Infection of Onion Leaves by Pseudomonas cepacia

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ABSTRACT

In artificial inoculations of onion leaf parts, *Pseudomonas cepacia* infected only wounded onion tissue. Inoculations involving wounding tissues by means of a needle (stab inoculations) resulted in small lesions; spray inoculations without wounds were unsuccessful. Lesions expanded very slowly unless free moisture was added to the inoculation site. Water-soaking the lesion by hard sprays caused rapid lesion expansion. The tissue at the juncture of the leaf blade and sheath (the leaf blade axil) was especially susceptible when stab inoculated and wetted. Rate of lesion development increased with more frequent wetting of leaves and/or as temp was elevated to 32 C.

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Pseudomonas cepacia Burkh. was described by Burkholder (3) as capable of causing decay of onion (*Allium cepa* L.) bulbs. He found that decay occurred when the bulb surfaces were cut and the wounds were artificially inoculated with the bacterium. He suggested that infection probably occurs naturally when the tops (leaves) of the plants are removed at harvest. However, our field observations in recent years in New York have shown that bacterial soft rots (caused by *P. cepacia* and other bacterial pathogens) also occur in onion plants before harvest. Soft-rotting of green leaves frequently has been associated with bacterial bulb decay in growing plants. These observations prompted this study on the role of factors such as the method and site of inoculation, temp, moisture, and light in relation to ingress and infection of *P. cepacia* on onion leaves and bulbs.

MATERIALS AND METHODS.—*General Procedures.—Pseudomonas cepacia* (culture 64-22) was utilized in all experiments. Inoculum was prepared from 18 to 24-h-old slant cultures grown on nutrient agar (NA) at 27 C. Plants were grown from seed and bulbs (cultivar 'Downing Yellow Globe'), or sets (cv. 'Ebenezer'). A mist chamber and moist chambers were utilized during incubation periods. The mist chamber consisted of a greenhouse bench enclosed by polyethylene sheeting to a height of 2 m (open on the top); mist was introduced to the chamber via nozzles operated for 10 s every 5 min. Temperatures ranged from 21-24 C in the mist chamber. Only daylight illumination was utilized. The moist chambers consisted of polyethylene bags (62×29.5 cm) used to enclose the plants which were grown in 10.2-cm (4-inch) pots; the bags were supported by stakes and secured to the pots by rubber bands.

Inoculations without wounds.—Leaves of 1-mo-old onion plants grown from bulbs were sprayed with a 10^7-10^8 cells/ml bacterial suspension. Other plants were sprayed with the same suspension containing Triton B-1956 (Rohm and Haas) spreader sticker (2 ml/liter). This was done at 0800, 1100, 1400, 1700, or 2300 hours. The plants were maintained in the greenhouse at 21-27 C; half of the plants of each treatment were kept uncovered on the greenhouse bench and half were placed in the moist chambers. The plants were examined daily for 2 wk for symptoms.

Wound inoculations of plants kept under greenhouse and growth chamber conditions.—Plants grown from seed or bulbs were stab-inoculated in the neck of the bulb; near the tip, middle, and base of the leaf blade; and at the juncture of the leaf blade and sheath (leaf blade axil). These plants were placed in mist chambers, moist chambers, or left exposed. With the exception of the plants in the mist chambers, these studies were conducted on the greenhouse bench or in Cornell University type controlled environment growth chambers. Temperature in the mist chamber was 21-24 C. Temperatures in the greenhouse ranged from 18 to 32 C. Temperatures in the growth chambers were 10, 21, 27, or 32 C. The growth chambers had a 14 h light period and a 10 h dark period. Relative humidity (RH) was 46-86% depending upon the chamber. Light intensity was 130-149 lux at leaf tip level

