Resistance of the Soybean Cultivar Archer to *Pythium* Damping-Off and Root Rot Caused by Several *Pythium* spp.

G. D. Bates, C. S. Rothrock, and J. C. Rupe, Department of Plant Pathology, University of Arkansas, Fayetteville, 72701

ABSTRACT

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Archer, a maturity group I soybean cultivar with demonstrated flood tolerance and resistance to *Pythium ultimum*, was compared with Hutcheson, a widely planted maturity group V cultivar in Arkansas, for resistance to *P. ultimum*, *P. irregulare*, *P. aphanidermatum*, *P. vexans*, and group HS. Emergence and establishment assays demonstrated that Archer had greater emergence and fewer disease symptoms after 10 days for all pathogenic species of *Pythium* than did Hutcheson. Archer also demonstrated higher root weights and fewer disease symptoms compared with Hutcheson in assays conducted for 6 weeks. Similar results were found using two different seed lots of the cultivars, suggesting that the results were not due to seed quality differences. This study indicates that resistance in Archer to *Pythium* damping-off and root rot compared with Hutcheson is robust, with efficacy over a number of *Pythium* spp. and a range of plant developmental stages.

During 2007, 1,255,000 ha were planted with soybean in Arkansas (15). The majority of those fields were alluvial soils with poor surface and subsurface drainage. Adequate soil drainage is essential for soybean production because temporary flooding and soil anoxia can reduce growth and yield of soybean plants compared with nonflooded conditions (6,12–14).

Research by Kirkpatrick et al. (10) indicated that the isolation frequency of *Pythium* spp. increased under soybean production subjected to temporary flooding at cracking (emergence) or at the V4 growth stage (4). Several species of *Pythium* were associated with soybean roots, including *Pythium ultimum* Trow, *P. aphanidermatum* (Edson) Fitzp., *P. irregulare* Buisman, *P. vexans* de Bary, *P. oligandrum* Drechsler, and group HS. Isolates in the group HS form hyphal swellings and lack oogonia (16). All species except *P. oligandrum*, a mycoparasite (2,3), were reported as pathogenic on soybean (10,18).

Greenhouse tests showed that Archer, a maturity group I cultivar with demonstrated flood tolerance (17), was significantly more resistant to *P. ultimum* than Hutcheson, a maturity group 5 cultivar commonly grown in Arkansas (9). Resis-

tance to *Pythium* spp. has rarely been reported in soybean and is not well understood (5,8). All reports of *Pythium* resistance for soybean have been observed using *P. ultimum* (5,8,9).

The objective of this study was to compare the reaction of the soybean cvs. Archer and Hutcheson to pathogenic *Pythium* spp. known to colonize soybean in Arkansas over a range of growth stages.

MATERIALS AND METHODS

Inoculum. Isolates of *Pythium* were collected and identified by Dr. M. T. Kirkpatrick in 1999 from the Pine Tree Branch Experiment Station, near Colt, AR (10) and included *P. ultimum* (isolate 124), *P. irregulare* (isolates 115 and 21), *P. aphanidermatum* (isolates 88, 64, and 16), *P. vexans* (isolates 140 and 182), *P. oligandrum* (isolates 125 and 120), and group HS (isolates 126 and 117). Isolates are available from the authors. Group HS isolate 117 was identified as *P. ultimum* based on restriction fragment length polymorphism using the mitochondrial COX II gene and sequence of internal transcribed spacers 1 and 4 (L. Rosso, *personal communication*). Isolates were grown on cornmeal agar (CMA; Difco Laboratories, Detroit) and incubated at room temperature for 3 days. Six 1-cm² plugs of 3-day-old CMA cultures of each isolate were placed in 500-ml flasks containing 200 ml of sand, 11.2 ml of corn meal, and 80 ml of deionized water that had been autoclaved for 40 min and then autoclaved again 24 h later. The isolates were allowed to colonize the sand-cornmeal medium for 9 days. The flasks were shaken every other day to ensure uniform colonization.

