Bacterial Blight of Peas in New York State

J. E. HUNTER and J. A. CIGNA, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456

ABSTRACT

Hunter, J. E., and Cigna, J. A. 1981. Bacterial blight of peas in New York State. Plant Disease 65: 612-613.

Bacterial blight of peas was found in New York State in 1979 after not being observed there for more than 25 years. The disease was not observed in 1980. The pathogen was identified as *Pseudomonas syringae* pv. *pisi* rather than *P. syringae* pv. *syringae*; both induce the same symptoms in the field. The disease was associated only with plants grown from one seed lot. The pathogen was not recovered from seeds assayed in the laboratory.

In the spring of 1979, diseased pea plants (*Pisum sativum* L.) suspected of being infected with *Pseudomonas syringae* pv. *pisi* (Sackett) Young, Dye, and Wilkie (PSP) were brought to our laboratory. The disease had not been a problem in New York since growers started using western-grown seed more than 25 years ago.

The disease, commonly referred to as bacterial blight, and the pathogen were first found in Colorado in 1916 (16). By 1928, more than 18% of the pea fields inspected in 15 states were infected (14). More recently, the disease has been found in widely scattered areas of the world (1,3,4,6,19,20,24). It has been known since 1927 (17) that the pathogen is seedborne, and this may account for its wide dissemination.

Diseased plants brought to our laboratory came from three fields in central New York State that had been planted with seeds from one seed lot. Many plants in these fields were infected. Symptoms on the pods, stems, and leaves of the diseased plants closely resembled those reported for bacterial blight caused by PSP (16,19).

MATERIALS AND METHODS

Isolations were made from water-soaked lesions on pods, stems, and leaves of several infected plants. Diseased plant parts were washed thoroughly, and small pieces of advancing margins of lesions were placed in sterile distilled water and crushed with a sterile rod. After 1 hr, the liquid was streaked onto plates of King's B (KB) medium (13). After 48-hr incubation at 28 C, fluorescent colonies were restreaked twice on KB medium to obtain pure cultures. Isolates were checked for oxidase activity by the method of Stanier et al (18) to screen for potential pathogens.

Pathogenicity tests were made on 4- to 5-wk-old plants of the cultivar Spring. Plants were placed in plastic bags overnight before inoculation. As each plant was removed, the leaves, stems, and pods were inoculated with a water suspension of bacteria (10^6–10^7 cells per milliliter) applied with a hand-operated DeVilbiss atomizer (DeVilbiss Co., Somerset, PA 15501). After inoculation, the plants were placed on a greenhouse bench and observed daily for disease development.

The scheme of Hildebrand and Schroth (11) was followed to identify pathogenic isolates. Bacteria used for these tests were grown for 2 days at 28 C on KB medium.

The presence of arginine dihydrolase was determined by Thornley's method (23); nutritional tests were conducted as described by Lukezic (15); pectolytic enzyme activity was determined at pH ranges 4.9–5.1 and 8.3–8.5 by Hildebrand's procedure (9). Formation of β-glucosidase was determined by using a medium containing 5 g of arbutin, 10 g of peptone, 3 g of yeast extract, 1 g of D-glucose, 0.5 g of ferric citrate, and 12 g of agar in 1 L of water adjusted to pH 7 before autoclaving. Each plate was spot inoculated on opposite sides of the plate and incubated for as long as 10 days at 25 C. Browning of the medium indicated β-glucosidase activity. To distinguish PSP from *P. syringae* pv. *syringae*, both of which

Accepted for publication 19 May 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/81/07061202/$03.00/0

produce the same symptoms on peas (12), cultures were grown on the minimal medium of Lukezic (15) with 0.2% DL homoserine (Sigma Chemical Co., St. Louis, MO 63178) added as the sole carbon source (10).

Three procedures were used in attempts to recover the pathogen from the seed lot associated with the disease. In the first procedure, approximately 5,000 seeds were tested by Taylor and Hewett's method (22) for detecting low levels of bean seed infected with *P. phaseolicola*. Two-hundred-gram lots (about 950 seeds) were added to 700 ml of sterile tap water and ground in a large Waring Blendor until the seeds were the consistency of flour. The mixture was incubated at room temperature for 1 hr and then filtered through four layers of cheesecloth. Five serial 1:5 dilutions were streaked onto KB medium.

