

Etiology

Bacterial Blight Incited in Parsnip by *Pseudomonas marginalis* and *Pseudomonas viridiflava*

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ABSTRACT

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Pseudomonas marginalis and *P. viridiflava* were isolated from soft rot lesions on parsnip petioles collected from a field in eastern New York in 1978 and 1979. In sections of the field, the disease was so severe that the tops of many plants were destroyed, making mechanical harvesting of the roots impossible. In 1979, *P. marginalis* was recovered from diseased petioles collected from two locations in Massachusetts. It was the only pathogen recovered from naturally infected roots in any location. Both species caused

soft rotting of inoculated petioles, but only *P. marginalis* moved into the roots and caused a hard brown rot. *P. viridiflava*, however, was recovered from symptomless roots of plants that had been inoculated at the crown. Only *P. viridiflava* was recovered from two lots of seeds and these isolates were pathogenic to parsnip petioles. The validity of *P. pastinacae*, a species described from parsnip in New York in 1960, is questioned.

In 1960 Burkholder reported a severe root rot of parsnip (*Pastinaca sativa* L.) caused on Long Island, NY, by a bacterium designated as a new species, *Pseudomonas pastinacae* Burkholder 1960 (3). The rot was first noticed in the spring when the roots were dug after overwintering in the soil. There had been no report of disease on the crop the previous fall. Following this

initial report, the disease has not been serious enough to have been brought to the attention of the extension vegetable pathologist in New York (A. F. Sherf, *personal communication*), nor has it been reported elsewhere.

In the fall of 1977 and 1978, parsnip plants with diseased foliage and roots were brought to our laboratory from a field in Schoharie, NY. The disease occurred again in this field in 1979 and it was also found in Westfield and Andover, MA. Symptoms on the tops of the plants were first noticed in September and they continued to worsen until the plants were harvested. In November 1979, every plant examined in the New York field was diseased. Root symptoms were noticed in the field in some cases, whereas in other

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instances they did not develop until after storage at 4 C. Symptoms consisting of soft, dark, sunken lesions occurred on the petioles, often just above the crown. Frequently the infected area was so extensive that the entire leaf collapsed (Fig. 1A). Discrete circular, brown lesions on the leaf blades also were noted. In some cases, the pathogen appeared to spread through the crown into the root where it caused a firm rot and browning of the vascular tissue (Fig. 1B). In sections of the field where foliage blight was severe, the leaves often were not sufficiently intact for the mechanical harvester to pull the roots out of the ground.

The extensive foliage blight and the absence of this in the report by Burkholder (3) prompted us to investigate the etiology of this disease.

MATERIALS AND METHODS

Bacteria were isolated from lesions on the foliage and roots following thorough washing and, in some cases, surface sterilization in 10% Clorox (0.52% sodium hypochlorite). Small pieces of tissue from margins of lesions were placed in sterile distilled water and crushed with a sterile glass rod. After soaking for 1 hr, the liquid was streaked onto plates of King's B (KB) medium (16). Individual colonies that fluoresced under ultraviolet light were streaked on the same medium to obtain pure cultures.

Pathogenicity tests were made initially by injecting parsnip petioles with a suspension of bacteria or by pricking the petiole with a needle carrying the bacteria. Inoculated plants were held in a plastic bag for ~48 hr, and then removed from the bags and held for four more days before disease incidence was recorded. In later tests, plants were inoculated by inserting a toothpick laden with bacteria into the petiole. The plants were then covered with a plastic bag and incubated for ~1 wk in a growth chamber at 16 C and 12 hr of fluorescent light daily. To determine if the bacteria could move from the petioles into the roots and cause disease symptoms, 2-, 5-, 7-, and 10-mo-old plants were inoculated by inserting a bacteria-infested toothpick into the petiole or by smearing bacteria on the crown where petioles had been pulled off. At 6 wk and 4 mo after inoculation, the roots were examined for vascular browning. Isolations were made from both healthy and diseased roots.

