Pseudomonas lachrymans Inoculum on Infected Cucumber Leaves Subjected to Dew- and Rain-type Wetting

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ABSTRACT

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The amount of readily available *Pseudomonas lachrymans* inoculum present on cucumber leaf surfaces with lesions was determined by vacuuming dry leaves and by dilution-plating of water on leaves subjected to various moisture conditions. No inoculum was lifted by vacuum from dry infected leaves unless the lesions were abraded. With no wetting period (except for a standard 10-minute wash prior to dilution plating), 2.8×10^4 colony-forming units (CFU) were obtained from leaves with one lesion. Increasing lesion numbers per leaf did not yield a comparable increase in CFU. On leaf surfaces wet with dew, both the pathogen and other bacteria increased for the first 12 hours, but the pathogen increased at a much more rapid rate. Following this rise there was a precipitous decline in the population of *P. lachrymans* and a very large increase in other bacteria on the leaf surface.

Similarly infected leaves subjected to continuous rain had increasing populations of both pathogen and nonpathogens for 8-16 hours until a relatively steady state was present. Inoculum production during discontinuous wetting was not additive; wet periods of 4 or 8 hours per day on successive days yielded pathogen and nonpathogen levels similar to those found with single 4- or 8-hour periods of wetting on one day. Also, the post-12-hour decline in pathogen population and increase in other bacteria found with continuous dew was not reproduced by several 4-, 8- or 12-hour wetting periods on successive days. Considering the *P. lachrymans* CFU available for dissemination from wet infected leaves, it seems likely that angular leaf spot epidemics are not limited by low inoculum levels but by factors that control the ingress of the pathogen to the intercellular leaf spaces.

Additional key words: epidemiology, bacteria, leaf-surface populations, *Cucumis sativus,* angular leaf spot.

Initial angular leaf spot infestations of cucumber crops usually is from seed-borne bacteria (3, 9, 15, 17). The first symptoms are lesions on cotyledons and the pathogen is then disseminated aerially to other leaves. The pathogen is not systemic in the stele (16) and although the bacteria can reproduce on the growing point and colonize the surface of several successive leaves (5), this phenomenon probably only occurs under constantly high relative humidity conditions and does not represent the normal pathway for inoculation of leaves. Dissemination through the air, whether from primary lesions on cotyledons or secondary lesions on other leaves, can take place without free water if diseased portions of leaves drop out (16) and are carried to other leaves. Still it is generally recognized that free-water is the most important vehicle for pathogen dispersal (2, 3, 9, 15).

Leaves may be wetted by dew or rain. Dew water is unlikely to be involved in splash dispersal but the water with the bacterium may be carried by insects or other animals, machinery, possibly wind, etc.

Rain water washes bacteria from leaves (7) but short precipitation periods may not be sufficient to bring many pathogen propagules into suspension. Crosse (4) has suggested that several hours of wetting may be required to bring most of the leaf-surface bacteria into suspension.

However, there are few other studies on this important phenomenon.

Wet infected leaves can not be considered merely an inert platform for inoculum production. Some of the characteristics of this microbiologically active surface have been reviewed recently (12, 13). Wet cucumber leaves support an active population of epiphytic bacteria even when healthy (11). Wet infected leaves presumably do also and in addition are more turgid than dry leaves; pathogenesis proceeds more quickly under these wet conditions (17).

The production and release of spores on lesions are important parameters in models of epidemics incited by fungi (14). These data need to be determined for bacteria-incited epidemics before the latter can be simulated. For phytopathogenic bacteria, multiplication rates within the suscept have been studied; the experiments we report here elucidate some effects of micro-environment on the release of bacteria from infected leaf tissue and (presumably) their availability for dissemination. We describe the gross effects of wetting periods on the epiphytic bacterial populations in this important ecological niche.

MATERIALS AND METHODS

The growing of the suscept *Cucumis sativus* L. 'Elem', and the pathogen, *Pseudomonas lachrymans* (Smith &

Bryan) Carsner has been described (8). Plants were inoculated when one to two true leaves had developed; inoculated leaves were 4 ± 1.7 cm^2. When variable numbers of lesions per leaf were required, inoculum was misted on leaves with an artist's air brush (10) and the leaf surface was rubbed gently with the finger. Varying the quantity of inoculum applied and natural variations in infectivity resulted in 0 to 40 lesions per leaf. To obtain exactly two lesions per leaf the sprayer was held to the abaxial leaf surface and inoculum released momentarily to form 1-mm^2 water-soaked spots. Three to four days later 4- to 8-mm^2 angular leaf spot lesions developed. *Pseudomonas lachrymans* contamination of noninoculated areas on the leaves was reduced by spraying through 2- to 3-mm diameter holes in a paraffin-coated cardboard template. These leaves also were incubated in situ for 7 days in growth chambers before treatments were imposed.

