Resistance and Seed Infection in Three Dry Bean Cultivars Exposed to a Halo Blight Epidemic

M. J. KATHERMAN, R. E. WILKINSON, and S. V. BEER, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

KATHERMAN, M. J., R. E. WILKINSON, and S. V. BEER. 1980. Resistance and seed infection in three dry bean cultivars exposed to a halo blight epidemic. Plant Disease 64:857-859.

Seeds from plants of the dry bean cultivars California Light Red Kidney, Redkloud, and Redkote, which had been exposed to a natural epidemic of halo blight, were harvested and assayed for the presence of *Pseudomonas phaseolicola*. Seed of the more susceptible cultivar California Light Red Kidney was infected and infested with the pathogen. Redkloud seed was only infested, and no *P. phaseolicola* was recovered from Redkote seed. These data suggest that a direct relationship exists between the susceptibility of a cultivar to halo blight and the likelihood of seed contamination by *P. phaseolicola*.

The major source of primary inoculum of the halo blight disease of *Phaseolus vulgaris* L. is seed contaminated by *Pseudomonas phaseolicola* (17). Although other sources of inoculum have been identified, their significance in the epidemiology of halo blight is not known. For example, Schuster (12) demonstrated that *P. phaseolicola* could survive in infested crop debris on the soil surface, but whether sufficient inoculum survives in debris to provide inoculum under natural conditions is not known. Epiphytic populations of *Xanthomonas phaseoli* var. *fuscans*, another bacterial pathogen of bean, have been found on healthy foliage of host and nonhost plants, and similar populations of *P. phaseolicola* may survive epiphytically (16).

Seed produced under semiarid conditions in the western United States is generally planted to reduce the amount of inoculum introduced into bean fields. The fear of increasing the level of the primary inoculum by using eastern-grown seed is based on the supposition that even resistant cultivars grown in the eastern United States may produce contaminated seed. However, recent studies (4) demonstrated a relationship between the susceptibility of cultivars and their potential to serve as sources of *P. phaseolicola* inoculum. Nevertheless, it is not known whether seed of a resistant cultivar would become sufficiently contaminated with *P. phaseolicola* when grown in the presence of the disease to serve later as a source of inoculum.

In this study, the level of seed infection and infestation was determined for the cultivars California Light Red Kidney (CLR Kidney), Redkloud, and Redkote when parent plants were exposed to a halo blight epidemic in the field. Whether seed contamination resulted from surface infestation or internal infection was also determined so that the efficacy of seed treatments to eradicate surface-borne bacterial pathogens (110,15) could be determined.

Present address of first author: CIAT, Apartado Aereo 6713, Cali, Colombia.

0191-2917/80/09085703/$03.00/0

MATERIALS AND METHODS

Seed source. Plants of CLR Kidney, Redkloud, and Redkote were harvested at maturity from a field of dry bean breeding material in Aurora, NY, where a severe epidemic of halo blight had occurred due to secondary spread from infected plants that grew from contaminated seed. These cultivars were chosen because they differ in susceptibility; CLR Kidney is the most susceptible, followed by Redkloud and then Redkote.

Sixteen 1.5-m long rows of each of the three cultivars were distributed uniformly throughout the field. All plants of each cultivar were harvested, threshed, and winnowed in bulk. The seed was stored in paper bags for 7 mo at room temperature. Seed harvest and processing procedures mimicked commercial seed production practices and permitted exposure to crop dust and debris which might contaminate the seed.

Determination of the presence of *P. phaseolicola*. Seed samples were assayed for *P. phaseolicola* by the procedures described by Taylor (13). Two lots from the bulked seed of each cultivar were assayed directly, and three were done after surface-disinfesting the samples in 1.5% sodium hypochlorite (w/v) for 5 min. Samples dried overnight at 30 C were ground in a Christy-Norris 8-in. laboratory hammer mill fitted with a 40-mesh screen (Christy and Norris, Ltd., Chelmsford, England).

Before the bean seed was ground, approximately 1 kg of heat-sterilized (170 C, 5 hr) wheat was passed through the mill to clean it. Bean seed samples were milled in reverse order of cultivar susceptibility; first Redkote, then Redkloud, and finally CLR Kidney. Each milled sample was collected in a new polyethylene bag. One liter of sterile distilled water was added to each 250-g sample of bean flour (from approximately 1,000 seeds). Taylor's method (13) was used to assay *P. phaseolicola* except that samples were taken from the top 1 cm of the suspension using a widemouth 10-ml pipet because even after 2-hr incubation, a sufficiently large supernatant layer for sampling had not formed.

Five serial 10-fold dilutions were made and triplicate 0.1-ml aliquots were removed from each dilution using an Eppendorf pipet (Brinkmann Instruments, Inc., Westbury, NY 11590). The 0.1-ml aliquots were spread onto petri plates containing medium B of King et al (5) (KB) amended with 40 ppm cycloheximide (Sigma Chemical Co., St. Louis, MO 63178).

