Evaluation for Bacterial Blight Resistance in Beans

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ABSTRACT

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Strains of *Xanthomonas* isolated from *Phaseolus vulgaris, P. coccineus, Vigna unguiculata*, and *Glycine max* were differentiated according to pathogenicity and serological relationships. Each strain was inoculated on primary leaves of young plants, trifoliolate leaves on plants in the flowering stage, and on detached trifoliolate leaves and pods. Several inoculation methods were compared. Inoculations at the vegetative stage of plant development were less reliable than those at the reproductive stage. *X. campestris* pv. *phaseoli* (*X.c.* pv. *phaseoli* and *X.c.* pv. *vignicola*, but not *X.c.* pv. *phaseoli* (from *P. coccineus*) and *X.c.* pv. *phaseoli* (var. *fuscans*) induced symptoms during the reproductive stage only. Inoculation of detached leaves did not produce reliable results. Of various inoculation methods, the needle-scratch on pods gave the quickest and most uniform results. In early tests, field selection 235-1 from an interspecific cross (*P. vulgaris* × *P. coccineus*) and several breeding lines from Cornell showed significantly higher resistance to all pathogen strains compared to susceptible local cultivars La Vega and W-117. Subsequent tests with *X.c.* pv. *phaseoli* (var. *fuscans*) and *X.c.* pv. *phaseoli* (from *P. coccineus*), chosen for virulence in a wide range of hosts, confirmed this high level of resistance. These highly resistant lines also showed field resistance in Puerto Rico.

Common bacterial blight caused in beans (*Phaseolus vulgaris* L.) by *Xanthomonas campestris* pv. *phaseoli* (E. F. Smith) Dowson (*X. phaseoli*) remains one of the most serious bean diseases in the world. Practically all the germ plasm that was evaluated by Centro Internacional de Agricultura Tropical (CIAT) for disease reactions during 1978 was susceptible (4). The bacteria usually affect the leaves, causing leaf spots that may coalesce and result in leaf blight, and are also capable of invading the vascular tissue of the plant and infecting stems, pods, and seeds (22).

Disease control is difficult because the pathogen is seed transmitted and has high survival capacity. Short-term control measures depend on such management practices as crop rotation and use of pathogen-free seed produced in dry regions where pathogen levels are low. Consequently, the most effective long-term control strategy is the use of genetic resistance. Unfortunately, high levels of resistance have not been reported. Only tolerant cultivars such as Great Northern, Jules, Tara, Star, and #1 Sel. 27, and PI 207262 have been developed (5,6). Even these lines can harbor low pathogen populations in various plant parts, including the seed (3,18).

Tepary bean (*P. acutifolius*) has long been suggested as a potential source of genetic resistance, because it has high tolerance to *X.c.* pv. *phaseoli* (7,13,18). In 1956, Honma (13) successfully crossed *P. vulgaris* with *P. acutifolius* and obtained segregants highly tolerant to the bacterium. Other investigators have recently obtained this cross as well (15,16).

P. coccineus has also been reported to have high levels of resistance to bacterial blight (7); in 1979, 11 breeding lines of that species identified by N. G. Vakili as potential sources of resistance were jointly released by the USDA and the University of Puerto Rico. One of these lines, Pc-H-46, was used by M. J. Bassett in the interspecific cross that produced resistant line 235-1. This line, also used in our studies, was first released jointly by the USDA, the University of Florida, and the University of Puerto Rico, and was subsequently registered as breeding germ plasm (11).

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Various methods of inoculation have been used to determine resistance to *X.c.* pv. *phaseoli*. However, information is lacking on the reliability and variability of results obtained by using these methods on leaves and pods of the test plant lines being inoculated with specific strains of bacteria (1,16,19).

The objectives of this investigation were to develop a simple, fast, and reliable method of inoculation for the different plant organs; to identify any differences that may exist between strains of *Xanthomonas* relative to cultivars attacked, incubation period, symptoms, and severity; and to identify host germ plasm with high levels of resistance to the tested strains of *Xanthomonas*.

MATERIALS AND METHODS

Seed source and plant culture. *Greenhouse.* Two standard breeding lines developed in Puerto Rico (La Vega, a somewhat variable black-seeded cultivar, and W-117, an advanced homozygous white-seeded line) were used as susceptible cultivars. Other lines used in the tests were received from CIAT, Oregon State University, Cornell University, the University of Illinois, and from the University of Puerto Rico (UPR)/U.S. Department of Agriculture—Tropical Agriculture Research Station (TARS) at Mayaguez, PR. Four hybrid lines from the interspecific cross *P. vulgaris* × *P. coccineus* were obtained from TARS/UPR. The seeds were disinfested in sodium hypochlorite (1% Cl) for 5 min prior to planting in 15-cm-diameter plastic pots containing a commercial sterilized greenhouse potting mix. Plants were grown in a screened, glass greenhouse that had been previously washed with a dilute sodium hypochlorite solution.

