Transmission of *Xanthomonas phaseoli* in Seed of Resistant and Susceptible *Phaseolus* Genotypes

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Cooperative investigations of AR, SEA, U.S. Department of Agriculture, and Michigan State University, East Lansing. Michigan Agricultural Experiment Station Journal Series Article 9095.

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We gratefully acknowledge typing of the manuscript by Ms. Patti Perkins.

Accepted for publication 13 December 1979.

ABSTRACT

CAFATI, C. R., and A. W. SAETTLER. 1980. Transmission of *Xanthomonas phaseoli* in seed of resistant and susceptible *Phaseolus* genotypes. Phytopathology 70:638-640.

A study was conducted on the possible transmission of the bean common blight pathogen *Xanthomonas phaseoli* (= *X. campestris*) (*Xp*) in seed of resistant *Phaseolus acutifolius* (tepary bean), and in *P. vulgaris* genotypes Great Northern (G.N.) Nebraska #1 selection 27, cultivar (G.N.) Valley, cultivar (G.N.) Jules, and breeding line MSU-51319 (all moderately resistant) and cultivars Tuscola and Seafarer (both susceptible). Field-grown plants were inoculated by scratching the dorsal suture of pods at the flat green stage of development with a syringe containing a suspension of R15-1 (an *Xp* mutant resistant to 50 ppm rifampin) at 10^7 colony-forming units per milliliter. At normal maturity, only susceptible Tuscola had typical bacterial blight symptoms. Internally borne blight bacteria were isolated in both solid and liquid rifampin-containing media. Mutant R15-1 was recovered from 40, 42, 46, 51, 42, and 70% of seeds with any type of visible symptoms of internal blight infection in the genotypes tepary, Nebraska #1 selection 27, Valley, Jules, MSU-51319, and Tuscola, respectively. Mutant R15-1 also was recovered from 1.3, 2.0, 1.3, 1.9, 2.0, and 10.4% of symptomless seeds of the same genotypes, respectively. Our data suggest that tests to detect seedborne bacterial blight should be included in production programs for certified, blight-free seed of all dry bean cultivars.

Seedborne inoculum is important in the survival and dissemination of plant pathogenic bacteria; most seedborne bacteria survive as long as the seed remains viable (8). Seed transmission is the primary means for dissemination of the bean (*Phaseolus vulgaris* L.)-pathogenic bacteria (12). Walker and Patel (9), Guthrie et al (6), and Wallen and Sutton (10) have indicated that low levels of infection with *Pseudomonas phaseolicola* (Burk.) Dows. or *Xanthomonas phaseoli* (E. F. Sm.)Dows. (= *X. campestris*) (*Xp*) in bean seeds are capable of initiating heavy field infections and causing severe crop losses under appropriate environmental conditions. Grogan and Kimble (5) reported that *P. phaseolicola* was transmitted through bean seeds harvested from a field in which the disease had not been detected during the growing season.

Both externally infested and internally infected seed have been mentioned as important sources of primary inoculum of *Xp* (7,12). For this reason disease control is based on seed certification programs designed for the maintenance of clean seed stocks. Copeland et al (3) described a process of producing Michigan-certified bean seed from breeder and foundation seed stocks. Seed raised from foundation seed is certified only after the crop has been field inspected for visible pod infection and the resulting seed tested for the presence of bacterial blight organisms. There is no doubt that such programs have reduced seed infection by the bacterial pathogens; nevertheless outbreaks of common and fuscous blights persist and each year seed from some fields is rejected for certification.

P. phaseolicola was detected in fields of halo blight-resistant bean cultivars and may cause serious problems for seed producers (4). Previous field experiments conducted by the authors (2) showed that *Xp* can multiply to high population levels in and on leaves and pods and can systemically colonize plants of bean genotypes with intermediate levels of resistance. Therefore, it was desirable to further investigate the possible transmission of *Xp* in seed of resistant bean genotypes.

MATERIALS AND METHODS

Experiments were conducted under both field and greenhouse conditions. Field experiments were done at the Botany and Plant Pathology Research Farm, Michigan State University, East Lansing, during the 1977 and 1978 growing seasons. In the greenhouse, plants were grown under controlled temperature (27 ± 2 C) and illumination (daylight supplemented with 14 hr of fluorescent lighting) in a standard soil mixture in 16-cm-diameter clay pots. They were watered as needed alternately with Rapid-Gro (2.45 g/L of water) and tap water.

Bacterial isolate. A spontaneous mutant (R15-1) of *Xp* resistant to 50 ppm rifampin was obtained by conventional selective plating methods and found to be as virulent as the parental wild type (*Xp* 15, a highly virulent Michigan isolate).

Greenhouse study. *Xanthomonas*-resistant tepary bean *Phaseolus acutifolius* A. Gray 'Arizona-Buff'; moderately resistant *P. vulgaris* W-117 (USDA, Puerto Rico); and susceptible navy bean *P. vulgaris* 'Seafarer,' were used in this experiment. When plants were at the flat green pod stage of development, about 50 pods of each genotype were inoculated by scratching along part of the dorsal suture with the needle of a sterile syringe containing 5.0 $\times 10^7$ R15-1 cells per milliliter.