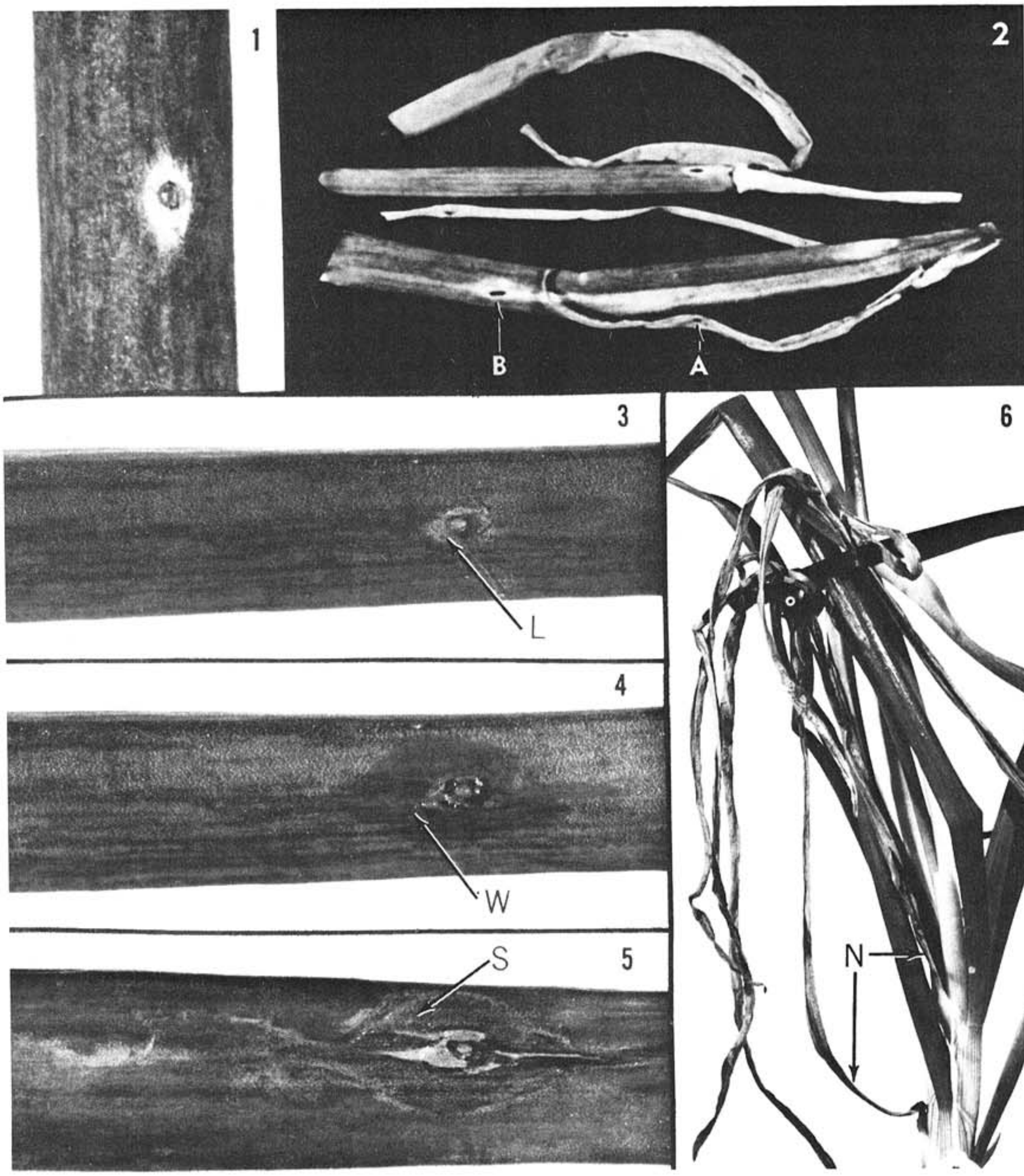

Fig. 1-6. Symptoms on onion leaves inoculated with and infected by *Pseudomonas cepacia*. **1)** Lens-shaped lesion resulting from stab-inoculation of onion leaf with *P. cepacia*. **2)** Dry leaf blight resulting from stab-inoculation of onion leaves with *Pseudomonas cepacia*. The blighting was caused by inoculation near the leaf tip (A); inoculation near the middle of the leaf resulted in restricted necrosis (B). **3-5)** Symptom development after water-soaking of lesion. **3)** Two-day-old lesion (L) before water-soaking. The lesion was produced by stab-inoculating the leaf with *P. cepacia*. **4)** Same lesion immediately after water-soaking. The water-soaked area (W) was produced by directing a hard spray of water at the lesion. **5)** Necrosis two days after water-soaking of a lesion. The necrosis extends beyond the water-soaked area, but water-soaking has left a distinct scar (S). **6)** Necrotic onion leaves (N) 4 days after lesions produced by *P. cepacia* were water-soaked.

