J. Amer. Soc. Hort. Sci. 102(2):182–185. 1977.

New Sources of Fusarium Root Rot Resistance in *Phaseolus vulgaris* L.[1]

A. G. Boomstra[2], F. A. Bliss, and S. E. Beebe
Department of Horticulture, University of Wisconsin, Madison, WI 53706

Additional index words. bean, *Fusarium solani* f. sp. *phaseoli,* hypocotyl lesions

Abstract. **A procedure using nutrient culture media was developed to screen bean seedlings on an individual plant basis for reaction to *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans. From over 800 accessions, 18 plant introductions and several cultivars of *Phaseolus vulgaris* were found to be either resistant or tolerant. Susceptible plants, grown with ammonium nitrate as the nitrogen source (210 ppm N) showed reduced symptoms compared to plants grown on nitrate-N only. Ammonium as the sole nitrogen source was toxic to bean seedlings at identical nitrogen concentrations. Results based on the nutrient culture technique were generally consistent with field reactions.**

Root rot of beans (*Phaseolus vulgaris*) occurs in all bean-growing areas of the world. Although the disease may be incited by several organisms, *Fusarium solani* f. sp. *phaseoli* is usually important. Yang and Hagedorn (19) found that *Fusarium* root rot caused widespread damage to beans grown in Wisconsin. However, later studies suggest that *Pythium* may also be important (8). Since improved cultural practices including use of chemicals has not provided adequate control, the development of resistant or tolerant cultivars is of prime importance.

No resistant snap bean cultivars have been developed, and only recently have tolerant dry bean cultivars been released in the U.S.[3]. Several factors have limited progress. Few good sources of resistance have been available, and PI 203958 (N203) which has been used most widely possesses many undesirable horticultural traits. The heritability of resistance is low due to complex inheritance and substantial influence of environmental factors. There are conflicting reports of the no. of genes involved and levels of dominance, depending on the parental strains studied and methods used to determine reaction to the pathogen (2, 6, 13, 15).

Methods to assess the reactions of host plants to the pathogen(s) have been used with varying degrees of success. Field testing provides more space to grow many experimental units, and the naturally occurring environmental conditions and pathogens may better represent the conditions encountered in commercial fields. However, it is difficult to study resistance to a single pathogen in the field due to the presence of several potential root rot-inciting pathogens in infested soils. Variability within the test plot and large seasonal variations often produce substantial non-genetic variation, with the result being low heritability, particularly on a single-plant basis. Since replicated comparisons permit better estimates of family performance, advancement of many families to near homozygosity prior to testing may be desirable. This is often time- and space-consuming and advancement by single seed descent should be used to minimize costs.

If greenhouse and laboratory seedling tests are to be useful, the results must be correlated to field results, and seedling tests must be indicative of the potential performance of the plant throughout its life cycle. Tests conducted under controlled conditions provide greater efficiency in no. of generations per year, ability to study individual plants and opportunity to determine inheritance of resistance to a single pathogen. An effective seedling test for reaction to a root rot-inciting pathogen may be difficult to employ since primary symptoms occur on the root and/or hypocotyl which are beneath the soil or growth medium. A non-destructive method is essential if superior plants are to be selected as parents.

Rapid, precise screening of seedlings and the production of seeds from selected plants would greatly improve the efficiency of a breeding program. A greenhouse testing procedure employing soil has been described by Wallace and Wilkinson (17). Burke and Silbernagel (5) have outlined a method for the identification of resistant individual plants in the field. Hydroponic culture techniques have been used for evaluating disease resistance and for studying different plant-pathogen interactions (1, 3, 10, 14). We developed a procedure in which plants are inoculated, then grown in nutrient culture solution to facilitate determining the reaction of bean seedlings to *F. solani.* Using this in combination with other testing methods, we identified new sources of root rot resistance. We also studied the effects of different forms of nitrogen on the expression of the disease in nutrient culture in an attempt to elucidate conflicting results (4, 11, 18).

Materials and Methods

Nutrient culture technique. Seeds were sown into containers filled with perlite and germinated in a growth chamber under light intensity of about 2.4 klx, with 25° (day) and 19°C (night) temp. The perlite was kept moist with tap water. Twelve days after sowing the plants were removed from the perlite, inoculated and immediately placed in continuously-aerated solutions, each tank containing an equivalent of one liter of nutrient culture solution per plant.