At normal maturity the pods were removed and the seed were separated into those without visible symptoms and those with some type of visible symptoms of blight infection. Internally borne blight bacteria were isolated from individual seeds surface-sterilized for 3 min in 2.5% NaOCl, and rinsed twice in sterile distilled water. Isolations were made in both solid (YCA-R: 10 g of yeast extract, 2.5 g of calcium carbonate, 50 mg of rifampin, 25 mg of cycloheximide, and 15 g of agar in 1 L of distilled water), and liquid (BYE-R: 10 g of yeast extract, 50 mg of rifampin, and 25 mg of cycloheximide in 1 L of 0.01 M phosphate buffer, pH 7.2) media. Individual seeds were first placed hilum-downward directly on YCA-R, incubated for 18 hr at room temperature, and transferred

TABLE 1. Recovery of the R15-1 mutant of *Xanthomonas phaseoli* from seeds harvested from symptomless pods on field grown plants of three bean genotypes inoculated at different stages of plant development[a]

Genotype and/or cultivar	Reported disease reaction	Colony-forming units per 100 seeds of plants inoculated at indicated stage of development				
		Seedling	Third trifoliolate	Blossom	Small flat pod	
					Surface[b]	Internal[c]
Tepary						
Arizona-Buff	Resistant	–[d]	–	–	1.0×10^1	–
Great Northern-type	Moderately resistant					
Valley		–	–	–	2.1×10^5	1.6×10^3
Tuscola (cultivar)	Susceptible	–	–	–	1.0×10^5	1.2×10^4

[a] Plants were inoculated by gentle spraying to run-off with a suspension of 1.0×10^7 R15-1 cells per milliliter.
[b] Blight bacteria borne on the surface of seeds were isolated by shaking the seeds in 0.01 M phosphate buffer (pH 7.2) for 1 min and plating the buffer on yeast extract, calcium carbonate agar containing rifampin and cycloheximide (YCA-R).
[c] Blight bacteria borne internally in seed were isolated from seeds surface sterilized for 3 min in 2.5% NaOCl, rinsed twice in sterile distilled water, ground in 0.01 M phosphate buffer (pH 7.2), and plated on YCA-R.
[d] Minuses (–) indicate that the isolation procedure yielded no blight bacteria from surface-sterilized bean seed.

TABLE 2. Incidence of internal infection in seed harvested from greenhouse-grown bean plants inoculated with the R15-1 mutant of *Xanthomonas phaseoli*[a]

Genotype	Reported disease reaction	Internal infections[b]			
		In seeds with visible symptoms		In seeds with no symptoms	
		Infected/total	%	Infected/total	%
Tepary					
Arizona-Buff	Resistant	23/50	46.0	9/168	5.4
Breeding line					
W-117 (USDA, Puerto Rico)	Moderately resistant	25/50	50.0	11/158	6.5
Navy					
Seafarer	Susceptible	34/50	68.0	17/144	11.8

[a] About 50 pods on greenhouse-grown plants of each genotype were inoculated by scratching the dorsal suture with the needle of a syringe containing 5.0×10^7 R15-1 cells per milliliter.
[b] For detection of bacteria, individual seeds were surface sterilized for 3 min in 2.5% NaOCl, placed hilum-downward directly into rifampin-containing media for 18 hr, and then incubated for 48 hr in liquid rifampin-containing media.

TABLE 3. Incidence of internal infection in seed harvested from field-grown bean plants inoculated with the R15-1 mutant of *Xanthomonas phaseoli*[a]

Genotype	Reported disease reaction	Internal infections[b]			
		In seeds with visible symptoms		In seeds with no symptoms	
		Infected/total	%	Infected/total	%
Great Northern-type					
Nebr. #1, sel. 27	Moderately resistant	42/100	42	6/303	2.0
Jules	Moderately resistant	51/100	51	8/420	1.9
Valley	Moderately resistant	46/100	46	5/396	1.3
Breeding line					
MSU-51319	Moderately resistant	42/100	42	7/350	2.0
Tepary					
Arizona-Buff	Resistant	40/100	40	6/450	1.3
Tuscola (cultivar)	Susceptible	70/100	70	32/307	10.4

[a] About 200 pods on field-grown plants of each genotype were inoculated by scratching the dorsal suture with the needle of a sterile syringe containing 1.0×10^7 R15-1 cells per milliliter.
[b] For detection of bacteria, individual seeds were surface sterilized for 3 min in 2.5% NaOCl, placed hilum-downward directly into rifampin-containing media for 18 hr, and then incubated for 48 hr in rifampin-containing liquid media.

to 7-ml test tubes containing 3 ml of BYE-R for 40 hr of shaker incubation. Bacteria from tubes that became turbid were streaked on YCA-R to confirm the presence of the R15-1 mutant. In this way, each seed was checked for internal *Xp* infection on two media.

