Variation, Virulence of *Pseudomonas syringae* pv *phaseolicola* on Beans in Colombia

R. A. Buruchara, M. A. Pastor-Corrales

CIAT
Cali, Colombia

Abstract

Thirty isolates of *Pseudomonas syringae pv. phaseolicola* (Burkholder, 1926) Young, Dye, and Wilkie, 1978 (ISPP List 1980), from Colombia were separated into race 1 and 2 on the basis of their pathogenicity for *Phaseolus vulgaris* cv. Red Mexican UI-3. Race determination was based on both leaf and pod reaction. Standard isolates of race 1 and 2 were used for comparison. Twenty Pasto isolates were classified as race 2, whereas 7 isolates from Popayán, 1 from Palmira, and 2 from Tenerife were identified as race 1.

The isolates were also classified in their order of virulence, by the diameter of watersoaking lesions caused on pods during a period of 5 days on cultivars Seminole, G. N. Nebraska #1 Sel 27, and Wisc HBR 72 upon inoculation using a needle. The most virulent isolates were from Pasto. Cultivar Wisc HBR 72 (with high foliar resistance) had a susceptible pod reaction (mean lesion diameter 3.1 mm; dispersion 0.5-5.3 mm) comparable to the susceptible cultivar Seminole (mean lesion diameter 2.8 mm; dispersion 0.5-4.8 mm). G. N. Nebraska #1 Sel 27 had a mean lesion diameter of 1.8 mm and a dispersion between 0.4 and 2.8 mm. The order of the isolates in virulence was similar in all three cultivars and gave a correlation coefficient of 0.87 between the 3 cultivars.

Introduction

Halo blight of beans (*Phaseolus vulgaris* L.) incited by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder, 1926) Young, Dye and Wilkie, 1978 (ISPP List 1980), is one of the bacterial diseases responsible for low bean yields in some of the bean growing regions (4). Occurrence of the disease has been reported in Africa, Australia, Canada, Europe, Latin America, New Zealand and United States (23, 24, 32). In Latin America the disease occurs in bean growing areas with moderate temperatures such as in some regions of Brazil (2), Chile (7), Colombia (31) and Guatemala (31).

Pathogenic variation in *Ps. syringae* pv. *phaseolicola* was for the first time demonstrated by Jensen and Livingstone (12). In 1964, Walker and Patel (29) reported the existence of 2 races (race 1 and race 2) in the United States. Their race distinction was based on bean cultivar 'Red Mexican UI 3' which is resistant to race 1 but susceptible to race 2. Since then, reports

on the occurrence of the two races in different countries have been documented (1, 8, 10, 17, 21, 30). Coyne *et al.* (5) in 1979 reported a more virulent strain than the previously reported race 2 of Walker and Patel (29). However, Schroth *et al.* (19) in 1971, suggested that, there exist many strains of *Ps. syringae* pv. *phaseolicola* with varying degrees of virulence. They also found that isolates of neither race 1 nor race 2 were homogenous with respect to virulence when tested on certain varieties. In 1979, Szarka and Velich (26) also observed that, *Ps. syringae* pv. *phaseolicola* does not consist of two races only but of a series of strains which can be ranged by their increasing pathogenicity.

The aim of this study was to determine the pathogenic variation and virulence of *Ps. syringae* pv. *phaseolicola* collected from selected bean growing regions of Colombia.

Materials and Methods

Sources, Isolation, and Verification of Isolates

Thirty isolates collected from some regions (Palmira, Pasto, Popayán, and Tenerife) of Colombia were used in these studies. In addition, race 1 and 2 isolates received from Dr. D. Hagedorn (University of Wisconsin), isolates HB 16 from Dr. M. L. Schuster (University of Nebraska), and isolate OHB from Dr. S. V. Beer (Cornell University) were included for comparison (Table 1).

To isolate bacteria from the host, lesions and parts bordering them were cut from leaves of naturally infected plants. They were surface sterilized with 0.5% sodium hypochloride for 2 min rinsed twice with sterile distilled water and comminuted in a test tube containing sterile distilled water. The bacteria suspension was streaked on nutrient agar (beef extract, 3 g; peptone (Difco), 3 g; agar (Difco) 15 g; and distilled water, 1000 ml), and incubated at 27°C.