The original isolate of *F. solani* was obtained from W. J. Virgin, Del Monte Corporation. Cultures of *F. solani* were grown on Difco PDA plates. Inoculum for this seedling test was prepared by placing 5 small agar discs from a 2-week-old sporulating culture into a 500 ml Erlenmeyer flask containing 150 ml of 'Armstrong's Fusarium medium' (16). The flasks were shaken vigorously, then incubated at 28C for 72 hr. Just prior to inoculation of seedlings, the growing cultures were homogenized using a blender and diluted with distilled water to the desired spore concn. The roots and lower hypocotyl of each seedling were immersed into 100 ml of inoculum to a uniform depth and plants were placed immediately into the tanks. To insure uniform infection, fresh inoculum was used for each group of 28 plants and then discarded. Approx 10^5 macroconidia/ml provided a high level of infection on susceptible check plants.

[1]Received for publication September 13, 1976. Research supported by the College of Agricultural and Life Sciences. This investigation is part of a thesis submitted by the senior author in partial fulfillment of the requirements for the PhD degree.

[2]Present address: Instituut De Haaff, Foundation for Agricultural Plant Breeding, Wageningen, The Netherlands.

[3]Burke, D. W. 1974. Naming and release of the Red Mexican bean cultivar 'Rufus'; naming and release of two pink bean cultivars, 'Viva' and 'Roza'. USDA-ARS and the Wash. Agr. Expt. Sta., Pullman; naming and release of the pink bean cultivar, 'Gloria'. USDA-ARS and The Wash. Agr. Expt. Sta., Pullman and Calif. Agr. Expt. Sta., Davis.

Table 1. Effects of nutrient concn and inoculum concn on the severity of hypocotyl reaction of bean seedlings (*Phaseolus vulgaris*) to *Fusarium solani* f. sp. *phaseoli*.

Bean strain	Inoculum concn macroconid./ml	Hypocotyl reaction[z] Full strength[y]	Half strength[y]
Falcon (Susceptible)	1.6×10^5	2.7 ± .11	2.9 ± .14
	$.8 \times 10^5$	2.4 ± .19	2.6 ± .20
PI 203958 (Resistant)	1.6×10^5	2.6 ± .28	2.0 ± 0
	$.8 \times 10^5$	1.7 ± .25	2.0 ± 0

[z]Concn of modified Hoagland's Soln Hoagland and Snyder (7).

[y]Average hypocotyl reaction of 24 plants based on visual observations 13 days after inoculation. Severity of reaction: 0-1.5, slight; 1.6-2.5, moderate; 2.6-4.0, severe.

Four homozygous plants provided a good estimate of disease reaction to *F. solani*. However, more plants were required to evaluate heterogeneous populations or segregating families. Two resistant (PI 203958) and 2 susceptible ('Tempo' or 'Cascade') check plants were included with each group of 24 test plants. Susceptible plants showed lesions on the hypocotyl and initial browning of the roots within 48-72 hr after inoculation. The development of the lesions was observed daily by simply raising the tank cover which supported the plants. Physical damage to the plants was avoided and they were allowed to grow until classification of each plant was made within 10-14 days.

Greenhouse pot test. A procedure similar to that described by Wallace and Wilkinson (17), except without the removable collar, was used to facilitate the detection of resistant strains. At planting time a suspension containing approx 2.5×10^6 macroconidia/ml was poured over 5 seeds per pot, the seeds were covered with soil to a uniform depth and the soil moistened with tap water. Disease reactions were based on severity of hypocotyl lesions and plant vigor. Percentage emergence was also recorded.

Table 2. Effect of nitrogen source in the nutrient solution and inoculum concn on the damage of 'Falcon' bean seedlings by *Fusarium solani* f. sp. *phaseoli*.

Infection type[x]	No. of plants NO_3^-[y] Inoculum concn[z] I	II	III	NH_4NO_3[y] Inoculum concn[z] I	II	III
slight	1	0	0	13	9	0
moderate	4	0	2	53	13	1
severe	39	48	16	4	26	23

[z]I = 0.53×10^5; II = 0.6×10^5; III = 1.1×10^5 macroconidia/ml.

[y]Nitrogen source, 210 ppm.

[x]Slight = no or few lesions on hypocotyl and vigorous growth; moderate = many lesions, some beginning to coalesce, and good growth; severe = lesions cover hypocotyl or completely rotted, growth severely reduced.

Field tests. Some entries, evaluated using the nutrient culture technique and the greenhouse pot test, were also grown in a field known to produce a high incidence of root rot, at the Hancock, Wisconsin Experiment Station. Seeds were sown 10 cm apart in rows spaced 1 m wide and the level of infection on mature plants evaluated near the end of the growing season.

