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Resistance to Pythium Seedling Disease in Soybean

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1. Introduction

Pythium spp. form one of the most important groups of seedling pathogens affecting soybean. Seedling diseases reduce stands, compromise plant vigor of surviving plants and may make replanting fields necessary (Yang, 1999). Yield reductions due to *Pythium* spp. have also been documented with the use of selective fungicide seed treatments (Poag, *et al*., 2005). While generally associated with cool, wet conditions, stand problems due to *Pythium* spp. can occur over a wide range of temperatures. At least seventeen species of *Pythium* are pathogenic to soybean and these species have a wide range of temperature optima. For example, *P. debaryanum*, *P. torulosum* and *P. ultimum*, infect soybean at low temperatures (20 °C or less) (Thomson *et al*., 1971; Yang, 1999) and affect early planted soybean. However, *P. aphanidermatum* and *P. myriotylum* infect soybean at high temperatures (30 °C or higher) (Littrell and McCarter, 1970; McCarter and Littrell, 1970; Thomson *et al*., 1971) and predominate in the southern United States, or when soybeans are planted late (Yang, 1999). Besides temperature, high soil water content is another important factor in seedling disease development. Saturated soils place plants under stress increasing loss of exudates from roots, diffusion of exudates away from the roots and, with many *Pythium* species, resulting in the production of motile zoospores that can swim in free water in the soil. All of the factors extend the distance from which these pathogens can recognize the presence of a host and infect the plant. These conditions are common in many soybean production areas, including Arkansas soybean fields.

Soybean is an important crop in the United States with more than 29 million hectares planted annually. Arkansas producers plant 1.2 to 1.4 million hectares annually with most soybean production in the state occurring on alluvial soils with poor internal and surface drainage. These soils are easily saturated favoring seedling diseases especially by *Pythium* spp. (Kirkpatrick *et al*., 2006b). In addition, soybean planting begins in April and often continues through June and into July, meaning that soybeans may experience a wide range of temperatures at planting. With the large number of *Pythium* spp. reported as pathogenic to soybean, there are often pathogenic species that are active at whatever soil temperatures occur at planting. The usual method of controlling seedling diseases caused by *Pythium* spp.

is to use fungicide seed treatments that include either metalaxyl or the active isomer mefenoxam (McGee, 1992). Plant resistance to Pythium damping-off is rare and has not been actively pursued in breeding programs; however we have identified a cultivar with high levels of resistance to Pythium damping-off that may be useful to reducing the impact of this disease in soybean (Rosso *et al*., 2008).

In 1996, we began investigating the effect of cultivars on stand when subjected to early flooding and which pathogens were favored by these saturated conditions. This was part of a larger project on flood tolerance supported by a grant from the United Soybean Board and headed by Dr. Tara VanToai. In this study, we compared the resulting stands of six cultivars with a range of flood tolerances subjected to no flood, flood at emergence or flood at the V4 growth stage. In addition we assayed roots to determine which pathogens were affected by flooding. Significant differences in stand among the cultivars occurred, especially with the flood at emergence. Of the pathogen groups isolated, *Macrophomina phaseolina*, *Fusarium* spp., and *Pythium* spp. responded to flooding with only the frequency of isolation of *Pythium* spp. increasing with flooding (Table 1) (Kirkpatrick *et al.*, 2006b). In the final year of the study, the cultivar Archer was included in the cultivar comparison.

Flood	*M. phaseolina*			*Pythium* spp.			*Fusarium* spp.		
	1996	1997	1998	1996	1997	1998	1996	1997	1998
No Flood	4.5	8.9	11.5	10.9	16.7	9.3	7.4	67.0	84.4
Emergence	3.0	3.0	10.0	20.9	27.3	55.3	10.4	62.4	53.9
LSD[c]		ns			18.5			17.8	
LSD[d]		ns			20.7			20.0	
No Flood	56.5	54.0	34.8	8.1	22.3	10.0	51.7	49.4	71.5
V4	29.9	24.8	24.8	42.8	31.1	45.9	37.4	56.7	56.7
LSD[c]		8.9			8.1			12.9	
LSD[d]		10.6			14.4			13.5	

[a]Plots were flooded for 3 days when the plants were emerging or for 7 days when the plants reached the four leaf growth stage (V4).
[b]Fifteen plants per plot of the cultivars Hartz 5164, Crowley, Asgrow 4715, NK S59-60, RVS-499, and TB881266 were sampled 4 days (1996) or 3 days (1997 and 1998) after removal of the flood and the roots were assayed for filamentous eukaryotic organisms.
[c]Least significant differences (LSD) to compare flood treatments within a year ($P = 0.05$).
[d]LSD to compare flood treatments among years ($P = 0.05$).