Cultures were purified by a series of single colony transfers and then verified as *Pseudomonas syringae* pv. *phaseolicola* by biochemical and pathogenicity tests (14). These included colony morphology, fluorescent pigment production on King's medium B (13), oxidate test, levan production, catalase reaction, production of hydrogen sulphide, arginine dihydrolase test, and pathogenicity test on susceptible bean cultivar 'Red Kidney'. The isolates were maintained on nutrient agar slants at 4°C and also in lyophilized form.

Seed Source

Seed of all bean cultivars and lines used were obtained from the Bean Pathology and the Genetic Resources Sections of Centro Internacional de Agricultura Tropical (CIAT). Seed increase was made from single plant selection. Plants were grown in the greenhouse in 15 cm pots containing a mixture of soil and sand (5:1) which had been sterilzed previously. Two to three plants were grown per pot.

Inoculation Procedures

In all inoculations, bacterial suspensions were prepared from 48 h old cultures, grown on yeast dextrose calcium carbonate agar (YDC) medium

Table 1. Source, host, and identifications of the *Pseudomonas syringae* pv. *phaseolicola* isolates.

Source	Host	Identification
Popayán (Colombia)	*Phaseolus coccineus*	CBP-177, PC-2, PC-3 PC-4, PC-5, PC-6
Popayán (Colombia)	*P. vulgaris*	CBP-178
Tenerife (Colombia)	*P. vulgaris*	CBP-172, CBP-173
Palmira (Colombia)	*P. vulgaris*	CBP-176
Pasto (Colombia	*P. vulgaris*	PPP-1, PPP-2, PPP-3 PPP-4, PPP-5, PPP-6 PPP-7, PPP-8, PPP-11 PPP-12, PPP-13, PPP-14 PPP-15, PPP-16, PPP-17 PPP-18, PPP-19, PPP-20 PPP-21, PPP-22
Dr. Hagedorn (Univ. of Wisc. USA)	*P. vulgaris*	CBP-196 (Race 1), CBP-197 (Race 2)
Dr. Schuster (Univ. of Nebraska, USA)	*P. vulgaris*	HB-16
Dr. S.V. Beer (Cornell Univ. USA)	*P. vulgaris*	CBP-198 (OHB)

(yeast extract (Difco), 10 g; dextrose (Difco), 20 g; $CaCo_3$, 3.5 g; agar (Difco), 20 g; and distilled water, 1000 ml). The suspension was then adjusted turbidimetrically using a spectronic 20 calorimeter (Bausch and Lomb Co.) to a concentration of 5×10^7 colony forming units (CFU) per milliliters.

Leaf reaction of bean cultivars was determined by using the water-soaking method as described by Schuster (20). The abaxial surface of either young unifoliate leaves or half-expanded first trifoliate leaves was sprayed with the bacterial suspension using a de Vilbiss atomizer attached to a compressed airline at 15 psi, until water-soaking appeared. Plants were kept in the cool part of the greenhouse where temperatures averaged 22°C.

Pod reaction was determined using the needle inoculation method (11, 18, 25, 28). Young growing green pods were used. A sterile needle was dipped into the bacterial suspension and then inserted at different points (3-5) along the pod's legth. The latter were placed in 250 ml Erlenmeyer flasks containing a small amount of distilled water and loosely plugged at the mouth with cotton wool to create a humid condition. The contents were left at room temperature (22 to 25°C) for 5 days. The diameter of water-soaking reaction around the point of inoculation was measured with the use of a stereoscope. Resistance was defined as the appearance of necrotic

spot at the point of inoculation. Fifteen to 30 readings were made for each isolate per cultivar. The experiment was repeated twice.

Seed inoculation was made by partial vacuum using a modification of Goth's method (9). Seeds in muslin bags were submerged in a bacterial suspension in a glass dessicator connected to a vacuum suction pump. They were then exposed to a partial vacuum of 415 mm of mercury for 5 minutes, after which, the negative pressure was released suddenly. The seeds were air-dried at room temperature for 3 days and then planted and grown in the growth chamber where temperature was maintained at 20°C.