and 93 lux at leaf blade axil ht. The temp of infected and uninfected leaf blade axils of plants enclosed or not enclosed in the moist chambers were measured with copper-constantan thermocouples made of 30-gauge wire. Air temp near the leaf blade axils of these plants were recorded continuously by a Tipptronic recorder (Environmental Growth Chambers, Chagrin Falls, Ohio). The thermisters of the recorder were shaded from light by aluminum foil. The response of lesions of different ages to water-soaking induced by a hard spray of water and the response of lesions to periodic wetting caused by gently sprinkling with water were determined

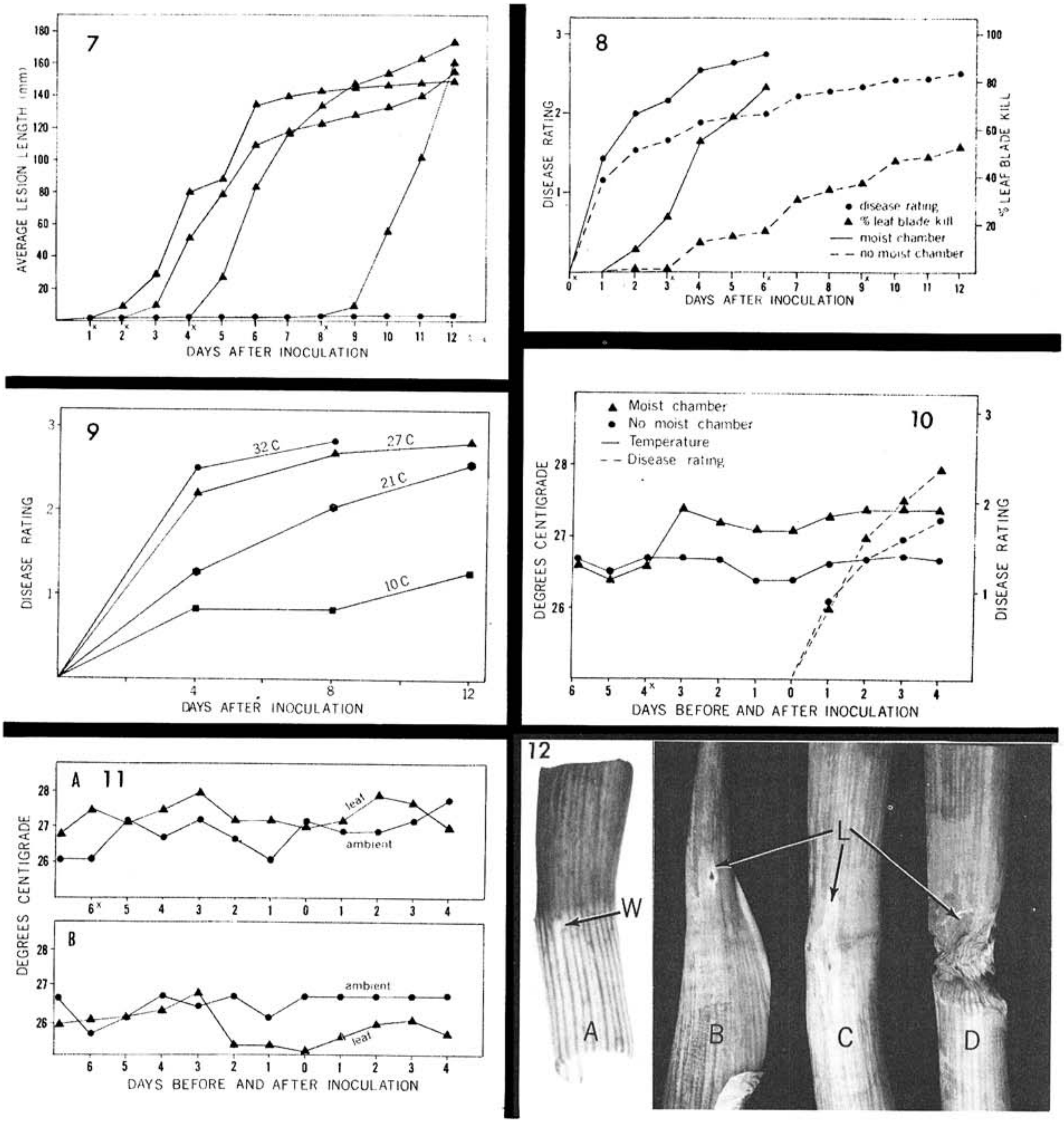

Fig. 7-12. Infection of onion leaves by *Pseudomonas cepacia*. **7)** Lesion response to water-soaking 1,2,4, and 8 days after inoculation (indicated by 1^x,2^x, etc). The lesions and the surrounding tissues were water-soaked by a hard spray of water directed from a No. 13 hypodermic needle connected to a Brewer automatic pipetting machine. **8)** Effect of moisture on disease development in onion leaves stab-inoculated in the leaf blade axil with *P. cepacia*. Disease ratings are based upon the symptoms described in Fig. 12. The leaves were sprinkled with water at the time of inoculation, and every third day thereafter (indicated by 3^x,6^x, etc.). The plants were incubated in a growth chamber at 27 C during the day and 24 C at night. The moist chambers consisted of 62 × 29.5-cm polyethylene bags placed over the plants in the controlled-environment chamber. **9)** Increase of disease in onion leaves stab-inoculated in the leaf blade axils with *P. cepacia* and incubated at different temp. Disease ratings are based upon the symptoms described in Fig. 12. The plants were covered with 62×29.5-cm polyethylene bags and incubated in controlled environment chambers. The inoculated leaves were sprinkled with water every 48 h. **10)** Average ambient air temp 2-3 cm from the plants, and disease increase of plants covered or uncovered with polyethylene-bag moist chambers. Disease ratings are based upon the symptoms described in Fig. 12. The onion plants were covered with polyethylene bags 4 days (indicated by 4^x) before inoculation. The onion leaves were stab-inoculated in the leaf blade axils with *P. cepacia*. The inoculated areas were wet once daily. **11)** Comparison of average leaf blade axil temp with air temp (ambient) 2-3 cm from the onion plant. Leaf blade axil temp were measured with copper-constantan thermocouples embedded in the base of the leaf blades, and ambient temp were measured with thermistors. (A) Leaf temp of an onion plant covered with 62 × 29.5-cm polyethylene bag 6 days before inoculation (indicated by 6^x) and the comparative ambient air temp. (B) Leaf temp of a plant not covered with polyethylene