Field. A single 5-m row of each line identified as resistant in greenhouse inoculations was planted 5 July 1980 at the University of Puerto Rico Fortuna Substation at Juana Díaz, PR. The plants were grown under soil, temperature, and humidity conditions that favored disease development. At midflowering, 52 days after planting, half of each row was inoculated by a sand-blast spray technique similar to the one described by Bohn et al (2). Daily overhead sprinkler irrigation was used to maintain a high moisture level during the week following inoculation. Size and incidence of leaf and pod lesions were recorded each week for 4 wk after inoculation.

Bacterial cultures and immunodiffusion. The cultures of *Xanthomonas* used in this study were obtained from the pathogen collection maintained by TARS. These cultures, which come from

several different hosts and localities, are maintained as pure strains in water and are renewed periodically.

Serological tests. The relationships between cultures were established by means of double diffusion tests in two dimensions and by intragel cross-absorption tests in agar (20). The antisera used for these tests were prepared at the Agricultural Experiment Station in Rio Piedras, PR, to isolates of *X.c.* pv. *vignicola* (ATCC 11648), *X.c.* pv. *glycines* (P.R. 360A), *X.c.* pv. *phaseoli* (var. *fuscans*) (ATCC 13464), and *X.c.* pv. *phaseoli* (P.R. X76) used as immunogens.

Inoculum preparation. *Greenhouse.* There are no reports in the literature on *Xanthomonas* that high inoculum concentrations result in hypersensitive reactions as is reported with *Erwinia* and *Pseudomonas* (10,14). A trial of five inoculum concentrations (10^4–10^9 colony-forming units (cfu) per milliliter of *X.c.* pv. *phaseoli* on a susceptible cultivar of *P. vulgaris* was performed under greenhouse conditions. No significant differences in symptom expression were found when the bacterial concentration was 10^6, 10^7, and 10^9 cfu/ml; the cultivar showed high susceptibility at these inoculum concentrations. On the other hand, the symptom intensity was significantly less when 10^4 and 10^5 cfu/ml were used and the reaction was rated from slightly susceptible to moderately susceptible, respectively.

For the study, bacterial cell suspensions were prepared in sterile distilled water from cultures grown 48 hr at 26 C. All concentrates were adjusted to 10^9 cfu/ml by using standard turbidimetric and dilution plating techniques. This method of standardizing inoculum concentration was used for all inoculations in the greenhouse.

Field. Leaves with visible blight lesions were collected from several plantings in the vicinity of the field test plots at the UPR Fortuna Substation. These were selected from many U.S. commercial cultivars of bush and vine types, PI accessions, and other test lines showing various degrees and types of bacterial blight infection at a stage somewhat later than initiation of flowering. The infected leaves were macerated in a high-speed blender with 10 ml of 0.01 M phosphate buffer (pH 7.0) per 1 g of infected tissue. The resulting suspension was used for field inoculation.

Inoculation techniques. *Environmental chambers.* Trifoliolate leaflets at a medium stage of development were removed from the plants and placed right side up, on two glass slides supported on a piece of filter paper in a petri plate to which 5 ml of sterile water was added to maintain a 100% relative humidity. A 0.5-cm-diameter cork borer was pressed into the upper surface (without cutting through the leaflet) to produce a circular wound, and then a 0.05-ml droplet of the bacterial suspension was deposited on the wound area. These inoculated leaflets were incubated in environmental chambers at 27 C with fluorescent lighting (3,220 lux) for 12 hr daily (9,12). Readings were taken daily. The most reliable readings were those taken at 7 days after inoculation; after that, the leaves began to deteriorate.

Pods for inoculation from each cultivar were carefully selected for medium maturity. They were disinfested in a dilute sodium hypochlorite solution (0.2% Cl), washed three times in tap water, and placed in petri dishes as described for leaves. Several inoculation techniques were compared. In the first technique, a 0.01-ml droplet of bacterial suspension was deposited near the ends of the pod. Two wounds were made in each pod, one from scratching the pod with a needle in a cross-hatch design, and the other from a 1.5-mm-deep puncture. After inoculation the pods were treated in the same way as leaves. In the second technique, 4-to 5-mm-long scratches (cutting through the epidermis) were made by two needles mounted 2 mm apart on all pods only at each end of the pod over a seed location. A 0.01-mm drop of inoculum was deposited only on the distal scratch marks. A comparison was then made between wounds on each pod, with or without inoculum.

In a further series of trials, a tray ($33 \times 23 \times 5$ cm) was used for maintenance of the inoculated pods instead of the petri dishes, since a larger number of pods could be placed in a given space, and it was more practical to cover and seal the trays with a transparent adhesive plastic sheet.

Screenhouse and greenhouse. Thirteen-day-old primary leaves from screenhouse-grown plants were inoculated with bacterial cultures 820, 303, and 113. The leaf was placed over a plastic sponge and perforated with a multiple-pin mount (prepared with 80 insect-mounting pins in a 25-mm-square piece of Styrofoam). The wounded area was then rubbed lightly while immersed in the bacterial suspension (1,18).

