Pseudomonas aeruginosa: Cause of Internal Brown Rot of Onion

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ABSTRACT

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From the results of biochemical tests and its utilization of 108 carbon sources, a pathogenic bacterium isolated from diseased onions was identified as *Pseudomonas aeruginosa*. In inoculation studies, lesions were formed only in the leaf bases near the center of the bulb. No lesions formed in immature bulbs, and no infection developed in wound-inoculated leaves. The pattern of disease development suggested that infection occurred at harvest, but in vitro experiments failed to support this. Glucose, fructose, and sucrose were the only soluble sugars in the leaf base tissue. Soluble sugars comprised up to 77% of the dry weight of the outer leaf bases; but only about 8% of the dry weight of the inner leaf bases. The importance of soluble sugars in disease development was examined and a potential mechanism of bulb infection is discussed.

About 95% of the annual onion production (36,000 tonnes or 35, 400 tons) in New South Wales (Australia) is grown in the Murrumbidgee Irrigation Areas (MIA) and it represents, in monetary terms, 30% of the total value of vegetable crops grown in that area. Onions grown in the MIA are harvested from early summer (November) to mid-autumn (April) and are stored in 0.5-tonne (12,000-lb) slatted wooden bins, stacked under cover six to eight bins high, for 3-4 months before distribution to market outlets. No controlled-environment storage is provided and ambient conditions of up to 30 C with varying degrees of humidity are common.

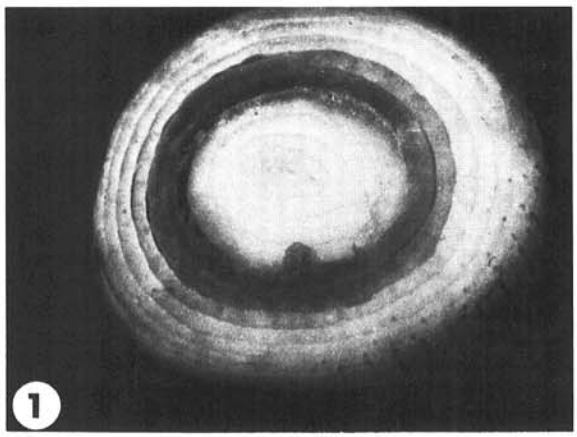
In March 1974, a breakdown and decay of onions resulted in a 2,000 tonne (1,968 tons) loss from one packing store. Inspection of internal tissues of apparently healthy looking bulbs (cultivar Cream Gold) revealed that one or two leaf bases were yellow to chocolate-brown while adjacent leaf bases were not visibly affected (Fig. 1-2). The discolored leaf bases were generally firm except in the neck region where a soft rot was usually present due to secondary infection. At an advanced stage of decay, the onion tissues collapsed and neighboring bulbs became infected. The high incidence of the disease and the delay in symptom development in bulbs arriving at overseas ports were viewed with considerable concern by the industry.

This paper reports studies on pathogen identification and some aspects of disease development.

MATERIALS AND METHODS

Anatomically, onion bulbs represent swollen leaf bases and the term "leaf base" will be used throughout rather than 'scales', 'scale layers', or 'rings'.

Cubes of tissue 2-mm square from individual leaf bases



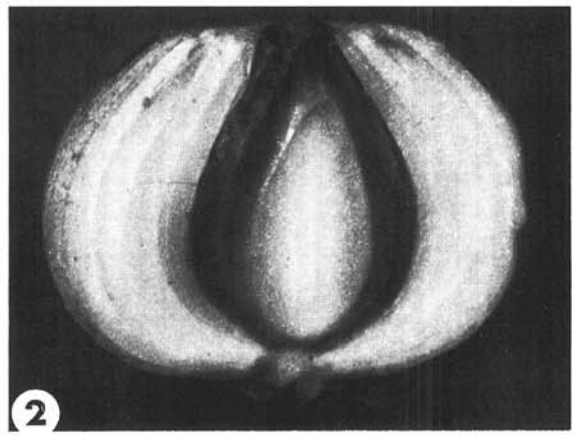


Fig. 1-2. Restriction of lesion development in onion bulbs infected with *Pseudomonas aeruginosa*. **1)** Transverse; and **2)** longitudinal section of naturally infected bulbs.

near the advancing margin of the lesions were placed in Oxoid nutrient broth or sterile distilled water for 2 hours, then a loopful of liquid was streaked on dry nutrient agar plates at 25 C. Tissue also was surface-sterilized for 60 seconds in sodium hypochlorite solution (1% available chlorine) and plated on potato-dextrose and nutrient agars. Only bacteria were isolated and pure colonies were obtained by repeated subculturing on nutrient agar.