Results

Biochemical Tests

On the basis of biochemical and pathogenicity tests, the isolates collected and isolated, were identified as *Pseudomonas syringae* pv. *phaseolicola*. On King's medium B, the cultures produced a diffusible fluorescent pigment, they caused levan formation on nutrient agar containing 5% (W/V) sucrose, gave positive catalase test, negative oxidase test, negative arginine dihydrolase test and did not produce hydrogen sulphide gas from nutrient broth. All the isolates were pathogenic when inoculated on to susceptible bean cultivar Red Kidney.

Race Determination and Pathogenicity Tests

Race determination was performed by inoculation of individual isolates on leaves and excised pods of cultivar Red Mexican UI3, which is resistant to race 1 but susceptible to race 2 (15). Cultivar Seminole was used as a susceptible (10) control and lines G. N. Nebraska #1 Sel 27 and Wisc HBR 72 resistant and highly resistant respectively to race 1 and 2 (16) were also included. Seed inoculation by partial vacuum was also used for comparison. A large amount of water-soaking at the site of inoculation indicated a susceptible reaction. Systemic chlorosis caused by toxin translocation was noted. Plants were rated as resistant if they showed brown necrotic lesions with some traces of water-soaking at the site of inoculation. This is the type of resistance referred to as tolerance by Patel and Walker (15). A highly resistant reaction was one where plants showed a brown necrotic (hypersensitive) reaction on the point of inoculation with no water-soaking.

All isolates except CBP 176 were pathogenic to cultivar Seminole. Large amount of water-soaking appeared on inoculated leaves 5 to 7 days after inoculation and some of the plants developed systemic chlorosis. The isolates that induced brown necrotic lesions on Red Mexican UI 3 were classified as race 1, but those that incited water-soaking were regarded as race 2. Twenty Pasto isolates were found to be race 2 whereas 7 isolates from Popayán, 1 from Palmira, and 2 from Tenerife belonged to race 1 (Table 2). Corresponding pod inoculation gave similar results of race characterization. However, isolate CBP 172 reacted as race 1 on plant inoculation but race 2 on pod inoculation.

The line G. N. Nebraska #1 Sel 27 was resistant (tolerant) to all race 1 isolates and most of the race 2 isolates. However, some Pasto isolates (PPP

Table 2. Race determination of 30 isolates of *Pseudomonas syringae* pv. *phaseolicola* from Colombia based on their pathogenicity to bean *(Phaseolus vulgaris* L.*)* cultivar "RED MEXICAN UI-3".

Isolates	Origin	Host	Race determination	
			Plant	Pod
CBP-177, PC-2	Popayan	*P. coccineus*	1	1
PC-3, PC-4, PC-5, PC-6	Popayan	*P. coccineus*	1	–[a]
CBP-178	Popayan	*P. vulgaris*	1	1
CBP-172	Tenerife	*P. vulgaris*	1	2
CBP-173			1	1
CBP-176	Palmira	*P. vulgaris*	1	1
PPP-1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16 17; 18, 19, 20, 21, 22	Pasto	*P. vulgaris*	2	2

[a] = not tested

7, PPP 18, PPP 20) incited a susceptible water-soaking reaction without any sustemic chlorosis. The line Wisc HBR 72 showed the highest degree of resistance for leaf reaction to all isolates tested. However, the line showed a susceptible pod reaction.

Most of the seeds of the cultivar Seminole inoculated by partial vacuum disintegrated in the soil whereas the controls (seeds inoculated with distilled water) germinated and grew normally. Cultivar Red Mexican UI 3 was resistant to race 1 isolates. Most of the race 2 isolates caused water-soaked lesions on the cotyledons, stems, and leaves. Plants were stunted and some of them showed systemic chlorosis.

Virulence Determination

Szarka and Velich (26) observed that virulence of *Pseudomonas syringae* pv. *phaseolicola* can be characterized by the diameter of water-soaked spots around the point of inoculation. In our studies, cultivar Seminole was used to determine the variation in virulence among the collected isolates. The lines G. N. Nebraska #1 Sel. 27, Wisc HBR 72, and Red Mexican UI 3 were also used for comparison.