At the flowering stage of plant development, fully developed trifoliolate leaflets were inoculated with the multiple-pin mount and cushion and by spray infiltration. In spray infiltration, a glass chromatogram sprayer was used to infiltrate the bacterial suspension into fully expanded leaflets from a distance of 5 cm through a 0.5-cm hole in a plastic shield into the leaf surface at a pressure of 34 kPa (5 psi) (18,19). After inoculation, high humidity was maintained by mist irrigation every few minutes from atomizers installed above the plants.

Field. A pneumatic sand-blasting pistol connected by an air hose to a portable air compressor was employed for inoculation in the field (Fig. 1). This inoculation technique closely simulates the natural method of inoculation from wind-driven infested soil particles and produces symptoms on inoculated plants very much like those that occur in the field (17). The bacterial suspension from field-collected leaves was placed in the reservoir of the pistol along with 28.38 g (1 ounce) of fine silica sand. This mixture was sprayed twice over one-half of each row of the test field plantings at 345 kPa (50 psi) with the pistol held 30–60 cm (1–2 ft) from the plants. As a result, close inspection revealed numerous minute lesions and perforations on the stems and leaves (Figs. 2 to 5). This method differs from the technique used by Webster et al (21) who sprayed a suspension containing 5×10^7 cfu/ml onto plants with a sprayer nozzle operated at 3 kg/cm^2 and held 3 m from plants at weekly intervals beginning 3 wk after planting and continuing for the duration of the experiment.

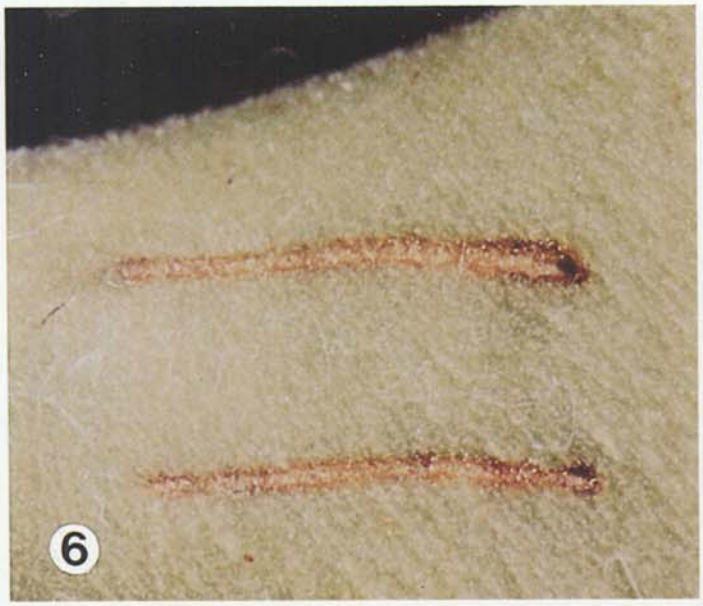
RESULTS

Bacterial culture relationships. The six bacterial isolates were first identified according to the host on which they were found and by the typical yellow pigmentation of the cultures in yeast dextrose carbonate agar. In addition, *X.c.* pv. *phaseoli* (var. *fuscans*) produced a brown pigment that diffused into the agar. However, the reactions observed by using the double diffusion tests without previous isolate adsorption showed that all the cultures were related. That this relationship was partial was shown by spur formations, which were verified with a second test by intragel isolate absorption in the agar before addition of the antiserum.

Inoculation of excised trifoliolate leaflets. Comparison of the results of testing 10 replicates of the standard susceptible cultivars La Vega and W-117 showed that the intensity of symptom expression was highly variable. The inoculation of excised leaflets involved difficulties that made the results relatively unreliable. In most cultivars, the excised leaves could be maintained apparently healthy for only about 15 days, but in some cultivars they turned yellow within 1–2 days after being placed in the petri dish. Apparently, the temperature that favored the physiological activity of the pathogen also accelerated the senescence of the leaves. It was also difficult to maintain high humidity in the petri dish, and it was necessary to add water to maintain the leaf tissue in a satisfactory state for infection. A paraffin strip was used to seal the edge of the dish to reduce drying and maintain adequate humidity, but this was troublesome and not always effective. At times, leaf tissue broke from the pressure of the wound, and the bacterial suspension was lost. When the leaf tissue did not break, the bacteria remained in contact with the tissue for a longer period of time.