Dew-type wetting conditions were initiated by misting all surfaces of the cucumber shoot with deionized water. The plants then were enclosed in clear polyethylene bags, the inside surfaces of which had been misted with water. Dew periods began at the start of the 8-hour dark/16-hour light cycle used in the growth cabinets. When 12 hours of dew were desired, the photoperiod was 12/12, dark/light. Remisting of plants was required near the beginning of each light period when leaves were scheduled to remain dew-covered continuously.

Rain-type wetting was accomplished in a 2×2 m temperature-controlled (25 ± 2 C) glasshouse compartment. A fine mist was produced 1.5 m above plants by dripping water onto a rotating horizontal disk. The speed of the disk and the water flow were adjusted to regulate the precipitation rate at 1.0-1.5 mm^3/mm^2/hour. A similar glasshouse compartment was employed to incubate plants under dry conditions. Natural lighting was used; the experiments were conducted during the months of March and April when day length was about 13 hours.

Pseudomonas lachrymans and other bacteria on the leaf surface were suspended in water and assayed on sucrose agar (SA), a semiselective medium (8). The collection and assay of dry inoculum from leaves is described below. All experiments were conducted at least twice with six replicate leaves per treatment. The data presented represent the means of all experiments.

RESULTS

Dry inoculum from leaves with lesions.—Naturally infected leaves from cucumber fields and commercial greenhouse crops, and artificially inoculated leaves from cucumbers grown in growth cabinets were collected. Typical angular leaf spot lesions were vacuumed to collect soil particles and loose segments of dried bacterial ooze which might have been present on the lesion. The vacuuming apparatus was a Pasteur pipet connected to a sintered glass membrane filter holder. The holder was loaded with a cellulose nitrate (0.45-μm pore size) filter and connected to a 1.0×10^5 N/m^2 (1-bar) vacuum source. After each set of lesions was vacuumed, the filter either was pressed momentarily onto SA or washed in sterile distilled water and the water (0.1-ml samples) spread on SA medium in petri plates. No *P. lachrymans* was recovered from these leaves with lesions unless the pipet tip was inadvertently touched to the leaf surface. When the lesions were purposely but gently abraded with the pipet tip, *P. lachrymans* was recovered. The number of colony-forming units (CFU) present on the filters was highly variable and could not be associated with leaf source or lesion age. Apparently, the bacteria present in the ooze covering all lesions could not be lifted from the surface without some abrasive action.

To simulate more closely wind disturbance of leaves with lesions, single infected lamina from field-grown plants were placed unfolded in paper sacks and shaken. The dislodged material (mostly soil particles) was rated for abrasive quality and 1 mm^3 quantities were sprinkled onto first true leaves previously moistened with a fine water spray. These leaves then were rubbed gently with the finger which increases lesion production tenfold (8). After incubation, typical angular leaf spot lesions developed on some leaves and there was a definite correlation between the abrasive quality of the inoculum and the number of lesions that developed (Table 1).

Inoculum availability on leaves with lesions.—*Ten-minute wetting period.*—To establish a baseline from which to measure the effect of longer wetting periods on inoculum availability, we determined the number of CFU on leaves wet for only short periods. Artificially inoculated leaves with 5- to 7-day-old lesions were washed in water for 10 minutes. The number of CFU per leaf on
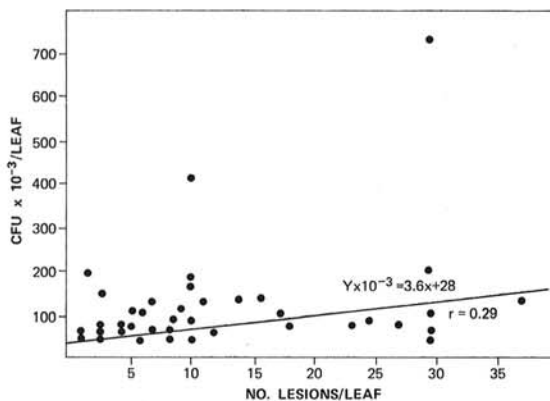

Fig. 1. The relation of number of angular leaf spot lesions and *Pseudomonas lachrymans* colony-forming units (CFU) on leaves artificially inoculated seven days previously.