Table 1. Effect of flooding at soybean emergence or V4[a] growth stage on recovery (%) of *Macrophomina phaseolina, Pythium* spp., and *Fusarium* spp. from soybean roots of six genotypes[b] sampled over three years (Kirkpatrick, *et al.* 2006b).

Archer was a maturity group I cultivar that another group on the project had identified as highly flood tolerant (Lark, 2001, VanToai, *et al.* 2001). While significant differences between cultivars in these tests have been observed previously for flooding at emergence, relative stands of Archer were much higher than any other cultivar evaluated during the study (Table 2).

Cultivar	Flood	No-flood
Archer	36.7	76.7
Asgrow 4715	3.3	63.3
Crowley	0.0	50.0
Hartz 5164	0.0	56.7

[a] Stands taken four weeks after planting and are the mean of four replications.
[b] Plots flooded for 24 hours at emergence.

Table 2. Stands (%)[a] of cultivars when flooded[b] or not flooded at emergence in the field (Kirkpatrick, *et al.* 2006b).

To determine if the greater stands in Archer than the other cultivars was due just to flood tolerance or if seedling pathogens were involved, greenhouse studies were conducted comparing Archer to the cultivar Hutcheson. Hutcheson was a commonly grown, maturity group V cultivar that was sensitive to flooding. These cultivars were either flooded for 24 hr at emergence or not flooded and the soil either infested or not infested with *P. ultimum*. *Pythium ultimum* was chosen as the seedling pathogen since *Pythium* spp. were the only group of pathogens that increased with flooding and *P. ultimum* was one of the principle species isolated in the field test. In the absence of the pathogen, stands of both cultivars were similar and did not appear to be affected by flooding (Table 3). However, stands of Hutcheson were significantly reduced in the presence of *P. ultimum* in the non-flooded treatment and were completely eliminated in the flooded/infested treatment. Stands of Archer, on the other hand, were not affected by the presence of *P. ultimum* in the non-flooded treatment and were lower in the flooded/infested treatment, but were significantly higher than those of Hutcheson in either the no flood/infested or flood/infested treatments. While not immune, Archer demonstrated a high level of resistance to *P. ultimum* under these conditions.

	Cultivar			
	Archer		Hutcheson	
Flood	Non-infested	Infested	Non-infested	Infested
-	9.83	8.83	9.17	1.50
+	8.83	3.67	8.67	0.00
LSD[b]	1.54			
LSD[c]	0.99			
LSD[d]	1.19			

[a] Means represent the combined data from two experiments, with ten seeds planted per experimental unit.
[b] LSD (least significant difference) to compare infestation treatments within the same cultivar and flood treatment (P=0.05).
[c] LSD to compare flood treatments within the same cultivar and same or different infestation treatments (P=0.05).
[d] LSD to compare cultivars within the same or different flood and infestation treatments (P=0.05).

Table 3. Plant stand for two soybean cultivars at seven days, after termination of flooding at emergence, when seed were planted directly into soil infested with *P. ultimum* or noninfested soil[a] (Kirkpatrick, *et al.* 2006a).

Besides *P. ultimum*, four other *Pythium* spp. pathogenic to soybean were isolated in the field study, *P. aphanidermatum, P. irregulare*, *P. vexans* (=*Phytopythium vexans*) (Bala, *et al*. 2010), and HS group (a group that does not produce oospores or sporangia that would allow it to be identified to species), and the non-pathogenic species *P. oligandrum*. The effect of these species on stand and root rot in Archer and Hutcheson were compared (Bates, *et al.* 2008). With each *Pythium* species, stands of Hutcheson were significantly lower than those of Archer (Table 4). Similarly, root rot was significantly higher with Hutcheson than with Archer with all of the species except *P. oligandrum* which did not cause damage on either cultivar.

		Plant stand[b]		Disease rating[c]	
Pythium spp.	Isolate	Archer	Hutcheson	Archer	Hutcheson
P. aphanidermatum	16	9.1 Ad[d]	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
P. irregulare	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
P. oligandrum	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
P. ultimum	124	9.5 Ab	7.0 Bb	1.0 Bde	2.8 Ad
P. vexans	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

[a] 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 each.
[b]Plant stand from 10 seeds planted.
[c]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, and analyzed as the mid-percentile value.
[d]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 differ significantly if followed by the same lower-case letter, protected LSD (*P*=0.05).

Table 4. Plant stand and disease rating of Archer and Hutcheson for the emergence assay[a] (Bates *et al*. 2008).