To determine the inoculum density, 10 g of sand-corn meal inoculum of each isolate were placed in an Erlenmeyer flask containing 95 ml of sterile distilled water and agitated on a wrist-action shaker for 20 min. Dilutions of 1/10 and 1/100 of original suspension were made and 1-ml portions of each were spread over the surface of five CMA plates. After 3 days, the number of *Pythium* colonies was counted and the number of propagules per gram of inoculum calculated.

Seed source. Seed of both Archer and Hutcheson were provided by D. Longer (University of Arkansas) and were produced in 1999 in a uniform environment at the Arkansas Agricultural Research and Extension Center in Fayetteville. Seed were harvested at approximately the same time and stored at 4°C until used. Germination at the start of the studies for both cultivars was 93% at 20°C by the rolled paper towel technique (1). To evaluate the influence of seed source on cultivar reaction, a seed lot assay was conducted using seed lots from 2001 of Archer from commercial seed from Minnesota and an Arkansas foundation seed lot of Hutcheson and the seed lots harvested in 1999.

Resistance assays. A Calloway silt loam soil obtained from the Pine Tree Branch Experiment Station near Colt, AR, was pasteurized for 30 min at 60°C prior to use for all experiments.

For emergence assays, a base layer of 250 g of pasteurized soil, oven dry weight equivalent (odw), followed by a layer of 12 g of *Pythium* inoculum, approximately 2 mm thick, were placed in Styrofoam cups (11 cm in diameter by 8 cm high) with bottom drainage. Ten seeds then were placed directly onto the inoculum. The seeds then were covered with 20 g of pasteurized soil (odw). The cups were placed into 15-cm-diameter plastic watering trays with water to keep a constant matric potential of -0.2 J/kg at planting depth, based on water level. The cups were placed in a growth chamber at 20°C with a 12-h photoperiod. The number of seedlings that emerged after 10 days was recorded. All seedlings and seed were separated from the soil and washed for 10 min under running water, and each seedling was rated for disease using a 0-to-10 scale. The ratings represented percent root discoloration, with 0 equaling no discoloration and 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 to 40, 5 = 41 to 50, 6 = 51 to 60, 7 = 61 to 70,

Corresponding author: C. S. Rothrock
E-mail: Rothrock@uark.edu

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8 = 71 to 80, 9 = 81 to 90, and 10 = 91 to 100% discoloration. Discoloration ratings were recorded for each seedling in a pot and were averaged for a pot using the midpoint of each rating prior to analysis. All of the seeds or roots then were plated on P₅ARP agar (7) to determine isolation frequency of *Pythium* spp. Each experiment consisted of five replications (cups) and this experiment was conducted five times.

An emergence assay was conducted to compare seed lots of Archer and Hutcheson using one species, *P. aphanidermatum* isolate 88, by placing seed on a layer of inoculum utilizing the same methods and collecting the same data as described above. Each experiment consisted of four replicate cups with 10 seeds per cup. The experiment was conducted twice.

An establishment assay was conducted in a manner similar to the above experiments except, in this experiment, seed were placed on a noninfested layer about 1.8 mm thick (10 g) of pasteurized soil (odw) that was on top of the layer of inoculum. Removal of inoculum from direct contact with the seed allowed the root rot phase and plant growth to be examined, rather than emergence only. In addition to plant stand, disease rating, and *Pythium* spp. recovery, fresh root weight also was recorded in this assay. Each test consisted of three replicate cups with five seeds per cup. The experiment was conducted three times.