Inoculation of excised pods. The methods used for inoculation of pods favored a rapid infection of susceptible pods; symptoms often appeared within 24 hr of inoculation. The needle-scratch method provided more uniform results, since the symptoms were visible from the beginning, whereas the puncture method gave less reliable readings because the bacteria could infect the interior of the pod without causing visible symptoms. Symptoms were observed for all the different bacterial cultures, and the water-soaked area

Fig. 1. Inoculation of bean foliage with a suspension of *Xanthomonas* spp. and fine sand delivered at 50 psi from a sand-blasting pistol in fields at the University of Puerto Rico's Fortuna Substation at Juana Diaz. **Figs. 2 and 3.** Lesions on upper leaf surface of susceptible beans (*Phaseolus vulgaris*) 7 days after inoculation with *X. campestris* pv. *phaseoli*. Note perforations made by sand grains and discolorations and necrotic tissue from bacterial invasion on **2,** cultivar La Vega and **3,** cultivar W-117. **Figs. 4 and 5.** Lesions on upper leaf surface of resistant beans 7 days after field inoculation with *X.c.* pv. *phaseoli*. Note absence of necrosis on **4,** *P. vulgaris* × *P. coccineus* line 235-1-1 and absence of necrosis and limited discoloration on **5,** *P. vulgaris*, Cornell line 79-1984-N. **Figs. 6–9.** Needle scratches on green pods. **6,** Lesion appearance (rated 1) on resistant *P. vulgaris* in the absence of inoculum at 9 days. **7,** Lesion appearance (rated 4) on susceptible *P. vulgaris* 1979 EP25 inoculated with culture 810 of *X.c.* pv. *glycines*. Note water-soaked tissue and bacterial ooze produced by this least virulent isolate of those tested. **8,** Lesion appearance (rated 1) on resistant *P. vulgaris* × *P. coccineus* line 235-1 9 days after inoculation with culture 810 of *X.c.* pv. *glycines*. **9,** Lesion appearance (rated 1) on resistant bean (*P. acutifolius*) line 76-TL-11 7 days after inoculation with culture 144 of *X.c.* pv. *phaseoli* (var. *fuscans*). Note raised and darkened tissue. **Figs. 10 and 11.** Leaves of *P. vulgaris* 7 days after inoculation by the multiple-pin method. **10,** Primary leaf inoculated with culture 144 of *X.c.* pv. *phaseoli* (var. *fuscans*). Note similarities with natural bacterial infection (rated 4). **11,** Trifoliolate leaflets inoculated with culture 144 *X.c.* pv. *phaseoli* (var. *fuscans*) on the left (rated 4) and with culture 820 of *X.c.* pv. *phaseoli* on the right (rated 3). Note differences between strains relative to necrosis and chlorosis of tissue.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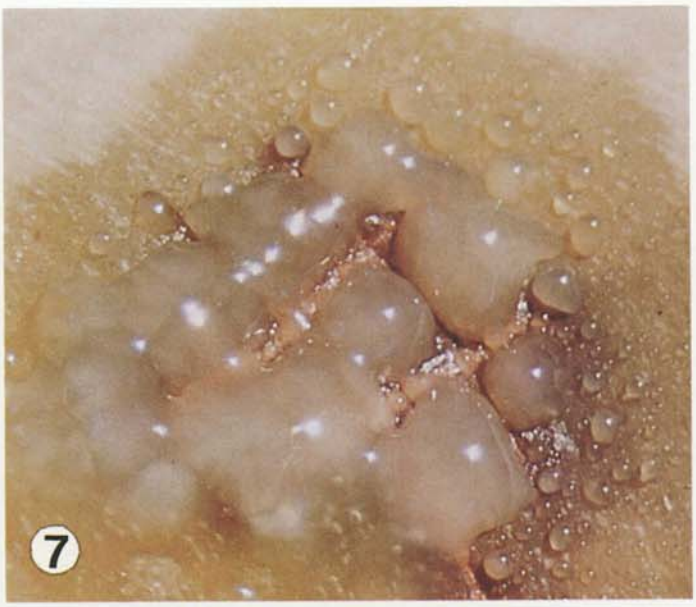
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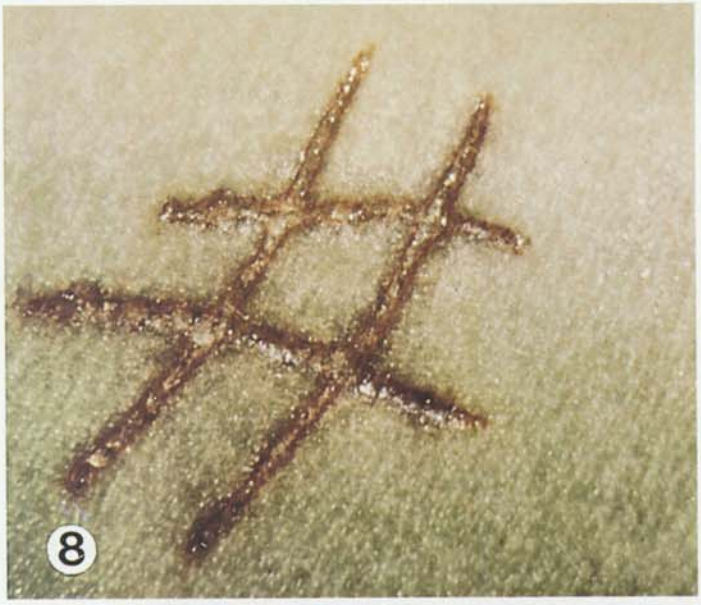
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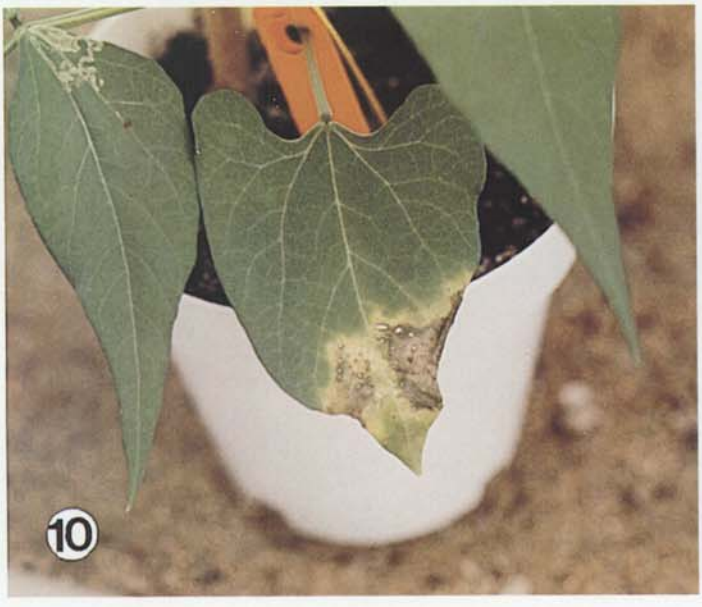
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bordering the scratch was rated with a scale of 1–5 as follows: 1 = no water-soaking, 2 = about 1 mm wide, 3 = 2–3 mm wide, 4 = coalescing over scratches, and 5 = reaching the sutures of the pod (Figs. 6–9).

