Multiplication of Pseudomonas cepacia in Onion Leaves

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ABSTRACT

Initial symptom expression in young onion leaf blades injected with different concentrations of *Pseudomonas cepacia* was delayed from 1 to 5 days as inoculum concentration was decreased from 10^8 cells/ml to 10^3 cells/ml. Concentrations less than 10^3 cells/ml failed to induce symptoms. Young leaves were highly susceptible; most mature leaves failed to develop symptoms. The population of *P. cepacia* increased to more than 10^9 cells/leaf disc from an initial population level of 10^4 cells/leaf disc. The initial symptoms of water-soaking and wilting were expressed when the peak population was attained. Bacteria declined in number after appearance of symptoms. None was reisolated once diseased leaves became dry and brittle. Air-dried diseased leaves apparently are not sources of inocula in the field.

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Onion bulbs infected with *Pseudomonas cepacia* often exhibit only one or a few inner bulb scales decayed while the outer fleshy bulb scales are sound. This pattern indicates the bacteria probably entered the bulb from either the neck or leaf blades. Stab-inoculation of green leaves with *P. cepacia* resulted in the production of small lesions within 24 to 48 hr, which then expanded very slowly even when incubated in moist chambers at 21 to 27 C. Injection of high concentrations of bacteria suspended in water (10^{7-8} cells/ml) into the lacunar cavity of the leaf blade resulted in wilting and decay of the leaf within 24 hr. These contrasting responses of the plant indicated that the symptoms produced by *P. cepacia* on these leaves may be a hypersensitive response. By using subepidermal injections of bacterial cell suspensions to inoculate plants, Klement (3) and Klement & Lovrekovich (5, 6) developed a procedure to test the pathogenicity of a bacterium and the susceptibility of the test plant. Studies on the changes in the population of phytopathogenic bacteria in non-host, resistant, tolerant, and susceptible plants (1, 2, 3, 4, 5, 6, 8) have shown that the bacterial growth patterns are dependent upon the susceptibility of the host and the amount of inoculum used. This paper reports the effects of inoculum concentration and population changes of *P. cepacia* on symptom expression.

MATERIALS AND METHODS.—*Pseudomonas cepacia* (isolate 64-22) was grown on a nutrient agar slant for 18 hr at 27 C. The cells were suspended in 10 ml of sterile distilled water, and 10 tenfold dilutions were prepared. The number of viable cells/ml in each dilution was determined by plate counts on nutrient agar containing 100 μg/ml streptomycin sulfate. The youngest leaf on 1-month-old plants (grown from onion bulbs) was inoculated by injecting 0.5 ml of each dilution into the lacunar cavity of the leaf. Three leaves were inoculated/dilution. The plants were incubated in a controlled environment chamber at 27 C, 72% relative humidity, and 16 hr light/day. The light source was a mixed bank of incandescent and fluorescent lights which produced 1,500 ft-c at leaf tip height. The plants were subirrigated.

A 20-hr-old culture of *P. cepacia* was suspended in 10 ml sterile distilled water and diluted 10^4 times. This suspension was injected into the youngest and oldest nonsenescent leaves of the plants. The injections were made ca. 1 cm from the tip of each leaf, and the lacunar cavities were filled with the cell suspension up to the point of injection. Discs 0.5 cm in diam were cut with a No. 2 cork borer from the base of the lacunar cavity (daily for 7 days). Each disc was ground up separately in a mortar with 10 ml sterilized distilled water; then decimal-dilutions of each disc were plated on streptomycin nutrient agar. Four plates/dilution were made. Four young leaves and four mature leaves were sampled in this manner daily.

RESULTS.—When the inoculum concentration of *P. cepacia* was decreased from 10^8 cells/ml to 10^4 cells/ml, the time interval between inoculation and initial symptom expression increased from 1 day to as much as 5 days (Table 1). The first symptom to appear was water-soaking at the base of the leaf blade. The blade wilted 1 to 3 hr later and decayed within 24 hr after wilting. The decayed leaf eventually became dry and brittle. The type and sequence of symptoms were the same regardless of the initial inoculum concentration. The time intervals between water-soaking, wilting, and maceration were independent of inoculum concentration.

TABLE 1. Effect of inoculum concentration of *Pseudomonas cepacia* on pathogenesis in young onion leaves

Inoculum concentration	Days required for wilt
ca. 10^8 cells/ml	1
ca. 10^7 cells/ml	1
ca. 10^6 cells/ml	5[a]
ca. 10^5 cells/ml	2-5
ca. 10^4 cells/ml	2-3
2.8×10^3 cells/ml	2-5
6.9×10^2 cells/ml	No symptoms
4.6×10^1 cells/ml	No symptoms

[a]Two of the three leaves showed no symptoms after 8 days.