Field studies. The following bean genotypes were selected for field studies on the basis of their reported reactions to bacterial blight: resistant tepary bean Arizona-Buff, moderately resistant; Great Northern (G.N.) Nebraska #1 selection 27, G.N. cultivars Valley and Jules, and breeding line MSU-51319 (all *P. vulgaris*); and susceptible *P. vulgaris* 'Tuscola'.

At the flat green pod stage of plant development, about 200 pods of each genotype were inoculated with a suspension containing 1.0×10^7 R15-1 cells per milliliter; the inoculum was prepared and administered as in the greenhouse study. Pods were collected at normal maturity, and the seeds were separated into groups either with visible symptoms or without symptoms. Internally seedborne bacteria were isolated from individual surface-sterilized seeds by the techniques described previously.

In a separate experiment, plants of Arizona-Buff, Valley, and Tuscola were inoculated by gently spraying to runoff with a suspension of R15-1 (1.0×10^7 cells per milliliter) at different stages of plant development; ie, seedling stage, third trifoliolate stage, blossom stage, and small flat pod stage. Symptomless pods were harvested at normal maturity, and internal seed infection was assayed by plating of seeds on the rifampin-containing medium, either directly or after 48 hr of incubation in BYE-R. Seeds were assayed for external infestation by shaking them in 0.01 M phosphate buffer (1 ml per seed) for 1 min and plating the resulting suspension on YCA-R.

RESULTS

When spray-inoculation of field plants was used, *Xp* mutant R15-1 was recovered from symptomless seed harvested from symptomless pods of the plants inoculated at the small flat pod stage of development, but not from plants inoculated earlier (Table 1). Cultivars Valley and Tuscola had similar surface bacterial populations, but susceptible Tuscola had the highest internal bacterial populations. Relatively few bacteria were detected on the surface of Arizona-Buff (tepary bean) seed.

When plants of resistant and susceptible bean genotypes were inoculated by scratching the dorsal suture of the pods at the flat green stage with a syringe containing the bacterial suspensions, different disease reactions were observed. At normal maturity, only pods of the susceptible Tuscola exhibited typical Xanthomonas blight symptoms extending beyond the inoculated areas. Great Northern Nebraska #1 selection 27, cultivars Jules, Valley, and breeding lines MSU-51319 and W-117 all had brown necrotic reactions and sometimes a few small water-soaked zones around the scratches. Arizona-Buff tepary bean had only a light-brown necrotic reaction.

Seeds with different degrees of Xanthomonas blight symptoms and seeds with no visible symptoms were harvested from inoculated

pods of plants of all the bean genotypes. In both experiments, *Xp* mutant R15-1 was recovered from about 40–50% of seeds with any type of visible symptoms of internal blight infection in resistant genotypes and from about 70% of those in susceptible bean genotypes. Also, in the greenhouse experiment, *Xp* was recovered from 5.4, 6.5, and 11.8% of symptomless seeds in the genotypes Arizona-Buff, W-117, and Seafarer, respectively (Table 2); and from 1.3, 2.0, 1.3, 1.9, 2.0, and 10.4% in the genotypes Arizona-Buff, Nebraska #1 selection 27, G. N. Valley, G. N. Jules, MSU-51319, and Tuscola, respectively, in the field experiment (Table 3).

DISCUSSION

Zaumeyer (12) indicated that *Xp* may invade the bean plant systemically under certain conditions and that the bacteria may pass into the developing seed through the vascular system of the plant without producing visible symptoms. The blight organism also may enter the pod cavity either via the stomata of the pod or by breaking through the vascular tissue of the pod suture; the bacteria then pass into the funiculus and the raphe or the micropyle leading into the seed.

In an early study, Burkholder (1) reported that bean seeds infected with common blight bacteria were obtained from symptomless pods. Recently, Weller (11) reported the production of symptomless navy bean seed containing low population levels of blight bacteria not only in visibly infected pods, but also in symptomless pods. In our studies, *Xp* was recovered from seeds harvested from symptomless pods of spray-inoculated plants only when they were inoculated at the small flat pod stage of development. This finding suggests that seed infection was primarily a sequel of pod colonization. Pods of tepary bean exhibited the highest level of resistance; only a few bacteria were recovered from the seed surfaces and none were recovered from surface-sterilized seed. Seed of moderately-resistant Valley and susceptible Tuscola had similar levels of external contamination, but internal seed infection was higher in the susceptible genotype. Pods of resistant and susceptible bean genotypes inoculated by scratching the dorsal suture with the needle of a syringe containing the bacterial suspension developed different disease reactions; however, seeds with and without disease symptoms from both susceptible and resistant genotypes carried blight bacteria internally, although bacterial populations were highest in the former.

The transmission of bean blight bacteria in symptomless seed from symptomless pods of both resistant and susceptible genotypes suggests that tests to detect seedborne bacterial blight should be included in production programs for certified, blight-free seed of all dry bean cultivars. With the present reliance on visible pod-infection symptoms, symptomless infections are not detected.

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