There appears to be two phases to seedling disease caused by *Pythium* spp., a seed rot and a root rot phase, and the resistance in Archer affects both phases. Placing seed directly on *P. ultimum* inoculum severely reduced stands of Hutcheson in non-flooded soils and there was no stand in flooded soils. (Kirkpatrick *et al*. 2006a). With Archer, placing seed directly on the inoculum did not affect stand unless the plots were flooded and then stands were reduced by almost 65%. Under these experimental conditions, most of the seedling disease was probably due to seed rot. In order to determine the effect of *P. ultimum* on root rot, Kirkpatrick *et al.* (2006a) separated the seed from the inoculum with 2 to 5 mm of sterile soil (Table 5). This thin layer of sterile soil allowed the seeds to germinate before contacting the pathogen. When this was done, most seeds of both cultivars emerged. *Pythium ultimum* significantly increased root rot over the non-infested treatments only in flooded soil. This increase was significant for both cultivars, but was significantly greater for Hutcheson than for Archer. The reduction in stand and the degree of root rot was also affected by seed

	Archer		Hutcheson	
P. ultimum	No flood	Flood	No flood	Flood
Non-infested	6.13	16.73	5.37	6.33
Infested	9.33	32.93	11.07	49.50
		LSD[c]= 9.09		
		LSD[d]= 8.21		
		LSD[e]=11.25		

[a] Root rating scale from 0 to 5; with 0 =no discoloration, 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75%, and 5 =76-100% discoloration, and analyzed as the mid-percentile value.
[b] Data represent combined over two experiments.
[c] LSD (least significant difference) to compare infestation treatments within same flood treatment (P=0.05).
[d] LSD to compare infestation treatments within the same cultivar and flood treatments (P=0.05).
[e] LSD to compare flood treatments within the same cultivar and same or different infestation treatments (P=0.05).

Table 5. The effect of *Pythium ultimum* and flooding at soybean emergence on root discoloration[a] for the two soybean cultivars Archer and Hutcheson when planted on a layer of pasteurized soil over the soil treatment[b] (Kirkpatrick, et al. 2004a)

quality. Nanayakkara (2001) using this inoculum layer technique with four *Pythium* species found that stands of Hutcheson were significantly lower with the lowest seed quality, but root rot increased significantly with the medium seed quality across all *Pythium* species (Table 6).

	Archer			Hutcheson		
Pythim spp.	High	Medium	Low	High	Medium	Low
Stand (%)						
Non-infested	93.3	86.7	80.0	100.0	93.4	93.3
P. vexans	86.7	73.3	53.3	100.0	93.3	73.3
P. aphanidermatum	86.7	66.7	20.0	93.3	100.0	66.7
P. ultimum	100.0	100.0	26.7	100.0	86.7	100.0
P. irregular	86.7	26.7	20.0	100.0	86.7	86.7
$LSD_{0.05}$=21.8						
Root Rot (%)						
Non-infested	0.3	2.3	3.0	0.6	1.7	1.7
P. vexans	42.3	53.7	81.7	8.0	15.0	54.7
P. aphanidermatum	6.7	32.7	50.5	3.7	6.7	10.7
P. ultimum	10.7	18.0	75.5	7.3	10.7	31.3
P. irregulare	6.7	22.3	63.0	5.0	10.0	22.3
$LSD_{0.05}$=15.0						

[a]High quality seed of each cultivar was artificially aged to create three seed quality levels: high, medium, and low. Standard germination for Archer were 88, 77,58% and for Hutcheson were 89, 78, and 58%, respectively.
[b] Vermiculite was used as the potting medium. Seed was separated from a layer of sand/corn meal inoculum by 1.5 cm of sterile vermiculite.

Table 6. The effect of *Pythium* spp. on stand and root rot of high, medium, and low quality seedlots[a] of the cultivars Archer and Hutcheson at 20 ^{0}C[b] (Nanayakkara, 2001).

		Root discoloration[b]		Fresh root weight (g)	
Pythium spp.	Isolate	Archer	Hutcheson	Archer	Hutcheson
P. aphanidermatum	16	0.0 Bc[c]	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
P. irregulare	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
P. oligandrum	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
P. ultimum	124	1.0 Bb	3.2 Ad	2.2 Ac	1.6 Bc
P. vexans	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

[a] 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.
[b] 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, and analyzed as the mid-percentile value.
[c] 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 ($P = 0.05$).