Pathogenicity of isolates was verified by stabbing dormant bulbs of white and brown onions with a needle that had been dipped into a bacterial colony on nutrient agar. In addition, sterile 3-mm-thick transverse sections through leaf bases were placed on moist sterile filter paper in petri dishes and inoculated in the center with bacteria. Control leaf bases received only water.

Characterization.—*Morphology.*—Cultures were maintained on nutrient agar. Stock cultures were maintained as 24-hour-old cultures in peptone yeast extract broth (bacteriological peptone, 10g; yeast extract, 5g; NaCl, 5g, per liter) in stoppered vials. Sterile glycerol was added (1:1, v/v) to the vials which were shaken and then stored at −18 C. Flagellation of 24-hour-old cultures and the presence or absence of poly-beta-hydroxybutyrate accumulation was determined by electron microscopy.

Biochemical and physiological tests.—Procedures to identify the bacteria included tests for production of levan, Kovac's oxidase, potato rot, and protease (16). Tests also were made for arginine dihydrolase, tobacco sensitivity, 2-ketogluconate, and lipase production (on Tween-80) (17); nitrate reduction and acid production from sugars (1); and synthesis of amylase, indole, and hydrogen sulfide (21). The methods used are outlined in these citations. Growth at 4 and 41 C on nutrient agar was determined after 2 days.

Nutritional tests.—A mineral-base agar (KH_2PO_4, 2.245 g; $Na_2HPO_4 \cdot 12\ H_2O$, 6.71 g; NH_4Cl, 1 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; ferric ammonium citrate, 0.05 g; $CaCl_2 \cdot 2\ H_2O$, 0.00625 g; Ionagar 10 g, per liter) served as the basic medium. The ability of the bacteria to utilize a range of carbon sources was determined as described by Stanier et al. (22) at the concentrations given by Palleroni and Doudoroff (18). The chemicals used were of analytical reagent grade. All compounds were prepared, where appropriate, as 1% solutions, filter sterilized, and added to the molten agar. Geraniol, naphthalene, and phenol were added to the lid of the petri dish. Nine-centimeter diameter glass petri dishes containing 30 ml of medium were poured 3-4 days before use to allow the agar surface to dry. Bacteria suspended in sterile distilled water (about 10^6 cells/ml) were streaked on the agar and bacterial growth was scored visually for growth after 2, 4, and 14 days at 25 C. Each compound was replicated twice, and the agar in each plate was streaked twice with the same bacterium. Bacterial growth on control plates containing only mineral-base agar was compared with that on agar containing an added carbon source.

Inoculation experiments.—*Intact bulbs.*—Normally roots and tops are removed manually from onions at harvest, and thus it was hypothesized that natural infection could occur at this stage. To test this possibility, intact onions were harvested at weekly intervals commencing 5 weeks before the anticipated maturity date. Each bulb was dipped up to the neck in a 0.2% solution of PCNB (pentachloronitrobenzene) to reduce growth of *Aspergillus* spp. during incubation. The leaves and roots were removed from 50 onions with scissors that were dipped into a 10^8 cells/ml suspension of *P. aeruginosa* before each onion was cut. Ten control bulbs were cut each week with clean scissors. One-half of the onions were placed in plastic bags for 48 hours to maintain a high humidity and then were stored in paper bags in the laboratory for 6 weeks. The remaining cut onions were stored in paper bags without exposure to high humidity. Ambient temperatures ranged from 20 to 27 C. At the end of the 6-week period each bulb was cut longitudinally and examined for infection.

Influence of maturity and inoculum concentration on lesion development.—Sterile onion slices were obtained by first wiping the bulb with 0.2% PCNB solution then dipping in 98% ethanol and flaming. Slices, 3-mm thick, were cut transversely through the bulb (containing six to eight leaf bases) and placed in sterile petri dishes. The outermost leaf base and an internal leaf base near the center of each slice were inoculated. Slices were incubated at 25 C for up to 10 days and observed for symptom development. Three onion cultivars were used, including immature Cream Gold bulbs at various stages of maturity as described above and mature bulbs of cultivars Hunter River White and Crystal Grano. The two inocula concentrations used were determined by dilution plating, and effectively placed 10^6 cells or 1.7×10^3 cells on the cut tissue surface. Control slices received only a drop of sterile distilled water.

Three days after inoculation, portions of slices adjacent to the margins of the lesions were fixed on Farmer's fluid (glacial acetic acid : absolute ethanol, 1:3), dehydrated in an ethanol series, and embedded in histological wax. Sections 10-μm thick were cut with a rotary microtome, affixed to slides using Haupt's adhesive, and stained with Harris' hematoxylin and Orange G (8).

