Screening for Resistance to *Fusarium oxysporum* in Soybean

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ABSTRACT

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Soybeans (*Glycine max*) infected with *Fusarium oxysporum* yielded up to 59% less than uninfected controls in field studies at Georgetown and Newark, DE. Of 10 cultivars tested, York was among the most resistant and gave the highest percentage of uninoculated yield at both locations. Resistance to *F. oxysporum* was detected using greenhouse screening techniques, and a rapid test tube screening method was developed that determined resistance levels in 16 days. Cultivars resistant to *F. oxysporum* were also detected using a mycelium production index. This new method is not destructive and permits saving resistant plants for use in a breeding program.

Various races of *Fusarium oxysporum* Schlecht. cause a wilt disease of soybean (*Glycine max* (L.) Merr.) that reduces yield (1,7,13,15). Since 1976, losses from Fusarium blight have occurred in Delmarva (the Delaware, Maryland, and Virginia peninsula). Losses can reach as high as 59% of average yield (7,13; S. Leath, *unpublished*). The pathogen occurs in most soybean-growing areas, and there is potential for widespread occurrence of the disease. In fields where *F. oxysporum* is present, it is common to find healthy, green plants interspersed among dead ones (7). This suggests that resistance to *F. oxysporum* may exist within cultivars. An attempt should be made to develop soybeans resistant to *F. oxysporum*. A rapid screening test in soybeans that would accurately predict field resistance to this pathogen is needed.

Many techniques have been used to evaluate plants for their reaction to fungal pathogens (3,4,8,10,12,14,18). Test tube culture of plants has been used extensively to test pathogenicity of pathogen isolates (1), to study cross-protection (4), and to determine *formae speciales* (1). The best techniques are those that result in rapid disease development. Also, nondestructive techniques are valuable to breeders who want to use single plant selections in segregating populations.

The purposes of this study were to determine reactions of soybeans to *F. oxysporum* in both field and greenhouse tests and to develop a rapid and nondestructive screening method in the growth chamber.

MATERIALS AND METHODS

Ten soybean cultivars from three maturity groups were used: Emerald, Verde, and Williams (group III); Miles, Union, and Ware (group IV); and Bedford, Essex, Forrest, and York (group V).

These cultivars were selected for several reasons. Essex and Williams are important in the Delmarva region. Verde and Emerald (green-seeded vegetable soybeans) appeared extremely susceptible to *F. oxysporum* in preliminary tests, and Ware has more resistance to *F. oxysporum* than other cultivars (13). Union and Miles are popular cultivars in the Delmarva region. York appears more resistant to root rots than other cultivars evaluated by Maryland researchers (11). Forrest and Bedford have resistance to races of the soybean cyst nematode (*Heterodera glycines*), and infection of soybeans by *F. oxysporum* is known to be affected by the feeding of *H. glycines* (15).

Field plot design at Newark and Georgetown, DE, was a randomized complete block with five and four replicates, respectively. All plots consisted of four 6.1-m rows with a row spacing of 0.76 m. Fields at Newark and Georgetown were plowed, disked, and harrowed, and trifluralin was incorporated at 0.43 kg/ha prior to planting. The Newark plots were planted 4 June with 640 seeds per plot or eight seeds per 30 cm of row, and alachlor and linuron were applied at 1.29 and 0.57 kg/ha on 5 June. The Georgetown field was planted on 10 June, and alachlor and linuron were applied at the Newark rates.

An isolate of *F. oxysporum* was obtained from a diseased, field-grown soybean plant in 1978, and its pathogenicity was confirmed in greenhouse tests (7). Inoculum was prepared using 1-cm plugs of 6-day-old cultures (grown on commercial potato-dextrose agar at 28 C in the light), which were transferred into 100 ml of a 1:9 solution (v/v) of V-8 juice and distilled water. These were placed on a rotary shaker at 150 rpm for 3 days, and contents of five flasks were combined with sterile distilled water to give final volumes of 1 L. This inoculum was poured over sterilized wheat (*Triticum aestivum* L.) kernels. After 5 days, the colonized wheat, which was free of contaminants, was incorporated into test plot soil at the rate of 147 and 131 kg/ha for the Newark and Georgetown plots, respectively. Inoculum was applied as a side-dressing using a tractor and was incorporated into the soil with a simultaneous close cultivation. Control plots (uninoculated) were adjacent to the inoculated plots and were of identical experimental design.