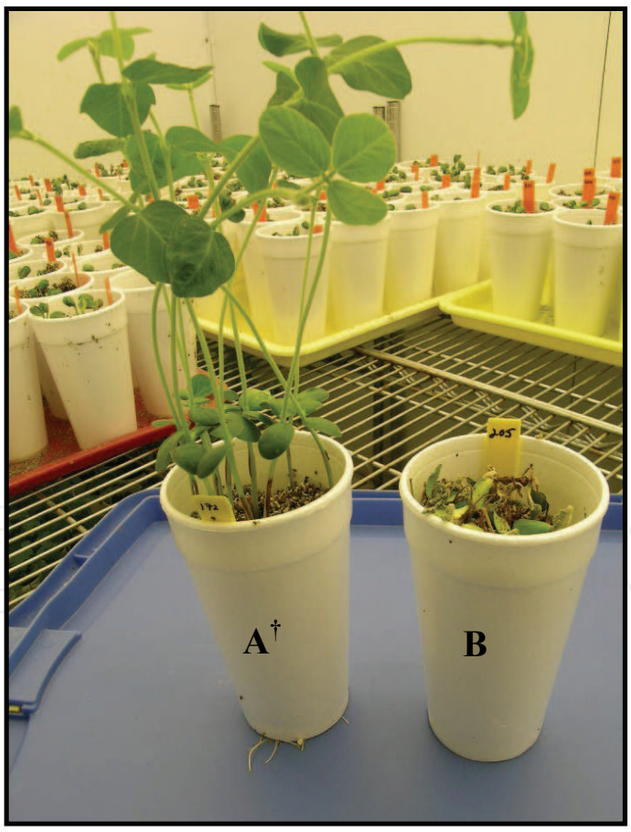
Table 7. Root discoloration and fresh root weight of Archer and Hutcheson for the plant growth assay[a](Bates *et al*. 2008).

Significant reductions in stand and increases in root rot only occurred at the lowest quality seed lot with Archer and not with all species. With low quality seed lots, Archer had significantly greater stands and less root rot than Hutcheson. Bates *et al*. (2008) found that root rot was lower and root weights were higher for Archer than Hutcheson when inoculated with *P. aphanidermatum*, HS group, *P. irregulare, P. ultimum*, or *P. vexans*. Reductions in root weights were observed in Hutcheson after two weeks, but not in Archer. After six weeks, reductions in root weights occurred in both cultivars but were greater for Hutcheson than Archer (Table 7). Root weights were not reduced with Archer after six weeks when inoculated with *P. vexans*, but were sharply reduced in Hutcheson with this species.

To determine if this resistance in Archer was effective in the field, Archer and Hutcheson were treated or not treated with metalaxyl and planted at five locations in Arkansas at three planting dates (April, May, and June) and flooded or not flooded at emergence. Stands were taken four weeks after planting. The test was conducted for three years and resulted in 140 comparisons. To determine the effectiveness of Archer resistance, the number of tests in which the metalaxyl treatment had a significantly greater stand than the non-treated control for each cultivar was determined. There were significantly more tests where Hutcheson responded to metalaxyl (41 tests) than did Archer (18 tests) showing that the resistance in Archer was reducing the impact of Pythium seedling disease under field conditions (unpublished data). In addition, the effect of metalaxyl occurred at all planting dates, with both seed qualities, and in flooded and non-flooded treatments clearly showing that

seedling diseases caused by *Pythium* spp. occurred across a wide range of environmental conditions and seed qualities.

The cultivar Archer was developed in Iowa and released in 1990, because of its resistance to specific races of *Phytophthora sojae* (Cianzio *et al*., 1991). 'Archer' has the resistance genes *Rps*6 and *Rps*1k, derived from 'PRX54-59' and 'Williams 82', respectively. Previous studies speculated that Archer resistance to *Pythium* could be associated with *Phytophthora* resistance genes (*Rps*), particularly to *Rps*1k (Bates *et al*., 2004). Bates *et al.* (2004) observed that when a set of differential cultivars containing specific resistance genes for *P. sojae* were planted in vermiculite infested with *P. aphanidermatum* and assessed for disease, the cultivar Williams 82 (*Rps* 1k) demonstrated resistance to *P. aphanidermatum* similar to Archer resistance. In addition, Pioneer (Pioneer Seed Co., Johnston, Iowa) cultivars 94M70 and 94M41 (with *Rps*1k) inoculated in the greenhouse with *P. aphanidermatum* using a hypocotyl inoculation technique showed significantly higher plant survival than cultivars 94B13 and 94M90 (without *Rps*1k) (Rosso *et al*., 2005). Pioneer cultivars with the *Rps*1k gene also had significantly less root discoloration and *Pythium* incidence in field trials than those without the gene (Rosso *et al*., 2005). However, the interpretation of the role of *Rps* 1k in resistance was complicated since Williams 82 was not only a parent of Archer, but in the background of other lines having *Rps* 1k and thus other genes from this parent.



†A. Resistant reaction in the resistant parent 'Archer'.
B. Susceptible reaction in the susceptible parent 'Hutcheson'.

Fig. 1. Reactions of 'Archer' and 'Hutcheson' plants seven days after inoculation with *P. aphanidermatum* by the hypocotyl inoculation technique (Rosso *et al*., 2008).