The resulting colonies were examined under long-wave UV light with a Mineralight UVSL-25 (Ultra-Violet Products, Inc., San Gabriel, CA 91778) 3 days and 5 days after plating. Fluorescent colonies were restreaked onto KB, incubated 4 days, then again examined for colony morphology and for fluorescence under long-wave UV light. Individual colonies from strains that produced a blue or greenish blue diffusible fluorescent pigment and had smooth, cream-colored colonies were transferred to nutrient agar (NA) slants, incubated at 25 C for 24 hr, and then subjected to Kovacs' oxidase test (7,8).

The pathogenicity on beans of all oxidase-negative strains was determined to confirm their identity as *P. phaseolicola* For each dilution plate from each sample, two oxidase-positive fluorescent colonies and one nonfluorescent colony were selected at random and included in pathogenicity tests.

Pathogenicity test. Cells from 24-hr NA slant cultures of candidate strains that had been incubated at 25 C were washed into sterile tubes with sterile distilled water and diluted to an optical density (620 nm) of 0.2 on a spectrophotometer (model Spectronic 20, Bausch and Lomb, Rochester, NY 14625). The suspensions then were

diluted further with sterile water to concentrations of approximately 10^5 colony forming units (cfu) per milliliter. This concentration was chosen to avoid possible confusion resulting from a hypersensitive response to an incompatible pathogen as would be expected for the other members of the *P. syringae* group inoculated into bean tissue at 10^6 cfu/ml or greater (6).

Eight-day-old CLR Kidney seedlings were inoculated with the bacterial suspensions by spray infiltration according to the method described by Schuster (11). The inoculated plants were incubated in a growth chamber at 63% RH and 22 C during the day and 18 C and 83% RH at night; day length was 16 hr with an intensity of 11.9 klux. Plants were watered daily. The appearance of typical halo blight symptoms 5–6 days after inoculation confirmed the identity of *P. phaseolicola.*

RESULTS

Fluorescent bacterial colonies were observed on the KB dilution plates 3 days after plating suspensions made from bean flour. These colonies had a green fluorescence under UV light and were large, mucoid, and creamy white. After 5 days, a second colony type was noted that was smaller, less mucoid, and creamy white and more blue under UV light relative to the first colony type. Blue fluorescent colonies were found only on dilution plates made from the CLR Kidney flour and from those Redkloud samples that had not been surface-disinfested before milling. All the blue fluorescent strains tested were oxidase-negative according to Kovacs' (7) test. All of the green fluorescent strains tested were oxidase-positive.

Typical symptoms of halo blight developed only on plants inoculated with the blue fluorescent, oxidase-negative strains. No symptoms were observed on CLR Kidney plants 14 days after inoculation with the nonfluorescent strains or with the green fluorescent strains.

P. phaseolicola was isolated from all CLR Kidney seed samples even at the highest dilution plated, which was equivalent to 0.25 μg of flour per plate (Table 1). The pathogen was isolated only from the lower dilutions from samples of Redkloud that were not surface-disinfested. *P. phaseolicola* was not isolated from any samples of Redkote.

Table 1. *Pseudomonas phaseolicola* in seed samples of *Phaseolus vulgaris* (dry bean) harvested from a field in which an epidemic of halo blight occurred

Cultivar	Treatment before grinding	
	Surface-disinfested[a]	Not surface-disinfested[b]
California Light Red Kidney	0.25[c]	0.25[c]
Redkloud	0.0	50.00
Redkote	0.0	0.0

[a] Average of three 250-g seed sample replicates. Seed was disinfested in 1.5% (w/v) sodium hypochlorite for 5 min, dried, ground to a fine flour, mixed with sterile distilled water, diluted, and plated onto King's B medium.
[b] Average of two 250-g replicates.
[c] Minimum weight (μg) of bean flour equivalent assayed that yielded *P. phaseolicola.*

DISCUSSION

The degree of resistance (or susceptibility) to *P. phaseolicola* of the three bean cultivars CLR Kidney, Redkloud, and Redkote is expressed in several interrelated aspects of the halo blight disease. The number of infection points (lesions) that develop on uniformly inoculated leaves is greatest on CLR Kidney and least on Redkote. The bacterial population per lesion is greatest in CLR Kidney and least in Redkote (F. H. Mahr, *unpublished data*; M. J. Katherman, *unpublished data*). Most secondary spread occurred from inoculated CLR Kidney plants to neighboring susceptible plants and least occurred from inoculated Redkote plants (4).

The pathogen has been shown to move further and more rapidly in susceptible plants, increasing the chance of systemic seed infection via the xylem (9). Thus, the more susceptible the plants, the more easily they are infected, the greater the buildup of inoculum at each infection point, and the greater the likelihood that infection will result in seed transmission and secondary spread.

