Systemic Invasion of Cucumber by Pseudomonas lachrymans

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ABSTRACT

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When hypocotyls of cucumber cultivar National Pickling were inoculated with *Pseudomonas lachrymans*, causal agent of angular leafspot, the pathogen was recovered from within surface-sterilized sections 10 or more cm above inoculation sites. When *P. lachrymans* was introduced into second-leaf midvein tissue, it was detected consistently in other parts of the plant, predominantly in sections taken from below the second-leaf node. The pathogen was recovered regularly from within symptomless tissues. Differences were observed in rate and extent of invasion in different cucumber cultivars by different *P. lachrymans* isolates. More extensive invasion occurred in susceptible cultivars Pickmore and GreenPak than in the field-resistant cultivars H3559 and Premier.

Additional key words: *Cucumis sativus*.

Angular leafspot, which is incited by the bacterium *Pseudomonas lachrymans* (Smith and Bryan) Carsner *(Pseudomonas syringae)* (1), is a serious problem in humid areas where cucumbers for production of pickles are grown. Improved fungicides and multiple-disease-resistant cultivars have greatly enhanced control of many other diseases of this crop. Consequently, angular leafspot has increased in importance as a limiting factor in cucumber cultivation.

This disease is characterized by prominent local lesions primarily on leaves. However, T. D. Miller (*personal communication*), working at the Ohio Agricultural Research and Development Center, detected the pathogen in various plant parts, presumably after systemic movement from inoculated leaves. These observations are contrary to those of Wiles and Walker (13), who reported that needle-puncture inoculations of stems of National Pickling plants consistently failed to produce "systemic infection".

In this study, Miller's observations were confirmed and extended. Specific objectives were to study the invasion of: (i) cucumber seedlings of one cultivar by different isolates of *P. lachrymans*, and (ii) different cucumber cultivars by systemic isolates of the pathogen.

MATERIALS AND METHODS

Cucumber seedlings (*Cucumis sativus* L. 'National Pickling'), in the second-leaf stage (second leaf about half expanded) were used in most experiments. Plants were grown in 10.2 cm (4-inch) pots in sterile soil in a greenhouse with an approximate temperature range of 19-30 C. Plants were watered at the pot rim with an automatic watering system to prevent splash dispersal of bacteria. Insects and powdery mildew were controlled with aldicarb (Temik, 10% granular, Union Carbide, New York, NY 10017) and dimethirimol (12.5% solution, Plant Protection, Ltd., Bracknell, England), respectively, by application to the soil surface. Both chemicals were effective systemically so that it was not necessary to apply sprays that could distribute *P. lachrymans*.

Isolates of *P. lachrymans* were maintained by storage (4 C) in dried diseased cucumber leaves and by serial transfer on agar slants. Inoculum was taken from 48-hr cultures grown on medium B of King et al. (6) plates at 24 C.

Hypocotyl inoculations.—Movement of *P. lachrymans* from sites of inoculation near the base of the stem was investigated. In two tests, a sterile cotton swab was used to apply the pathogen, taken from agar plate colonies, to a 95% ethanol-sterilized area of the hypocotyl about 2 cm above the soil surface. A large needle (dissecting needle, approximately 0.9 mm diameter) was then thrust through this area; several separate wounds were made around the circumference of the hypocotyl cylinder. One min later a second application of inoculum was made to the wounded region. In a modified hypocotyl-inoculation experiment, a small needle (No. 10 sharp sewing needle, approximately 0.4 mm diameter) was coated lightly with *P. lachrymans* cells from agar plates and thrust into the lower hypocotyl several times equidistantly around the hypocotyl. Wounds were covered with sterile petroleum jelly. Control plants were wounded similarly with a sterile needle.

Inoculation of leaf vascular tissue.—Since it was

difficult to determine which specific tissue was being exposed to the pathogen during hypocotyl inoculations, a system similar to that described by Pennypacker et al. (12) was used. Cells of *P. lachrymans* were deposited, by the small needle described above, into vascular tissue in the under side of the midvein of the second leaf about 2 cm from the junction of the lamina and petiole. Care was taken to guide the needle toward the petiole in the plane of the leaf blade so that most of the bacterial cells were deposited inside the vein. Controls were wounded the same way with a sterile small needle.