An advantage of using nearly mature green pods for inoculation tests is that they persist for some time before physiological deterioration begins. Inoculation of pods attached to the plant was not investigated because the use of the excised pods gave good results. On the basis of mean pod severity readings among cultivars, the cultures were classified in descending order of virulence as follows: 820, 144, 866, 303, 113, and 810 (Table 1).

Cultivar W-117, which is moderately tolerant to some Michigan

TABLE 1. Average lengths (millimeters) of the water-soaked areas developed in pods of *Phaseolus vulgaris* inoculated with six isolates of *Xanthomonas* by the needle-scratch method

Host		Bacterial isolate number[a]						
Species and cultivar or line	Origin[b]	820	144	866	303	113	810	$\bar{x}$[c]
P. vulgaris								
La Vega	TARS/UPR	3.5[d]	1.8	2.0	2.8	1.0	1.0	2.00
W-117	TARS/UPR	4.3	2.5	4.0	2.8	3.3*	2.9	3.15**
79-1981-N	Cornell	1.0**	2.3	1.0**	1.0	1.0	1.0	1.21**
79-1987-N	Cornell	1.3**	2.0	1.8	1.3	1.0	1.0	1.26**
79-1982-N	Cornell	1.5**	2.0	1.5	1.0	1.0	1.0	1.33*
79-1984-N	Cornell	2.3	3.0	1.3*	1.0	1.5	1.5	1.52
4117 OSU	Oregon	3.3	2.0	3.5**	2.0	1.5	1.0	2.21
4091 OSU	Oregon	3.0	3.8*	2.8*	1.5	1.5	1.5	2.33
4886 OSU	Oregon	3.5	3.3	2.0	2.3	1.3	1.0	2.17
4911 OSU	Oregon	4.8*	3.0	4.3**	1.0	1.0	1.0	2.50
1979 EP 46	CIAT	4.5*	3.1	1.3*	1.5	1.3	1.4	2.17
1979 EP 25	CIAT	4.1	2.3	3.3	1.7	4.3**	2.5**	3.07*
1979 EP 9	CIAT	5.0**	3.0	3.8	1.3	1.3	1.8	2.63
1979 EP 146	CIAT	5.0**	2.8	3.8*	2.8	1.9	2.5	2.89**
IBRN 5 (27R)	TARS/UPR	1.5**	2.0	1.9	2.0	1.0	1.0	1.56
Bountiful	TARS/UPR	2.8	1.9	2.3	1.6	1.0	1.3	1.77
P. acutifolius								
AC-2	Oregon	1.0**	1.5	1.0	1.0	2.5	1.0	1.11**
76-TL-11	TARS/UPR	1.0**	1.0	1.0**	1.0	1.0	1.0	1.00**
Glycine max								
Line H	Illinois	1.0**	1.0	1.0**	1.0	1.1	1.3	1.02**
P. vulgaris × *P. coccineus*								
235-1	TARS/UPR	1.0**	1.3	1.0**	1.0	1.0	1.0	1.04**
206-1	TARS/UPR	5.0*	2.0	3.3	1.5	1.0	1.0	2.29
106-1	TARS/UPR	2.0	2.0	1.0	1.0	1.0	1.0	1.33
16-1	TARS/UPR	3.8	2.0	1.5	1.4*	1.8	1.2	1.92
	$\bar{x}$[e]	2.88**	2.24**	2.19	1.94	1.34	1.34	