Parents/ population[a]	No. of Plants[b]			Chi-square 1:2:1 ratio (df=2)	
	R	I	S	Value	*P*-value
'Archer' (R)	34		6		
'Hutcheson' (S)	2		38		
'Archer' x 'Hutcheson'	21	48	17	1.53	0.5-0.25

[a]Each experimental unit contained 10 plants and was repeated 4 times. Experiment performed under 12-h day length at 28 °C. The experiment was run twice.
[b]R= resistant (≥ 70% plant survival); I= intermediate reaction (69 to 31 %, plant survival); S= susceptible (≤ 30% plant survival).

Table 8. Reactions to *Pythium* damping-off of parents and $F_{2:4}$ lines from an 'Archer' (R) x 'Hutcheson' (S) cross in hypocotyl inoculations[a] with *Pythium aphanidermatum* . (Rosso *et al*., 2008).

Rosso *et al.* (2008) made a cross between Archer and Hutcheson, and 86 $F_{2:4}$ lines were developed. Resistance in these lines as well as the parents was tested by using a hypocotyl inoculation method with *P. aphanidermatum*. The hypocotyl inoculation method was developed for identifying major resistance genes to *Phytophthora sojae* (Dorrance, *et al*. 2004; Gordon, *et al.* 2007). The results showed a clear separation between the parents by phenotype. When plants were inoculated in the hypocotyl with *P. aphanidermatum* nearly all seedlings were killed in the susceptible parent Hutcheson whereas most of the plants remained healthy in the resistant parent Archer (Fig. 1). Using this method to screen the 86 $F_{2:4}$ lines, resulted in a ratio of resistant:segregating:susceptible lines that fit a 1:2:1 ratio consistent with a single gene for resistance (Table 8). These lines were then screened with race 7 of *Phytophthora sojae* to determine which lines carried the *Rps*1k gene. These results also indicated a single gene as expected, but linkage analysis indicated that resistance to *Pythium* was regulated by a separate gene, not linked to *Rps*1k. The Pythium resistance gene was designated *Rpa*1.

Resistance to *Pythium* spp, conferred by a single dominant gene has been also reported in other crops. Yang *et al*. (2005) found that resistance to *P. inflatum,* the causal agent of stalk rot in maize, was controlled by a single dominant gene, (*Rpi1)* in the cross of the maize inbred lines 1145 (resistant) and Y331 (susceptible). In periwinkle (*Catharanthus roseus*), resistance to dieback, caused by *P. aphanidermatum* was reported to be governed by a single gene and inherited independently of genes governing dwarfness and stem pigmentation (Kulkarni and Baskaran, 2003). Otsyula *et al.* (2003) indicated that the nature of resistance to *P. ultimum* root rot, in the bean genotypes, MLB 49-89A and 1062 and RWR 719 is controlled by a single dominant gene. Likewise, Mahuku *et al.* (2005) reported that a single dominant gene conditioned resistance to *P.ultimum* var. *ultimum* in *Phaseolus vulgaris.*

The location of *Rpa*1 was determined using 88 SSR primers from the 20 major linkage groups (MLG) in soybean (Rosso, *et al.* 2008). These primers were tested against the parents, Archer and Hutcheson, and a resistant bulk made up of 12 of the resistant $F_{2:4}$ lines and a susceptible bulk composed of 10 of the susceptible $F_{2:4}$ lines. Two markers, Satt114 and Satt 510, were polymorphic between the parents and the resistant and susceptible bulks.

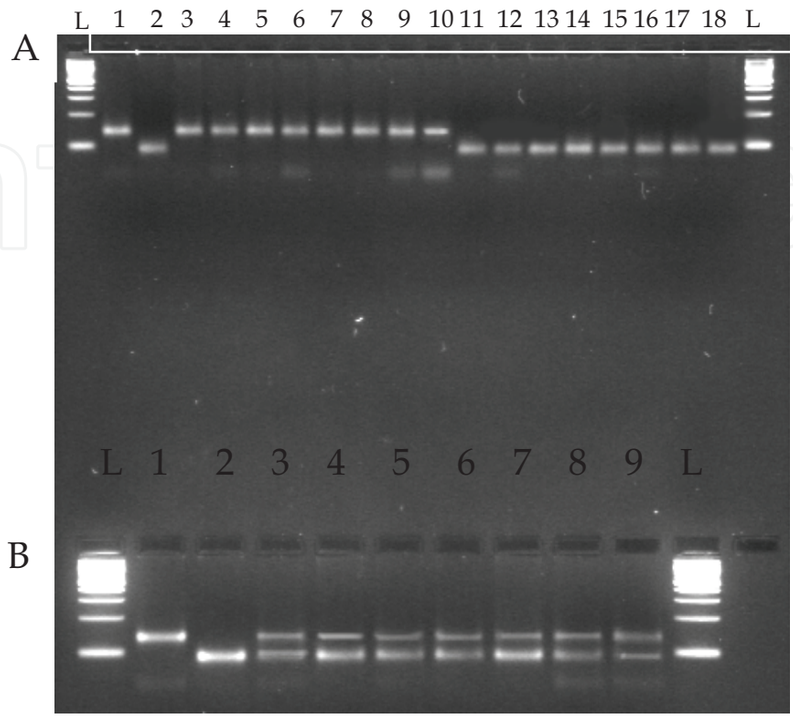
Evaluating the 86 $_{F2:4}$ lines, it was found that some lines had the same bands as Archer, or Hutcheson or both (Fig. 2). In the inoculation tests, these lines were classified as resistant,