In preliminary studies, isolates that were fluorescent on KB

medium were screened for ability to rot potato slices and induce a hypersensitive reaction in tobacco leaves (17). Those that were positive for either test were checked for pathogenicity on parsnip petioles. In later studies, fluorescent isolates that were recovered were screened for pectolytic enzyme activity by using the procedure of Hildebrand (13). Isolates that were pathogenic or exhibited pectolytic enzyme activity at pH 8.5, were identified according to the determinative scheme of Hildebrand and Schroth (14) and also the scheme of Lelliott et al (18) for plant-pathogenic pseudomonads. Cultures used for these physiological tests were grown for 2 days at 28 C on KB medium. The method of Stanier et al (23) was used for the oxidase test; the presence of arginine dihydrolase was determined by the method of Thornley (24); nutritional tests were conducted as described by Lukezic (19); production of 2-ketogluconate was determined using Haynes' method (12); and the method of Lelliott et al (18) was used to check for reduction of nitrate. Formation of β -glucosidase was determined using a medium containing 5 g arbutin, 10 g peptone, 3 g yeast extract, 1 g D-glucose, 0.5 g ferric citrate, and 12 g agar in 1 L of water adjusted to pH 7 before autoclaving. Each plate was spot-inoculated at two distant points and incubated for up to 10 days at 25 C. Browning of the medium indicated β -glucosidase activity. Rotting of carrot slices was tested because of the importance of this criterion in Burkholder's description of *P. pastinacae*.

The optimum temperature for growth of five isolates each of *P. marginalis* (Brown 1918) Stevens 1925 and *P. viridiflava* (Burkholder 1930) Dowson 1939 was determined by inoculating tubes containing 10 ml of nutrient broth with 0.5 ml of a 48-hr culture. Prior to inoculation, the culture was adjusted to 70% transmittance at 620 nm on a Bausch and Lomb Model 20 spectrophotometer. Tubes were incubated at 22, 24, 26, 28, and 30 C and the amount of growth was determined spectrophotometrically after 28 and 44 hr. Growth at low temperature was determined by streaking plates of KB medium with a dilute suspension of bacteria and incubating them at 2, 4, and 6 C, and in a cold room in which the temperature ranged from -1 to 2 C. These were observed for growth after 1 and 2 wk.

One seed lot used in Massachusetts, where the disease occurred in 1979, and a seed lot produced for the 1980 planting were assayed