Leaf inoculations.—Immature onion plants (cultivars Cream Gold, Hunter River White, and Crystal Grano) about 8 weeks from harvest and growing in 30-cm diameter plastic pots were maintained in a glasshouse at 25 C day/20 C night and about a 14-hour photoperiod. The three youngest leaves on 10 bulbs were inoculated 4 cm above the bulb with 0.2 ml of a 10^6 bacteria/ml suspension delivered from a syringe. One milliliter of suspension was syringed into the leaf cavity of the youngest leaf on another 10 bulbs. One-half of the plants were covered with wet plastic bags to maintain a high humidity for 2 days. Leaves and bulbs were inspected 2, 10, and 14 days later. Plants not inoculated with bacteria, but otherwise treated similarly, served as controls.

Extraction and analysis of soluble sugars.—Transverse sections 5-mm thick were cut through a mature onion bulb containing 8-10 leaf bases. Leaf bases were numbered from the outer mature base to the inner immature base, and were separated, weighed, and extracted for 3 hours with 95% ethanol in a Soxhlet apparatus. Total soluble sugar was determined using the phenol-sulfuric method (5).

A Hewlett-Packard 5700A gas chromatograph with a hydrogen flame ionization detector was used to determine individual sugars. One milliliter samples of the extract were dried and silylated with 100 μliter of TriSil Z. Ten microliters of the silylated sugar solution were injected

into the chromatograph and the sugars were separated on a 1.8-m (6-foot) column of 3% SE-30 on 177-149 μm (80- to 100-mesh) Supelcoport. An initial temperature of 140 C was maintained for 4 minutes, followed by an increase of 2 C/minute to 190 C, after which the heating control was set isothermally at 250 C to elute sucrose. Flow rates used were; nitrogen 20 cc/minute at 413 kPa (60 psi), hydrogen 30 cc/minute at 103 kPa (15 psi), and air 24 cc/minute at 165 kPa (24 psi). Sorbitol was used as the internal standard.

The ability of pathogenic bacteria to grow on the ethanol extract, after removal of ethanol, was determined by incorporating filter-sterilized aliquots in molten mineral-base agar at concentrations of 1:2, 1:3, 1:5, 1:10, 1:20, and 1:40.

RESULTS

Isolations.—Three bacterial types of different colony morphology were isolated on nutrient agar and potato-dextrose agar. One type predominated in all onions sampled. No fungi were isolated. Two bacteria were identified by Bradbury's key (1) as *Pseudomonas* spp. and one as an *Erwinia* sp.

Pathogenicity.—Pathogenicity tests with whole and sliced onions produced symptoms ranging from a colorless soft rot to the dark-brown discoloration characteristic of this disease. The latter symptom was produced only by one *Pseudomonas* sp. that was dominant in all isolations. Tissue surrounding the puncture wound was discolored, but the rot and/or discoloration was more advanced in the younger leaf bases towards the center of the bulbs. The dark-brown discoloration appeared in both onion types, although it was more pronounced in the brown-skinned cultivar. The control bulbs were unaffected.

The necrosis in the inoculated onion slices was limited to the one inoculated leaf base only and did not progress into adjacent leaf bases.

Characterization of the bacteria.—Only the one *Pseudomonas* sp. that produced characteristic symptoms in pathogenicity tests was used in these experiments. Two isolates were examined.

Morphology.—The pathogenic *Pseudomonas* sp. was motile with 1-4 (mainly 1-2) polar flagella having a definite wavelength pattern of 1.1 μm. The cells ranged in size from 0.9 to 2.0 μm (average 1.1 μm), and they did not accumulate poly-beta-hydroxybutyrate as a cellular reserve material. Colonies on nutrient agar were initially pale dirty-white, slightly raised with smooth edges. Within about 3 days, the agar turned a dull dark reddish-gray color and the colonies became translucent and flat with irregular edges. Within 14-21 days, the agar was golden-gray in color.

Biochemical and physiological tests.—Both isolates produced yellow-green diffusible fluorescent pigments on King's medium B and blue-green diffusible pigments on King's medium A. The pigments were soluble in water, but not in hexane or ethanol. The bacterium was Kovac's oxidase-positive, levan-negative, and did not produce a soft rot in potatoes. No hypersensitive reaction was observed in tobacco. Gelatin liquefaction and nitrate reduction were rapid and complete within 3 days. The cultures were arginine dihydrolase-positive and produced 2-ketogluconate. Lipase production was rated as positive, but the diffuse margins of the colonies and coloring of the agar tended to obscure the area adjacent to the colony, which made the presence of an opaque halo usually difficult to detect. No amylase, indole, or H_2S were produced. Glucose was not utilized anaerobically. Acid was not produced from sucrose, trehalose, maltose, lactose, raffinose, sorbitol, inositol, adonitol, dextrin, or rhamnose, but it was produced from glucose, mannose, fructose, galactose, mannitol, and glycerol. Both isolates grew at 41 C, but not after 5 days at 4 C.

