A Greenhouse Screening Protocol for Fusarium Root Rot in Bean

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Abstract. Root rot, caused by Fusarium solani f.sp. phaseoli, is a serious disease of bean for which successful control has been elusive. Genetic resistance to the pathogen is considered quantitative and is strongly influenced by environmental factors. To reduce environmental variation and facilitate selection in earlier generations, an accurate, consistent, and nondestructive greenhouse screen was developed for the evaluation of Fusarium root rot resistance in bean. We describe a protocol that involves the germination of seedlings in perlite, inoculation of roots and hypocotyls 10 days after planting and evaluation within 4 weeks. The accuracy of this greenhouse screen was confirmed by demonstrating significant correlations between greenhouse and field ratings. Two experiments that included 24 and 21 diverse bean genotypes, respectively, were performed in the greenhouse and the ratings were correlated with field ratings over two growing seasons. Correlation coefficients between the greenhouse and field ratings were significant and as high as 0.99. Numerous genotypes can be evaluated within a short time for relatively minimal costs and labor. Furthermore, once roots have been rated and dipped in fungicide, plants can be transplanted for production of seed. This simple, rapid, and inexpensive protocol reduces environmental variation inherent to field ratings, thereby more accurately representing physiological resistance while maintaining a close association with observed field ratings.

Fusarium root rot, caused by the soilborne fungal pathogen Fusarium solani f.sp. phaseoli (Berk. & Snyder. & Hans.), has been reported to reduce bean yield in California, Colorado, Wisconsin, Washington, Nebraska, North Dakota, New York, Minnesota, and Michigan (Burke and Hall, 1991; Burke and Silbernagel, 1965; Estevez de Jensen et al., 1998; Keenan et al., 1974; Saettler and Anderson, 1978; Sippell and Hall, 1982; Smith and Houston, 1960; Steadman et al., 1975). The pathogen invades underground roots and stems directly through the epidermis, stomates and wounds (Christou and Snyder, 1962). Infection results in characteristic red streaks along the base of the hypocotyl, discoloration and deterioration of the main taproot and laterals. Severely diseased roots cannot sustain growth and development of the above-ground plant, resulting in visible symptoms such as chlorosis, defoliation and stunting (Abawi and Pas- tor Corrales, 1990). Fusarium root rot is a particular problem in dark red kidney beans due, in large part, to the lack of any degree of genetic tolerance to this pathogen. Compounding this problem, stresses such as soil compaction and drought conditions, aggravated by the sandy soils on which this bean class is produced, inhibit root growth and vigor and may ultimately lead to increased susceptibility to disease.

Genetic resistance to F. sp. f.sp. phaseoli exists in P. vulgaris and root rot–resistant genotypes have been documented (Silbernagel, 1987; Wallace and Wilkinson, 1966). Progress toward improving root rot resistance, however, has been limited. Polygenic inheritance, compounded by strong environmental effects, has limited improvement of physiological disease resistance under field conditions (Beebe et al., 1981; Boomstra et al., 1977; Bravo et al., 1969; Hassan et al., 1971; Silbernagel, 1990; Smith and Houston, 1960; Tu and Park, 1993; Wallace and Wilkinson, 1965). Furthermore, evaluating roots is difficult and not amenable to large-scale population analysis. Many researchers examining Fusarium root rot resistance in bean have utilized greenhouse screening methods with variable results (Baggett and Fraizer, 1959; Baggett et al., 1965; Beebe et al., 1981; Boomstra et al., 1977; Hassan et al., 1971; Tu and Park, 1993; Wallace and Wilkinson, 1965). In most cases, an association between greenhouse and field data was not established because a field trial with genotypes similar to those used in the greenhouse screen was not conducted. Soil medium, form and concentration of inoculum, hypocotyl vs. root ratings, number of ratings, labor, and cost must be considered in the development of a useful greenhouse screening method. Our objective was to develop a simple, controlled greenhouse screen to evaluate large numbers of bean genotypes for resistance to Fusarium root rot while eliminating environmental variation inherent in field experiments. The screening method presented will ultimately be valuable in the identification and evaluation of physiological resistance mechanisms present in bean.