Isolation procedures.—The intent of this study was to determine whether or not *P. lachrymans* was inside cucumber plant sections. Therefore, a type of "baiting" technique was used in which tissue sections were placed on agar plate surfaces and time was allowed for *P. lachrymans* to multiply to a population sufficient for detection.

Host tissue was plated on medium M72, a boric acid medium, devised by T. D. Miller (*personal communication*). This medium consisted of 2 g boric acid plus the following "TTCC" basal medium ingredients: peptone, 10 g; casein hydrolysate, 1 g; glucose, 5 g; cycloheximide, 0.05 g; triphenyl tetrazolium chloride, 0.05 g; agar, 17 g; water, 1 liter. Medium M72 was based on M71 (8); doubling the amount of boric acid enhanced selectivity. The plating efficiency of M72 compared to M71 averaged 89% in two tests.

Plant parts were cut aseptically from plants. They were rubbed vigorously with a fresh paper towel under running deionized water to remove the epidermal hairs. A plant part was immersed in 70% ethanol for 2 min and then transferred to a beaker containing 500 ml of 20-25% (v/v) aqueous Clorox (5.25% sodium hypochlorite) plus one drop of Tween 20 (Emulsion Engineering, Elk Grove Park, IL 60007). The plant part was agitated in this mixture for 3 min and then rinsed in sterile water and dried on sterile paper. Ends of the parts were aseptically removed, the remaining tissue cylinder was split longitudinally (usually three to five pieces), and the pieces placed on M72 plates. The pathogen was detected more readily when multiple pieces were prepared than when

TABLE 1. Recovery of *Pseudomonas lachrymans* from surface-sterilized plant sections of cucumber plants inoculated in the hypocotyl with a systemic isolate[a]

	Recovery (%)[d]						
Inoculation method	Hypocotyl	Petiole 1	Petiole 2	Internode 1	Internode 2	Internode 3	Internode 5
Large-needle[b] (29 days postinoculation)	100	25	13	93	87	53	0
Small-needle[c] (21 days postinoculation)	93	21	7	86	50	36	0

[a]Wound-inoculated in the lower hypocotyl with *P. lachrymans*; several wounds made around circumference of lower hypocotyl cylinder.
[b]Based on 15 National Pickling cucumber plants in each of two experiments, wound-inoculated with a dissecting needle.
[c]Based on 14 National Pickling cucumber plants, wound-inoculated with a fine sewing needle.
[d]Plant section designation key: Petiole 1 = petiole of leaf 1, at the cotyledonary node; internode 1 = internode between leaf 1 and leaf 2; leaf 1 is at the cotyledonary node.

TABLE 2. Recovery of three *Pseudomonas lachrymans* isolates (PL23, PL24, and PL11) from plants inoculated in the vascular tissue of the second-leaf midvein[a]

	Number of plants (out of four) in which *P. lachrymans* was detected														
	6 days			10 days			14 days			18 days			48 days		
Plant sections assayed[b]	PL23	PL24	PL11	PL23	PL24	PL11	PL23	PL24	PL11	PL23	PL24	PL11	PL23	PL24	PL11
Transition zone	0	0	0	1	3	1	2	2	0	2	3	0	0	3	0
Hypocotyl	0	1	0	2	4	1	3	3	0	4	4	0	2	4	0
Internode 1	4	4	0	3	4	2	3	3	0	4	4	0	3	4	0
Proximal half of second-leaf petiole	4	4	4	4	4	4	4	4	3	4	4	4	...	...	...
Distal half of second-leaf petiole	4	4	4	4	4	4	4	4	3	4	4	4	...	...	...
Internode 2	1	2	0	1	1	0	3	3	0	4	3	0	2	3	0
Petiole of third-leaf	0	0	0	0	1	0	0	1	0	2	1	0	0	0	0
Internode 6	...	...	...	...	...	...	...	0	0	0	0	0	0	0	0

[a]Each isolate was used to wound-inoculate 20 National Pickling cucumbers with cells from agar plate colonies by the small-needle method (see text). No entry (...) indicates plant section not available.
[b]Plant designation key: Internode 1 = internode between first and second leaves. First leaf is at cotyledonary node.

only halves were plated. Reported distances of movement were measured from the inoculation site to the midpoint of the plant part furthest away in which the pathogen was found.