Nutritional tests.—Of the 108 carbon sources tested, 76 could be utilized by the bacterium as sole sources of energy. The two isolates had a similar reaction in the majority of cases. Details on carbon-source utilization are available from the authors.

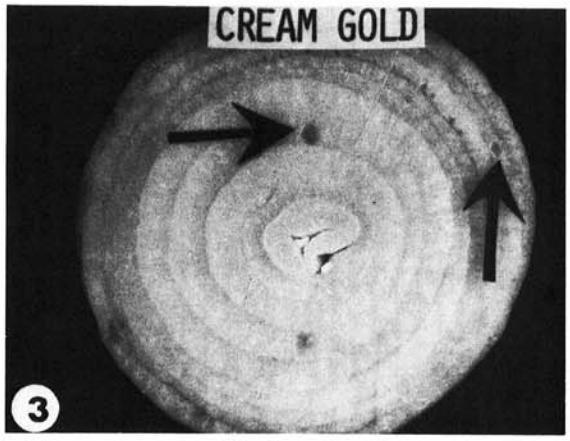
From the work of Sands et al. (19) it was clear that this bacterium could not be classified within the majority of phytopathogenic pseudomonads (Group One) but it could be grouped with *P. aeruginosa* and *P. putida* in Group Two; (the arginine dihydrolase-positive, oxidase-positive, hypersensitive-negative, green-fluorescent pseudomonads). According to the data of Stanier et al. (22), the onion isolates had nutritional spectra similar to those of *P. aeruginosa* and *P. fluorescens*. A comparison of 10 characters selected by Stanier et al., to permit unambiguous separation of these two species indicated that the onion isolates were most similar to *P. aeruginosa* (Table 1).

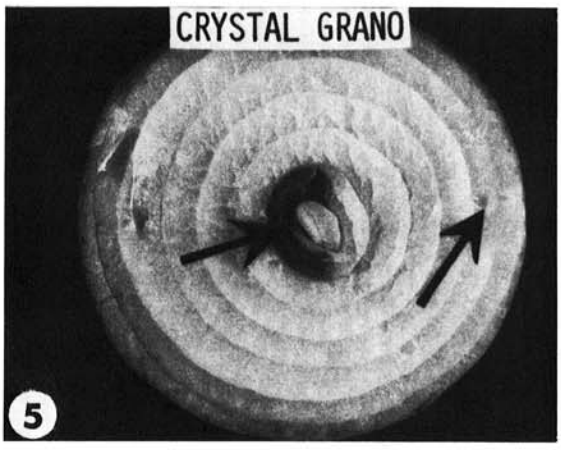
Inoculation experiments.—*Intact bulbs.*—Of the 300 bulbs inoculated over the 5-week period, only three showed symptoms of infection and all of these had been

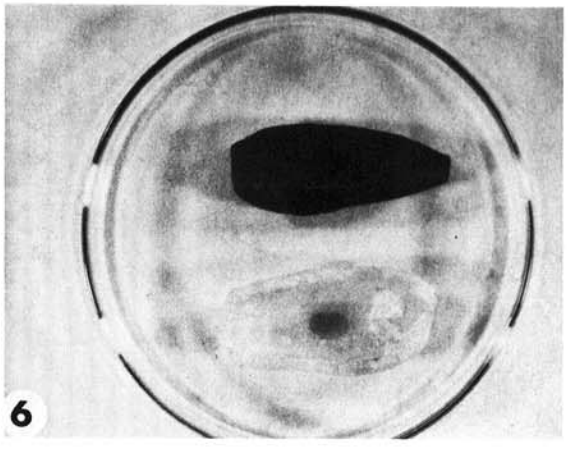
TABLE 1. Selected characters for the differentiation of fluorescent *Pseudomonads*

	Onion isolate	*P. aeruginosa*	*P. fluorescens*
Monotrichous flagellation	−	+[b]	−[b]
Pyocyanine production	+	+	−
Growth at 4 C	−	−	+
Growth at 41 C	+	+	−
Egg yolk reaction	nt[a]	−	+
Levan formation from sucrose	−	−	+
Utilization of trehalose	−	−	+
Utilization of inositol	−	−	+
Utilization of geraniol	+	+	−
Utilization of acetamide	+	+	−

[a]nt = not tested.
[b]From data of Stanier et al. (22).