[a]Bacterial isolate numbers: *X. campestris* pv. *phaseoli*, (820 and 866), *X.c.* pv. *phaseoli* var. *fuscans* (144), *X.c.* pv. *phaseoli* (303 type), *X.c.* pv. *vignicola* (113), and *X.c.* pv. *glycines* (810).

[b]Origin: TARS/UPR—Tropical Agriculture Research Station in collaboration with the University of Puerto Rico; Cornell—Cornell University, Ithaca, NY; Oregon—Oregon State University, Corvallis; CIAT—Centro Internacional de Agricultura Tropical, Cali, Colombia; Illinois—University of Illinois, Urbana.

[c]Comparison of the host response to the bacterial isolates with the response of the cultivar La Vega.

[d]Scale: 1 = no water-soaking, 2 = about 1 mm wide, 3 = 2–3 mm wide, 4 = coalescing over scratches and forming a round spot, and 5 = reaching the sutures of the pod. Significantly different than value for cultivar La Vega according to the Student's *t*-test. Asterisk * and ** indicate statistically significant difference at $P = 0.05$ and $P = 0.01$, respectively.

[e]Average comparison of the bacterial isolates with the type-isolate 303.

TABLE 2. Average length of the water-soaked area on bean pods inoculated with two virulent strains of *Xanthomonas* by the needle-scratch method

Host	*X. campestris* pv. *phaseoli* Isolate 820				*X. campestris* pv. *phaseoli* var. *fuscans* Isolate 144			
Species/line/family	N[a]	$\bar{x}$	SD	SE	N	$\bar{x}$	SD	SE
P. vulgaris								
La Vega (Standard)	12	3.25	0.97	0.28	12	1.75	0.72	0.21
W-117	9	3.78	1.48	0.49	9	2.06	1.26	0.42
79-1981-N	8	1.00**[b]	0.00	0.00	8	1.75	0.65	0.23
79-1987-N	7	1.14**	0.38	0.14	8	1.88	0.58	0.21
79-1982-N	8	1.25**	0.46	0.16	8	1.81	0.37	0.13
79-1984-N	8	1.88	1.36	0.48	7	2.29	1.22	0.46
P. vulgaris × *P. coccineus*								
Sel. No. 235-1								
F_6 Family -1	16	1.03**	0.13	0.03	14	1.14*	0.53	0.14
F_6 Family -2	13	1.08**	0.28	0.08	15	1.00**	0.00	0.00
F_6 Family -3	6	1.33**	0.41	0.17	5	1.40	0.42	0.19
F_6 Family -4	18	1.08**	0.26	0.06	18	1.14**	0.38	0.09

[a]N = number of observations, $\bar{x}$ = average length (millimeters) of water-soaked area, SD = standard deviation, and SE = standard error.

[b]Significantly less than the value for cultivar La Vega according to the Student's *t*-test. Asterisks * and ** indicate statistically significant difference at $P = 0.05$ and $P = 0.01$, respectively.

strains (A. W. Saettler, *personal communication*), showed highly significant differences in its reponse to all the strain inoculations when compared to La Vega, the more tolerant standard cultivar. Of the lines inoculated with all six cultures, high levels of resistance were found in several Cornell lines, in some lines derived from crosses of *P. vulgaris* × *P. coccineus*, and in *Glycine max* and *P. acutifolius* (Table 1).

The interspecific cross 235-1 showed good resistance to the six strains of *X.c.* pv. *phaseoli* (Table 1). The general resistance of this line, as measured by the average of the responses of pods to inoculation with all strains, was highly significant (Student's *t*-test at $P = 0.01$) compared with similarly inoculated cultivar La Vega. The individual response to each strain was highly resistant, without any evidence of water-soaking symptoms developing, except for one of the four replicates inoculated with *X.c.* pv. *phaseoli* (var. *fuscans*). This replicate was observed to be a much younger pod than those generally chosen for the test; thus, very young tissues may be somewhat more susceptible to disease. In this case, the water-soaking was observed to progress only approximately 0.1 mm from the scratch. In these tests, the highly significant differences of the responses of interspecific hybrid 235-1 to the most virulent strains of *X.c.* pv. *phaseoli* 820 and 866, which were isolated from *P. coccineus*, probably resulted from the uniformity of the results of these strains (standard deviation of zero). Comparisons of resistance for 235-1 for the other strains were not significant compared to that of La Vega, but were significant compared with that of W-117.