In the second procedure, 25,000 seeds were tested using the "Dome test" developed by J. R. Venette (*personal communication*). Seeds were soaked in water for 1 day to increase any bacteria present, and then this water was vacuum-infiltrated into other seeds that had imbibed water for 24 hr. The infiltrated seeds were subsequently planted in sterile vermiculite in a moist chamber. Using KB medium, isolations were made from water-soaked lesions.

The third procedure consisted of soaking 4.5 kg of seeds in water, and an equal amount of seeds in an enrichment broth similar to the selective media used by Burr and Hurwitz (2) for isolating *P. syringae*. An aquarium pump was used to aerate both liquids. Aliquots were removed after 0, 6, 12, 24, 48, 72, and 96 hr and streaked onto KB medium and also used to inoculate plants using the procedure previously described for pathogenicity tests.

For all three procedures, fluorescent colonies that developed on KB medium were tested for oxidase activity. Those that were negative or questionable for oxidase were transferred to plates containing homoserine. Any isolates that were negative or questionable on homoserine were checked for pathogenicity and for their reaction on the differential media used by Hildebrand and Schroth to identify *Pseudomonas* spp. (11).

Fields throughout the main pea-growing area of central New York were observed periodically for recurrence of the disease in 1980. Plants with symptoms that could have been due to bacteria were checked in the laboratory by the diagnostic procedures used in 1979.

RESULTS

Nearly all isolations from diseased pods, stems, and leaves collected in 1979 yielded pure cultures of a blue-white-fluorescent pseudomonad. Eleven of these isolates, which were oxidase negative, were used to inoculate plants. All of them induced symptoms characteristic of bacterial blight of peas.

All 11 isolates reacted identically in tests used to determine the species of the pathogen. The following test results indicate that the pathogen was PSP: oxidase (−), arginine dihydrolase (−), β-alanine (−), trehalose (−), mannitol (+), sorbitol (+), erythritol (−), L-tartrate (−), D-tartrate (−), DL-lactate (−), sucrose (+), DL-homoserine (+), sodium polypectate pH 5 (−) and pH 8.5 (−), and β-glucosidase (−). The only discrepancy between our results and those of Hildebrand and Schroth (11) for PSP is that our isolates did not utilize DL-lactate. This may not be significant, however, since Hildebrand and Schroth found only a few colonies of PSP on plates streaked with a large amount of inoculum.

Fluorescent pseudomonads were recovered frequently from seeds. Some isolates produced a questionable oxidase-negative reaction. These were checked for their reaction on the diagnostic media of Hildebrand and Schroth (11) and for their ability to utilize homoserine. None of the isolates was PSP.

We found no evidence from our field and laboratory studies to indicate that the disease recurred in 1980.

DISCUSSION

By calling attention to this unusual outbreak of bacterial blight of peas in New York State, we hope to increase awareness that this disease can recur anytime and to focus attention on the need to strive continuously to produce pathogen-free seeds. Fortunately, the disease is not often severe (8), even in fields planted with infected seeds. This may be because the pathogen does not move systemically through the vascular system as does *P. phaseolicola*, the pathogen inciting halo blight of beans (5). Continuous cool, wet weather is considered essential for the disease to continue developing in infected plants. For this reason, field inspection of the crop may not reveal the disease even though the plant is infected (24).

The pathogen can be carried on the seed surface as well as inside the seed (17). Surface contamination might occur during seed harvest as reported for *P. phaseolicola* in beans (7). Taylor and Dye (21) used a sensitive method to detect PSP and found that one infected seed per 10,000 in commercial seed lots would be a high incidence. This may explain our failure to detect the bacterium in seeds. We also may have failed because the organism did not survive the interval between planting and 6–20 mo later when we tried different procedures to recover it from seeds.