The mean diameter and range of the water-soaked spots for each cultivar are presented on Table 3. The line Wisc HBR 72 (with high foliar resistance) had susceptibility as comparable to that of Seminole. The frequency distribution of the isolates on the basis of mean diameter of water-soaked spots of the 4 lines is shown in Figure 1. Isolate PPP 20, had the highest mean diameter value, followed by PPP 18, PPP 16 and PPP 22. The order (high to low) for most of the isolates in virulence was similar in the lines G.N. Nebraska #1 Sel 27, Wisc HBR 72 and Seminole. A high correlation coefficient (r) between the lines on the order of isolates virulence with

Table 3. Average diameter and range (mm) of watersoaked spots induced by 30 isolates of *Pseudomonas syringae* pv. *phaseolicola* on pods of 3 tested bean *(Phaseolus vulgaris* L.*)* lines.

Line	G. N. Nebraska No. 1 Sel 27	Seminole	Wisc HBR 72
Average	1.9	2.8	3.1
Range	0.4 - 2.8	0.5 - 4.8	0.5 - 5.3

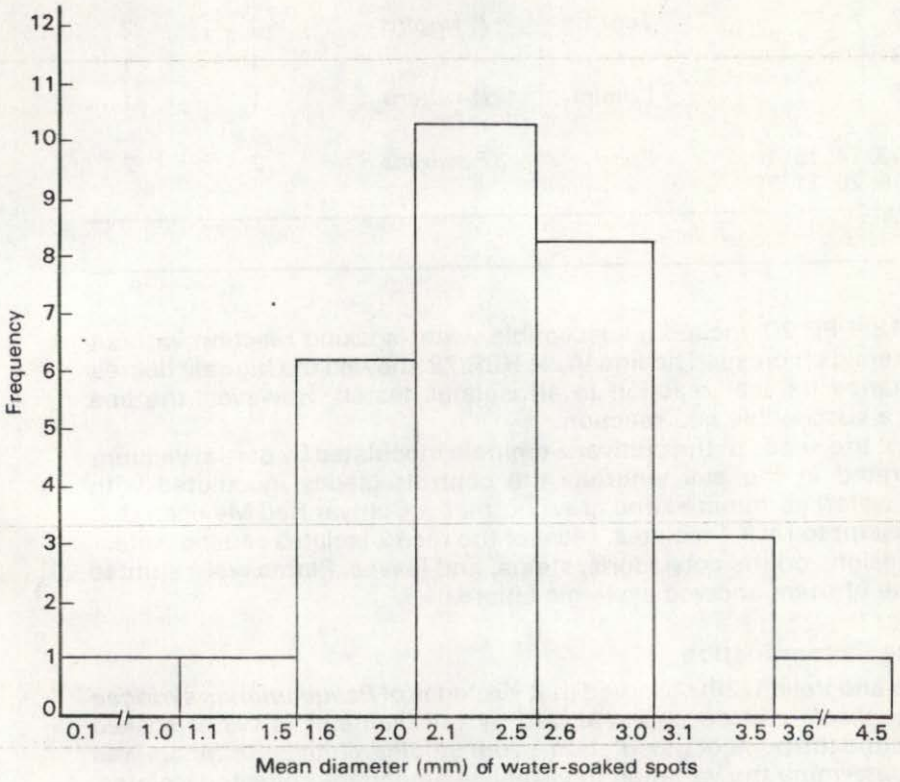

Fig. 1. Histogram showing frequency distribution of 30 isolates of *P. syringae* pv. *phaseolicola* in relation to diameter of watersoaked spots caused on 4 bean (*Phaseolus vulgaris* L.) lines.

respect to the means of the diameter of water-soaked spots was observed (Table 5).

The virulence of the isolates varied in two ways. Some of the isolates were more (or less) virulent than others on the 3 lines; isolate PPP 20 was consistently more virulent than isolates PPP 12 (Figure 2). This type of

variation was observed with most of the isolates. But, virulence of other isolates depended on the cultivar, isolate PPP 22 was less virulent that isolate PPP 18 on the line G. N. Nebraska #1 Sel 27 but was more virulent on Wisc HBR 72. These types of variations were observed both within and between the isolates of the 2 races identified (Figure 2).