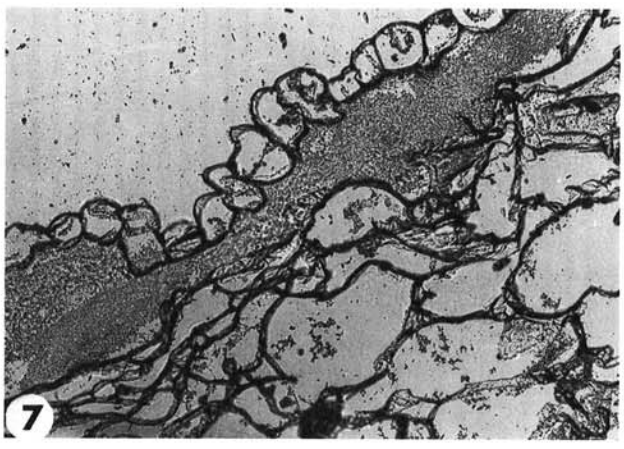


Fig. 3-7. 3) Section of an immature Cream Gold onion bulb inoculated at four points with 10^6 viable units/ml of *Pseudomonas aeruginosa*. Only water-soaked spots developed; **4)** and **5)** Transverse sections of mature Cream Gold and Crystal Grano onion bulbs inoculated as in Fig. 3 showing lesion development at inoculation point near center of the bulbs. No lesions developed on outer leaf bases. Arrows denote points of inoculation; **6)** Portions of mature Cream Gold onion leaf bases inoculated with about 1,800 viable units/ml of *Pseudomonas aeruginosa*. Upper leaf base portion had epidermis removed and rotted within 6 days at 25 C. Lower portion with intact epidermis developed only water-soaked area; **7)** Transverse section of inoculated leaf base showing development of bacteria beneath intact epidermis (× 250).

inoculated at maturity. One infected bulb came from the batch maintained at high humidity after inoculation. Tissue discoloration was confined to two leaf bases towards the center of each onion. The bacteria were reisolated from discolored tissue. The other inoculated bulbs and controls showed no signs of bacterial infection. Seven bulbs from various maturity times were infected with *Aspergillus* sp.

Influence of maturity and inoculum concentration on lesion development. —The effect of maturity on lesion development was particularly evident in the experiments with onion slices. In the cultivar Cream Gold, no lesions occurred until the bulbs were fully matured. Slices from immature bulbs developed only water-soaked spots at the points of inoculation (Fig. 3). In the mature slices, lesions developed only in the inoculated leaf bases near the center of the onion; except for a small water-soaked spot there were no symptoms of infection in the inoculated outer leaf bases (Fig. 4). In the immature onion slices neither 10^6 nor 1.7×10^3 cells produced lesions within 10 days.

The reaction of mature bulb slices of cultivars Hunter River White and Crystal Grano was similar to that shown by those of mature Cream Gold. The outer leaf bases showed no symptoms of infection (Fig. 5), but advanced lesion development occurred in the inner inoculated bases. After 10 days, however, viable bacterial cells still were present in the inoculated outer leaf bases of all slices. Five days after inoculation, bacteria were isolated from tissue up to 5 mm in advance of the visible margin of the lesion. It is likely that in these in vitro experiments, bacteria moved via the water film on the surface of the slice. A similar experiment with immature bulbs gave results identical to those achieved in the experiment with slices of mature bulbs; i.e., no lesions developed in the inoculated immature tissue.

Leaf bases adjacent to the infected bases generally did not become infected. In inoculated slices, adjacent infection occurred only when the inoculated leaf base eventually collapsed and a liquid film developed on the petri dish base, which provided a medium for transport of bacteria to adjacent leaf bases. It appeared from examination of inoculated slices that the lower and upper epidermis that separated the leaf bases may prevent lateral bacterial movement from leaf base to leaf base. This possibility was examined by quartering mature Cream Gold bulbs and carefully separating the slices. The distinctive lower epidermis on the concave side of each leaf base near the center of the bulb was removed. Portions of each leaf base were placed in petri dishes and surface-inoculated in the center of the concave side with 3 μliters of a suspension containing about 6×10^5 cells/ml. Additional slices with epidermis intact were similarly inoculated and control portions of each received only sterile distilled water. After 6 days at 25 C inoculated leaf base portions lacking an epidermis were dark brown and slightly shrivelled, whereas those with an epidermis had only a water-soaked spot at the point of inoculation (Fig. 6).

Leaf inoculations.—No infection or hypersensitive reaction was observed in inoculated leaves. Leaves were sectioned at the point of inoculation but there was no evidence of infection. Bulbs whose leaf cavities had been inoculated were cut longitudinally; however, no sign of bacterial infection was evident.