In those portions of plant stem and petiole where a white or amber ooze appeared on the surface, assays were done by streaking some of this material on M72. The ooze always contained high numbers of *P. lachrymans* cells.

Plates were incubated for 3 days at 24 C and then examined for *P. lachrymans* colonies with a dissecting microscope illuminated with tangentially-directed light from beneath the plate. Plates were kept at 22 C for an additional 3 days and examined for additional bacterial growth. Correct pathogen identification was verified in each experiment by pathogenicity tests of 22-28% of the representative colonies.

Although all precautions were taken to ensure that *P. lachrymans* was not spread on the surface of the test plants, the efficacy of the surface sterilization was checked periodically by making cotton swab smears of surface-sterilized sections on M72.

RESULTS

Hypocotyl inoculations.—After 7 days, plants inoculated with the large needle showed extensive damage in the vicinity of the punctures. All plants were water-soaked and necrotic up to the cotyledonary node. Bacterial exudate (usually amber-colored) often was observed on hypocotyls above the inoculation site. In 20% of the plants, browning and water-soaking of tissue and bacterial exudate developed in stem sections above the cotyledonary node. Experiments in which 15 test plants and five controls were used (Table 1) show that *P. lachrymans* was detected in nearly 100% of the first internode sections and in over 50% of the third internode sections 29 days after inoculation. The average maximum distance the bacteria moved was 10.7 cm (range = 6.1 - 13.8 cm, SD = 2.4 cm).

In the small-needle modification of the above experiments (based on 14 test plants and five control plants), indications of systemic invasion were observed in 35.9% of the plants at 7 days, and symptoms consisted of water-soaking and light necrosis. At 14 days, however, symptoms were seen in over 90% of the plants. Tissue had collapsed around wounds and necrosis extended the length of the hypocotyl. As in the large-needle tests, the pathogen was recovered from plant sections above the inoculation sites (Table 1). The average maximum distance the bacteria were recovered above the point of inoculation was 13.8 cm (range = 6.4 - 17.9 cm, SD = 7.9 cm, based on 13 plants; the bacterium did not appear to move in one plant in this experiment).

Pseudomonas lachrymans was isolated regularly from epicotyl sections of inoculated plants showing no disease symptoms. Since the cotton swab checks for surface populations of *P. lachrymans* on surface-sterilized samples were always negative, and controls in all experiments always were devoid of the pathogen, we concluded that angular leafspot bacteria moved systemically within stems.

Rate and extent of pathogen spread from inoculated leaves.—The rate and distance of systemic movement of *P. lachrymans* cells were studied by means of the midvein-inoculation technique. Three different isolates were used to inoculate 20 plants per isolate with the small needle. A fourth treatment consisted of plants wounded with a sterile needle. Four plants of each isolate treatment were assayed at 6, 10, 14, 18, and 48 days after inoculation. This experiment was done twice, first in early winter and then in late spring. Symptoms of systemic movement developed 4 to 6 days later. Along second-leaf veins, other than the midvein, there was chlorosis on the upper side and water-soaking on the underside. White to amber exudate yielding nearly pure cultures of *P. lachrymans* appeared on the surface of second-leaf petioles, and in some plants was observed on the surface of the internode, just below the diseased petiole. Although *P. lachrymans* was recovered from hypocotyls, transition zones, and second internodes of plants inoculated with PL23 or PL24, no symptoms were observed on these sections.