The resistance expressed by a plant therefore results not only in reduced symptom expression but also in decreased likelihood of secondary spread in both the current season and in future seasons through seed transmission. A similar relationship has been reported for common blight of beans. Seed from plants inoculated with *X. phaseoli* become contaminated in proportion to their susceptibility; seed of only the susceptible cultivars become infected internally (2).

Because *P. phaseolicola* was detected in the surface-disinfested seed samples only of CLR Kidney, this cultivar alone is considered to have been infected internally. Because surface-disinfestation eliminated *P. phaseolicola* from the seed of Redkloud, the pathogen was likely to have been present only on the surface of the seed. With appropriate seed treatment (as with streptomycin), our seed of Redkote and Redkloud probably could have been used without danger of introducing seedborne halo blight inoculum. It must be recognized that surface contaminants that lodge in the micropyle, hilum, or cracks of the seed coat may not be eliminated by surface-disinfestation (3). Also, a negative result in our assay does not necessarily mean that the seed lot is free of the pathogen. Taylor (14) calculated that as much as 0.1% infection in a sample of 3,000 seeds might not be detected by this test. Furthermore, in recent experiments, internally infected seed of Redkote and Redkloud was detected after several inoculations of the seed-mother plants in the field (R. E. Wilkinson, M. J. Katherman, and M. E. Lyons, *unpublished data*). Thus, the chances of seed infection of cultivars with the intermediate levels of resistance as possessed by Redkote and Redkloud are increased as inoculum pressure is increased.

Although the chance for seed transmission is greatly reduced with intermediate levels of halo blight resistance as in Redkote and Redkloud, some transmission may be possible. In addition, other seedborne pathogens may be transmitted when bean seed is produced under nonarid conditions (17). Until high levels of resistance to all seedborne pathogens are incorporated in bean cultivars, seed production in nonarid regions should be done under rigorous programs of inspection and certification.

ACKNOWLEDGMENT

We thank D. H. Wallace for providing the seed used in this study.

LITERATURE CITED

1. ANDERSON, A. L. 1970. What to do about bacterial blights in 1970. Plant Pathol.-Dis. Rep. 4. Coop. Ext. Serv., Michigan State Univ., East Lansing. 2 pp.
2. COYNE, D. P., M. L. SCHUSTER, and S. MAGNUSON. 1976. Effect of tolerant and susceptible dry bean germplasm on seed transmission of *Xanthomonas phaseoli.* Bean Improv. Coop. Annu. Rep. 19:20.
3. GROGAN, R. G., and K. A. KIMBLE. 1967. The role of seed contamination in the transmission of *Pseudomonas phaseolicola* in *Phaseolus vulgaris.* Phytopathology 57:28-31.
4. KATHERMAN, M. J., R. E. WILKINSON, and S. V. BEER. 1980. The potential of four dry bean cultivars to serve as sources of *Pseudomonas phaseolicola* inoculum. Plant Dis. 64:72-74.
5. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
6. KLEMENT, Z., G. L. FARKAS, and L. LOVREKOVICH. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
7. KOVACS, N. 1956. Identification of *Pseudomonas pyocyonae* by the oxidase reaction. Nature 179:703.
8. MISAGHI, I., and R. G. GROGAN. 1969. Nutritional and biochemical comparison of plant pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
9. OMER, M. E., and R. K. S. WOOD. 1969. Growth of *Pseudomonas phaseolicola* in susceptible and resistant bean plants. Ann. Appl. Biol. 63:103-116.
10. RALPH, W. 1976. Pelleting seed with bactericides; the effect of streptomycin on seedborne halo blight of French bean. Seed Sci. Technol. 4:325-332.
11. SCHUSTER, M. L. 1955. A method of testing the resistance of beans to bacterial blights. Phytopathology 45:519-520.
12. SCHUSTER, M. L. 1967. Survival of bacterial pathogens in the field and greenhouse under

different environmental conditions. Phytopathology 57:830.
13. TAYLOR, J. D. 1970. The quantitative estimation of the infection of bean seed with *Pseudomonas phaseolicola* (Burkh.) Dowson. Ann. Appl. Biol. 66:29-36.
14. TAYLOR, J. D. 1978. The detection of seed-borne bacteria. 16th Int. Workshop on Seed Pathol., Karlsruhe, Germany. 8-14 August 1978.
15. TAYLOR, J. D., and C. L. DUDLEY. 1977. Seed treatment for the control of halo blight of beans, *Pseudomonas phaseolicola*. Ann. Appl. Biol. 85:223-232.
16. WELLER, D. M., and A. W. SAETTLER. 1977. Growth of *Xanthomonas phaseoli* variety *fuscans* in field grown navy (pea) beans. Proc. Am. Phytopathol. Soc. 4:137.
17. ZAUMEYER, W. J., and H. R. THOMAS. 1957. A monographic study of bean diseases and methods for their control. USDA Tech. Bull. 868. 255 pp.