Analysis of soluble sugar.—Glucose, fructose, and sucrose were the only soluble sugars in the leaf-base tissues. Sugars were highest in the outer, and lowest in the inner, leaf bases (Fig. 8). Soluble sugars accounted for about 77% of the total dry weight of the outer leaf bases, whereas in the inner bases soluble sugars comprised only 8% of the material present. These results suggest that because the bacterium is unable to utilize sucrose this sugar may suppress bacterial growth. However, in separate in vitro experiments the bacterium grew on a sucrose-containing medium if an alternative carbon source (e.g., glucose) was present.

Ethanol extracts of individual leaf bases from the center and outside of a mature bulb were reduced to 25 ml. Each sample was filter-sterilized and aliquots were added to cooled (but still molten) mineral-base agar to give final concentrations of extracts rnaging from 1:2 to 1:20. Control plates contained the extracting liquid similarly treated. Plates were streaked with bacteria, incubated at 25 C, and examined for growth after 48 hours. Growth occurred on all dilutions of the extracting liquid (controls) but did not occur until inner or outer leaf base tissue extracts were diluted 1:10. The bacterium grew on dilutions higher than this for both leaf bases. This experiment was repeated three times with variable results that ranged from no inhibition of growth at a 1:2 dilution to inhibition by dilutions as high as 1:15.

Histology.—A transverse section of a lesion on a leaf base inoculated with 10^6 bacterial cells is shown in Fig. 7. Extensive bacterial development occurred immediately below the epidermis and appeared to progress intercellularly between the epidermis and the adjacent cylindrical cells. The epidermis remained intact when

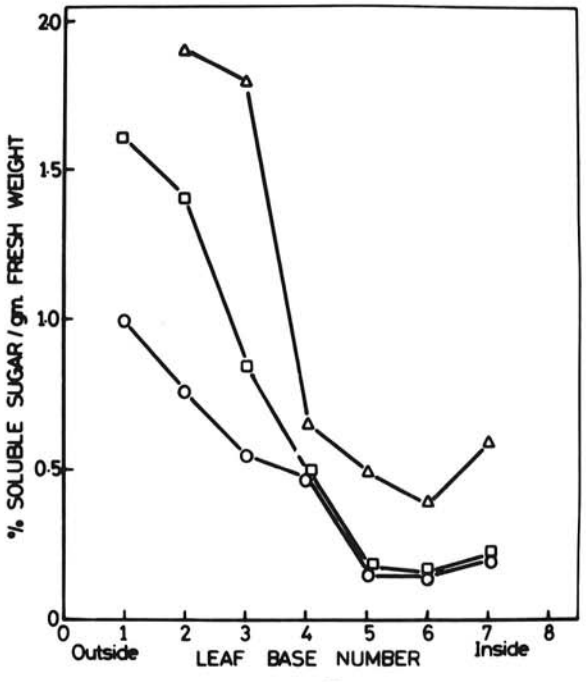


Fig. 8. Concentration of glucose ○, fructose □, and sucrose Δ across the leaf bases of mature onion bulbs of cultivar Cream Gold.

separated from the parenchyma. Of the few bacteria observed within host cells, the majority were associated with cell walls. Scattered bacteria were observed in advance of the main mass of bacteria and the development of the lesion. After surface sterilization to eliminate bacteria from the cut surface of the leaf base, bacteria could be isolated from portions of inoculated tissue taken up to 5 mm from the margin of the discoloration. These results indicate that *P. aeruginosa* moved through the intercellular spaces, a mode of advance that was described for *P. cepacia* in onion leaf bases (13).

DISCUSSION

The onion bacterium resembles *P. aeruginosa*. The ability to utilize specific compounds differs in seven instances (D-arabinose, galactose, caprate, L+tartrate, citraconate, erythritol, and creatinine) but only two of these (erythritol and creatinine) are considered to be of any importance in distinguishing between species.

Based on the similarity of physiological tests and substrate utilization, the pathogenic pseudomonad isolated from onions was identified as *Pseudomonas aeruginosa* (Schroeter) Migula. This is confirmed by the abridged key of Schroth and Hildebrand (20) and the species definition of Jessen (11). The only striking dissimilarity between the onion isolate and *P. aeruginosa* is the number of flagella possessed by the onion isolate. The 8th edition of Bergey's Manual mentions that two or more polar flagella may occur infrequently, and 3% of the 122 strains examined by Lautrop and Jessen (15) had more than one flagellum.

