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Susceptibility to *Calonectria ilicicola* in Soybean Grown in Greenhouse and Field

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ABSTRACT

Susceptibility of soybean cultivars to *Calonectria ilicicola* was evaluated in a greenhouse by inoculating seedlings with mycelium in agar discs placed on the stems at the soil line. A range of responses was detected among cultivars following inoculation with a virulent isolate of *C. ilicicola*. Rankings of cultivars between greenhouse tests 1 and 2 were similar for disease severity and areas under the disease progress curves (AUDPC). In addition, rankings of cultivars for Final disease severity were highly correlated with AUDPC in test 1 ($r_s = 0.88, t = 5.48, p < 0.001$), test 2 ($r_s = 0.99, t = 22.10, p < 0.001$), and when tests were combined ($r_s = 0.89$, $t = 5.82$, $p < 0.001$). Final disease severity and AUDPC consistently identified Asgrow 7986, Braxton, Cajun, and Forrest as soybean cultivars least susceptible to red crown rot. In 1993 and 1994 field tests, a range in disease susceptibility was observed for tested cultivars but none was completely resistant. Soybean cultivars Braxton, Cajun, and Forrest, which were least susceptible to red crown rot in greenhouse tests, also ranked among cultivars with the lowest disease incidence and AUDPC in field tests. Comparisons between rankings of the eight cultivars common to greenhouse and field tests showed a correlation between final disease severity from combined greenhouse tests and both final disease incidence ($r_s = 0.63, t = 1.99, p < 0.1$) and AUDPC ($r_s = 0.60, t = 1.82, p < 0.2$) from the combined field tests. However, AUDPC from greenhouse tests did not correlate with either final disease incidence or AUDPC from field tests. The greenhouse screening method provided consistent results between greenhouse and field tests and successfully identified the least susceptible cultivars Braxton, Cajun, and Forrest.

Key words : *Calonectria crotalariae, Calonectria ilicicola, Cylindrocladium crotalariae, Cylindrocladium parasiticum*, disease screening, *Glycine max*, red crown rot.

Red corwn rot of soybean [*Glycine max* (L.) Merr.] is caused by the soilborne fungus *Calonectra ilicicola* Boedijn & Reitsma [anamorph: *Cylindrocladium parasiticum* Crous, Wingf. & Alfenas (Crous et al., 1993), syn., *C. crotalariae* (Loos) Bell & Sobers (Bell & Sobers, 1966)]. The disease is also known as Cylindrocladium root rot, black root rot, and Calonectria root rot (Bell & Sobers, 1966; Berggren & Snow, 1989; Rowe et al., 1974). Since its description on peanut in 1966 (Bell & Sobers, 1966), *C. ilicicola* has been reported as pathogenic on soybean (Rowe et al., 1973), koa (Aragaki et al., 1972), papaya (Nishijima & Aragaki, 1973), blueberry (Milholland, 1974), leea (Ko et al., 1981), alfalfa (Ooka & Uchida, 1982), indigo (Berner et al., 1988), and palm (Uchida & Aragaki, 1992).

Symptoms of red crown rot on soybean include chlorosis, yellowing, interveinal necrosis, wilting, and defoliation as well as stem necrosis and root decay. Reddish-orange perithecia, a distinctly diagnostic sign of the disease, normally develop on the stem (Bell & Sobers, 1966; Berggren & Snow, 1989; Rowe et al., 1973) at late growth stages (R_3-R_4) (Fehr et al., 1971). Microsclerotia (Krigsvold et al., 1977; Phipps et al., 1976) are primary inocula to infect host plants in spring. In the United States, the disease was first reported on soybean in North Carolina in 1973 (Rowe et al., 1973). It was observed in St. John the Baptist Parish, Louisiana USA in 1976 (Berggren et al., 1985), and red crown rot is one of the major fungal diseases of soybean in the state (Berner et al., 1988). Roy et al. (1989) reported the disease on soybean in Mississippi USA and estimated 25~30% yield loss in affected fields.