Isolation from root tissue. Two soybean plants with wilt symptoms were collected from the middle two rows of all plots biweekly starting 9 July and continuing through October for a total of seven sampling times. Plant material below the second internode was placed in polyethylene bags and transported to the laboratory in an ice chest. One root from each pair was selected at random, rinsed in running tap water to remove soil, cut into 0.5-cm sections, surface-disinfested for 45 sec in 70% (v/v) ethanol and 1 min in 10% bleach (5.25% sodium hypochlorite, w/v), and rinsed in sterile distilled water.

Five sections from each selected plant were placed in 100-mm-diameter petri plates on commercial acidified potato-dextrose agar and peptone-pentachloronitrobenzene agar. Sections were incubated in the dark for 8 days at 28 C. Isolations were made from a total of 630 plants in 1980. Macrocultural characteristics were used to identify *F. oxysporum* recovered from diseased plant tissue. When identification was difficult, cultures were exposed to northern sunlight to induce sporulation.

Yield data. Group III soybeans were harvested on 21 October at Newark and 23 October at Georgetown, whereas group IV and V soybeans were harvested on 30 October at Newark and 6

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November at Georgetown. From the center rows of each plot, the middle 4.9 m was hand harvested, threshed, and weighed.

Greenhouse studies. Shake cultures were started as previously described except that the growth medium was a commercial Czapek-Dox broth plus micronutrients. The micronutrient solution was prepared by autoclaving the solution developed by Hoagland and Arnon (9). One-tenth of one milliliter of solution was added to each flask of inoculum.

After 1 wk, every inoculum flask was diluted to 1 L with sterile distilled water, and 1 ml of micronutrient solution was added to each flask. The inoculum was then blended for 30 sec at low speed in a Waring Blendor and refrigerated until use.

Wooden greenhouse flats, 55.9 × 35.6 × 7.5 cm, were filled with a 1:1 (v/v) mixture of sand and greenhouse potting mix (2:3:4 vermiculite:peat moss:perlite, v/v). These were autoclaved twice for 1 hr at 118 C with 24 hr between autoclavings, then planted with seeds of the 10 test cultivars. Cultivars were randomized in a complete block design with four replicates with five subsamples per experimental unit. Six seeds were planted 2.5 cm deep per row and later thinned to five to make the five subsamples; there were four control flats. After planting, all plants were watered lightly and then thoroughly wetted with 1 L of micronutrient working solution (1 ml of micronutrient stock solution diluted to 1 L with sterile distilled water). After 2 wk, plants in flats were inoculated with 1.5 L of inoculum. Immediately after inoculum was applied, a knife was used to induce root injuries by slicing through the soil 1 cm from plant stems (3). The experiment was terminated after 4 more weeks. Plant roots were washed under running tap water, split longitudinally to reveal the vascular system, and evaluated using a disease index of 1–5 based on symptoms of Fusarium blight as follows: 1 = healthy root, no evidence of infection; 2 = slight cortical necrosis or vascular discoloration; 3 = moderate cortical necrosis or vascular discoloration; 4 = extensive cortical and/or vascular tissue destroyed; and 5 = plant wilted and dying.

In another experiment, 100 cc of the infested wheat kernels was mixed with a standard greenhouse potting mix, placed in 10.2-cm-diameter pots, and planted with four seeds of each test cultivar. There were 10 pots (replicates) for each of the 10 cultivars. They were watered regularly for 60 days, after which roots were removed, washed, and evaluated for symptoms of Fusarium blight.

Growth chamber studies. Inoculum was grown in Czapek-Dox broth as described earlier. Isolates transferred from the original isolate of *F. oxysporum* were used, as were isolates obtained from plants infected in the greenhouse. Two other isolates from field-grown soybeans were also tested in separate experiments. Inoculum concentration was adjusted to 1.3×10^6 propagules per milliliter of sterile distilled water.