Pseudomonas aeruginosa is a natural pathogen of lettuce, banana, and tobacco but is pathogenic when artifically inoculated onto potatoes, tomatoes, onion, avocado, chilli, cucumber, and pea (4). It has not been previously reported naturally infecting onions in field or storage.

The symptoms of this disease are remarkably similar to those of slippery skin caused by *P. alliicola* described in the USA by Stewart (23) and Vitanov in Bulgaria (24). It was assumed that *P. alliicola* infects mature bulbs at harvest (2) although no reports of that have been published.

Infection of onions by *P. cepacia* is similar to that described above for *P. aeruginosa*. Kawamoto and Lorbeer (13) reported that only one or a few of the inner leaf bases of infected onions are decayed whereas the outer fleshy bases are sound. The disease frequently occurs in the field before harvest and *P. cepacia* is capable of infecting, and multiplying within, wounded onion leaves under high-moisture conditions (14). The association of soft rotting of green leaves and bacterial bulb decay in the field is attributed to early infections by this bacterium.

The failure of *P. aeruginosa* to infect leaves and topped bulbs under laboratory conditions, may be due to inability of the bacterium to infect cells except under extremely specific conditions. The possibility that *P. aeruginosa* is not an homologous or compatible pathogen in onion seems likely from our results. Leaf tissue maintained in a water-soaked condition allows reproduction of nonpathogenic bacteria in plant tissues; otherwise the growth of nonpathogens may be restricted by low nutrient concentration and limited free water (25). Water-soaking may cause cell anoxia, which would be associated with subsequent increase in permeability of plant-cell membranes to the nutrients and water required for bacterial growth, or it may dilute preformed or induced inhibitors in the tissue.

Although no definite conclusions on the mode of infection can be drawn from these results, the possible source of inoculum and ease of infection can be explained by prevailing cultural practices. In seasons of higher-than-average summer rainfall and lower-than-average temperatures (e.g., in 1973-74), considerable weed growth occurs within the onion rows. Bulbs do not dry readily after rain, irrigation or heavy dew and the moist environment around the bulb would be conducive to the maintenance and increase of bacterial populations. Onions are grown on sandy soils with little or no rotation or fallow periods between crops. Residues from the previous crop still may be present at the next sowing. On these soils, crops are irrigated by overhead sprinkler systems or self-propelled travelling irrigators. The splash impact from the high-velocity, high-volume drops produced by this latter type of equipment projects soil and particulate organic matter onto the bulbs and leaves with possible abrasion of the fleshy tissue, and could cause water-congested areas in the neck region and leaves of the onions. Bacteria in the soil thus may be conveyed onto the wet plant and infection then could occur through water-soaked and/or wounded tissue. *P. alliicola* and *P. cepacia* have been isolated from onion field soil and surface drainage water both of which were implicated as inoculum sources for those pathogens when they caused increasing losses of bulbs in both field and storage (10, 12). Green et al. (7) showed that *P. aeruginosa* was fairly common in agricultural soils and could multiply and persist within plant tissue. Recently Cho et al. (3) reported isolating this bacterium from potted ornamental plants. Using acetamide broth (9) and centrimide agar (7) we were unable to isolate this bacterium from an onion field in which diseased bulbs had been detected 16 months previously. This may indicate the absence of *P. aeruginosa* in the soil, but the failure was more likely a result of mid-winter sampling or insufficient samples being taken.

The difference in lesion occurrence in inner and outer inoculated leaf bases suggests that some factor in the outer bulb tissue prevents lesion development even from bacterial concentrations as high as 10^6 cells/ml at the inoculation point. A somewhat similar situation must exist throughout the leaf bases of an immature onion. The variable growth on the ethanol extracts of leaf bases also suggests the presence of compounds that may be inhibitory to bacterial growth in the outer leaf bases. These data are consistent with the disease symptoms wherein the lesions are limited to one or two leaf bases near the bulb center. Microscopic observations of inoculated leaf bases indicate that the bacterium is unable to break down the constituents of the cuticle and thus is limited to the leaf bases it initially infects.

Pseudomonas aeruginosa generally is regarded as a plant saprophyte and the few reports of its pathogenicity have not been accompanied by a detailed description of the bacterium involved nor have Koch's postulates been

satisfied. Friedman (6) argued that "until such time as *P. aeruginosa* is definitely isolated from naturally diseased plants and Koch's postulates of pathogenicity fulfilled, the role of *P. aeruginosa* as a plant pathogen is doubtful".

The pathogenicity of *P. aeruginosa* to plants has been demonstrated (3, 7) and the bacterium is considered to be a "quasi-pathogen" (3) with a pathogenic potential in certain environments. From these reports and the data presented above, *P. aeruginosa* may be considered a successful plant pathogen under limited specialized conditions.