Reactions of plants grown in pots and the field were evaluated for hypocotyl lesion severity and root damage using the following scale: 0 = no lesions or damage; 20 = small lesions, slight damage; 50 = moderate; 80 = extensive lesions and damage; 100 = hypocotyl completely rotted, plants dead or missing. A disease index was computed as the mean reaction of all plants in a family. Two indices were computed in 1975. Index 1 differed from Index 2 described above, in that Index 1 excluded plants that did not emerge or died early in the season.

Ratings of individual seedlings evaluated using the nutrient culture technique were based on damage to the hypocotyl and

Table 3. Comparisons of reactions of 11 *Phaseolus vulgaris* cultivars to *Fusarium solani* f.sp. *phaseoli* when evaluated by the nutrient culture technique, greenhouse pot test and performance in a root rot-infested field at Hancock, Wisc.

Cultivar on line	Testa color[v]	Seedling tests: Nutrient culture index[w] Mean	Range	Pot test index[y]	Field tests: 1973[y]	1974[z]	1975 Emergence (%)	1975 Index 1[x]	1975 Index 2[y]
Resistant									
N203 (PI 203958)	B	1.2	.5-2	33	18	H	71	19	41
Tolerant									
Black Turtle Soup	B	2.4	2-3	73	52	H	74	26	45
State Half Runner	W	2.2	2-3	69	25	H	81	29	42
Slightly tolerant									
Resist. Asgrow Valentine	B	2.5	2-3	51	40	I	–	–	–
Bush Romano 14	Br	2.3	2-3	71	–	H	65	42	62
Cherokee Wax	B	2.6	2-3	71	45	I	80	27	42
Bush Romano 14	W	2.3	2-3	100	–	I	–	–	–
Provider	B	2.8	2-3	80	47	I	80	49	58
Susceptible									
Cascade	W	2.8	2-3	98	67	S	36	43	80
Tempo	W	3.5	3-4	–	55	S	–	–	–
Tenderette	W	3.4	3-4	–	60	S	–	–	–
LSD 5%								15	16

[z]Visual ratings of foliage vigor (3 reps): S = stunted; I = intermediate; H = healthy.

[y]Disease index based on damage to hypocotyl and roots: 0 = no damage; 20 = slight damage; 50 = moderate; 80 = severe damage; 100 = hypocotyl rotted, plants dead or missing.

[x]Disease index same as y, except only plants standing at end of season were included in index.

[w]Mean index based on hypocotyl and foliage ratings: 0-1.5 = slight hypocotyl damage and vigorous growth; 1.6-2.5 = moderate damage and growth; 2.6-4.0 = severe damage and stunted growth.

[v]B = black; Br = brown; W = white.

Table 4. Reactions of *Phaseolus vulgaris* lines to *Fusarium solani* f.sp. *phaseoli*, as determined by the nutrient culture technique, greenhouse pot test and performances in a root rot-infested field at Hancock, WI.

Line	Testa color[v]	Seedling tests: Nutrient culture index[w] Mean	Seedling tests: Nutrient culture index[w] Range	Seedling tests: Pot test index[y]	Field tests: 1974[z]	Field tests: 1975 Emergence (%)	Field tests: 1975 Index 1[x]	Field tests: 1975 Index 2[y]
Resistant								
PI 325619	B	1.5	(1-2)	–	H	60	2	41
PI 311991	B	1.0	(1)	57	H	70	4	33
PI 311987	B	1.0	(1)	55	H	73	5	30
PI 311989	B	1.5	(1-2)	61	H	78	7	28
PI 312033	B	1.2	(1-1.5)	73	H	76	7	29
PI 312028	B	1.2	(1-1.5)	49	H	61	7	42
PI 224737	W	1.2	(1-1.5)	–	H	43	7	59
PI 312043	B	1.2	(1-1.5)	81	H	80	11	29
PI 311917	PPu/Mo	1.0	(.5-1.5)	50	H	51	11	36
PI 312077	B	1.1	(.5-1.5)	50	H	68	13	41
PI 310607	Ppu/Mo	1.2	(1-1.5)	–	H	80	15	33
PI 309726	PPu/Mo	1.5	(1-2)	49	H	75	17	37
PI 319606	Pu	1.7	(1-2)	36	H	66	17	44
PI 203958 (N203)	B	1.2	(.5-2)	33	H	71	19	41
PI 309801	B	1.2	(1-1.5)	54	H	60	23	53
PI 312041	B	1.2	(1-1.5)	56	H	78	25	41
PI 312062	B	2.1	(1-2.5)	42	H	76	25	43
PI 224730	W	1.5	(1-2)	63	H	70	34	53
Tolerant								
W73-507	W	–	–	–	–	66	21	47
W73-501	W	–	–	–	–	69	35	51
W73-510	W	–	–	–	–	71	40	57
PI 311975	B & Br	1.2	(1-2)	77	H	66	41	60
Susceptible								
Cascade	W	2.8	(2-3)	98	S	36	43	80
LSD 5%							15	16

[z]Visual ratings of foliage vigor (3 reps): S = stunted; I = intermediate; H = healthy.