Seeds of all test cultivars were placed into 100-mm-diameter petri plates on two sheets of Whatman No. 5 filter paper disks. Paper was wetted with sterile water and seeds incubated in darkness at 28 C for 3 days. Seedlings were tested on a variety of substrates including filter paper, cotton, and different types of agars before the filter paper cones were chosen (1,4,10,12). Healthy seedlings were then transferred with forceps onto inverted cones of 12.5-cm filter paper disks at the bottom of 25 × 200 mm test tubes. Radicles were pointed down, and tubes were then replugged with nonabsorbent cotton. Prior to the transfer of seedlings, 8 ml of micronutrient solution was added to the tubes fitted with their filter paper cones point up, plugged, and sterilized by autoclaving. Tubes with seedlings of 10 cultivars were arranged in a completely randomized design in a growth chamber and grown at 29 ± 1C with light intensity of 190 μE/sec per square meter PAR (photosynthetically active range, 400–700 nm) during 14-hr days.

Seedlings were inoculated on their fourth or fifth day in the chamber using a technique reported by Keeling (10). Seedlings and their filter paper substrates were pulled to the top of the tubes with forceps. The largest part of each taproot was wounded once with a sterile dissecting needle, and plants were then repositioned in the tubes. The micronutrient starter solution was drained by inverting tubes and replaced with 8 ml of inoculum plus micronutrient solution. Control plants were wounded once, and 8 ml of sterile distilled water was added to each tube.

Tubes were replugged and returned to the chamber. After 1 day, the cotton plugs were removed. When establishing plants for screening, 20–30% more seeds were started than necessary and 5–10% more tubes were planted. Seeds and tubes were selected on the basis of uniformity and health.

Seven days after inoculation, plants were rated for the presence of mycelium on an index of 1–4, where 1 = no mycelium and 4 = a mycelial mat. Roots were also scored for lesion development on a scale of 1–4, where 1 = a healthy plant and 4 = presence of a well-developed lesion. These evaluations were made with plants remaining in the tubes. Mortality counts were made 1 or 2 days after ratings for mycelial production and lesion development were determined. Disease continued to develop, and when a high percentage (about 80%) of the population was dead, mortality counts were made. Dead plants were scored as 2 and all other plants as 1.

Statistical analysis. All field data were handled as raw data and analyzed for differences between cultivars with regard to the frequency of isolation of *F. oxysporum* and yield. Analyses were conducted with a University of Delaware computer library program, NAOVMAIN, capable of conducting N-way analysis of variance.

Field data were considered as from a fixed-effect, randomized complete block design with a two-way factorial treatment combination. Weeds were considered as a factor, and the mean square error values for blocks and block-interaction effects were all pooled into an error term.

Greenhouse data were analyzed as a fixed-effect, randomized complete block design with four replicates and five subsamples per replicate.

Mycelial production indices and lesion development ratings from growth chamber screenings were analyzed as completely randomized, fixed-effect designs. Mortality data were considered to be binomially distributed, and cultivars were compared using a chi-square test of independence for binomial populations; proportions were compared using a test to compare two proportions found in the text by Walpole and Myers (17).

RESULTS

Isolations from root tissue. Of the 3,500 root sections from Newark, 44.9% were infected with *F. oxysporum*. Of the 2,800 Georgetown sections, 63.6% were infected with the fungus. In Newark but not Georgetown samples, there was a significant difference between cultivars with regard to frequency of isolation of *F. oxysporum* from diseased roots. Frequency of recovery of *F. oxysporum* was higher from plants grown at Georgetown than at Newark (Table 1).

Yield. Yield data from inoculated plots

Table 1. Isolation of *Fusarium oxysporum* from roots of soybeans in test plots at Georgetown and Newark, DE

Cultivar	Isolation frequency (%)[a]	
	Georgetown	Newark
Group III		
Emerald	6.6	3.5
Verde	6.4	4.5
Williams	6.1	3.8
Group IV		
Miles	5.8	4.6
Union	6.9	4.4
Ware	6.1	5.2
Group V		
Bedford	7.0	5.2
Essex	6.1	3.6
Forrest	6.4	3.9
York	6.2	5.0
FLSD[b] ($P = 0.05$)	n.s.	1.2

[a] Means from 10 sections per plant averaged over seven sampling dates, four replicates per date at Georgetown and five replicates per date at Newark.