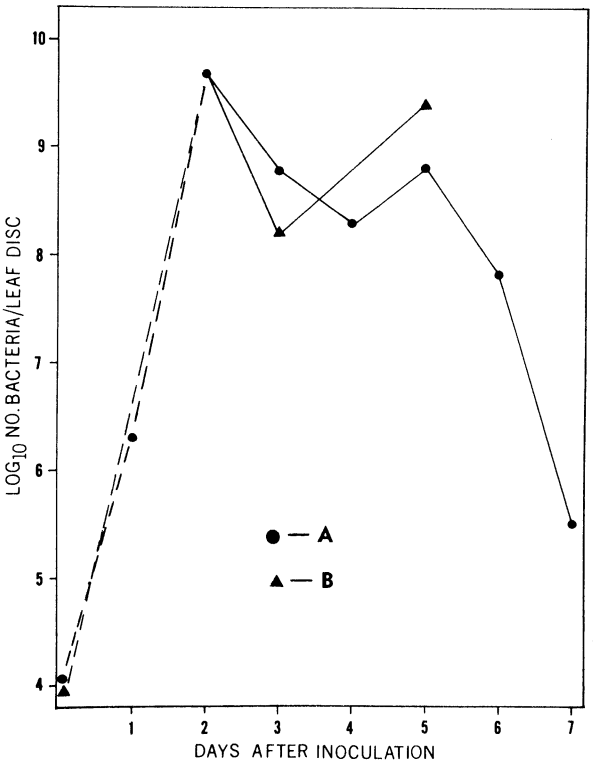

Fig. 1. Multiplication of *Pseudomonas cepacia* in young and mature onion leaves as related to symptom production. The appearance of symptoms on day 2 coincided with the maximum bacterial population. A = bacterial population in young leaves; B = bacterial population in mature leaves; - - - = bacterial population prior to the appearance of symptoms; ——— = bacterial population after the appearance of symptoms.

Population changes in those leaves which developed symptoms are presented in Fig. 1. The growth curve of the bacteria in young leaves was similar to a normal growth curve of bacteria in vitro. From an initial level of 10^4 cells/leaf disc, the population increased to a maximum of 4.8×10^9 cells/leaf disc. The bacteria reached this peak at approximately the same time symptoms appeared on the leaves. Nearly all the young leaves developed symptoms on the 2nd day after inoculation, but several did not produce symptoms until the 3rd or 4th day. The bacteria declined in number after the production of symptoms. No bacteria were recovered from leaves which had become dry and brittle.

Only 7 of 28 mature leaves developed symptoms, excluding those leaves sampled on the first 2 days after inoculation. The population changes in the diseased mature leaves were similar to the population changes in the young leaves. The maximum population in the diseased mature leaves was 4.7×10^9 cells/leaf disc, and occurred concurrently with the time of initial development of symptoms. The population varied greatly in those leaves which failed to develop symptoms, but did not exceed 4×10^5 cells/leaf disc (Table 2). When four of the leaves not showing symptoms were refilled with sterilized distilled water 12 days after inoculation, they developed the symptoms described earlier within 3 days and *P. cepacia* was reisolated.

TABLE 2. Number of cells of *Pseudomonas cepacia*/leaf disc from symptomless mature onion leaves

Days after inoculation	Bacteria/disc
0	5.5×10^3-1.9×10^4
3	1.0×10^4-8.0×10^4
4	2.6×10^2-1.4×10^3
5	3.5×10^4
6	< 10 - 1.0×10^2
7	8.6×10^4-3.9×10^5

DISCUSSION.—The interaction between *P. cepacia* and young onion leaves fits the pathogen-congenial host combination described by Klement & Lovrekovich (6) or the "eusymbiotic relationship" described by Klement (3) and Klement et al. (4). The time interval between inoculation and the appearance of symptoms increased as the inoculum concentration of *P. cepacia* decreased. The appearance of symptoms was correlated with the population level within the leaf and was independent of time. These results, when compared to the systems described by Klement (3) and his co-workers (4, 5, 6), suggest that *P. cepacia* is pathogenic on young onion leaves. Therefore, the young green leaves of onion appear to be a favorable site of ingress for this bacterium prior to infection of the bulb.

The behavior of *P. cepacia* in mature onion leaves was more complex. In some cases, the multiplication of the bacteria and the host responses were identical to those in the young leaves. In other cases, the bacteria behaved more as saprophytes. The bacterial population fluctuated widely, but did not exceed 4×10^5 cells/disc, and the leaves failed to develop symptoms. It appears that moisture was a limiting factor in the latter situation because when such leaves were refilled with sterilized distilled water a few days after inoculation, all developed symptoms. It is not known why the bacteria failed to cause disease immediately after inoculation, since the inoculum also was suspended in water.

The phenomenon of bacteria surviving and multiplying in symptomless plants has been observed before (1, 2, 6, 8). In the case of phytopathogenic bacteria in a noncongenial host, Klement & Lovrekovich (6) hypothesized that there is a postinfection-induced reaction which inhibits the bacteria, and that this reaction induces visible symptoms only when the inoculum level is sufficiently high. In the case of *Xanthomonas vesicatoria* and its congenial host, pepper, Sasser et al. (7) hypothesized that the high osmotic potential of the intercellular fluid of the pepper leaves prevented the multiplication of the bacteria, and that water-congestion reduced the osmotic potential so that the bacteria could multiply and cause disease. Neither hypothesis explains why the second addition of water enabled *P. cepacia* to cause disease and the first failed, or why the young leaves are more susceptible under these conditions than the mature leaves.

The population level of *P. cepacia* decreased after the leaves developed symptoms. This decrease became most pronounced as the leaves dried out following the soft-rot stage. Bacteria could not be isolated when the leaves became dry and brittle, suggesting that air-dried diseased leaves are not sources of inocula in the field.

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