bag and the comparative ambient air temp. **12)** Onion leaves stab-inoculated with *P. cepacia* with lesions (L) showing different disease-rating stages. (A) Disease rating of "0"; with very little necrosis surrounding the stab wound (W). (B) Disease rating of "1"; lens-shaped lesion surrounding the inoculation wound. (C) Disease rating of "2"; larger, irregularly-shaped lesion. (D) Disease rating of "3"; lesion has girdled the leaf, causing it to topple.

by a disease rating system of 0-4 (Fig. 12).

Infection of onion bulbs from stab-inoculation of leaf blade axils was studied using large field-grown onions and onions grown from sets in the greenhouse. The dead leaves of the field-grownonions were removed and the leaf blade axils of the remaining leaves inoculated. The plants were incubated in the mist chamber for 5 days, then transferred to the greenhouse bench where the inoculated leaves were wetted (light spray) once daily for 20 days, after which the bulbs were examined for decay. All the leaf blade axils of plants grown from sets were stab-inoculated, and the plants were grown in the moist chambers for 5 days. The moist chambers then were removed and the plants grown under greenhouse conditions for 25 additional days. The inoculated foliage was wetted once daily while the plants were in the moist chambers and twice daily while on the greenhouse bench. The plants then were cut in half and infected bulb scales traced back and identified with their leaf blades.

Wound inoculations of plants in the field.—Four-mo-old plants grown in commercial onion fields in Orange County, New York, were inoculated while the leaf surfaces were still wet from a 0.5 cm rain the previous night. For one series, the onions were stab-inoculated with *P. cepacia* at the leaf blade tip, leaf blade axil, or in the leaf sheath (neck) above the bulb. For the second series, a bacterial suspension (10^8-10^9 cells/ml) was injected into the lacunar cavity of leaves. For the third series, the leaves were severely bruised and then either covered or not covered with organic soil. Uninjured plants or those stabbed with a sterile needle or injected only with distilled water served as controls. Three leaves (young, medium-aged, and fully mature) on each of approximately 30 plants were inoculated for each treatment; each treatment was replicated eight times. There was 0.5 and 1.9 cm of rainfall 2 and 4 days after inoculation, respectively. The plants were observed for symptoms at 5 and 13 days, and harvested at 31 days after inoculation. The bulbs were cut and examined for decay 34 days after inoculation. Relative humidity ranged from 28 to 98%, and temp from 9 to 32 C, between inoculation and harvest. Maximum and minimum temp generally occurred between 1400 and 1600 hours and 0500 and 0700 hours, respectively.