Soil fumigation (Rowe et al. 1974) for control of red crown rot is not cost effective (Berner et al., 1988). Cultural controls, such as crop rotation with non-legume crops, also do not control this disease very effectively (Berner et al,. 1986). Crop rotation sometimes reduces microsclerotia populations in soil but not to levels necessary for acceptable disease reduction (Phipps & Beute, 1979). Delayed planting can reduce disease severity in certain cultivars (Berner et al., 1988), but this option is not always practical. Therefore, host resistance appears the most practical approach for control. Resistance to *C. ilicicola* in peanut (Bailey & Matyac, 1989; Coffelt & Garren, 1982; Pataky et al., 1983; Wynne et al., 1975), and soybean (Berggren et al., 1985; Berner et al., 1986; 1988) has been evaluated. However, screening for resistance in soybean has been difficult because of the uneven distribution of inoculum in most fields, the irregular appearance of symptoms, i.e., leaf and/or stem symptoms, and the fact that diseased plants are difficult to detect because leaves showing symptoms are shed quickly (Berner

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et al., 1986; 1988). Little progress has been made toward evaluating disease reaction under controlled greenhouse conditions as well (Pataky et al., 1983). The objectives of this study were to evaluate red crown rot disease reaction in soybean cultivars under greenhouse conditions and to compare these reactons to the results of the field.

MATERIALS AND METHODS

Greenhouse tests

For test 1, 11 soybean cultivars (Table 1) were grown in 22-cm-diameter plastic pots containing a mixture of methyl bromide-treated soil (80% sand, 5% silt, 15% clay), peat moss, and perlite (3:2:1, v/v/v) in the greenhouse for 10 days. Three seeds were planted in each pot. Virulent isolate SG915 of *C. ilicicola* was used in this experiment. Preliminary tests (Kim, 1994) demonstrated a wide range of virulence among isolates of *C. ilicicola* and showed that isolates with low levels of virulence could not distinguish between cultivars with different susceptibilities to red crown rot. Soil was removed gently from a 1 cm^2 area near the crown of each plant (V_1 stage) (Feht et al., 1971). One disc (10 mm in diameter) of mycelium in potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) from a 10-day-old culture of the fungus was placed in direct contact with the crown, then covered with soil. Control plants received PDA discs without mycelium. The plants were watered for 3~5 days with care to prevent dislodging inoculum discs from crowns; afterwards plants were watered normally. Disease severity based on a 0~5 scale was recorded as follows: 0 = no visible symptoms; 1=small necrotic lesions (<10 mm in length) on stems, no leaf symptoms; 2 = stem lesions 10~20 mm, and/or slight yellowing and wilting; 4 = stem lesions > 20 mm and/or desiccation of upper stems, extensive yellowing and wilting of all leaves, and lower leaves dropped; 5 = stem completely necrotic and desiccated, entire plant wilted and collapsed. Disease severity was evaluated for 33 days following inoculation. Areas under the disease progress curves (AUDPC) for disease severity were calculated from 6 observations during the period. The design of this experiment was a completely randomized design with 9 replicates per treatment.

Greenhouse test 2 was a duplicate of test 1 with the following changes. Disease severity was evaluated for 46 days following inoculation. AUDPC based on disease severity was calculated from 13 observations during the period. AUDPC was determined using the formula described by Shaner and Finney (1977). The design of this experiment was a completely randomized design with 12 replicates per treatment.

Table 1. Final disease severity and areas under disease progress curves (AUDPC) caused by isolate SG915 of *Calonectria ilicicola* on 11 soybean cultivars in greenhouse tests.