susceptible, or segregating, respectively. Satt114 and Satt510 were 15.5 cM and 10.6 cM from *Rpa*1, respectively, and were located on the MLG F of the soybean genome (Fig. 3). Besides *Rpa*1, MLG F contains many other single-gene disease resistance loci and QTL's. For example, there are two resistance genes that confer resistance to *P. sojae* (*Rps*), *Rps*3 and (Burnham *et al*., 2003a, Demirbas *et al*., 2001, Sandhu *et al*. 2005), one resistance gene that confers resistance to *Phomopsis* seed decay (*Rpsd*1) (Jackson, *et al.* 2005). The linkage group F also contains additional disease resistance loci conferring bacterial disease resistance (*Pseudomonas syringae* pv. *glycinea*) and two viral resistance alleles, *Rsv*1 (resistance to *Soybean mosaic virus*) and *Rpv*1 (resistance to *Peanut mottle virus*) (Ashfield *et al*., 1998; Ashfield *et al*., 2004; Gore, 2000; Hayes *et al*., 2004; Koning *et al*., 2002; Schmittehener, 1999). A QTL for partial resistance to *P. sojae* was reported on MLG F between marker Satt252 and Satt423 (Burnham *et al*., 2003b) and QTL's conferring resistance to *Meloidogyne javanica, M. arenaria*, *Heterodera glycines*, and soybean sudden death syndrome (SDS) have been mapped on the MLG F (Concibido *et al*., 1994; Hnetkovsky *et al.,* 1996; Tamulonis, *et al*., 1997a; Tamulonis *et al.,* 1997b; Webb *et al*., 1995).

Nutrient availability is a limiting factor for microbial growth and activity in agricultural soils, as a result plant pathogenic fungi and oomycetes propagules survive in soils in a state of exogenous dormancy or fungistasis (Lockwood, 1977). In order for a successful host-pathogen interaction to be initiated, fungistasis must be overcome by external stimulants (Curl and Truelove, 1986; Mitchell, 1976). Soluble and volatile exudates from germinating seeds and developing roots are the primary stimuli for such responses. Some compounds inhibit pathogen growth, thus preventing seed/root infection (Rose *et al*., 2006), while others have a direct beneficial effects on germination itself (Barbour *et al*., 1991). The primary groups of compounds released from seed and root exudates are soluble sugars, amino acids, organic acids, flavonoids, sterols and proteins (Casey, *et al.*, 1998: Nelson, 1990; Terras, *et al*. 1995).

Soilborne *Pythium* species are among the most responsive group to germinating seeds and developing roots in diverse host systems. For example, sporangia of *P. ultimum* and oospores of *P. aphanidermatum* germinate within 1.5 to 3 h after exposure to bean or pea exudates (Lifshitz *et al*., 1986; Stanghellini and Burr, 1973; Stanghellini and Hancock, 1971a; 1971b). Increased germination of encysted zoospores of *P. aphanidermatum* was observed by the addition of three-week-old pea root exudates (Chang-Ho, 1970). Exudates collected from 6-day-old red pine seedlings stimulate the germination of sporangia and promote growth of *P. ultimum* and *P.irregulare* (Agnihotri and Vaartaja, 1967a, 1970). The stimulatory effect of plant exudates on *Pythium* was earlier described by Agnihotri an Vaartaja (1967a; 1967b; 1970) who found mixtures of sugars and amino acids to be stimulatory to *P. ultimum* and *P. irregulare* while Chang-Ho (1970) found mixtures of glucose and organic acids to be most stimulatory to zoospore cysts of *P. aphanidermatum.* Furthermore, both Flentje and Saksena (1964) and Keeling (1974) observed more seed rot caused by *Pythium* spp. among pea and soybean cultivars that released more sugars and amino acids during germination than among those cultivars that release less. Mathew and Bradnock (1968), also observed a direct correlation between carbohydrates exudation *in vitro* and decreased emergence of pea seedlings due to Pythium seed rot.