Further tests with two of the most virulent bacterial strains and an F_6 generation of the four families derived from interspecific hybrid 235-1 showed significantly more resistance than the susceptible standard to both strains. In the case of family 1-3, there was no significant difference from the standard for resistance to *X.c.* pv. *phaseoli* (var. *fuscans*) 144, possibly because of the relatively few pods tested and the relatively high degree of resistance shown by the susceptible line, La Vega. The lines from Cornell were highly resistant to *X.c.* pv. *phaseoli* 820 with highly significant differences, but there was no significant difference from the standard for resistance to *X.c.* pv. *phaseoli* (var. *fuscans*) 144 (Table 2).

The resistance derived from the Cornell lines and from *P. coccineus* is probably different from the resistance shown by *Glycine max* and *P. acutifolius*. Both of these showed a marked discoloration around the inoculation site, which was absent in both the Cornell and the *P. coccineus* hybrid lines (Figs. 8 and 9).

Inoculation of attached leaves in the greenhouse. The multiple-pin puncture method has the advantage that larger numbers of lesions appear at one time, and the symptoms expressed seemed to be more like those seen in the field. The method, however, involved the risk of transmitting other diseases from plant to plant. Nevertheless, it gave good results and there were no complications with other diseases (Figs. 10 and 11). Spray infiltration of bacteria gave results similar to those achieved with the multiple-pin method, and had the advantage of no possibility of transmitting other pathogens. However, it was more laborious, since plants had to be moved to the spray unit for inoculation.

Primary leaves of susceptible cultivars La Vega and W-117 inoculated with culture 820 by the multiple-pin puncture method, showed clear symptoms of the disease 11 days after inoculation, whereas those inoculated with cultures 303 and 113 developed symptoms after 31 days, when the plants were flowering.

Some of the Cornell lines (79-1945-N, 77-3845-3, and 77-3860-2) showed high levels of resistance (Table 3).

Inoculation of plants in the field. Observations were recorded at 7, 14, and 33 days after sand-blast spray inoculation with a mixed bacterial suspension, and compared with the natural incidence of the disease. In the field, several different symptoms (pustules, small black greasy lesions, burn lesions with and without yellow borders, and chlorotic lesions) developed in the foliage.

The response of the resistant material represented by the four families of selection 235-1 was compared with the responses of the standard cultivars, La Vega and W-117 (Table 4). Symptom expression was rated similarly to that for attached leaves in greenhouse-grown plants as explained above.

By 7 days after inoculation, responses of leaves to artificial and natural inoculation were similar in susceptible La Vega and W-117 check cultivars. Both showed susceptible leaves with black greasy borders. La Vega leaves showed necrotic lesions with yellow borders and chlorotic lesions, and those of W-117 showed necrotic lesions both with and without yellow borders. Pods of W-117 showed resistance, and those of La Vega, susceptibility.

Leaves of selection 235-1, and of families 1-1, 1-2, and 1-4 were resistant, while leaves of family 1-3 showed slightly susceptible reactions. Family 1-3 showed a few black greasy lesions and a few small chlorotic spots and family 1-1 showed small chlorotic spots. No pods had developed on these lines at this time. Under natural conditions, the foliage of all families was resistant.

Cornell lines 79-1981-N, 79-1984-N, and 79-1987-N showed no symptoms and 79-1982-N showed a slight susceptibility to leaf infection. No symptoms were observed on pods.

Control cultivars showed heavy infection with more or less the

TABLE 3. Response of attached leaves of bean inoculated by the multiple-pin method with three isolates of *Xanthomonas*

	Primary leaves						Trifoliolate leaves		
	11 days[a]			31 days			9 days		
Lines	303[b]	113	820	303	113	820	303	113	820
Standards									
La Vega	1[c]	1	3	4	3	5	3	4	5
W-117	1	1	3	3	3	4	3	2	5
Cornell lines									
79-2015-N	1	1	1	1	1	4	1	1	4
79-2002-N	1	1	1	1	1	4	1	2	2
79-1995-3	1	1	1	1	2	3	1	3	3
79-1979-N Blk	1	1	2	1	1	3	1	1	3
79-1963-N	1	1	2	1	3	3	1	1	2
79-1957-N	1	1	3	1	2	3	1	2	2
79-1953-N	1	1	2	2	2	3	3	2	3
79-1945-N	1	1	1	1	1	3	1	1	1
77-6941-1	1	1	1	1	3	3	1	3	2
77-3838-3	1	1	1	1	1	3	1	1	3
77-3841-2	1	1	1	1	2	2	1	2	2
77-3845-3	1	1	1	1	1	2	2	1	2
77-3855-2	1	1	1	1	1	3	2	2	3
77-3860-2	1	1	1	1	1	2	1	2	4

[a] Days after inoculation.
[b] Bacterial strain designation: 303 = *X.* campestris pv. *phaseoli* (type), 113 = *X.c.* pv. *vignicola*, 820 = *X.c.* pv. *phaseoli* (PR).
[c] Scale: 1 = resistant, 2 = slightly susceptible, 3 = moderately susceptible, 4 = susceptible, and 5 = highly susceptible.