TABLE 1. Angular leaf spot lesion development on cucumber inoculated[a] and gently rubbed with the finger

Relative abrasive quality	Leaves inoculated (no.)	Lesions per leaf		
		Min. (no.)	Mean (no.)	Max. (no.)
0	17	0	1.2	4
1	20	0	3.4	7
2	14	1	3.8	7
3	20	3	7.6	13

[a]Inoculum was from field-grown naturally infected crops and contained soil particles which imparted an abrasive texture (rated 0 = no abrasion noted at time of inoculation; 3 = distinctly abrasive).

leaves with 1 to 37 lesions is presented in Fig. 1. There was a significant ($P = 0.5$) correlation between the number of lesions and the CFU per leaf. However, lesion numbers had to increase greatly in order to get a small increase in *P. lachrymans* populations. Neither transformation of the data onto linear-log or log-log scales, nor exclusion of the six largest outlying values increase the significance of the correlation.

Dew-type wetting periods.—Because of the positive correlation of lesion number and CFU, leaves with two lesions per leaf were used to test the effect of continuous

dew periods and alternate dew and dry conditions on the production of *P. lachrymans* inoculum.

When previously nonwetted infected leaves were washed, low populations of *P. lachrymans* were detected. Their number was similar to that found in the previous experiment. Other bacteria also were present on the SA plates but in very low numbers. Leaves that were dew covered and sampled during the first 12 hours after wetting had increasing numbers of both the pathogen and the other bacteria but the pathogen population increased most rapidly (Fig. 2). During the succeeding 12 hours of
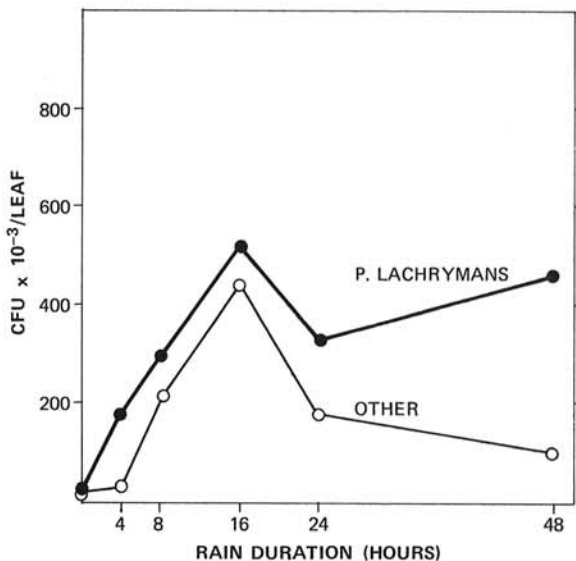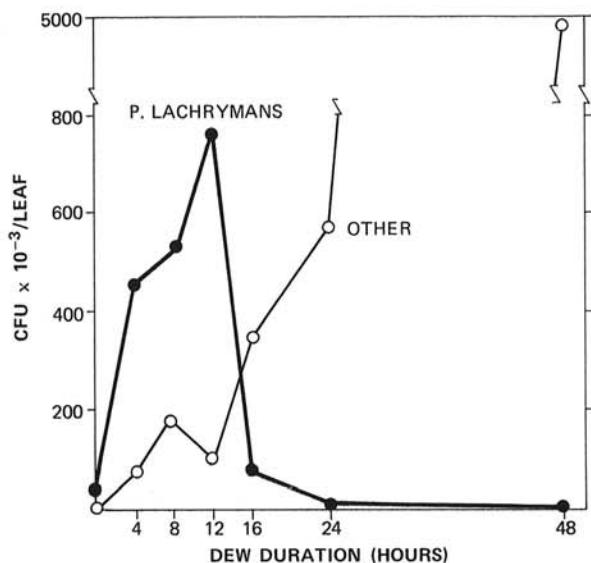