LITERATURE CITED

1. Boelema, B. H. 1972. Bacterial blight (*Pseudomonas pisi* Sackett) of peas in South Africa with special reference to frost as a predisposing factor. Meded. Landbouwhogesch. Wageningen. 72:1-87.
2. Burr, T. J., and Hurwitz, B. 1980. A selective medium for the isolation of *Pseudomonas syringae*. (Abstr.) Phytopathology 70:460.
3. Chakravarti, B. P., and Hegde, S. V. 1972. Bacterial blight of pea. FAO Plant Prot. Bull. 20:21-22.
4. Cirulli, M., and Ercolani, G. L. 1969. Identificazone di *Pseudomonas pisi* Sackett in Italia. Phytopathol. Mediterr. 8:234-237.
5. Dowson, W. J. 1957. *Pseudomonas pisi*. Page 121 in: Plant Diseases Due to Bacteria. Cambridge University Press, London. 231 pp.
6. Gorlenko, M. V. 1961. Bacterial Diseases of Plants. (Translated from Russian) M. Monson, publisher, Jerusalem. 174 pp.
7. Grogan, R. G., and Kimble, K. A. 1967. The role of seed contamination in the transmission of *Pseudomonas phaseolicola* in *Phaseolus vulgaris*. Phytopathology 57:28-31.
8. Hagedorn, D. J., and Patel, P. N. 1965. Halo blight and bacterial brown spot of bean in Wisconsin in 1964. Plant Dis. Rep. 49:591-595.
9. Hildebrand, D. C. 1971. Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial pathogens. Phytopathology 61:1430-1436.
10. Hildebrand, D. C. 1973. Tolerance of homoserine by *Pseudomonas pisi* and implications of homoserine in plant resistance. Phytopathology 63:301-302.
11. Hildebrand, D. C., and Schroth, M. N. 1972. Identification of the fluorescent pseudomonads. Pages 281-287 in: H. P. Maas Geesteranus, ed. Proc. Third Int. Conf. Plant Pathogenic Bacteria. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands. 365 pp.
12. Hoitink, H. A. J., Hagedorn, D. J., and McCoy, E. 1968. Survival, transmission, and taxonomy of *Pseudomonas syringae* van Hall, the causal organism of bacterial brown spot of bean (*Phaseolus vulgaris* L.). Can. J. Microbiol. 14:437-441.
13. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
14. Linford, M. B. 1929. Pea diseases in the United States in 1928. Plant Dis. Rep. Suppl. 67. 14 pp.
15. Lukezic, F. L. 1979. *Pseudomonas corrugata*, a pathogen of tomato, isolated from symptomless alfalfa roots. Phytopathology 69:27-31.
16. Sackett, W. G. 1916. A bacterial stem blight of field and garden peas. Colo. Agric. Exp. Stn. Bull. 218. 43 pp.
17. Skoric, V. 1927. Bacterial blight of pea: Overwintering, dissemination, and pathological histology. Phytopathology 17:611-627.
18. Stanier, R. Y., Palleroni, N. J., and Douderoff, J. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:159-211.
19. Stapp, C. 1961. *Pseudomonas pisi*. Pages 204-208 in: Bacterial Plant Pathogens. Oxford University Press, London. 292 pp.
20. Taylor, J. D., and Dudley, C. L. 1975. Bacterial blight of peas. Page 105 in: 26th Annu. Rep. 1975. Natl. Veg. Res. Stn., Wellesbourne, England. 158 pp.
21. Taylor, J. D., and Dye, D. W. 1976. Evaluation of streptomycin seed treatments for the control of bacterial blight of peas (*Pseudomonas pisi* Sackett 1916). N.Z. J. Agric. Res. 19:91-95.
22. Taylor, J. D., and Hewett, P. D. 1978. The detection of seed-borne bacteria. Pages 28-29 in: Rep. 16th Int. Workshop on Seed Pathology, Int. Seed Testing Assoc., Karlsruhe-Durbach, German Federal Republic. 63 pp.
23. Thornley, M. J. 1960. The differentiation of *Pseudomonas* from other gram negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol 23:37-52.
24. Young, J. M., and Dye, D. W. 1970. Bacterial blight of peas caused by *Pseudomonas pisi* Sackett, 1916 in New Zealand. N.Z. J. Agric. Res. 13:315-324.