage in the sampler. A battery powered air pump drew air at the rate of 0.028m^3/minute (one cubic foot per minute) through the sampler which was placed beneath an open-sided rain shield and operated in a vertical position with sampler orifice 124 cm above the soil surface. Precautions taken to minimize ballistic particle contamination included loading and unloading the sampler in a closed vehicle outside the field plots, and disregarding colony counts from sampler stage 1 ($>$ 7.0μm), where ballistic droplets would probably be deposited.

Bacteria were collected on Kado's D-4 medium (9) as modified by Mew and Kennedy (11) supplemented with 50 μg/ml cycloheximide to inhibit fungal growth. After 48 hours at 27 C on this selective medium, *P. glycinea* formed smooth, bluish gray, translucent colonies and all suspected colonies were identified by gram stain, fluorescence on King's B medium (10), and pathogenicity on soybean (cultivar Acme) seedlings.

RESULTS AND DISCUSSION.—Sampling methods enabled us to determine numbers of viable bacteria as aerosol particles (Table 1). It is probable that many particles were made up of more than one cell and thus numbers are probably most conservative with regard to total numbers of bacteria per unit volume of air. One might expect rainstorms to be efficient in aerosol generation because of high incidence of raindrops striking infected plants and, in contrast, sprinkler irrigation to be less effective because only a few plants are impacted at any one time. Data (Table 1) generally support this hypothesis ($\overline{X}$ = 151 bacteria/m^3 during rainstorms compared to $\overline{X}$ = 94 bacteria /m^3 during sprinkler irrigation) but differences were not statistically significant. Sprinkler irrigation was applied during calm periods and rainstorms generally were accompanied by higher wind velocities. Increased wind velocity could quickly remove aerosols from the sampling area, thus

TABLE 1. Populations of *Pseudomonas glycinea* detected as aerosols within a plot of infected soybeans during rain and sprinkler irrigation

Date (1974) (month/day)	Wind speed (km/hour)	Sampling period (24-hr times)	Samples collected (no.)	Viable particles per sample (no./m^3)
7/8 (Sprinkler)	11-18[a]	0200-0320	6[b]	153[c]
7/9 (Sprinkler)	10-13	0100-0200	4	44
7/13 (Rain)	19-45	2130-2330	6	59
7/17 (Rain)	13-26	0800-1000	6	188
7/23 (Sprinkler)	6-10	0100-0210	6	77
7/23 (Rain)	22-24	1830-1935	8	221
8/2 (Rain)	19-22	1400-1450	3	71
8/9 (Sprinkler)	5	1930-1950	4	80

[a]Estimated wind speed from National Weather Service data, Minneapolis airport.
[b]One-minute samples taken at the rate of 0.028 m^3/minute with Andersen sampler.
[c]Analysis of variance on square-root transformed data showed no significant differences between means.

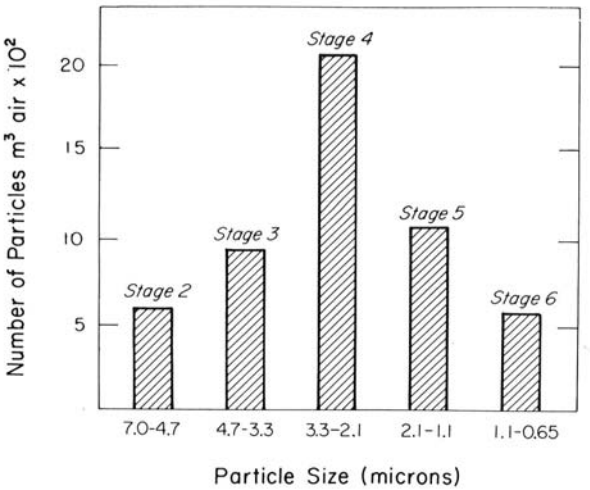

Fig. 1. Size distribution of *Pseudomonas glycinea* aerosol particles generated during rainstorms and sprinkler irrigation on a plot of infected soybeans. Particle size was determined by Andersen sampler separation.

reducing the population; in addition, rainout (8) may have reduced concentrations during rainstorms, but probably would not have been an important factor in reducing particle numbers during sprinkler generation of aerosols.

Frequency distribution of the combined data (Fig. 1) indicated the predominate particle size was 2.1-3.3μm (stage 4 of the Andersen sampler). Why this particle size should be most common is not known. One possibility is that larger and smaller particles were not generated as readily; or if they were, they may have had different patterns of movement in the air.

The presence of *P. glycinea* as aerosols naturally formed under field conditions is important in view of recent data which have shown that artificially generated aerosols of *P. glycinea* can transmit the pathogen to susceptible plants (J. R. Venette and B. W. Kennedy *unpublished*). Southey and Harper (12) have shown that artificially generated aerosols of *Erwinia amylovora* can survive up to 3 hours under laboratory conditions, and we have shown in laboratory studies (*unpublished*) that *P. glycinea* can survive in aerosols over 8 hours. Since airborne *P. glycinea* can remain viable for some time, can infect susceptible plants after deposition, can be generated under field conditions during rain and sprinkler irrigation, and can probably be transported long distances (3), we conclude that aerosols are potentially important in the epidemiology of bacterial blight of soybeans.

LITERATURE CITED

1. ANDERSEN, A. A. 1958. New sampler for the collection, sizing and enumeration of viable airborne particles. J. Bact. 76:471-484.
2. ANONYMOUS. 1974. Andersen 2000 Inc. Catalog for Viable Samplers. 4 p.
3. BROOKS, F. A. 1947. The drifting of poisonous dusts applied by airplanes and land rigs. J. Agric. Eng. 28:233-239.
4. BUCHANAN, R. E., and N. E. GIBBONS (eds.). 1974. Bergey's Manual of Determinative Bacteriology, 8th ed. Williams and Wilkins, Baltimore, Maryland. 1246 p.
5. DAFT, G. C., and C. LEBEN. 1972. Bacterial blight of soybeans: Epidemiology of blight outbreaks. Phytopathology 62:57-62.
6. DIMMICK, R. L. 1969. Mechanics of aerosols. Pages 3-21 *in* R. L. Dimmick and A. B. Akers, eds. An introduction to experimental aerobiology. Wiley-Interscience, New York. 494 p.
7. FLESCH, J. P., C. H. NORRIS, and A. E. NUGENT, JR. 1967. Calibrating particulate air samplers with monodisperse aerosols: Application to the Andersen cascade impactor. Am. Ind. Hyg. Assoc. J. 28:507-516.
8. GREGORY, P. H. 1973. The microbiology of the atmosphere, 2nd ed. John Wiley and Sons, New York. 377 p.
9. KADO, C. I., and M. G. HESKETT. 1970. Selective media for isolation of Agrobacterium, Corynebacterium, Erwinia, Pseudomonas, and Xanthomonas. Phytopathology 60:969-976.
10. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
11. MEW, T. W., and B. W. KENNEDY. 1971. Growth of Pseudomonas glycinea on the surface of soybean leaves. Phytopathology 61:715-716.
12. SOUTHEY, R. G. W., and G. J. HARPER. 1971. The survival of Erwinia amylovora in airborne particles: Tests in the laboratory and in the open air. J. Appl. Bact. 34:547-556.