Fig. 2-3. Bacterial populations on angular leaf spot-infected leaves wet for 0-48 hours. Population assay was by dilution plating on a modified sucrose agar medium semiselective for *Pseudomonas lachrymans*. 2) Dew-type wetness. 3) Rain-type wetness.
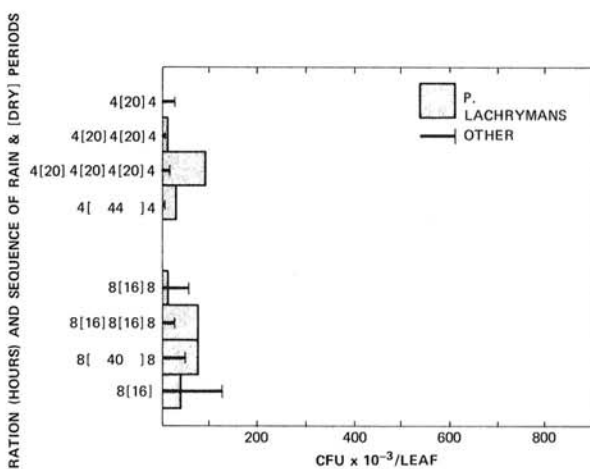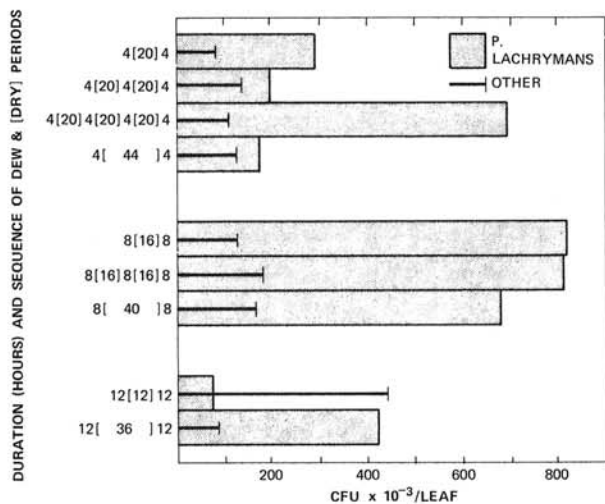

Fig. 4-5. The effect of discontinuous wetting on bacterial populations on angular leaf spot-infected cucumber leaves. Plants were wet for 4-12 hours, allowed to dry for the remainder of the day, and rewet the following day. The sequence was repeated as indicated. Population assay was performed at the end of the last wet period (except for one treatment). Colony-forming units (CFU) were counted on dilution plates of modified sucrose agar, a medium semiselective for *Pseudomonas lachrymans*. 4) Dew-type wetness. 5) Rain-type wetness.

dew there was a precipitous decline in *P. lachrymans* CFU on the leaves; the other bacteria continued to increase as before. The experiment was repeated three times with similar results; in one of these tests the peak pathogen population was found at 8 hours but the large decline in CFU was still not found until after 12 hours of wetting. After 2 days of dew-type wetting, no *P. lachrymans* could be detected on the leaves but the saprophytic bacteria had increased to at least 1,000 times their original number. It was not possible to accurately determine the CFU of non-*P. lachrymans* because of the large number of colonies with even the highest serial dilution used; it is therefore possible that some of the *P. lachrymans* cells were present but were not detected.

Alternate dew and dry conditions were established in a circadian rhythm; e.g., 4 hours dew followed by 20 hours dry or 8 hours dew and 16 dry, etc. The various treatments are indicated in Fig. 4. Leaf washing always was performed at the conclusion of a wetting period while the plants were still wet.

Two and three consecutive days with 4-hour dew periods and also wetting on day 1 followed by dryness until a 4-hour dew period on day 3 yielded similar populations of *P. lachrymans*. When four consecutive days with 4-hour wetting periods were investigated, there was a significant increase in *P. lachrymans*. The character of the lesions also was different; they had increased in size and were more watersoaked.

Eight-hour dew periods interrupted by one or two 16-hour dry periods or by 40 hours of dryness all resulted in high levels of *P. lachrymans* and low levels of other bacteria. The pathogen was present in somewhat higher numbers when discontinuous dew periods were used as opposed to the single 8-hour wetting described above (Fig. 2). With 12 hours of dew followed by 12 hours dryness and then another 12-hour wetting, *P. lachrymans* populations were low and those of the saprophytes were high. The reverse situation prevailed when a 12/36/12-hour, wet/dry/wet regime was investigated.

Rain-type wetting periods.—Continuous rain resulted in increasing pathogen populations for the first 16 hours followed by a small decrease in inoculum levels thereafter (Fig. 3). Other bacteria similarly increased and decreased in numbers. The precipitous drop in pathogen populations noted with dew-type wetting did not occur.