RESULTS.—*Inoculations without wounds.*—None of the unwounded leaves sprayed with *P. cepacia* produced symptoms within 2 wk of inoculation, regardless of time of inoculation or conditions of incubation. *P. cepacia* was isolated from surfaces of two of 36 leaves 3 days after spray-inoculation, but was not isolated from 48 and 29 leaves 11 and 18 days after inoculation, respectively.

Wound inoculations of plants kept under greenhouse and growth chamber conditions.—Leaf blades stab-inoculated with *P. cepacia* and incubated in moist chambers, developed small lens-shaped lesions in 2 days. The lesions were approximately 3 mm long and 2 mm wide (Fig. 1). Lesion expansion was slow and differed from leaf to leaf. Lesions expanded more longitudinally than laterally. Leaf blades inoculated approximately 3 cm from the leaf tip usually developed a dry leaf blight (Fig. 2) when incubated continuously in moist chambers for 2 wk. Leaves inoculated near the middle or 2 cm from the base of the blade seldom developed leaf blight. After 2 wk, *P. cepacia* was isolated from the small lesions and from the margins of the freshly blighted area, but not from the dry, blighted portions. When the neck of the onion bulb was stab-inoculated, each leaf sheath comprising the neck developed small lens-shaped lesions. These lesions were similar to those on the leaf blades; those on the inner sheaths were more moist in appearance. Lesions on the sheaths usually expanded very little in 2 wk; a few expanded to the shoulders of the bulb.

When small, 2- to 8-day-old lesions on the leaf blades were sprayed with water so that the surrounding healthy tissue became water-soaked, the lesions expanded into the water-soaked area within 1 day and then continued to spread beyond (Fig. 3,4,5,7), frequently killing the leaves (Fig. 6). Wounded (stab-inoculated) onion leaves were readily water-soaked with a spray of water; unwounded leaves were more difficult to water-soak. Larger and older leaves became water-soaked more readily than younger, smaller leaves and the base of the leaf blade became water-soaked less readily than upper portions of the blade. Attempts to water-soak unwounded leaves frequently resulted in small cracks in the leaf. When water-soaked leaves were sprayed with a cell suspension of *P. cepacia,* only a few of the water-soaked areas became necrotic. Leaves, which were water-soaked and then immediately stab-inoculated, developed lesions within 24-48 h in the water-soaked area.

Plants stab-inoculated in the leaf blade axils and incubated without moist chambers developed small, lens-shaped lesions. These increased slowly in size or not at all. A few leaves developed a soft rot in a few days on plants similarly inoculated and incubated in moist chambers. These leaves first developed small, lens-shaped lesions, which later increased in size, became irregularly-shaped, and eventually girdled the leaf blade, causing it to topple and die. The number of leaves killed increased markedly when the leaves were wetted (Fig. 8). Wetting combined with incubation in moist chambers, further increased the rate of disease development (Fig. 8). Lesion-size also increased as the temp of incubation was increased (Fig. 9). The difference between the leaf and air temp within the polyethylene bags was slight (Fig. 10,11).

The leaf blade and the leaf blade axil differed only slightly in susceptibility when an abundance of water was provided (Table 1). There also was little difference between the amount of disease at the two inoculation sites when neither site was wet (Table 1).

Bulb decay resulted from leaf blade axil inoculation (Table 2) and *P. cepacia* was reisolated from the decayed bulbs. The actual rate of success of bulb infection as related to leaf blade axil inoculation was rather low. In plants incubated in moist chambers, only 18 of 94 leaves developed a decay extending down into the fleshy leaf sheath bases (bulb scales) as the result of leaf blade axil inoculation. However, when these 18 leaves were grouped according to relative age, 14 were classed as young leaves, two as medium-aged leaves, and two were fully mature leaves at the time of inoculation. Thirty of the 94 leaves were young leaves; thus the bacterium invaded the bulb in 47% of the young leaf inoculations.