Cultivar	Final disease severity†			AUDPC‡		
	Test 1	Test 2	Mean	Test 1	Test 2	Mean
Riverside 699	3.7§	4.1	3.9	79	148	114
Hartz 7126	3.6	3.5	3.6	82	117	100
Riverside 677	3.4	3.2	3.3	69	110	90
Centennial	3.3	2.6	3.0	78	102	90
Deltapine 726	4.1	2.2	3.2	91	70	81
Bedford	4.3	2.1	3.2	96	62	79
Hartz 6200	3.9	1.7	2.8	82	38	60
Forrest	2.7	1.6	2.2	63	39	51
Braxton	3.1	1.4	2.3	61	31	46
Cajun	2.8	1.5	2.2	56	33	45
Asgrow 7986	3.4	1.3	2.4	61	26	44
LSD (0.05)	1.5	1.0		37	33	

† Disease severity was rated on a 0~5 scale in which 0=no visible symptoms and 5=dead plants 33 days and 46 days after inoculation of 10-day-old plants in tests 1 and 2, respectively.

‡ AUDPC was determined based on disease severity for 6 and 13 observations over 33 and 46 days following inoculation in tests 1 and 2, respectively.

§ Values are means of 9 and 12 replicates in tests 1 and 2, respectively.

Field tests

Soybean cultivars were evaluated for resistance to red crown rot during 1993 and 1994 in a field at the Ben Hur Research Farm, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana USA. In 1993, 19 soybean cultivars in maturity groups Ⅳ~Ⅶ (Table 2) were used, including eight of the 11 cultivars used in greenhouse tests 1 and 2. The remaining three cultivars, i.e., Hartz 6200, Hartz 7126, and Riverside 677, were not available when field tests were established. Soybean cultivars were planted on May 7 on a row spacing of 76 cm. Plots were 12.2 meters long by two rows wide and were arranged in a randomized complete block design with five replications. Red crown rot was evaluated on all plants in each plot on August 10, August 17, August 27, and September 10. Disease evaluations were initiated when symptoms appeared on cultivars with earliest maturity.

Plants were considered diseased if they exhibited any of the following reactions: perithecia on stems, leaf symptoms, or a reddish discoloration on stem bases (Berggren & Snow, 1989). Incidence of red crown rot was determined as the percentage of plants in plots showing disease symptoms or signs. AUDPC for disease incidence in each cultivar was determined. Final stand in each plot was estimated by counting numbers of plants in six l-meter-long sections of each plot.

The 1994 field test was a duplicate of the 1993 test with the following changes. Soybean cultivars Asgrow 6297 and Pioneer 9791 were excluded because they were no longer available commercially, and planting date was delayed until May 23 because of excessive rain. Plots were 7.6 meters long and were replicated six times. Red crown rot was evaluated on August 17, August 27, September 6, and September 16.

For both greenhouse and field tests, statistical analyses were done using the General Linear Models procedure of SAS (1988). Analysis of rank correlation between field and greenhouse results was done using Spearman's method (Be-

Table 2. Final incidence of red crown rot and areas under disease progress curves (AUDPC) for soybean cultivars in four maturity groups at the Ben Hur Research Farm, Baton Rouge, Louisiana, USA in 1993 and 1994.