[b] Fisher's least significant difference.

Table 2. Soybean yield (hl/ha) in inoculated and uninoculated plots at Georgetown and Newark, DE

Cultivar	Georgetown		Newark	
	Inoculated[a]	Uninoculated	Inoculated	Uninoculated
Group III				
Emerald	23.8	...[b]	11.8	...
Verde	19.5	...	12.7	...
Williams	23.7	39.2	12.3	20.7
Group IV				
Miles	25.1	39.7	12.4	21.4
Union	20.1	38.9	10.6	26.8
Ware	25.7	40.3	7.2	26.9
Group V				
Bedford	27.0	44.6	18.3	21.6
Essex	20.6	39.4	11.1	21.4
Forrest	20.8	41.1	11.6	19.7
York	29.6	30.0	18.9	21.9
FLSD[c] ($P = 0.05$)	7.0	7.8	5.4	NA[d]

[a] Mean of four replicates at Georgetown and five replicates at Newark from 1980 season.
[b] Not included in uninoculated plots.
[c] Fisher's least significant difference.
[d] No FLSD obtained for Newark uninoculated plots.

Table 3. Relative severity of Fusarium blight in inoculated greenhouse soybeans

Cultivar	Disease index[a]
Group III	
Emerald	2.8[b]
Verde	3.0
Williams	3.0
Group IV	
Miles	2.6
Union	3.2
Ware	3.1
Group V	
Bedford	3.4
Essex	2.7
Forrest	2.9
York	2.8
FLSD[c] ($P = 0.10$)	0.4

[a] 1 = Healthy plant and 5 = plant wilted and dying.
[b] Mean of four replicates (five plants each).
[c] Fisher's least significant difference.

Table 4. Production of mycelia and survival of soybean seedlings infected by *Fusarium oxysporum* in test tube culture

Cultivar	Production index[v]	Survival ratio[w]
Miles	1.64[x]	1:43 a[y]
Williams	1.64	6:38 b
Bedford	1.66	19:35 c
Essex	1.70	6.38 b
Composite	2.00	15:29 c
FLSD[z] ($P = 0.05$)	0.35	

[v] 1 = No mycelia and 4 = mycelial mat.
[w] Ratio of surviving plants to dead plants per cultivar.
[x] Mean of 44 single-plant replicates.
[y] Proportions followed by same letter do not differ significantly according to the Walpole and Myers hypothesis test for proportions.
[z] Fisher's least significant difference.

in Georgetown and Newark are compared with the control plots in Table 2. The Fisher statistic for the Newark inoculated plots is unprotected. York had the smallest percentage of decrease in yield at both locations (14 and 22%) of all cultivars tested.

Greenhouse studies. Plants in all control flats were free of symptoms of Fusarium blight, but inoculated plants had enough symptoms to permit root disease evaluations. Cultivars were separated with the root disease index at the 0.1 level of significance (Table 3). Fisher's least significant difference separated the best cultivar—Miles—from the three poorest cultivars—Union, Ware, and Bedford. All cultivars were susceptible, and only eight roots received a rating of 1 out of 200 examined.

Growth chamber studies. Results of evaluations based on a four-point scale of lesion development were inconclusive, and there were no significant differences between cultivars. The mean scores for all populations tested varied little. Results did not vary with time, and no separations could be obtained whether scoring was done earlier or later than the usual time of 16 days. There were significant differences between cultivars based on a four-point scale of total mycelia in each tube (Table 4).

A comparison between field and greenhouse studies was made. Regression analysis did not prove satisfactory, and the R^2 value obtained when regressing Georgetown yield depression on greenhouse root ratings was 14.7. However, results of greenhouse tests are still valuable and could be used to determine how a cultivar would perform in the field. Miles, the most resistant cultivar in greenhouse tests, was also the top group IV cultivar in the field. Cultivars that were significantly (0.10 level) worse than Miles in greenhouse tests—Verde, Union, and Bedford—all did poorly in field tests. However, Bedford yielded well, indicating that results of greenhouse tests may not be suitable for detecting tolerance. Plots of greenhouse ratings versus yield depression showed that top-performing cultivars and poor-performing cultivars fell on a straight line, but other cultivars did not fall on this line.