When seed exudates were examined, total sugar and organic acid concentrations were less in seed exudates of the Pythium resistant cultivar Archer than those in the susceptible cultivar Hutcheson (Table 9)(Nanayakara, 2001). The levels of these exudates increased as the seed quality of each cultivar was reduced, but those increases were greater with



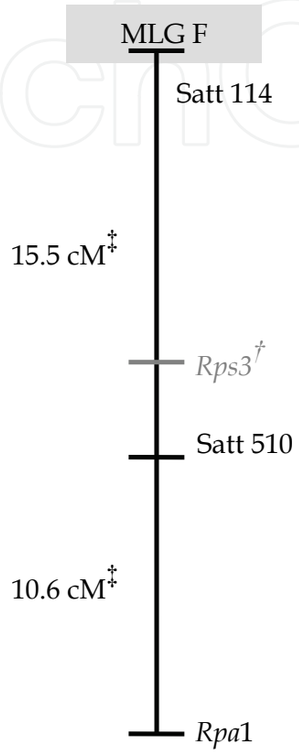
A – L, 100 bp DNA ladder. Lane 1, resistant parent ('Archer'); lane 2, susceptible parent; lanes 3-10, resistant plants; lanes 11-18, susceptible plants.
B- L, 100 bp DNA ladder. Lane 1, resistant parent ('Archer'); lane 2, susceptible parent; lanes 3-9, heterozygous plants.

Fig. 2. PCR amplification of SSR marker Satt 510 linked to *Pythium* damping-off (caused by *P. aphanidermatum*) resistance in 'Archer' x 'Hutcheson' $F_{2:4}$ populations(Rosso *et al*., 2008).

	Total Organic Acids		Total Sugars	
Seed quality[a]	Archer	Hutcheson	Archer	Hutcheson
High	16.97	79.03	292.89	405.69
Medium	68.69	349.07	326.24	1,229.29
Low	115.40	380.32	422.63	1,433.67

[a]High quality seed of each cultivar was artificially aged to create three seed quality levels: high, medium, and low. Standard germination for Archer were 88, 77, and 58% and for Hutcheson were 89, 78, and 58%, respectively.

Table 9. Concentrations (µg/seed/72hr) of total sugars and organic acids in seed exudates of Archer and Hutcheson at three levels of seed quality (Nanayakara, 2001).



‡ Distance in cM from the SSR markers to the resistance gene *Rpa*1.
†*Rps*3: Resistance gene to *Phytophthora sojae* (Gordon *et al.*, 2007).

Fig. 3. Proposed genetic linkage map of *Pythium* damping-off (caused by *Pythium aphanidermatum*) resistance gene in soybean. *Rpa*1 is a proposed designation for the resistance gene according to the standard nomenclature for plant disease resistance genes (Rosso *et al.*, 2008).

Hutcheson than Archer. Fewer zoospores of *P. aphanidermatum* were detected in preference assays for seed exudates from Archer than exudates from Hutcheson seed. However, evidence suggests this response may be the result of inhibitory compounds rather than stimulatory compounds. Radial hyphal growth of Pythium isolates was inhibited by exudates from Archer seed compared to no exudates or seed exudates from Hutcheson. In addition, an 'exchange exudates' experiment treating imbibed Archer seed with Hutcheson exudates reduced plant stands in infested soil compared to Archer seed treated with Archer exudates while treating imbibed Hutcheson seed with Archer exudates increased plant

stands compared to Hutcheson seed treated with Hutcheson exudates (*unpublished* data). Current research is being conducted to determine the major components of seed and seed exudates of Archer and Hutcheson cultivars and their roles in the resistant/susceptible responses.

Differences in seed exudates do not appear to be the full story for the resistance in Archer to *Pythium* spp. Resistance in Archer was expressed in surviving plants in soil infestation experiment and more importantly using hypocotyls inoculations where mycelium of the pathogen is placed directly into the hypocotyls of seedling. It is unlikely that seed exudates affect the infection process under these conditions and an induced defensive response is likely. The nature of this response is not known at this time.

2. Conclusions

The cultivar Archer possesses a resistance gene, *Rpa*1 that is located on MLG F which confers a high level of resistance to *Pythium aphnidermatum* and possible a number of other *Pythium* spp. pathogenic to soybean. This resistance is expressed under field conditions and should be useful in reducing the risk of stand loss due to this group of pathogens. In addition to protecting the plants during stand establishment where seed treatment fungicides are effective, resistance may continue throughout the season. Resistance appears to be due both to differences in pre-existent defense barriers due to compounds in seed exudates and to the induction of active defense pathways in the plant. Future work will try to better understand how these defense pathways work and if they are conferred by the same gene.