Cultivar	Maturity group	Final disease severity†			AUDPC‡		
		1993	1994	Mean	1993	1994	Mean
Sharkey	Ⅵ	26.8§	32.8	29.8	454	651	553
Deltapine 726	Ⅵ	12.7	37.9	25.3	196	891	544
Davis	Ⅵ	20.3	27.3	23.8	272	657	465
Centennial	Ⅵ	16.6	28.4	22.5	269	605	437
Pioneer 9641	Ⅵ	23.3	24.2	23.8	374	472	423
Northrup King / Ring Around 452	Ⅳ	18.9	16.4	17.7	393	379	386
Terra Vig 5452	Ⅴ	11.8	23.8	17.8	207	563	385
Asgrow 7986	Ⅶ	18.3	28.1	23.2	231	523	377
Hyperformer HSC 557	Ⅴ	21.6	19.9	20.8	410	277	344
Bedford	Ⅴ	11.5	20.0	15.8	179	355	267
Asgrow 6297	Ⅵ	17.1	–¶	17.1	252	–	252
Pioneer 9791	Ⅶ	17.6	–	17.6	235	–	235
Riverside 699	Ⅵ	14.3	9.5	11.9	225	173	199
Braxton	Ⅶ	6.6	11.6	9.1	82	223	158
Hyperformer HSC 741	Ⅶ	9.6	8.3	9.0	163	150	157
Cajun	Ⅵ	4.6	12.2	8.4	56	249	153
Hyperformer HSC B2J	Ⅶ	9.5	8.1	8.8	134	164	149
Forrest	Ⅴ	1.8	2.8	2.3	21	36	29
LSD(0.05)		12.0	10.1		222	251	

† Mean percentages of plants with stem and leaf symptoms were evaluated on September 10, 1993 and September 16, 1994, respectively.
‡ AUDPC was determined based on disease incidence from 4 observations over 31 and 30 days in 1993 and 1994, respectively.
§ Values are means of five and six replicates in 1993 and 1994, respectively.
¶ Not tested.

nder et al., 1989).

RESULTS

Greenhouse tests

A range of responses was detected among the 11 soybean cultivars inoculated with virulent isolate SG915 of *C. ilicicola* (Table 1). Rankings of cultivars between tests 1 and 2 were similar for disease severity ($r_s = 0.41$, $t = 1.39$, $p < 0.2$) and AUDPC ($r_s = 0.58$, $t = 2.15$, $p < 0.1$). Furthermore, rankings of cultivars for final disease severity were highly correlated with AUDPC in test 1 ($r_s = 0.88$, $t = 5.48$, $p < 0.001$), test 2 ($r_s = 0.99$, $t = 22.10$, $p < 0.001$), and when tests were combined ($r_s = 0.89$, $t = 5.82$, $p < 0.001$). Both final disease severity and AUDPC consistently identified Asgrow 7986, Braxton, Cajun, and Forrest as soybean cultivars least susceptible to red crown rot.

Field tests

Red crown rot was evenly distributed throughout the field (data not shown), which suggests that none of the cultivars escaped exposure to fungal inoculum. A range in disease susceptibility was observed for tested cultivars but none was completely resistant (Table 2). Final disease incidence for all 18 cultivars was highly correlated with AUDPC in 1993 ($r_s = 0.98$, $t = 17.97$, $p < 0.001$), 1994

Table 3. Final incidence of red crown rot and areas under disease progress curves (AUDPC) for soybean cultivars in maturity groups Ⅴ, Ⅵ, and Ⅶ, Ben Hur Research Farm, Baton Rouge, Louisiana, USA in 1993 and 1994†.

Maturity group	Final disease severity‡			AUDPC§		
	1993	1994	Mean	1993	1994	Mean
Ⅴ	11.7	16.3	14.0	204	311	258
Ⅵ	16.1	22.7	19.4	248	487	368
Ⅶ	13.0	15.7	14.4	178	298	238
LSD (0.05)	NS¶	NS		NS	176	

† Maturity group Ⅳ was not included in analysis because only one cultivar in this group was tested.
‡ Mean percentages of plants with stem and leaf symptoms were evaluated on September 10, 1993 and September 16, 1994, respectively.
§ AUDPC was determined based on disease incidence from 4 observations over 31 and 30 days in 1993 and 1994, respectively.
¶ NS indicates no significant difference among maturity groups.