Distances of movement from the inoculation point differed for the various isolates (Table 2). Within 14 days six of eight plants inoculated with isolates PL23 and PL24 yielded the bacterium from the hypocotyl, whereas in those inoculated with isolate PL11 the pathogen was not recovered from the hypocotyl.

The initial direction of movement was basipetal. With PL23 and PL24, recovery percentages were larger at the earlier sampling dates in sections below the cotyledons than in sections above the second leaf. As the plants became older, an endophytic bacterium grew out from the lower plant sections faster than *P. lachrymans*, making it more difficult to detect the pathogen. This interference probably was responsible for some of the decreases in the recovery percentages from the transition zone and hypocotyl at later sampling dates.

The test was confirmed when repeated in late spring, and results were similar except that the maximum recovery percentages at the later sampling dates were 15% lower (based on hypocotyl and transition zone assays). Pathogenic bacteria never were recovered from control plants in either test.

Systemic movement was demonstrated using other inoculation methods. In one test, the second-leaf midvein was wounded by rubbing vigorously with a cotton swab coated with *P. lachrymans* cells. Two of 20 plants inoculated by this method had populations of *P. lachrymans* in the stems. In another test, wounds were made in second-leaf vascular tissue by rasping leaves with a triangular file. Pathogen cells were applied to the wound with a cotton swab. *Pseudomonas lachrymans* was found in the first internode in nine of the plants and in the hypocotyl in seven.

Tests of commercial cultivars.—Cultivar National Pickling is no longer grown commercially in Ohio. Consequently, cultivars now widely used were studied: field-resistant cultivars Premier and H3559, and susceptible GreenPak and Pickmore. Fourteen plants of each cultivar were inoculated by the small-needle method in the second-leaf midvein with a mixture of isolates PL23 and PL24. Isolations were made from seven plants of each cultivar 13-15 days and 29-30 days later.

We observed that *P. lachrymans* moved systemically in all four cultivars, but quantitative differences were observed (Table 3). The most extensive systemic invasion occurred in GreenPak, and the pathogen was detected the

TABLE 3. Systemic invasion of field-resistant and field-susceptible cultivars by *Pseudomonas lachrymans*; recovery at two times of *P. lachrymans* from plant sections inoculated in the vascular tissue of the second-leaf midvein[a]

Resistance and cultivar	Percent recovery											
	Plant section[b] sampled at 13-15 days						Plant section[b] sampled at 29-30 days					
	TZ	Hyp	Int 1	Pet 2	Int 2	Int 6	Int 8	TZ	Hyp	Int 1	Pet 2	Int 2	Int 6	Int 8
Field resistant:														
Heinz 3559	14	14	43	71	29	...	...	14	43	71	100	43	0	0
Premier	14	14	29	86	29	...	...	14	29	29	100	29	0	0
Susceptible:														
Pickmore	14	29	57	100	29	...	...	71	71	86	100	57	0	0
GreenPak	29	57	100	100	71	...	...	33	83	100	100	83	0	0

[a]Based on 14 plants of each cultivar inoculated by the small-needle method in second leaf vascular tissue with a mixture of isolates PL23 and PL24. Seven replications of each cultivar were assayed at each sampling time. No entry indicates plant section not available at that time.

[b]Plant section designation key: TZ = transition zone between stem and roots; Hyp = hypocotyl; Int = internode; Pet = petiole. Internode 1 = internode between first and second leaves. First leaf is at cotyledonary node.

fewest number of times in the various stem sections of Premier. If the data for recovery from all stem sections are combined for both sampling times, we find that the bacteria moved into the stem in 57.1% of the H3559 plants, 35.7% of Premier (both field-resistant), 71.4% of Pickmore, and 100% of GreenPak (both susceptible). A chi-square test indicated that differential response among cultivars to systemic invasion was highly significant ($P \leqslant .01$), invasion being more extensive in the susceptible cultivars.