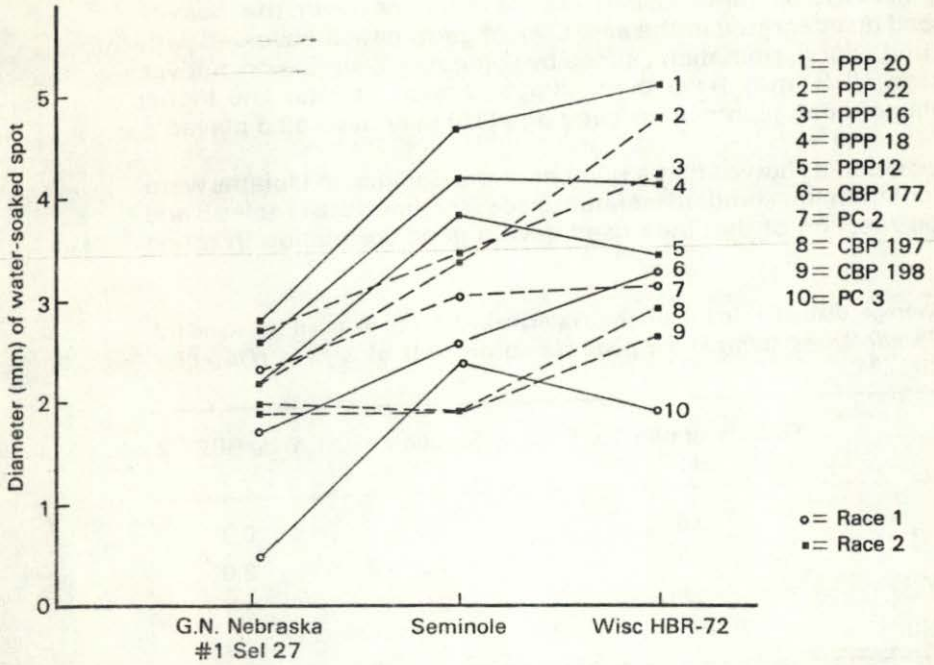

Fig. 2. Variation in diameter of watersoaked spots on pods of 3 bean (*Phaseolus vulgaris* L.) lines inoculated with isolates of *P. syringae* pv. *phaseolicola*.

Discussion

The resistance of the line G. N. Nebraska #1 Sel 27 to races 1 and 2 of *Pseudomonas syringae* pv. *phaseolicola* was previously reported by Coyne and others (6). But the water-soaking reaction incited by some of the Pasto strains in our studies suggested that they were able to overcome some of the genes' controlling resistance to race 2. Similar observations have been made by Coyne and others (5) in U.S.A. and Poryazos (16) in Bulgaria. The line, however, showed some degree of pod resistance (Table 3).

Line Wisc HBR 72 was highly resistant to all isolates and gave a hypersensitive leaf reaction, a form of induced resistance (4). The independent reactions of the plant components observed stress the importance of evaluating and selecting plants with both leaf and pod resistance.

The race studies showed that both race 1 and 2 occur in Colombia. There was a close correlation between the leaf and pod methods used to determine races on cultivar Red Mexican UI 3. The designation of isolate CBP 172 as race 1 by leaf reaction and 2 by pod reaction may be due to an intermediary character of the isolate's virulence, which is not uncommon (10).

Goth (9) observed that seed inoculation by partial vacuum was superior to soaking them in a bacterial suspension. He found, however, that heavily infested seed disintegrated in the soil. Lack of germination observed with Seminole and poor germination caused by some race 2 strains on cultivar Red Mexican UI 3, may have been due to similar effects. The higher susceptibility of bean plants at an early age (15) may have also played a role.

Virulence studies showed that a number of the Colombian isolates were more virulent than the standard isolates used for comparison (Tables 3 and 4). The pod reaction of the lines used gave a good correlation in rating

Table 4. Average diameter (mm) of the watersoaked spots induced by standard isolates of *Pseudomonas syringae* pv. *phaseolicola* on pods of 3 bean *(Phaseolus vulgaris* L.*)* lines.[a]

Line	G. N. Nebraska No. 1 Sel 27	Seminole	Wisc HBR 72
Race 1	0.6	1.0	0.9
Race 2	1.9	1.9	3.0
HB-16	1.6	2.0	2.1
CBP 198 (OHB)	2.0	1.9	2.7

[a] Average of no less than 20 values

Table 5. The correlation coefficients (r) between 4 bean *(Phaseolus vulgaris* L.*)* lines on the order of isolates in virulence with respect to the mean diameters of water-soaked spots on pods.