LITERATURE CITED

1. BRADBURY, J. F. 1970. Isolation and preliminary study of bacteria from plants. Rev. Plant Pathol. 49:213-218.
2. BURKHOLDER, W. H. 1942. Three bacterial plant pathogens: Phytomonas caryophylli sp. n., Phytomonas alliicola sp. n., and Phytomonas manihotis (Arthaud-Berthet et Bondar) Viegas. Phytopathology 32:141-149.
3. CHO, J. J., M. N. SCHROTH, S. D. KOMINOS, and S. K. GREEN. 1975. Ornamental plants as carriers of Pseudomonas aeruginosa. Phytopathology 65:425-431.
4. COMMONWEALTH MYCOLOGICAL INSTITUTE. 1968. Review of Applied Mycology: Plant Host-Pathogen Index to volumes 1-40 (1922-1961).
5. DUBOIS, M., K. A. GILLES, J. K. HAMILTON, P. K. REVERS, and F. SMITH. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
6. FRIEDMAN, B. A. 1960. Status of synonymy and plant pathogenicity of Pseudomonas marginalis and P. aeruginosa. Int. Bull. Bacteriol. Nomencl. Taxon. 10:197-204.
7. GREEN, S. K., M. N. SCHROTH, J. J. CHO, S. D. KOMINOS, and V. B. VITANZA-JACK. 1974. Agricultural plants and soil as a reservoir for Pseudomonas aeruginosa. Appl. Microbiol. 28:987-991.
8. GURR, E. 1965. The rational use of dyes in biology and general staining methods. Leonard Hill. London. 422 p.
9. HEDBURG, M. 1969. Acetamide agar medium selective for Pseudomonas aeruginosa. Appl. Microbiol. 17:481.
10. IRWIN, R. D., and E. K. VAUGHAN. 1972. Bacterial rot of onion and the relation of irrigation water to disease incidence. Phytopathology 62:1103 (Abstr.).
11. JESSEN, O. 1965. Pseudomonas aeruginosa and other green fluorescent pseudomonads. A taxonomic study. Munksgaard. Copenhagen. 244 p.
12. KAWAMOTO, S. O., and J. W. LORBEER. 1964. Selective isolation of soft-rot bacteria of onion. Phytopathology 54:897 (Abstr.).
13. KAWAMOTO, S. O., and J. W. LORBEER. 1972. Multiplication of Pseudomonas cepacia in onion leaves. Phytopathology 62:1263-1265.
14. KAWAMOTO, S. O., and J. W. LORBEER. 1974. Infection of onion leaves by Pseudomonas cepacia. Phytopathology 64:1440-1445.
15. LAUTROP, H., and O. JESSEN. 1964. On the distinction between polar monotrichous and lophotrichous flagellation in green fluorescent pseudomonads. Acta Pathol. Microbiol. Scand. 60:588-598.
16. LELLIOT, R. A., E. BILLING, and A. C. HAYWARD. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bact. 29:470-489.
17. MISAGHI, I., and R. G. GROGAN. 1969. Nutritional and biochemical comparisons of plant pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
18. PALLERONI, N. J., and M. DOUDOROFF. 1972. Some properties and taxonomic subdivisions of the genus Pseudomonas. Annu. Rev. Phytopathol. 10:73-100.
19. SANDS, D. C., M. N. SCHROTH, and D. C. HILDEBRAND. 1970. Taxonomy of phytopathogenic pseudomonads. J. Bact. 101:9-23.
20. SCHROTH, M. N., and D. C. HILDEBRAND. 1971. Current taxonomic thinking on the genus Pseudomonas, with emphasis on the plant pathogens. Proc. 3rd Int. Conf. Plant Pathogenic Bacteria, Wageningen, The Netherlands. 365 p.
21. SHINDE, P. A., and F. L. LUKEZIC. 1974. Isolation, pathogenicity and characterization of fluorescent pseudomonads associated with discolored alfalfa roots. Phytopathology 64:865-871.
22. STANIER, R. Y., N. J. PALLERONI, and M. DOUDOROFF. 1966. The aerobic pseudomonads:a taxonomic study. J. Gen. Microbiol. 43:159-271.
23. STEWART, F. C. 1899. A bacterial rot of onion. N. Y. Agric. Exp. Stn. Bull (Geneva) 164:209-212.
24. VITANOV, M. 1970. Slippery skin of onion caused by Pseudomonas alliicola Burkholder. Gradinar lozar Nauka. 7:83-90.
25. YOUNG, J. M. 1974. Effect of water on bacterial mutliplication in plant tissue. N. Z. J. Agric. Res. 17:115-119.