Wound inoculations of plants in the field.—All treatments involving stab inoculation of plants in the field, regardless of inoculation site, resulted in 5-mm diam, lens-shaped lesions in 5 days. After 13 days there was more variation between the different stab-inoculation treatments. The leaves of plants stab-inoculated in the necks were dead but the bulbs were firm. Plants stab-inoculated in the leaf blade axils had chlorotic streaks extending up and down from the tips of the lesions and a few inoculated leaves were dead. Several of the leaves stab-inoculated near the tip of the blade were completely necrotic, but the majority still had only the small lens-shaped lesions exhibited on the fifth day. Some leaves injected with the bacterial suspension had died back to the neck of the bulb in 5 days and nearly all leaves so inoculated had died back at least to the neck of the bulb by the 13th day. Plants in which leaves were bruised and placed in contact with the soil showed extensive wound injury in 5 days. The leaf blades of more than half of these plants died back to the neck in 13 days. Necrosis of the remaining plants was confined to the original injured area. Very few of the bulbs of any treatment had bacterial decay when examined 34 days after inoculation, despite the abundance of leaf blades with symptoms (Table 3).

DISCUSSION.—*P. cepacia* appears to be a wound pathogen on onion plants. The experiments conducted in this study did not produce evidence that *P. cepacia* can enter onion leaves directly through natural openings which are not water-soaked and cause disease. Even in water-soaked areas a wound probably is required for infection to occur. In a number of experiments when *P. cepacia* was sprayed on leaves with many water-soaked areas, produced previously by water spraying, very few lesions developed. Most of the lesions which developed occurred where the water-soaked areas had macroscopic wounds produced by the sprays. The remaining lesions in the water-soaked areas probably were related to the presence of microscopic wounds. The failure of the pathogen to induce lesions in the water-soaked areas could be explained also on the failure of *P. cepacia* to gain ingress into the water-soaked areas. This, however, is unlikely because Allington (1), Diachun et al. (5), and Johnson (7) have shown that uninjured water-soaked leaves will absorb a liquid with suspended particles from the leaf surface through the stomates.

P. cepacia spreads much more rapidly in water-soaked tissue than in uncongested tissue. Clayton (4) noted that *P. tabaci* was scattered widely in water-soaked tissue, with no indication of zoogloea. He suggested that the bacteria spread rapidly in the liquid as free swimming cells. In stab-inoculated, uncongested tissue, *P. tabaci* was in the form of zoogloea, implying a rather slow penetration. In addition to the difference in rate of spread of *P. cepacia* in congested onion leaf tissue, there was a difference in the type of symptoms. The symptoms in the water-soaked tissue were typically a soft rot, and those in nonwater-soaked tissue a dry leaf blight.

The gentle application of free moisture to the surface of a lesion caused by *P. cepacia* may result in limited nonvisible water-soaking of the surrounding tissue (diseased or healthy). The water probably enters the tissue through the wound and spreads by capillarity through the intercellular spaces. Johnson (7) has found this to occur with wounded tobacco leaves, although the area and amount of congestion are less than that resulting from external or physiological water-soaking. Histological observations of *P. cepacia* inoculated into onion leaf blade axils wet once daily suggested that *P. cepacia* moves through the intercellular spaces as free swimming cells or small clumps of bacteria (8). In addition to aiding bacterial movement, free moisture may serve to dilute materials toxic to bacteria and also make nutrients more readily available to the bacteria.

Water in vapor form alone was insufficient to cause much increase in the rate of disease development on onion leaves. Inoculated plants incubated in moist chambers usually developed little disease in the absence of free moisture at the site of inoculation. When free moisture

TABLE 1. Susceptibility of onion leaves stab-inoculated with *Pseudomonas cepacia* at the axil or middle of the leaf blade and incubated 12 days on the greenhouse bench or in the mist chamber

Treatment	No. Samples	Disease Rating[c]	Leaf kill (%)
Greenhouse[a]			
Leaf base (leaf axil)	42	1.1	5
Leaf middle	50	1.0	2
Mist chamber[b]			
Leaf base (leaf axil)	52	2.3	58
Leaf middle	43	2.2	58

[a]Temperature was 21-27 C. Plants sub-irrigated to avoid wetting the site of inoculation.
[b]Temperature was 21-24 C. Plants misted for 10 s every 5 min.
[c]Disease rating system used was as follows: 0 = small, circular necrosis, such as that which would be produced by wounding with a sterile needle; 1=small-sized lens-shaped lesion; 2 = larger, irregularly-shaped lesion; and 3 = lesion girdling the leaf (See Fig. 12 for illustration).