DISCUSSION

This research demonstrated that when *P. lachrymans* was introduced into the vascular system, it moved to plant parts distant from the inoculation site. This movement is called "systemic invasion", following the terminology of Wilson and Magie (14), to describe the movement from an initial infection site to other locations within the host. (This usage neither implies that the entire plant is invaded nor that extensive blighting develops.) We suggest that in the field wounding that breaks the vascular system, such as the damage produced by a severe storm, cultivation, or by turning vines to pick fruit, may well introduce the pathogen into the plant. How important subsequent systemic invasion is in nature, however, is not known. If it takes place, our work suggests that different cultivars are likely to be invaded to different extents by one isolate of the pathogen, and that different isolates will differ in the extent of invasion of a given cultivar. The two widely used field-resistant cultivars were invaded less extensively than the two susceptible cultivars in these experiments. The phenomenon of systemic invasion may be useful in evaluation of resistance in different cucumber lines, by reducing the time necessary for selection of promising genotypes.

Pseudomonas lachrymans was recovered mostly from inside healthy-looking parts of plants; these bacteria are "latent" (4) or "internal residents" (9). The occurrence and epidemiological importance of plant pathogenic bacteria inside symptomless plant tissue is well documented (2, 4, 7, 10, 11).

Pseudomonas lachrymans was detected more readily in stem sections below the inoculated leaf than in sections above it. El Khalifa et al. (3) and Pennypacker et al. (12) reported similar trends in recoveries of pathogenic bacteria introduced into leaves of other hosts. The initial basipetal movement of *P. lachrymans* in cucumber may be related to the vascular anatomy of cucurbits (5). Leaf traces extend downward for at least two internodes before anastomosing with other bundles. Once the bacteria enter a bundle that extends up through the stem, they are more likely to move to plant parts above the inoculated leaf.

LITERATURE CITED

1. BUCHANAN, R. E., and N. E. GIBBONS, (eds). 1974. 8th ed. Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore. 1,246 p.
2. CROSSE, J. E., R. N. GOODMAN, and W. H. SHAFFER, JR. 1972. Leaf damage as a predisposing factor in the infection of apple shoots by Erwinia amylovora.

Phytopathology 62:176-182.
3. EL KHALIFA, M. D., E. E. EL NUR, B. B. LIPPINCOTT, and J. A. LIPPINCOTT. 1973. Crown gall on castor bean leaves. II. Formation of secondary tumors. J. Exp. Bot. 24:1117-1129.
4. HAYWARD, A. C. 1974. Latent infection caused by bacteria. Annu. Rev. Phytopathol. 12:87-97.
5. HAYWARD, H. E. 1938. The structure of economic plants. Macmillan, New York. 674 p.
6. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
7. KNAUSS, J. E., and J. W. MILLER. 1974. Etiological aspects of bacterial blight of Philodendron selloum caused by Erwinia chrysanthemi. Phytopathology 64:1526-1528.
8. LEBEN, C. 1972. The development of a selective medium for Pseudomonas glycinea. Phytopathology 62:674-676.
9. LEBEN, C. 1974. Survival of plant pathogenic bacteria. Ohio Agric. Res. Dev. Cent. Res. Circ. 100. 21 p.
10. MENELEY, J. C., and M. E. STANGHELLINI. 1974. Detection of enteric bacteria within locular tissue of healthy cucumber. J. Food Sci. 39:1267-1268.
11. MOORE, L. W., H. B. LAGERSTEDT, and N. HARTMAN. 1974. Stress predisposes young filbert trees to bacterial blight. Phytopathology 64:1537-1540.
12. PENNYPACKER, B. W., P. E. NELSON, and R. S. DICKEY. 1974. Histopathology of chrysanthemum stems artificially inoculated with Erwinia chrysanthemi. Phytopathology 64:1344-1353.
13. WILES, A. B., and J. C. WALKER. 1951. The relation of Pseudomonas lachrymans to cucumber fruits and seeds. Phytopathology 41:1059-1064.
14. WILSON, E. E., and A. R. MAGIE. 1964. Systemic invasion of the host plant by the tumor-inducing bacterium, Pseudomonas savastanoi. Phytopathology 54:576-579.