Materials and Methods

Isolation and preparation of inoculum. Fusarium solani was isolated from kidney bean plants collected from production fields in Presque Isle County, Mich. Roots infected with F. sp. f.sp. phaseoli sampled from these fields were stored at 4°C until isolation. Stem and root sections were bisected longitudinally and then cut into 0.5-cm pieces. Segments from each plant were wrapped in cheesecloth and soaked under running deionized water for 24 h. Stem sections were then surface sterilized in 95% ethanol, soaked in 10% Clorox® for 5 min, and dried on a paper towel moistened with 10% Clorox®. Sections were placed in a petri dish containing water agar (15 g agar in 1 L of water) and left under continuous light. Four to 5 d later, mycelia emanating from plant sections were removed at the growing point and transferred to potato dextrose agar (PDA) medium containing 100 mg L–1 ampicillin. These isolates were grown at room temperature under continuous light for 4 to 5 d and then replated on fresh PDA plus ampicillin. Ten milliliters of distilled water were added to spore cultures after 4 to 5 d of growth, and mycelia and conidia were scraped into solution for storage. Two milliliters of this solution was then added to a 20-mL screw cap test tube with 3 mL of sterilized potting soil. The test tubes were held under continuous light for 4 to 5 d and then transferred to the refrigerator. One milliliter of this inoculum was grown on PDA plus ampicillin plates as needed. Isolates were characterized by conidial shape, blue spore color on PDA, and pathogenicity on bean (Burke and Hall, 1991). Continued culture of the fungus and verification of pathogenicity was performed by inoculating several plants with the specific isolate and reisolating the fungus using the above procedure.

Greenhouse screen. Several greenhouse screening tests were attempted and the following protocol was adopted. Using perlite-filled, 72-well greenhouse flats, a single seed was germinated in each well, using three to six seedlings per cultivar per replication. The perlite was saturated with half-strength Hoagland’s solution at planting and flats were fertilized once every week thereafter with the same solution. Ten days after planting, 10 mL of a 2 × 106 spore suspension of F. sp. f.sp. phaseoli macroconidia was applied over the base of the hypocotyl using a 4-L hand pump sprayer. Inoculum was prepared by scraping PDA plates of F. sp. f.sp. phaseoli macroconidia into distilled water quantifying with a hemocy-
tomato, and adjusting to the proper spon concentration. The Hawks 2b isolate of *F. sp. f.sp. phaseoli* was used for all inoculations. Fourteen days after inoculation, seedlings were removed from flats, cleaned of excess Perlite and rated on a scale from 1 to 7 (Table 1). When seed was desired from inoculated seedlings, roots were dipped in a fungicidal solution of benomyl and transplanted to Bacto potting soil (Michigan Peat Co., Houston).

The aforementioned greenhouse screen was used to evaluate two groups of bean genotypes differing in genetic background. The first experiment (GH96) included 24 cultivars and advanced breeding lines, some of which were previously reported as possessing good levels of resistance to Fusarium root rot. The 24 genotypes were replicated four times and three plants were sown per replication (Table 2). The second greenhouse evaluation (GH97) consisted of 21 of the most popular Michigan-grown bean cultivars and advanced breeding lines. Six of the seven commercial market classes (except cranberry) were represented in this experiment (Table 3). Greenhouse evaluation was similar to that used in GH96 and consisted of four replications with three plants per replication. GH97 was repeated three times (Sept. 1997, Feb. 1998, and Dec. 1998) to verify reproducibility of this greenhouse screening protocol.

**Field trials.** Field trials were conducted on a Grace very fine sandy loam soil (coarsely, mixed, frigid, Typic Hapludalfs) in Presque Isle County, Mich., where previous *F. solani* f.sp. *phaseoli* infections had been reported and from where the Hawks 2b isolate was collected. The first field trial was conducted in 1996 and included the same 24 cultivars and advanced breeding lines described for GH96 (Table 2). Two-row plots, 7.6 m long, were hand planted on 17 June 1996 and replicated four times. Between-row spacing was 0.76 m. Two weeks after planting, plots were thinned to 13 plants/m. Plots were arranged in a randomized complete-block design with four replications. Weeds were controlled chemically and manually. Twice during the growing season at pod fill (51 and 70 d after planting (DAP)), five random plants from each plot were carefully removed from the soil using a shovel and rated for Fusarium root rot symptoms using the root rating scale described in Table 1. Yield was recorded on a total harvestable area of 10 m² per plot. Seed moisture samples were also taken for each plot and yields were adjusted to 18% moisture (Table 4). Field root rot evaluations were averaged over the two ratings for each genotype to provide an average score.