Lines/Cultivars	Correlation coefficients (r)
Red Mexican UI3 : Seminole 1	0.53
Red Mexican UI3 : G.N. Nebraska No. 1 Sel 27	0.57
Red Mexican UI3 : Wisc HBR 72	0.53
Seminole : G. N. Nebraska No. 1 Sel 27	0.86
Seminole : Wisc HBR 72	0.87
G. N. Nebraska No. 1 Sel 27 : Wisc HBR 72	0.87

isolates in their order of virulence although some reactions with some of the isolates showed to be host dependent. The data obtained indicated that differentiated race 1 and 2 consisted of isolates which were not homogeneous when tested with different lines. These results agree with those obtained by previous workers (19, 26). It may suffice to develop and standardize a method that can determine the whole range of virulence variability among the isolates or strains of *Pseudomonas syringae* pv. *phaseolicola*. This is important, because the information obtained thereof is essential for a successful breeding program.

Acknowledgment

This is a portion of the Ph.D. thesis submitted to the University of Nairobi by the senior author who gratefully acknowledges the grant support by the Centro International de Agricultura Tropical (CIAT) and the Ford Foundation.

Literature Cited

1. Bannerot, N., 1964. Repport thactivite, SCAP Versailles, France.
2. Costa, A. S., 1972. Investigacoes sobre molestias do feijoeiro no Brazil. En Anais do I Simposio Brazilero de Fiejao, Vol. 2, pp. 337-338. Univ. Fed. Vicosa, Minas Gerais, Brazil.
3. Coyne, D. P., and M. L. Schuster. 1974. Breeding and genetic studies of tolerance to several bean (*Phaseolus vulgaris* L.) bacterial pathogens. Euphytica 23:651-656.
4. Coyne, D. P., and M. L. Schuster. 1979. Bacterial diseases of legumes: A summary of breeding and resistance. *In:* M. H. Dickson (ed.) Annual Report Bean Improvement Coop. 22:21-22. (Mimeo). N. Y. Agri. Expt. Sta. Geneva, N.Y.
5. Coyne, D. P., M. L. Schuster and Carol Erwin. 1979. Reaction of *Phaseolus vulgaris* germplasm to a new virulent strain *Pseudomonas phaseolicola*. *In* M. H. Dickson (ed.) Annu. Rept. Bean Improvement Coop. 22:20-21 (Mimeo.), N.Y. Agr. Expt. Sta. Geneva, N.Y.
6. Coyne, D. P., M. L. Schuster and C. C. Gallegos. 1971. Inheritance and linkage of the halo blight systemic chlorosis and leaf water-soaked reaction in *Phaseolus vulgaris* L., variety crosses. Plant Dis. Rep. 55:203-207.
7. Dubin, H. J., and L. R. Ciampi. 1974. *Pseudomonas phaseolicola* en Chile. Fitopatología 9:91-92.
8. Epton, H. A. S., and B. J. Deverall. 1965. Physiological races of *Pseudomonas phaseolicola* causing halo blight of beans. Plant Pathology 14:53-54.
9. Goth, R. W., 1966. The use of partial vacuum to inoculate bean seeds with phytopathogenic bacteria. Plant. Dis. Rept. 50:110-111.
10. Hale, C. N., and J. D. Taylor. 1973. Races of *Pseudomonas phaseolicola* causing halo blight of beans in New Zealand. J. Agr. Res. 16:147-149.
11. Hill, K., D. P. Coyne, and M. L. Schuster. 1972. Leaf, pod and systemic chlorosis reactions in *Phaseolus vulgaris* to halo blight controlled by different genes. J. Amerc. Soc. Hort. Sci. 97:494-498.
12. Jensen. J. H., and J. E. Livingstone. 1944. Variation in symptoms produced by isolates of *Phytomonas medicaginis* var. *phaseolicola*. Phytopathology 34:471-480.