A long-term plant growth assay was conducted in pots constructed from PVC pipes (15 cm in diameter by 35 cm high) with a fiberglass screen on the bottom. A base layer of 4,000 g of pasteurized soil (odw) was covered with a layer of 20 g of *Pythium* sand-cornmeal inoculum, approximately 1.5 mm thick. The inoculum was covered with 20 g (odw) of pasteur-

ized soil to provide a noninfested layer, similar to the establishment assay. Ten seeds were placed on this layer of soil and covered with 30 g of pasteurized soil. The pipes were placed into a 20-cm-diameter plastic watering tray to keep a constant matric potential of -0.5 J/kg at planting depth based on water level. Plants were thinned to five representative plants per pipe at the V1 growth stage. The plants were grown in a greenhouse for 6 weeks at 20 to 30°C. Data recorded included disease rating, fresh root weight, and *Pythium* spp. recovery. Each experiment consisted of five replications and the experiment was conducted two times.

Statistical analyses. All experiments used a randomized complete block design. A factorial treatment arrangement of the two cultivars and *Pythium* isolates was used when more than one isolate was examined. Data from the multiple experiments were combined and analyzed by GLM with SAS. Experiments were treated as random factors and interactions of experiment-treatment were used as the error term for comparing treatments to determine the repeatability of the experiments. The repeatability of experiments was determined to be not significant for each experiment type; therefore, experiments were combined. Means were separated using Fisher's protected least significant difference (LSD) at $P = 0.05$.

RESULTS

Inoculum ranged from 820 to 1,340 propagules/g for isolates over all assays. For all experiments using a factorial treatment arrangement, there was a significant interaction between isolates and cultivars; thus, interaction means are presented for isolates and cultivars and LSDs were calculated to compare the appropriate interaction means. Plant stand was not signifi-

cantly different for the pathogenic *Pythium* spp. compared with *P. oligandrum* for Archer, and ranged from 9.1 to 10 seedlings emerged from the 10 seeds that were planted. Plant stands for Hutcheson were reduced significantly from 8.8 plants for soils infested with *P. oligandrum* to 3.6 for *P. irregulare* (Table 1). Isolates of *P. aphanidermatum*, *P. vexans*, and *P. irregulare* caused the greatest stand reduction at 20°C compared with the isolates of the other *Pythium* spp. examined. Root discoloration was statistically higher for Hutcheson for all potentially pathogenic isolates compared with Archer (Table 1). Disease rating for Archer ranged from 0 for *P. oligandrum* to 2.0 in group HS. For Hutcheson, disease rating ranged from 0 for *P. oligandrum* to 4.8 for *P. irregulare*. Disease ratings were significantly lower for *P. oligandrum* than all other species in both Archer and Hutcheson. The *Pythium* sp. used to infest each soil was recovered from all plated plants of that treatment (*data not shown*).

The seed lot emergence assay demonstrated that there were no significant differences between seed lots for a cultivar for stand or root discoloration. However, there were significant differences between cultivars. Archer had a plant stand of 9.1 for the 2001 seed lot and 8.9 for the 1999 seed lot, with a disease rating of 0.5 for both seed lots. Hutcheson had a plant stand of 3.1 for the 2001 seed lot and 2.9 for the 1999 seed lot, with disease ratings of 2.8 for the 2001 seed lot and 2.5 for the 1999 seed lot. Both seed lots of Archer demonstrated statistically higher stand than either seed lot of Hutcheson ($P = 0.05$). Root discoloration for Hutcheson was statistically higher than Archer ($P = 0.05$). *Pythium* spp. were recovered from all plated plants.

For the establishment assay, where seed were separated from inoculum by a layer of noninfested pasteurized soil, plant stand was high and statistically the same between the two cultivars, except for isolate 126 of group HS, where Hutcheson had a significantly lower stand than Archer (Table 2). Within Hutcheson, there were statistical differences for root discoloration among isolates within a given species for *P. vexans*, *P. aphanidermatum*, and group HS. There were significantly higher disease ratings in Hutcheson compared with Archer for all species except *P. oligandrum* (Table 2). Root weights were reduced significantly for Hutcheson compared with Archer for *P. aphanidermatum* isolate 88 and group HS isolate 126. Isolates of all pathogenic species did not reduce root weight in Archer compared with the isolates of *P. oligandrum*. Isolates for group HS, *P. ultimum*, and *P. vexans* isolate 140 significantly reduced root weight for Hutcheson compared with isolates of *P. oligandrum*. *Pythium* spp. were recovered from all plated plants.