Comparisons between field results and growth chamber results are similar to those discussed for the comparison of field and greenhouse tests. Comparisons using all 10 cultivars and the four cultivars listed in Table 4 were made. Comparisons of yield depression data from field studies and data on mycelium production from growth chamber studies with a composite of susceptible cultivars are good. The top field cultivars were significantly different from the composite with regard to a mycelium production index. Mortality data separated Bedford as having good survival ability, and Bedford appeared most tolerant of *F. oxysporum* infection in field tests.

DISCUSSION

Emerald proved to be an excellent control cultivar for these studies because it did poorly in many other measurements of disease intensity (S. Leath, *unpublished*) and showed the greatest yield loss.

Applications of herbicides were not considered as factors in disease development because of previous work on interactions between alachlor, linuron, and *F. oxysporum* (J. P. Smith, *unpublished*). Newark plots were inoculated at a higher rate than the Georgetown plots because of the presence of a heavier, and possibly more *Fusarium*-suppressive, soil at Newark (16).

Plant samples were also taken from rows throughout the season. Thinning after the V4 stage of development (6) is known to affect yield (2). However, sampling amounted to just one plant per meter of row for samples taken after the V4 stage of development. Because all plots were treated identically, sampling was not considered to have an influence on yield.

Weather during the 1980 growing season was excellent for the development of Fusarium blight. Blight symptoms are influenced by stresses caused by high temperatures (5) and unusually dry weather, the type present at both locations during 1980.

Greenhouse techniques were after those developed by Crill et al (3). Micronutrients were used extensively to ensure consistency of results and to enhance pathogenicity of isolates (3). The soil-drench inoculation method provides more time for roots to be in contact with infective propagules than the root-dip method. Roots are susceptible to *F. oxysporum* infection when they are young, and the soil drench method is an effective method for establishing disease in young celery roots (8). Under the conditions used in this study, the soil-drench method was very effective. It was determined that inoculation method, age of plants at time of inoculation, and time of exposure to inoculum were important

factors in disease development (4,8,10).

Inoculum levels were similar to those used by other researchers (12,14,18). The greenhouse test was repeated until a desirable inoculum level was determined. A level was desired that would produce rapid symptom development in soybeans inoculated while in the V2 growth stage as defined by Fehr and Caviness (6); field-grown soybeans were also inoculated at the V2 stage. Plants were water stressed and control plants were allowed to wilt on afternoons of sunny days before they were watered, to simulate conditions observed in the field.

Use of filter paper cones to screen seedlings in test tubes allowed a clear view of root systems, and filter paper was easier to separate from root systems than other substrates. This was important when a seedling was to be saved and transplanted. The technique described in our study proved as reliable as other methods (1,4,10,12) and was more efficient and consistent than other methods tested. Isolates of *F. oxysporum* were used separately to prevent cross-protection (4).

Consistency in growth raises the possibility that control plants should be compared with inoculated plants to determine whether hypocotyl elongation is inhibited as observed with other diseases of soybean (18). However, observations did not indicate that this was true with *F. oxysporum* infection of soybean seedlings. In preliminary tests to ensure accuracy, mortality data were taken for several days. It was then determined that meaningful mortality counts could be taken once on day 16.

Bedford yielded well in spite of heavy *F. oxysporum* infection (S. Leath, *unpublished*). As there were no nematode problems at either location, Bedford was considered tolerant to *F. oxysporum* infection.

These studies confirmed that soybean cultivars differ in susceptibility to *F. oxysporum*. Differences can be detected with field, greenhouse, and growth chamber studies. It is possible to use a rapid growth chamber screening test to sort out plants resistant to *F. oxysporum*. Therefore, this procedure should be useful to soybean breeders in developing a soybean resistant to *F. oxysporum*.

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