TABLE 2. Onion bulb decay after stab-inoculation of leaf blade axils with *Pseudomonas cepacia*[a]

Treatment	Misted every 5 min	Wet once daily	
	Decayed bulbs (%)	Decayed bulbs (%)	Decayed bulb scales (%)
Inoculated	68	48	19
Control	18	5	2

[a]Three to six leaf blade axils inoculated per plant.

TABLE 3. Incidence of bacterial decay in onions resulting from different methods of artificial inoculation at different leaf sites with *Pseudomonas cepacia* under field conditions[a]

Inoculation site	Inoculation method	Decay (%)
Leaf tip	Stab inoculation	0
Control	Sterile needle	0
Blade axil	Stab inoculation	3
Control	Sterile needle	0
Neck	Stab inoculation	3
Control	Sterile needle	0
Lacunar cavity	Hypodermic needle	4
Control	Sterile water injected	0
Bruised leaves	Dipped in soil[b]	2
Control	Not dipped in soil (leaves bruised)	1
Control	Unwounded	1

[a]Approximately 240 plants/treatment.
[b]Soil was natural soil in which the plants were growing.

was added to the inoculation sites of plants in the moist chamber or on the greenhouse bench, disease incidence was higher for plants in the moist chambers (Fig. 8) than for those kept on the bench. The higher disease incidence in the moist chambers may have been due to a slower rate of evaporation of the water droplets on the inoculation sites and not directly due to higher humidity. A slow rate of evaporation would allow the water droplets more time to enter and permeate the diseased tissue.

The leaf blade axil was more susceptible than other parts of the leaf blade except when both sites were wet frequently (in the mist chamber) or not at all. This may be the result of the difference in morphology between the two sites. The leaf blade axil catches and holds water, whereas the leaf blade readily sheds water sprinkled upon it. The ability of the leaf blade axils to catch and hold water varies from leaf to leaf. The young leaves are upright and water striking these leaf blades will often flow down the leaf and collect in the leaf blade axils. Older leaves of mature plants bend downwards and are less efficient in collecting water at their bases.

As expected, in leaf blade axils the amount of disease increased as the temp was increased (Fig. 9). Unfortunately, growth chambers above 32 C were unavailable, and the complete temp range at which *P. cepacia* will cause leaf disease in onion was not determined. The maximum temp for pathogenesis probably is somewhat higher than 32 C since isolate 64-22 multiplied in nutrient broth up to 41 C. Others (2,9) have found that *P. cepacia* (syn. *P. multivorans*) will grow at 41-42 C.

The importance of the age of the leaf, the portion of the leaf inoculated, and the need for free moisture and warm temp would seem to restrict the incidence of onion soft rot caused by *P. cepacia*. *P. cepacia* is not very invasive in onion leaves unless all conditions are favorable, yet it is one of the predominant soft rot bacteria of onions grown on organic soil in New York. Bacterial soft rot of onions usually is associated with rainstorms and flooding in New York. These conditions favor water-soaking (4, 6) as well as wounding and localized congestion of onion leaves.

LITERATURE CITED

1. ALLINGTON, W. B. 1945. Wildfire disease of soybeans. Phytopathology 35:857-869.
2. BALLARD, R. W., N. J. PALLERONI, M. DOUDOROFF, and R. Y. STANIER. 1970. Taxonomy of the aerobic pseudomonads: Pseudomonas cepacia, P. marginata, P. alliicola and P. caryophylli. J. Gen. Microbiol. 60:199-214.
3. BURKHOLDER, W. H. 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology 40:115-117.
4. CLAYTON, E. E. 1936. Water soaking of leaves in relation to development of the wildfire disease of tobacco. J. Agric. Res. 52:239-269.
5. DIACHUN, S., W. D. VALLEAU, and E. M. JOHNSON. 1944. Invasion of water-soaked tobacco leaves by bacteria, solutions, and tobacco mosaic virus. Phytopathology 34:250-253.
6. JOHNSON, J. 1937. Relation of water-soaked tissues to infection by Bacterium angulatum and Bact. tabacum and other organisms. J. Agric. Res. 55:599-618.
7. JOHNSON, J. 1945. Infection experiments with detached water-congested leaves. Phytopathology 35:1017-1028.
8. KAWAMOTO, S. O., and J. W. LORBEER. 1972. Histology of onion leaves infected with Pseudomonas cepacia. Phytopathology 62:1266-1271.
9. STANIER, R. Y., N. J. PALLERONI, and M. DOUDOROFF. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:159-271.