Table 1. Plant stand and disease rating of Archer and Hutcheson for the emergence assay^x

<i>Pythium</i> spp.	Isolate	Plant stand ^y		Disease rating ^z	
		Archer	Hutcheson	Archer	Hutcheson
<i>P. aphanidermatum</i>	16	9.1 Ad	5.0 Bf	0.7 Bef	2.9 Acd
	64	9.2 Acd	5.0 Bf	0.6 Bf	3.0 Acd
	88	9.2 Ad	5.2 Bf	0.4 Bf	2.9 Acd
Group HS	117	9.4 Abc	6.1 Bd	2.0 Ba	3.2 Ac
	126	9.4 Abc	6.4 Bc	1.8 Bab	3.1 Acd
<i>P. irregulare</i>	21	9.5 Ab	3.6 Bg	1.6 Bbc	4.8 Aa
	115	9.3 Acd	3.6 Bg	1.6 Bbc	4.8 Aa
<i>P. oligandrum</i>	120	10.0 Aa	9.0 Ba	0.0 Ag	0.0 Ae
	125	10.0 Aa	8.8 Ba	0.0 Ag	0.0 Ae
<i>P. ultimum</i>	124	9.5 Ab	7.0 Bb	1.0 Bde	2.8 Ad
<i>P. vexans</i>	140	9.4 Abc	5.6 Be	1.1 Bd	4.6 Aab
	182	9.1 Ad	5.9 Bd	1.2 Bcd	4.4 Ab

^x Seed were placed in direct contact with an inoculum layer of *Pythium* isolates in pots and grown for 7 days at 20°C. Means represent five combined experiments with five replications. Cultivars for a *Pythium* isolate did not significantly differ if followed by the same capital letter, protected least significant difference (LSD; $P = 0.05$). Within a cultivar, *Pythium* isolates did not significantly differ if followed by the same lower-case letter, protected LSD ($P = 0.05$).

^y Plant stand from 10 seeds planted.

^z Disease rating based on scale of 0 to 10, where 0 = healthy and 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 to 40, 5 = 41 to 50, 6 = 51 to 60, 7 = 61 to 70, 8 = 71 to 80, 9 = 81 to 90, and 10 = 91 to 100% root discoloration.

In the plant development assay, Hutcheson had significantly higher root discoloration than Archer for all isolates of pathogenic *Pythium* spp. (Table 3). Archer had the most root discoloration with isolates of *P. irregulare* followed by *P. ultimum* and *P. vexans*. Isolates of the other species were not significantly different from *P. oligandrum*. Hutcheson had the most root discoloration with isolates of *P. vexans*, followed by group HS, *P. aphanidermatum*, *P. irregulare*, and then *P. ultimum*. All species caused significantly more root discoloration than *P. oligandrum* for Hutcheson (Table 3). Isolates of a given species reacted similarly.

Root weight in soil infested with isolates of *P. oligandrum* was greater for Hutcheson than Archer (Table 3). However, when soil was infested with isolates of pathogenic species, root weights were significantly less for Hutcheson compared with Archer for isolate 16 of *P. aphanidermatum* and all isolates of group HS, *P. irregulare*, *P. ultimum*, and *P. vexans*. All species reduced root weight in both cultivars when compared with *P. oligandrum*, except for *P. vexans* for cv. Archer. Both Archer and Hutcheson demonstrated the lowest numerical root weight with *P. ultimum*. *Pythium* spp. were recovered from all plated plants.