A second field trial consisting of the same 21 cultivars and advanced breeding lines described for GH97 was conducted in 1997 on the same field in Presque Isle County, Mich. A randomized complete-block design experiment with three replications was planted on 13 June 1997 with plot area and design similar to those used in 1996. Weeds were controlled manually. Yields were determined twice during pod fill, 51 and 70 d after planting, (DAP), and the scoring procedure was identical with that used in 1996.

**Statistical analysis.** Analyses of variance for all experiments were performed using the GLM procedure of SAS (SAS Institute, 1994). Pearson rank correlation coefficients between all ratings and yield data were calculated using the PROC CORR procedure of SAS.

### Results

Significant genetic variation for root rot ratings was observed for all greenhouse and field experiments conducted, and for yield and 100-seed weight in both field trials. Root rot ratings ranged from 1.9 to 4.8 with a mean of 3.0 in greenhouse experiments (Table 2); the black bean breeding line B95219 had the lowest rating, followed by N203, a traditional source of resistance (Wallace and Wilkinson, 1966). The large-seeded genotype FR266, which derives its resistance from N203, had one of the lowest root rot ratings in the field but ranked higher in greenhouse experiments. Black bean genotypes tended to be the most resistant, followed by navy beans. Pinto and pink-seeded genotypes scored moderately well (2.6 to 3.1), and the most susceptible types were the large-seeded white, dark, and light red kidney beans. 'Isles' and 'Montcalm', both dark red kidney beans, had the highest rating.
Table 3. Genotype, seed type, greenhouse rating (GH97), first (RRR1) and second (RRR2) field ratings, and average field root rot ratings (AVG), seed yield, and 100-seed weight (SW) for 21 genotypes grown in Presque Isle County, Mich., in 1997.

| Genotype  | Seed type | GH97  | RRR1  | RRR2  | AVG  | Yield (kg·ha⁻¹) | SW (g) |
|-----------|-----------|-------|-------|-------|------|----------------|-------|
| Avanti    | Navy      | 2.4   | 2.0   | 2.8   | 2.4  | 2410           | 19.0  |
| Newport²  | Navy      | 2.4   | 2.3   | 2.5   | 2.4  | 1849           | 19.3  |
| T39³      | Black     | 2.4   | 2.6   | 2.4   | 2.5  | 953            | 18.0  |
| A300 shielding | Cream   | 2.5   | 2.5   | 2.4   | 2.5  | 2267           | 20.1  |
| Phantom    | Black     | 2.5   | 2.9   | 2.2   | 2.5  | 1739           | 18.0  |
| Mackinac³ | Navy      | 2.6   | 2.0   | 3.2   | 2.6  | 1740           | 19.1  |
| N94082⁴    | Navy      | 2.6   | 2.7   | 2.6   | 2.6  | 1529           | 18.2  |
| Mayflower  | Navy      | 2.8   | 3.2   | 2.4   | 2.8  | 1091           | 19.5  |
| Vista      | Navy      | 2.9   | 2.7   | 3.0   | 2.9  | 1378           | 14.8  |
| Alpiner    | GN        | 3.2   | 2.9   | 3.5   | 3.2  | 997            | 29.5  |
| Huron ⁵    | Navy      | 3.3   | 2.8   | 3.7   | 3.3  | 1635           | 20.5  |
| Kodiak     | Pinto     | 3.4   | 3.7   | 3.2   | 3.4  | 937            | 30.2  |
| Matterhorn | GN        | 3.7   | 3.1   | 4.3   | 3.7  | 791            | 30.1  |
| K93629     | LRK       | 4.1   | 3.8   | 4.5   | 4.2  | 740            | 55.8  |
| Aztec      | Pinto     | 4.1   | 3.6   | 4.7   | 4.1  | 1153           | 33.7  |
| Red Hawk⁶  | DRK       | 4.4   | 4.1   | 4.6   | 4.4  | 1565           | 55.1  |
| Chinook 2000⁶ | LRK     | 4.5   | 3.8   | 5.1   | 4.5  | 1717           | 54.4  |
| Isles⁷     | DRK       | 4.5   | 4.1   | 4.9   | 4.5  | 638            | 61.8  |
| K93613     | LRK       | 4.7   | 4.0   | 5.3   | 4.7  | 1797           | 63.7  |
| Chinook⁷   | LRK       | 5.0   | 4.5   | 5.5   | 5.0  | 493            | 47.0  |
| Montcalm    | DRK       | 5.1   | 4.6   | 5.5   | 5.1  | 1279           | 52.0  |
| Mean       |           | 3.5   | 3.2   | 3.7   | 3.5  | 1369           | 33.2  |
| LSD0.05    | LSLD0.05  | 0.8   | 1.3   | 1.1   | ---  | 873            | 2.2   |
| cv (%)     |           | 14.5  | 24.3  | 17.9  | ---  | 30.5           | 3.2   |

