## Colonization of Cucumber Buds by Pseudomonas lachrymans in Relation to Leaf Symptoms

A. de Lange and Curt Leben

Department of Plant Pathology, Ohio Agricultural Research and Development Center and The Ohio State University, Wooster, Ohio 44691.

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## ABSTRACT

Cucumber seedlings grown at high relative humidity and inoculated with *Pseudomonas lachrymans* on the buds, showed angular leaf spot symptoms on four successive unfurling leaves. The pathogen multiplied in some buds and was isolated from the buds of 48 out of 112 plants 19 days after inoculation. Phytopathology 60:1865.

Additional key word: Epiphytic bacterium.

During the investigation of a possible resident phase of *Pseudomonas lachrymans* (Smith & Bryan) Carsner, special study was given to the bud of cucumber plants as a site of multiplication of the pathogen. Under conditions of high humidity, the colonization of buds of other plants by bacterial pathogens has been demonstrated (1, 2), and it seemed possible that cucumber buds are colonized by *P. lachrymans* in a similar way.

Plants (Cucumis sativus L. 'Green Prolific') were grown at 25-28 C in plastic film-covered chambers containing air at a relative humidity of 85-96%. A seed was planted 2 cm deep in dry vermiculite in a styrofoam cup. The vermiculite was covered with water for 2 hr, then drained through a hole punched in the cup bottom. Cups were placed in another vessel containing sufficient water to bring the water level to 2.5 cm below the seed. After plants emerged, they were watered once a week with a soluble fertilizer. Light was provided (450 ft-c at plant height) for 16 hr/day by fluorescent lamps over the chamber. A small amount of air was introduced into the chambers for 2 of every 12 min. Leaves appeared to be dry, except for guttation drops observed 4-6 hr after the chambers were lighted.

When the bud exposed after the cotyledons unfolded was 2-3 mm long, it was inoculated with a water drop

(0.01 ml) containing 10<sup>4</sup> viable cells of an isolate (628) of *P. lachrymans* obtained from a diseased cucumber leaf in northern Ohio. The inoculum was applied with a pipette, with care not to touch the bud or cotyledons with the pipette. Cells were taken from a 1-day-old slant of nutrient-sucrose agar (1) grown at 24 C. Control plants were treated in a similar way with sterile water. Plants were not disturbed during the 19-day test period.

In three successive experiments, the unfurling first leaf of 108 of 112 inoculated plants showed angular leaf spot symptoms a few days after inoculation. Symptoms varied from a leaf bearing a few lesions to a completely malformed leaf with numerous lesions. With 39 of these plants, no further symptoms appeared on leaves that were produced later. With the remaining 69 plants, one to three of the new leaves bore one or more small lesions. Fifty-five of the 69 plants showed symptoms on the third leaf, and 38 of the 69 plants also had lesions on the fourth leaf, which was the youngest. The continuous production of diseased leaves indicated that the buds were colonized by *P. lachrymans* for at least 19 days.

At the conclusion of each experiment, when four leaves had expanded and the fifth was < 1 cm long, the terminal bud (including the fifth leaf) was assayed in vitro. The tissues were crushed in 20 ml sterile water, and 0.02-0.04 ml of this suspension was spread on the surface of tetrazolium agar plates (1). After 40-48 hr, *P. lachrymans* produced characteristic colonies when viewed with a dissecting microscope (×40) with tangential light directed at the bottom of the plate. Reliability of the identification was assessed by inoculating cucumber seedlings with progeny from 53 representative colonies from a number of buds. All incited symptoms typical of plants inoculated with *P. lachrymans*.

The pathogen was isolated from the terminal buds of all 38 plants showing symptoms on the fourth leaf, and also from the terminal buds of 10 other plants. In 36 buds, the number of P. lachrymans cells was 10-200 times that applied in the inoculum. In the remaining buds, mostly from plants without symptoms on the fourth leaf, 104 or fewer P. lachrymans cells were found. The pathogen was not detected in buds of plants without symptoms or with symptoms only on the first leaf. It was not found in buds of control plants. Probably some P. lachrymans colonies were not detected because of the large number of other bacterial species in almost all of the 112 buds examined. The influence of the age of the plant on numbers of diseased leaves and terminal buds bearing the pathogen are being further investigated.

These results indicate that under suitable conditions some buds colonized by *P. lachrymans* produce a series of leaves bearing lesions. If this happens in the field,

a substantial amount of inoculum could be provided by one plant.

## LITERATURE CITED

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