13. King. E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
14. Lelliot, R. A., E. Billing and A. C. Hayward. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
15. Patel, P. N., and J. C. Walker. 1965. Resistance of *Phaseolus* to halo blight. Phytopathology 55:889-894.
16. Poryazov, I., 1975a. About the disease reaction to *Pseudomonas phaseolicola* (Burkh) Dowson of some tolerant or resistant bean lines. *In* D. P. Coyne (ed.), Annu. Rept. Bean Improvement Coop. 18:58-60. (Mimeo), Univ. of Nebraska, Lincoln.
17. Poryazov, I., 1975b. Physiological races of *Pseudomonas phaseolicola* (Burkh) Dowson in Bulgaria. *In* D. P. Coyne (ed.), Annu. Rpt. Bean Improvement Coop. 18:57-58. (Mimeo). Univ. of Nebraska, Lincoln.
18. Russel, P. E., 1976. Potential sources of resistance to halo blight of beans. *In* M. H. Dickson (ed.) Annu. Rpt. Bean Improvement Coop. 19:65-67. (Mimeo.), Univ. of Nebraska, Lincoln.
19. Scroth, M. N., V. B. Vitanza, and D. C. Hildebrand. 1971. Pathogenic and nutritional variation in the halo blight group of fluorescent pseudomonads on beans. Phytopathology 61:757-882.
20. Schuster, M. L., 1955. A method of testing resistance of bean bacterial blights. Phytopathology, 45:519-520.
21. Schuster, M. L., D. P. Coyne, and E. D. Kerr. 1965. New virulent strain of halo blight bacterium overwinters in the field. (Abstr.) Phytopathology 55:1075.
22. Schuster, M. L., D. P. Coyne, and E. D. Kerr. 1966. Pathogenicity tests of Nebraska isolates of halo blight bacteria in legumes. *In* D. P. Coyne (ed.), Annu. Rpt. Bean Improvement Coop. 9:35-36. (Mimeo). Univ. of Nebraska, Lincoln.
23. Schuster, M. L., and D. P. Coyne. 1981. Biology, epidemiology, genetics and breeding for resistance to bacterial pathogens of *Phaseolus vulgaris* L. *In* J. Janick (ed.) Hort. Rev. Vol. 3. 468 pp.
24. Schwartz, H. F., 1979. Halo blight and miscellaneous bacterial diseases. *In* H. F. Schwartz and G. E. Gálvez (eds.), Bean Production Problems: diseases, insects, soil and climatic constraints of *Phaseolus vulgaris* L. CIAT, Cali, Colombia. 424 pp.
25. Szarka, J., and I. Velich. 1976. Virulence of Hungarian and standard strains of *Pseudomonas phaseolicola* on beans (*Phaseolus vulgaris* L.). *In* M. H. Dickson (ed.), Annu. Rpt. Bean Improvement Coop. 19:81-83. (Mimeo.), Univ. of Nebraska, Lincoln.
26. Szarka, J., and I. Velich. 1979. A study of the aggressivity of isolates belonging to the *Pseudomonas phaseolicola* (Burkh.) Dowson. *In* M. H. Dickson (ed.), Annu. Rpt. Bean Improvement Coop. 22:64-65. (Mimeo). N. Y. Agr. Exp. Sta. Geneva, N.Y.
27. Szarka, J., and I. Velich. 1980. Classification of isolates of *Pseudomonas phaseolicola* (Burkh.) Dowson by means of the diameter of watersoaked lesions caused on pods of beans. *In* M. H. Dickson (ed.), Annu. Rpt. Bean Improvement Coop. 23:105. (Mimeo.), N.Y. Agr. Exp. Sta, Geneva, N.Y.
28. Taylor, J. D., 1970. Bacteriophage and serological methods for the identification of *Pseudomonas phaseolicola* (Burkh.) Dowson. Annals. Appl. Biol. 66:387-396.
29. Walker, J. C., and P. N. Patel. 1964. Inheritance of resistance to halo blight of beans. Phytopathology 54:952-954.
30. Wharton, A. L., 1967. Sources of infection by physiological races of

Pseudomonas phaseolicola in dwarf beans. Plant Pathology 16:27-31.
31. Zaumeyer, W. J., 1973. Goals and means for protecting *Phaseolus vulgaris* in the tropics. *In* P. H. Graham (ed.) Potentials of field beans and other food legumes in Latin America. CIAT Ser. Seminars 2E, Feb. 26-March 1, 1973. CIAT, Cali, Colombia.
32. Zaumeyer, W. J., and R. H. Thomas. 1957. A monographic study of bean diseases and methods for their control. USDA Tech. Bul. 255 pp.