Table 4. Pearson rank correlation coefficients for yield, 100-seed weight (SW), first (RRR1) and second field (RRR2) root rot ratings, average field root rot rating (AVG), and greenhouse (GH) evaluations for each group of genotypes (GH96 and GH97). GH96 values are presented in the upper right-hand diagonal, whereas the GH97 values are printed in the lower left-hand diagonal.

|          | Yield | SW  | RRR1 | RRR2 | AVG  | GH96 |
|----------|-------|-----|------|------|------|------|
|          |       |     |      |      |      |      |
| Yield    | 0.69**| 0.63**| 0.74***| 0.72***| 0.61**|     |
| SW       | 0.33  | 0.72***| 0.89***| 0.85***| 0.87***|     |
| RRR1     | 0.38  | 0.86***| 0.87***| 0.95***| 0.77**|     |
| RRR2     | 0.54**| 0.87***| 0.84***| 0.98***| 0.83**|     |
| AVG      | 0.47**| 0.91***| 0.94***| 0.97***| 0.73**|     |
| GH97     | 0.46**| 0.91***| 0.94***| 0.97***| 0.99**|     |

³,⁴,⁵,⁶,⁷Significant at *P = 0.05, 0.01, or 0.001, respectively.

Discussion

Our objectives were to develop a simple greenhouse screen to evaluate the reaction of lateral bean roots to inoculation with Fusarium root rot and correlate that response with field ratings. The current perlite-based greenhouse...
screen was inexpensive, required little main-
tenance, and permitted the evaluation of large
populations, as 12 to 24 genotypes can be
grown in one 54 × 27 × 6-cm flat. Minimal
requirements for greenhouse space ensure that
large populations can be evaluated with lim-
ited resources. Time and labor constraints
were also minimized since the time from plant-
ing to evaluation took 4 weeks and only daily
watering and weekly fertilization were neces-
sary. Using perlite, an inexpensive soil me-
dium, roots could be cleared of adhering
particles with relative ease so that ratings
were not based on hypocotyl symptoms alone.
Burke and Barker (1966) demonstrated that
hypocotyl ratings are not adequate indicators
of root rot damage. Using infested and noninfested
islands of soil surrounding the
hypocotyl and taproot, they found that sev-
erely diseased hypocotyls could support
adequate plant growth and development. Our
observations confirmed that lateral root dam-
age was the more important contributor to
yield reductions. The greenhouse screening
presented in this paper provides a means to
evaluate both hypocotyl and lateral root dam-
age. The reddish discoloration typical of
F. sp. f.sp. phaseoli infection in highly susceptible
genotypes is striking and can be easily scored
using the visual rating system for both hypo-
cotyl and roots (Table 1).

Positive and highly significant correlations
between greenhouse and field ratings for 11
bean genotypes common to both field trials,
ensure confidence in this screening technique
(Tables 4 and 5). These positive and signifi-
cant relationships between the proposed green-
house screening method and replicated field
ratings prove that a controlled greenhouse screen
method that would reduce the
influence of environmental factors.

In addition to offering a more controlled
screening environment for root rot, this proto-
col is nondestructive and allows for the testing
of early generation material for which ad-
vanced generation seed is desired. Once rated,
plants can be dipped in fungicide solution and
transplanted. Considering the aforementioned
criteria for a simple, consistent, accurate, in-
expensive, nondestructive and rapid green-
house screen to evaluate bean genotypes for
Fusarium root rot resistance, the proposed
protocol accurately and consistently satisfies
these requirements.

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