[y]Disease index based on damage to hypocotyl and roots: 0 = no damage; 20 = slight damage; 50 = moderate; 80 = severe damage; 100 = hypocotyl rotted, plants dead or missing.

[x]Disease index same as y above, except only plants standing at end of season were included in index.

[w]Mean index based on hypocotyl and foliage ratings: 0-1.5 = slight hypocotyl damage and vigorous growth; 1.6-2.5 = moderate damage and growth; 2.6-4.0 = severe damage and stunted growth.

[v]B = black; Br = brown; W = white; PPu/Mo = purple mottled; Pu = purple; TStr = tan striped.

vigor of the foliage. Plants having slight hypocotyl damage and vigorous foliage received ratings of 0-1.5, those with moderate hypocotyl damage and good foliage, 1.6-2.5; and those with severe damage and stunted growth, 2.6-4.0. The disease index of the line was computed as the mean value of individual plant ratings.

Plant materials. In 1972, 106 cultivars and breeding lines from the USA and 25 foreign accessions were studied for their reaction to the single isolate of *F. solani* using the nutrient culture technique. During 1972-73, 700 plant introductions (PI) originating in Mexico were obtained from the Western Regional Plant Introduction Station, Pullman, Washington and screened for *Fusarium* resistance using the nutrient culture technique. Based on superior seedling resistance, 50 PI's were selected for further evaluation. Because some accessions produced little or no seed due to daylength sensitivity, only 18 promising PI's were evaluated in the field in 1974 and 1975, and 15 PI's in the pot test.

Results and Discussion

The nutrient culture technique provided a means to distinguish between reactions of bean seedlings having different levels of root rot resistance. It was possible to alter the severity of the test by varying the concn of the inoculum and/or that of the nutrient solution (Table 1). Both resistant and susceptible plants showed a severe reaction when grown in full strength soln at the high inoculum concn. When grown in either half- or full-strength nutrient soln and subjected to a concn of 0.8×10^5 macroconidia/ml resistant and susceptible plants showed differences in hypocotyl lesion severity. The form of nitrogen in the nutrient solution had a significant effect upon expression of the disease (Table 2). The symptoms of susceptible plants were less severe when nitrogen was supplied as ammonium nitrate compared to nitrate as the sole nitrogen source. This response was observed when low inoculum concn were used, but at high concn plants grown on either nitrogen source died within 10 days. Uninoculated plants grew better when supplied with ammonium nitrate rather than nitrate only, but when ammonium was the sole nitrogen source at similar concn (210 ppm N), growth was inhibited.

Our results differ from those of Weinke (18), Huber et al. (9) and Maurer and Baker (12), who found that nitrate-N decreased *Fusarium* root rot severity compared to ammonium-nitrogen. However, in those studies ammonium concn were not specified and it could have been supplied at levels which were toxic to the plants, with the results being a supposed increase of root rot severity. Our results cannot be compared directly with those of Burke and Nelson (4) who found no significant differences between the effects of different forms of nitrogen fertilizer on root rot severity in field experiments where other factors such as nitrogen fixation and the presence of numerous micro-organisms and other pathogens in addition to *F. solani* often play an important role. The possibility that root rot damage can be reduced somewhat by proper and timely application of certain forms of nitrogen fertilizer deserves further investigation. In other experiments we found that a potassium

level of 390 ppm increased root rot severity in nutrient culture tests.

Although the degree to which lesions developed on the hypocotyls of seedlings evaluated using the nutrient culture technique depended in part on several external factors, differences between bean cultivars and accessions were also evident. Susceptible plants were usually dead within two weeks after inoculation. Resistant plants developed vigorous roots, had few lesions on the hypocotyl, and showed good foliage development. The resistant plants were maintained for seed production by transplanting into pots filled with soil.