TABLE 4. Symptom expression on leaves and green pods of bean inoculated with a *Xanthomonas* suspension prepared with naturally bean blight infected foliage and compared with natural incidence of the disease in Juana Díaz, PR

	7 days				14 days				33 days			
	Artificial		Natural		Artificial		Natural		Artificial		Natural	
Host cultivar or line	L[a]	P	L	P	L	P	L	P	L	P	L	P
Standards												
La Vega	4[b]	4	4	4	5	4	5	4	5	5	5	3
La Vega	5	4	5	4	5	5	5	5	5	5	5	5
W-117	3	1	3	1	4	5	3	3	5	5	5	5
W-117	3	1	3	1	4	4	3	3	4	4	4	4
P. vulgaris × *P. coccineus*												
F_7 Fam. 1-1	1	–[c]	1	–	2	–	1	–	2	1	2	1
Fam. 1-2	1	–	1	–	1*	–	1*	–	2	1	1	1
Fam. 1-3	2	1*[c]	1	–	2	1	1	1	2	1	2	1
Fam. 1-4	1	–	1	–	2	–	1	–	3	1	2	1
P. vulgaris – Cornell												
79-1981-N	1	1	1	1	1	1*	1	1	2–4	1,4	2–4	1,4
79-1982-N	2	1	2	1	2	1*	2	1	3	1,4	?[c]	1
79-1984-N	1	1	1	1	1	1*	1	1	–	–	2	1
79-1987-N	1	1	1	1	1	1	1	1	–	–	1,2	1

[a] Response on leaves (L) and pods (P) under artificial and natural infection.
[b] Scale of symptom expression: 1 = resistant, 2 = slightly susceptible, 3 = moderately susceptible, 4 = susceptible, and 5 = highly susceptible.
[c] Symbols: – = no pods present; ? = reading confounded by disease symptoms caused by *Macrophomina* and * = these lines are segregating, showing a slight susceptibility to the bacteria.

same types of symptoms as those that developed 7 days after inoculation. Under natural conditions, leaves and pods of W-117 were less susceptible than those of La Vega.

Leaves of the four families of selection 235-1 showed a slight susceptibility. Families 1-2, 1-3, and 1-4 developed chlorotic lesions, while those of family 1-1 developed black greasy lesions with pustules. Under natural conditions, the leaves were resistant. No pods had set at this time, except on family 1-3, which had a few pods that were resistant.

Cornell line 79-1987-N had developed no symptoms at this date, line 79-1984-N had developed many bacterial necrotic symptoms without yellow borders, 79-1981-N had developed small (1–2 mm) chlorotic spots, and 79-1982-N had developed chlorotic spots (4–13 mm).

No difference in susceptibility was observed between check cultivars either on the leaves or pods 33 days after inoculation. Both were more or less defoliated, La Vega having only 0.5%, and W-117 15–20%, green foliage remaining.

The 235-1 families had between 50 and 90% green foliage remaining with only slightly more foliage remaining under natural conditions than in the inoculated area. Under both artificial and natural inoculation, the leaf responses were similar, showing a reaction from resistant to moderately susceptible, and the pod response was resistant.

In general, it was observed that the families of selection 235-1 showed a differential response in which the pod had higher resistance than the foliage and both were highly superior in resistance to the susceptible controls.

At the final reading, plants of the Cornell lines, which were very early, were either dead or defoliated, except 79-1981-N, which retained much of its foliage. Some variability in pod response was shown by lines 79-1981-N and 79-1982-N.

DISCUSSION

Two types of inoculations are recommended for evaluation of resistance to *Xanthomonas* blight of beans: inoculation of the trifoliolate leaf on the plant at the flowering stage with either the multiple-pin and cushion method or the spray infiltration method, and inoculation of green pods by parallel needle scratches and placing them in controlled environments. Pod inoculation is practical, since many lines can be tested at one time and the results are uniform and rapidly obtained.

There may be many genes involved in the resistance of the Cornell lines because of the breeding system used, which favors the accumulation of minor genes. Polygenic inheritance was proposed by Coyne in 1966 (8). In the selections from hybrids of cross *P. vulgaris* × *P. coccineus*, there may be only a few major genes for resistance since the resistance was evident in *P. coccineus* and in a high proportion of the progeny in each succeeding generation.

At Cornell, primary leaf inoculation in environmental chambers has been successful for the identification and recombination of minor genes for resistance. We found no evidence that this resistance is limited to the primary leaf; it also seemed to be effective against all strains tested, but we did find that symptom expression for some strains was delayed until flowering.

Where environmental chambers are not available, inoculation of trifoliolate attached leaves at flowering of the plant is recommended since there are strains of the bacteria that do not colonize or induce pronounced symptoms until the flowering stage is reached.

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