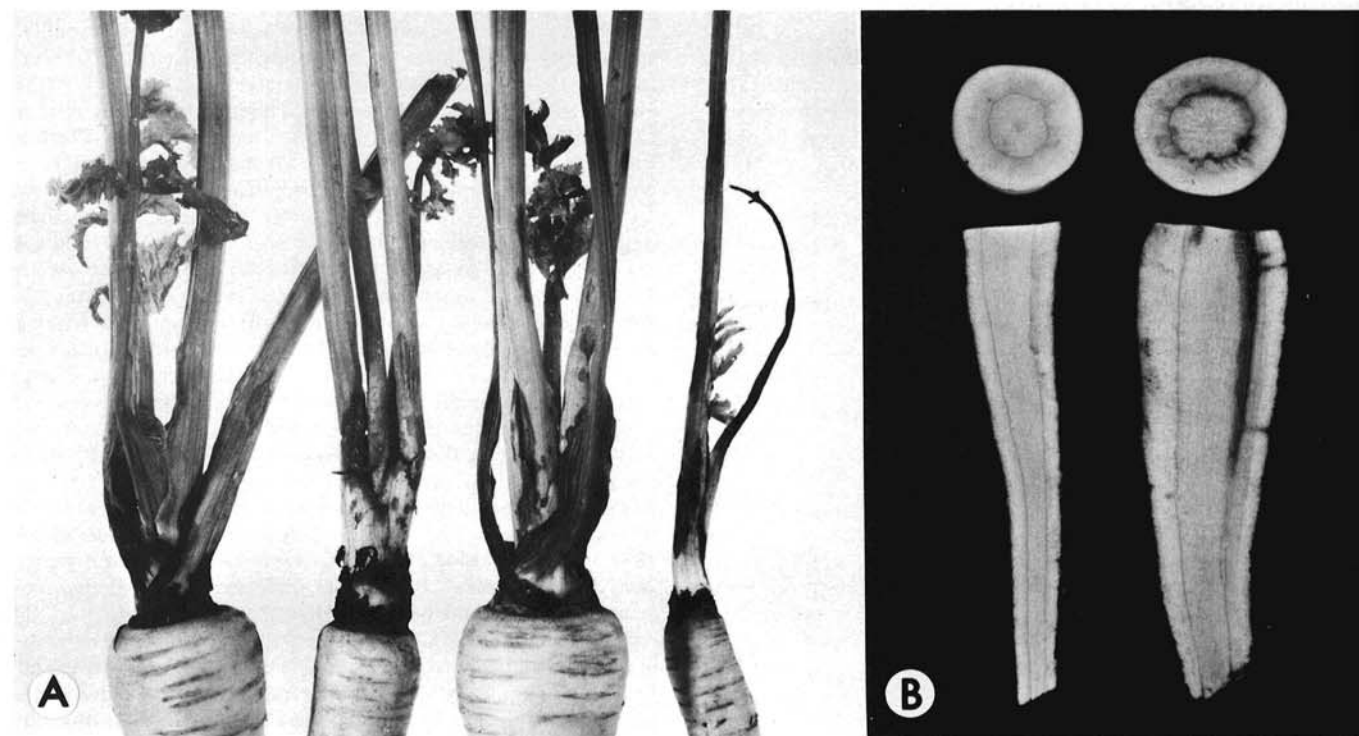


Fig. 1. Symptoms of bacterial blight of parsnip. A, Soft, dark, sunken lesions with complete rotting and collapse of some petioles; may be caused by either *Pseudomonas marginalis* or *P. viridiflava*. B, Left: healthy root. Right: firm rot of roots, caused by *P. marginalis* and characterized by browning of the vascular tissue beginning at the crown and extending downward.

for *P. marginalis* and *P. viridiflava*. For each lot four samples of 5 g each were placed in flasks with 100 ml of sterile tap water and shaken for 6 hr at room temperature. Five serial 1:5 dilutions were plated on KB medium and incubated at 28 C. Fluorescent colonies were tested for pectolytic enzyme activity following the method described previously. Those that caused pitting of the medium were identified to species and checked for pathogenicity by inoculating parsnip petioles.

RESULTS

Both pathogenic and nonpathogenic fluorescent pseudomonads were isolated from diseased plants. The pathogens were identified as *P. marginalis* and *P. viridiflava* (Table 1), according to the determinative schemes of Hildebrand and Schroth (14), and Lelliott et al (18). A variable hypersensitivity was obtained in tobacco with *P. marginalis*, whereas *P. viridiflava* consistently induced a positive reaction. Both species produced an abundance of pectolytic enzymes, which was evidenced by extensive pitting of sodium polypectate medium, and both caused rotting of potato and carrot slices. The optimum temperature for growth of both species was 28 C. All isolates showed abundant growth after incubation for 2 wk at -1 and 2 C.

P. marginalis and *P. viridiflava* were both recovered from diseased petioles collected in New York in 1978 and 1979. Only *P. marginalis*, however, was isolated from roots of these plants. In addition, it was the only pathogen recovered from either leaves or roots of samples sent to us from Massachusetts in 1979. On four occasions both species were recovered from a single petiole lesion on a plant collected in New York. In two instances roots that had been considered healthy, packaged for sale, and put in cold storage were examined for disease symptoms. One group was removed from cold storage in March and held at room temperature for 1 wk after which time dark-brown, firm rot symptoms were evident. The other group showed symptoms after storage for 3 mo at 4 C. *P. marginalis* was recovered from roots of both groups.

No obvious difference in symptoms was noted in petioles naturally infected with either species. Also, artificial inoculation of petioles with both species resulted in the production of lesions that were indistinguishable.

Isolations made from the roots of parsnip plants inoculated in the petiole with either *P. marginalis* or *P. viridiflava* yielded only *P. marginalis*. However, both species were recovered from the roots of plants that had been inoculated by smearing inoculum on the crown where the petioles had been broken off. Symptoms in the roots were evident only in plants inoculated with *P. marginalis*; the organism could be recovered before symptoms developed. Neither species was recovered from uninoculated check plants or plants inoculated with the other species.

P. viridiflava, but not *P. marginalis*, was recovered from both

seed lots tested. Parsnip petioles inoculated with these isolates produced characteristic soft rot symptoms.

DISCUSSION

Both *P. marginalis* and *P. viridiflava* caused soft rotting and collapse of parsnip petioles, but only *P. marginalis* was found to move down into the roots and cause a rot. *P. marginalis* has been reported to be primarily a soft-rotting organism that attacks storage tissues, although it can invade soft leaf and stem tissue (18,20). For many years it was known to cause only a disease of lettuce leaves (2), but more recently it has been reported to infect endive, chicory, dahlia, Bok Choy, rhubarb, pea, Chinese cabbage, and tobacco (4-6,9-11,15,21). A similar organism has been recovered also from discolored alfalfa roots (22). Misaghi and Grogan (20) stated that it seems unlikely that this organism would cause severe outbreaks under normal conditions. The disease has been very serious over a 3-yr period in a parsnip field in New York, and we have been told that the disease is very severe in the two fields in Massachusetts where specimens were collected for us.