Using the nutrient culture technique, only 7.2 m^2 of greenhouse space was required to screen more than 800 entires in about 4 months. Several cultivars were found to have a useful level of resistance to the culture of *Fusarium* we used and field tolerance to root rot. 'State Half Runner' is the most promising for use in snap bean improvement because of no pigmentation in the seed and foliage, determinate growth habit and desirable pod types (Table 3). 'Resistant Asgrow Valentine', 'Cherokee Wax' and 'Bush Romano' also show promise as breeding materials. Seventeen plant introductions of Mexican origin showed levels of resistance comparable to N203 (Table 4). Although the results based on the nutrient culture technique, the pot test and field tests do not agree completely, these introductions appear to be promising sources of resistance or tolerance to *Fusarium* root rot. Since pure cultures of *F. solani* f.sp. *phaseoli* were used in the nutrient culture technique and the pot test, and several other pathogens are present in the field some discrepancies are expected. These differences in performance may indicate that pathogens other than *Fusarium* are important in the field and that all accessions are not uniformly resistant to the major root rot-inciting pathogens. However, the performances under different conditions were consistant enough to allow identification of potentially useful sources of resistance.

Literature Cited

1. Armstrong, G. M. 1941. A solution culture infection method used in the study of Fusarium wilts. *Phytopathology* 31:549-553.
2. Azzam, H. A. 1958. Inheritance of resistance to *Fusarium* root rot in *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. *Diss. Abstr.* 18:32-33.
3. Broadfoot, W. C. and L. E. Tyner. 1938. Studies in foot and root rot of wheat. VI. Methods of securing infection of wheat seedlings for study in nutrient solutions. *Canad. J. Res.* 16:253-261.
4. Burke, D. W. and C. E. Nelson. 1968. NH_4^+ versus NO_3^- fertilization of dry beans on *Fusarium* infested land. *Wash. Agr. Expt. Sta., Cir.* 490.
5. _____ and M. J. Silbernagel. 1965. Testing beans for field tolerance to *Fusarium* root rot. *Phytopathology* 55:1045-1046.
6. Hassan, A. A., D. H. Wallace, and R. E. Wilkinson. 1971. Genetics and heritability of resistance to *Fusarium solani* f. *phaseoli* in beans. *J. Amer. Soc. Hort. Sci.* 96:623-627.
7. Hoagland, D. R. and W. C. Snyder. 1933. Nutrition of strawberry plants under controlled conditions: (a) Effects of deficiencies of boron and certain elements, (b) Susceptibility to injury from sodium salts. *Proc. Amer. Soc. Hort. Sci.* 30:288-294.
8. Hock, H. C., D. J. Hagedorn, D. L. Pinnow, and J. E. Mitchell. 1975. Role of *Pythium* spp. as incitants of bean root and hypocotyl rot in Wisconsin. *Plant Dis. Rptr.* 59:443-447.
9. Huber, D. M., R. D. Watson, and G. W. Steiner. 1965. Crop residue, nitrogen and plant disease. *Soil Sci.* 100:302-308.
10. Jordan, V. W. L. 1973. A procedure for the rapid screening of strawberry seedlings for resistance to *Verticillium wilt. Euphytica* 22·367-372.
11. Maier, C. R. 1968. Influence of nitrogen nutrition on *Fusarium* root rot of pinto beans and on its suppression by barley straw. *Phytopathology* 58:620-625.
12. Maruer, C. L. and R. Baker. 1965. Ecology of plant pathogens in soil. II. Influence of glucose, cellulose, and inorganic nitrogen amendments on the development of bean root rot. *Phytopathology* 55: 69-72.
13. McRostie, G. P. 1921. Inheritance of disease resistance in the common bean. *J. Amer. Soc. Agron.* 13:15-32.
14. Mussell, H. and F. E. Fay. 1971. A method for screening strawberry seedlings for resistance to *Phytophthora fragariae. Plant Dis. Rptr.* 55:471-472.
15. Smith, F. A. and B. R. Houston. 1960. Root rot resistance in common beans sought in a breeding program. *Calif. Agr.* 14(9):8.
16. Tuite, J. 1969. Plant pathological methods, fungi and bacteria. Burgess, Minneapolis.
17. Wallace, D. H. and R. E. Wilkinson. 1965. Breeding for *Fusarium* root rot resistance in beans. *Phytopathology* 55:1227-1231.
18. Weinke, K. E. 1962. The influence of nitrogen on the root disease of beans caused by *Fusarium solani* f.sp. *phaseoli. Phytopathology* 52:757 (Abstr.).
19. Yang, S. M. and D. J. Hagedorn. 1966. Root rot of processing bean in Wisconsin. *Plant Dis. Rptr.* 50:578-580.