DISCUSSION

Archer was resistant to *P. ultimum*, *P. aphanidermatum*, *P. irregulare*, *P. vexans*, and group HS isolates compared with Hutcheson in the emergence assay, as well as establishment and plant growth assays, indicating that the resistance in Archer is robust over numerous *Pythium* spp. and over a range of plant development stages. Resistance was demonstrated in Archer as greater plant stand and less root discoloration compared with Hutcheson. Isolates showed some variation against the cultivars in the assays, especially Hutcheson in

the establishment assay. The variation among isolates against Hutcheson suggests some differences in aggressiveness of isolates within a species. However, these differences were minor compared with differences among species. In the plant growth assay, which was conducted over 6 weeks, differences between a maturity group I and a maturity group V cultivar seemed to play a larger role than in the seedling assays. Hutcheson was still growing vigorously and vegetatively at the end of 6 weeks, while Archer was setting pods. Thus, root weight for Hutcheson was much greater at 6 weeks than Archer in soil infested with *P. oligandrum*. However, Hutcheson root weight decreased dramatically in soil infested with any isolate of a pathogenic *Pythium* spp. compared with Archer in this longer assay, again demonstrating Archer's resistance.

P. oligandrum, reported to be a mycoparasite (2,3), worked effectively as a con-

trol. *P. oligandrum* frequently is associated with soybean roots in the field (10). In experiments where noncolonized sand-cornmeal also was used as a control, *P. oligandrum* had less variation than noninfested soil, with noncolonized sand-cornmeal medium negatively affecting plant growth in some cases (*data not shown*). This nutrient source in the control may have led to greater variation because of microbial activity or compounds in the medium affecting plant growth.

This research demonstrated that, for the isolates and the *Pythium* spp. examined, Archer is more resistant than Hutcheson to *Pythium* damping-off and root rot. Differences in virulence were evident among species. *P. irregulare*, *P. vexans*, and *P. aphanidermatum* appeared to be more important than *P. ultimum* and group HS in the seed rot and preemergent phases of the disease. *P. ultimum* and group HS demonstrated increasing virulence in the root rot

Table 3. Root discoloration and fresh root weight of Archer and Hutcheson for the plant growth assay^y

<i>Pythium</i> spp.	Isolate	Root discoloration ^z		Fresh root weight (g)	
		Archer	Hutcheson	Archer	Hutcheson
<i>P. aphanidermatum</i>	16	0.0 Bc	4.5 Ac	4.6 Ac	3.0 Bb
	64	0.1 Bc	4.3 Ac	4.5 Ac	3.1 Ab
	88	0.0 Bc	4.1 Ac	4.5 Ac	3.1 Ab
Group HS	117	0.0 Bc	5.8 Ab	9.2 Ab	2.6 Bb
	126	0.0 Bc	6.0 Ab	8.9 Ab	2.4 Bbc
<i>P. irregulare</i>	21	1.6 Ba	4.6 Ac	4.1 Ac	2.3 Bbc
	115	1.5 Ba	4.2 Ac	4.0 Ac	2.4 Bbc
<i>P. oligandrum</i>	120	0.0 Ac	0.0 Ae	20.8 Ba	40.9 Aa
	125	0.0 Ac	0.0 Ae	20.6 Ba	40.1 Aa
<i>P. ultimum</i>	124	1.0 Bb	3.2 Ad	2.2 Ac	1.6 Bc
<i>P. vexans</i>	140	0.9 Bb	6.1 Aab	21.8 Aa	2.4 Bbc
	182	0.9 Bb	6.6 Aa	21.4 Aa	2.4 Bbc

^y Inoculum was separated from seeds in pots by a layer of pasteurized soil. The experiment was terminated after 6 weeks. Means represent two combined experiments with five replications. Each pot was thinned to five plants. Cultivars for a *Pythium* isolate did not differ significantly if followed by the same capital letter, protected least significant difference (LSD; $P = 0.05$). Within a cultivar, *Pythium* isolates did not differ significantly if followed by the same lower-case letter, protected LSD ($P = 0.05$).

^z Disease rating based on scale of 0 to 10, where 0 = healthy and 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 to 40, 5 = 41 to 50, 6 = 51 to 60, 7 = 61 to 70, 8 = 71 to 80, 9 = 81 to 90, and 10 = 91 to 100% root discoloration.