It is not known what is unique about the field in New York where the disease has been severe. For several years it has been used to grow parsnips, carrots, peas, and spinach, but generally parsnips have not been grown on the same section of the field for more than two consecutive years. The field is located on a flood plain next to a river that frequently overflows in the spring, and which is used for irrigation water. The disease has been most severe in low areas of the field near the river. The same grower has two fields a few miles away where the disease has not occurred. Only a very small section of one of these fields lies within the flood plain and it has been planted to corn until recently. The other field had never been used to grow parsnips before and it was irrigated from a different river. The lack of a long rotation may have contributed to a buildup of inoculum in the flood plain field. Muntañola (21) reported that in Argentina a foliar disease of lettuce, endive, and chicory caused by *P. marginalis* can be controlled by cultural practices; viz, wide row spacing, minimal use of irrigation water, and annual crop rotation. The latter is clearly something that should be tried in fields with a history of this disease.

Studies were not conducted to determine the source of either pathogen, except for the seed assays where *P. viridiflava* was recovered. The significance of this is uncertain because this organism has an extremely wide host range and it is probably an epiphyte on many plants (1,25). Cuppels and Kelman (7) recently reported that *P. marginalis* can overwinter in field soil in Wisconsin and Connecticut, and they also recovered it from lake mud and river water. Similar observations were made earlier by Clark and Paton (6) who found *P. marginalis* on unaffected plants, in greenhouse soil, and in large numbers in a greenhouse water tank. Later, Clark and Graham (5) reported that pond water used for irrigation of rhubarb contained *P. marginalis*, which caused a soft rot of petioles similar to that which we observed on parsnip. Transmission of inoculum apparently could have occurred by splashing of infested soil onto petioles (8). It is not known how the organism would have gained entry into the parsnip petioles, but on one occasion the plants were heavily infested with aphids. Possibly they provided wounds through which the bacterial cells entered.

It has been suggested that *P. marginalis* may be synonymous with *P. pastinacae*, the species described in 1960 by Burkholder as the cause of a rot of parsnip roots on Long Island, NY (18). Burkholder considered but rejected this possibility for the following reasons. The optimum temperature for *P. pastinacae* is 18 C, whereas that for *P. marginalis* is considered to be higher; eg, 28 C in our studies. Burkholder understood that *P. marginalis* utilizes tartrates and he found that *P. pastinacae* did not. This reason for separating the two species may no longer be valid as *P. marginalis* is now known to be unable to utilize L(+)tartrate and to be variable for utilization of D(+)tartrate (14). Burkholder also was persuaded to name a new species because parsnip roots inoculated with *P. marginalis* did not become infected; however, our isolates did infect parsnip roots. The only other basis for separation is that Burkholder reported that *P. pastinacae* did not rot carrot slices,

TABLE 1. Reaction to differential tests of isolates of *Pseudomonas marginalis* and *P. viridiflava* recovered from parsnip plants

Tests	<i>P. marginalis</i> (26 isolates)	<i>P. viridiflava</i> (35 isolates)
Oxidase	26 ^a	0
Arginine dihydrolase	26	0
Balanine	26	0
Trehalose	26	1
Yellow fluorescent pigment	26	35
Mannitol	26	35
Sorbitol	26	35
Erythritol	25	35
L(+)Tartrate	1	0
D(-)Tartrate	13	35
DL-Lactate	26	35
Sucrose	26	0
Sodium polypectate, pH 5	0	0
Sodium polypectate, pH 8.5	26	35
β -Glucosidase formation	25	35

^aNumber of isolates positive for test.

whereas our isolates of *P. marginalis* from parsnip caused an extensive soft rot of carrot.

Burkholder had no opportunity to observe the plants for foliar symptoms in the field. He did observe, however, that in many instances the disease symptoms appeared to have progressed down from the crown. We demonstrated experimentally that this can occur with *P. marginalis*. He felt that the low optimum temperature of 18 C indicated that the disease may have developed during the cool months when the roots were in the soil. With our isolates of *P. marginalis*, which have an optimum temperature of 28 C but grow at a temperature between -1 and 2 C, it is likely that infection occurs on petioles during the summer or fall and progresses into the roots, where the organism continues to grow at cool temperatures.

P. marginalis pv. *pastinacae* (Burkholder 1960) Young, Dye & Wilkie 1978 is now the correct name for the organism Burkholder recovered from parsnips (26). However, Lelliott et al (18) suggested that *P. marginalis* and *P. pastinacae* are possibly synonyms because no differences were found when isolates of both species were compared in 12 physiological tests. Our results also suggest that *P. pastinacae* is synonymous with *P. marginalis*. However, before a firm proposal can be made concerning the synonymy of these two nomenclatures, Burkholder's original culture should be compared with authentic cultures of *P. marginalis* pv. *marginalis* for reaction on host plants and in a complete series of cultural and biochemical tests.

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