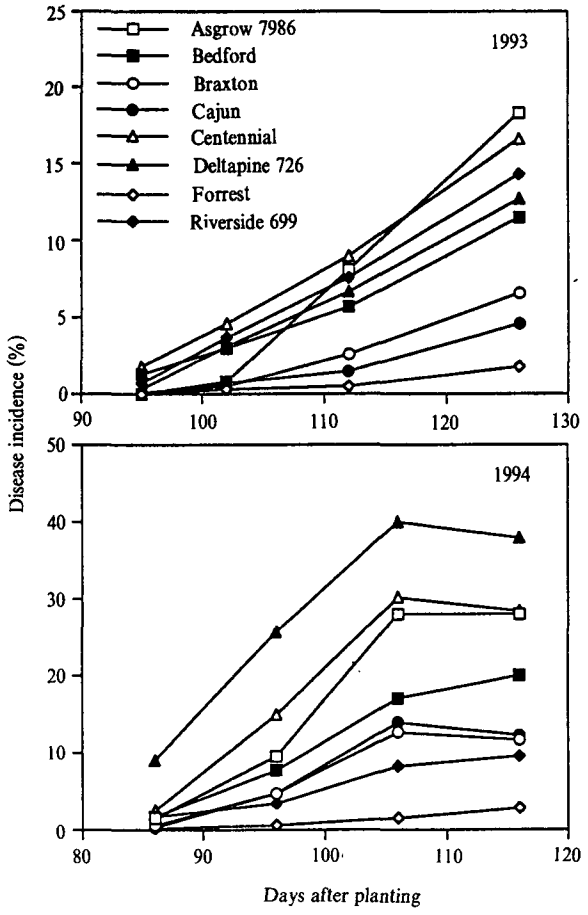

Fig. 1. Progress curves for incidence of red crown rot disease caused by *Calonectria ilicicola* on eight soybean cultivars in field tests at Ben Hur Research Farm, Baton Rouge, Louisiana, USA in 1993 and 1994. Values are means of five and six replicates in 1993 and 1994, respectively.

($r_s = 0.96$, $t = 12.63$, $p < 0.001$), and in both years combined ($r_s = 0.95$, $t = 11.83$, $p < 0.001$). Soybean cultivars Braxton, Cajun, and Forrest, which were least susceptible to red crown rot in greenhouse tests (Table 1), also ranked among cultivars with the lowest disease incidence and AUDPC in field tests (Table 2). Hyperformer HSC B2J and Hyperformer HSC 741, previously untested in greenhouse, also were among the least susceptible cultivars in the field (Table 2).

Comparisons between rankings of the eight cultivars common to greenhouse and field tests showed a correlation between final disease severity from the combined greenhouse tests and both final disease incidence ($r_s = 0.63$, $t = 1.99$, $p < 0.1$) and AUDPC ($r_s = 0.60$, $t = 1.82$, $p < 0.2$) from the combined field tests. However AUDPC from greenhouse tests did not correlate with either final disease incidence ($r_s = 0.25$, $t = 0.62$, $p < 0.5$) or AUDPC ($r_s = 0.34$, $t = 0.93$, $p < 0.4$) from field tests.

Disease progress curves for 1993 and 1994 field tests are shown in Fig. 1. These figures include only those eight cultivars that were tested in the greenhouse. Disease incidence increased over time in all cultivars but the relative rankings of most cultivars were consistent across all evaluation dates (Fig. 1). Disease incidence was consistently lowest on cultivars Braxton, Cajun, and Forrest. Disease incidence increased consistently across all evaluation dates in 1993 but decreased slightly in certain cultivars by the last evaluation date in 1994. This resulted from loss of diseased leaves from plants without perithecia or red discoloration at stem bases. Differences in disease incidence between cultivars were easily detectable 110~120 days after planting in both years. A comparison of final disease incidence in 1993 and 1994 showed no difference in red crown rot susceptibility among soybean cultivars in maturity groups V~Ⅶ (Table 3). AUDPC for group Ⅵ cultivars was higher than that for groups V and Ⅶ in 1994 (Table 3).