Interrupted rain periods had little effect on either *P. lachrymans* or the saprophytes remaining on the leaf surface after the rain (Fig. 5). Four days of 4-hour wetting periods resulted in slightly higher pathogen populations than 3 or 2 days. Also, the pathogen was more abundant on leaves wet for 8 hours per day compared with the 4-hour wetting treatments. The differences between treatment means, however, were not statistically significant.

DISCUSSION

Inoculum dissemination of bacterial leaf spot pathogens typically is believed to take place under wet conditions. The pathogen becomes suspended in moisture on an infected leaf surface and is then readily available for dissemination. Rain or dew can supply the moisture and may have different effects on the pathogen on the leaf either directly, by promoting more or less leaching of surface nutrients, or indirectly by affecting the activities of other microorganisms on the phylloplane.

In our experiments, cucumber leaves with 1- to 3-day-old angular leaf spot lesions were always a potential source of inoculum. Dry leaves yielded *P. lachrymans* whenever the lesions were abraded or the leaves were shaken vigorously. The pathogen also was found in water on leaves wet for only 10 minutes. With short wetting periods, the colony-forming units (CFU) obtained from leaves with "young" lesions was relatively constant whether one or many lesions were present. We believe that the *P. lachrymans* propagules obtained were the residual population surviving from the time of inoculation 5-8 days earlier. The slight positive linear correlation between surface populations and lesion numbers reflected the variation in inoculum applied to achieve different lesion numbers per leaf and the subsequent natural inoculum attrition to a relatively constant pathogen population per leaf. We have shown previously that *P. lachrymans* is capable of surviving at least 6 days on cucumber leaves (8). The present experiments suggest that there is a maximum resident pathogen population which these leaves can support.

Leaves with lesions when wet with dew for four hours have 10 times more pathogen CFU than after 10 minutes. Some portion of the increase may be due to multiplication on the leaf surface (4) but most probably comes from the dissolution of macerated epidermal cells or dried exudate from the lesion. Further evidence for the nonsignificant involvement of bacterial growth was the smaller rate of CFU increase between four and 12 hours of continuous dew, as compared with the rate during the first 4 hours of wetting.

The effects of rain-type wetting could be expected to vary from those of dew-type. Bacteria released from lesions would wash off the leaf along with the excess water. The difference in surface populations after dew- and rain-type wetting is a measure of the pathogen population lost by run-off. From Fig. 2 and 3 we estimate 46% of the population is washed off leaves at anytime during the first 12 hours of wetting. These bacteria falling on other leaves could serve as inoculum for further infections. Under continuous rain-type wetting the large diminution in CFU found in the dew-type treatments did not occur. The increase of "other" bacteria which accompanied this decrease in *P. lachrymans* suggests a causal relation; after long dew periods, pathogen populations may be suppressed by lack of nutrients or the presence of bacteriocins, phages, or other agents.

Wetting by dew for more than 16 hours is rather uncommon. However, shorter dew periods can occur on successive days and, with some fungal pathogens, can be as effective in promoting inoculum production as an equivalent duration of wetness in one continuous period (1). This is not the case with *P. lachrymans;* the population found after two or three wettings interrupted by dryness is similar to that found after the first wetting. The fate of the inoculum released during each wetting period is unknown. The bacteria may all die or some may remain viable and survive on the leaf. After four discontinuous wettings more bacteria are present than after three such wettings. We believe this is an effect of several wet-dry cycles affecting the integrity of the epidermal cells and the dried bacterial ooze covering the

lesion. Also, moisture increased lesion activity (17) and after 4 days may be responsible for the presence of significantly greater populations of the pathogen per lesion.

Considering the CFU available for inoculum, *P. lachrymans* must be an inefficient pathogen. It is always available on infected leaf surfaces and large numbers quickly become suspended in moisture films on leaves. Still, a relatively small proportion of leaves in a crop may become infected and many will have only one lesion. Also, substantial numbers of the pathogen can be present on noninfected leaves without having symptoms produced (5, 7). Few bacteria in a leaf are required to incite an infection (6). This suggests that once the initial inoculum is present in a crop, the epiphytic population of the pathogen is not a limiting factor in epidemic development. Other factors, which limit the ingress of the pathogen into the leaf, actually control the rate of disease progress. Perhaps the general recommendation of controlling bacteria-incited diseases by avoiding cultural operations in crops when leaves are wet may not be as effective in preventing inoculum dissemination as in preventing wounds on the leaf surface.

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