Table 2. Plant stand, disease rating, and root weight of Archer and Hutcheson in the establishment assay^x

<i>Pythium</i> spp.	Isolate	Plant stand ^y		Disease rating ^z		Fresh root weight (g)	
		Archer	Hutcheson	Archer	Hutcheson	Archer	Hutcheson
<i>P. aphanidermatum</i>	16	4.3 Aa	3.4 Abc	1.4 Bde	3.8 Abc	2.3 Ab	1.8 Aabc
	64	4.2 Aa	4.7 Aa	1.8 Bbcd	4.5 Ab	2.0 Ab	1.9 Aabc
	88	4.6 Aa	4.0 Aabc	1.9 Bbcd	3.6 Ac	2.6 Aab	1.8 Babc
Group HS	117	4.2 Aa	4.3 Aab	2.2 Bbc	6.6 Aa	1.9 Ab	1.5 Abc
	126	4.4 Aa	3.1 Bc	1.6 Bcde	4.7 Ab	2.5 Aab	1.4 Bbc
<i>P. irregulare</i>	21	4.6 Aa	4.2 Aab	1.1 Bef	3.2 Ac	2.0 Ab	2.0 Aab
	115	4.3 Aa	4.3 Aab	1.1 Bef	3.4 Ac	2.4 Ab	2.0 Aab
<i>P. oligandrum</i>	120	4.6 Aa	4.4 Aab	0.2 Ag	0.3 Ad	2.8 Aa	2.3 Aa
	125	4.7 Aa	4.6 Aa	0.6 Afg	0.4 Ad	2.1 Ab	2.3 Aa
<i>P. ultimum</i>	124	4.6 Aa	3.4 Abc	1.9 Bbcd	7.1 Aa	1.8 Ab	1.3 Ac
<i>P. vexans</i>	140	4.6 Aa	4.1 Aabc	2.3 Bab	6.5 Aa	1.9 Ab	1.4 Abc
	182	4.2 Aa	4.4 Aab	2.9 Ba	4.1 Abc	1.9 Ab	1.7 Aabc

^x Inoculum was separated from seeds in pots by a layer of pasteurized soil. Means represent three combined experiments with three replications. The experiment was conducted at 20°C for 10 days. Cultivars for a *Pythium* isolate did not differ significantly if followed by the same capital letter, protected least significant difference (LSD; $P = 0.05$). Within a cultivar, *Pythium* isolates did not differ significantly if followed by the same lower-case letter, protected LSD ($P = 0.05$).

^y Plant stand from 5 seeds planted.

^z Disease rating based on scale of 0 to 10, where 0 = healthy and 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 to 40, 5 = 41 to 50, 6 = 51 to 60, 7 = 61 to 70, 8 = 71 to 80, 9 = 81 to 90, and 10 = 91 to 100% root discoloration.

phase of the disease. In addition to root discoloration, isolates of pathogenic *Pythium* spp. caused dramatic reductions in root weight in the plant growth assay, in some cases in the absence of appreciable root discoloration. It appears that, by colonizing primarily juvenile tissue near the root tips or by causing sloughing off of cortical tissue as the root matures over the 6 weeks of this assay, much of the damage from *Pythium* root rot was not associated with root rot symptoms. Seed lot was not a factor in the resistance observed with Archer, indicating that differences in disease reaction between cultivars were not due to differences in seed quality. Nanayakkara (11) also demonstrated the consistency of resistance in Archer compared with Hutcheson using artificially deteriorated seed.

The relationship between inheritance of *Pythium* resistance in Archer and its reported flood tolerance is unknown. Characterization or identification of the nature of the resistance would aid in breeding because other more adapted genotypes or cultivars may have this resistance. The nature of other cultivars believed to have resistance to *Pythium* spp. such as Dare, V81-141, (5), and Semmes (8) should be compared with Archer.

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