DISCUSSION

Efforts have been made to identify soybean cultivars resistant to *C. ilicicola* in the field (Berggren et al., 1985; Berner et al 1986; 1988). However there has been little consistency in results from cultivars tested at different locations in different years. This may be due to several factors. Uneven distribution of natural inoculum in the test field may allow cultivars to escape disease and be incorrectly identified as resistant. Such uneven distribution also was recognized in peanut field soils by Pataky et al. (1983). In addition, levels of virulence within the fungal population in a given field also generally are not identified. Kim (1994) indicated that only isolates with a high level of virulence could reliably separate cultivars with different levels of susceptibility. Inconsistent field data also may result from different methods of disease evaluation. Berner et al. (1988) evaluated disease incidence after plant senescence and considered only plants supporting perithecia as diseased. However there are other symptom reactions on soybean and development of perithecia is dependent on moisture conditions in the field (Rowe et al., 1973).

In an earlier study, Pataky et al. (1983) screened peanut lines resistant to *C. ilicaicola* in the greenhouse but their results from greenhouse inoculations did not correlate with those from microplot and field tests. The greenhouse screening technique described in the current report, however, provided consistent rating for red crown rot severity between greenhouse and field tests and successfully identified the least susceptible soybean cultivars Braxton, Cajun, and Forrest. Therefore, repeated greenhouse tests for measurement of disease reaction may be desirable. Such an approach would allow proper evaluation of susceptible cultivars such as Asgrow 7986, Bedford, Deltapine 726 or Hartz 6200 which showed high disease severtiy and AUDPC in greenhouse test 1 but

moderate or low in test 2.

In addition, examination of individual cultivar rankings showd that the lack of correlation for AUDPC between greenhouse and field tests was due to inconsistent results from two cultivars, i.e., Riverside 699 and Asgrow 7986. Riverside 699 was identified as susceptible to red crown rot in both greenhouse tests and the 1993 field test but was among the least susceptible cultivars in the 1994 field test. This may have resulted form delayed planting in 1994 which is known to reduce disease severity in certain cultivars (Berner et al., 1988). Asgrow 7986 was among the least susceptible cultivars in greenhouse test 2 but was susceptible in field tests. Examination of disease progress curves for this cultivar showed low levels of disease relative to other cultivars during early evaluation dates but much greater disease at later dates. That this cultivar did not demonstrate susceptibility until later stages of plant development may have contributed to incorrect identification of this cultivar as less susceptible in greenhouse tests. These two cultivars also affected the correlation of disease severity between greenhouse and field tests. However the correlation became highly significant between final disease severity from both tests combined in the greenhouse and both final disease incidence and AUDPC ($r_s = 0.89$, $t = 3.82$, $p = 0.02$) from both years combined in the field when the analysis excluded Riverside 699 and Asgrow 7986.

In field tests, soybean cultivars in maturity groups Ⅴ ~ Ⅶ did not differ in final disease incidence, but cultivars in maturity group Ⅵ showed greater AUDPC in 1994. Maturity group Ⅳ was not included in this analysis because only a single cultivar was used in this study. Field results of Berner et al. (1988) also indicated that cultivars of maturity group Ⅵ were more susceptible to red crown rot than cultivars in groups Ⅴ and Ⅶ. In this study, planting all cultivars at the same time was designed to evaluate all genotypes under the same conditions and thus minimize the possibility of escape. Increased disease incidence in group Ⅵ indicates that this phenomenon may be related to genetic factors for disease susceptibility.

The greenhouse inoculation method described herein, which involves direct placement of mycelium in PDA discs on seedling stems, induced visible disease symptoms within 3~5 days after inoculation and allowed evaluation of disease reactions within a few weeks. This method may apply for screening large numbers of soybean cultivars in a relatively short period of time. From these screening, a small number of less susceptible cultivars could be selected and examined more thoroughly under field conditions. This procedure could reduce time and labor required for direct field screening of large numbers of genotypes and potentially identify soybean cultivars resistant to red corwn rot.

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