3. References

Agnihotri, V.P., and Vaartaja, O. 1967a. Root exudates from red pine seedlings and their effects on *Pythium ultimum*. Can. J. Bot. 45:1031-1040

Agnihotri, V.P., and Vaartaja, O. 1967b. Effects on amendments, soil moisture contents, and temperature on germination of *Pythium* sporangia under the influence of soil mycostasis. Phytopathology 57:1116-1120

Agnihotri, V.P., and Vaartaja, O. 1970. Effect of seed exudates from *Pinus resinosa* and their effects on growth and germination of *Pythium afertile.* Plant and Soil 32:246-249

Ashfield, T., Danzer, J.R., Held, D., Clayton, K., Keim, P., Sanghai Maroof, M. A., Webb,D.M., and Innes, R.W. 1998. *Rpg*1, a soybean gene effective against races of bacterial blight, maps to a cluster of previously identified disease resistance genes. Theor. Appl. Genet. 96: 1013-1021

Ashfield, T., Ong, L. E., Nobuta, K., Schneider, C.M., and Innes, R.W. 2004. Convergent evolution of diseased resistance gene specificity in two flowering plant families. Plant Cell 16: 309-318

Bala, K., Robideau, G.P., Lévesque, A, Cock de, A.W.A.M., Abad, Z.G., Lodhi, A.M., Shahzad, S., Ghaffar, A., Coffey, M.D., 2010. *Phytopythium* Abad, de Cock, Bala, Robideau & Lévesque, gen. nov. and *Phytopythium sindhum* Lodhi, Shahzad & Levesque, sp. nov. Persoonia 24: 136-137

Barbour, W.M., Hatterman, D.R., and Stacey, G. 1991.Chemotaxis of *Bradyrhizobium japonicum* to soybean exudates. Appl. Environ. Microbiol. 57:2625-2639

Bates, G.D., Rothrock, C.S., Rupe, J.C. and Chen. P. 2004. Resistance in soybean cultivars to *Pythium* damping-off and root rot. Phytopathology 94:S7.

Bates, G. D., Rothrock, C. S., and Rupe, J. C. 2008. Resistance of the soybean cultivar Archer to Pythium damping-off and root rot caused by several *Pythium* spp. Plant Dis.92:763-766

Burnham, K.D., Dorrance, A.E., Francis, D.M., Fioritto, R.J., and St. Martin, S.K. 2003a. *Rps*8,A new locus in soybean for resistance to *Phytophthora sojae*. Crop Sci. 43 101-105

Burnham, K.D., Dorrance, A.E., Van Toai, T.T., and St Martin, S.K. 2003b. Quantitative trait loci for partial resistance to *Phytophthora sojae* in soybean. Crop Sci. 43: 1610-1617

Casey, C.E., O'Sullivan, O.B., O'Gara, F., and Glennon, J.D. 1998. Ion chromatographic analysis of nutrients in seed exudates for microbial colonization. J. Chromatogr. 804:311-318

Chang-Ho, Y. 1970. The effect of pea root exudate on the germination of *Pythium aphanidermatum* zoospore cysts. Can. J. Bot. 48:1501-1514

Cianzio, S.R., Shultz, S.P., Fehr, W.R., and Tachibana, H. 1991. Registration of 'Archer' Soybean. Crop. Sci. 31: 1707

Concibido, V.C., Denny, R.L., Boutin, S.R., Hautea, R., Orf, J.H., and Young, N.D. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci. 34: 240-246

Curl, E.A., and Truelove, B. 1986. 'The Rhizosphere', Springer-Verlag, New York, 288 p.

Demirbas, A., Rector, B.G., Lohnes, D.G., Fioritto, R.J., Graef, G.L., Cregan, G.L., Shoemark, R.C., and Specht J.E. 2001. Simple sequence repeat markers inked to the soybean Rps genes for *Phytophthora* resistance. Crop Sci. 41: 1220-1227

Dorrance, A. E., Jia, H., and Abney, T. S. 2004. Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. Online. Plant Health Progressdoi:10.1094/PHP-2004-0309-01-RS.

Flentje, N.T. and Saksena, H.K. 1964. Pre-emergence rotting of peas in South Australia. III.Host-pathogen interaction. Aust. J. Biol. Sci. 17:665-675

Gordon, S.G., K. Kowitwanich, W. Pipatpongpinyo, S.K. St. Martin, and A.E. Dorrance. 2007. Molecular marker analysis of soybean plant introductions with resistance to *Phytophthora sojae*. Phytopathology 97:113-118

Gore, M.A. 2000. High-resolution mapping of the region around the soybean virus resistance genes, *Rsv*1 and *Rpv*1. M.S. thesis. Virginia Tech, Blacksburg, VA

Hayes, A.J., Jeong, S.C., Gore, M.A., Yu, Y.G., Buss, G.R., Tolin, S.A., and Saghai Maroof, M.A. 2004. Recombination within a nucleotide-binding-site/leucine-rich-repeat gene cluster produces new variants conditioning resistance to *Soybean mosaic virus* in soybeans. Genetics 166: 493-503

Hnetkovsky, N., Chang, S.C., Doubler, T.W., Gibson, P.T., and Lightfoot, D.A. 1996. Genetic mapping of loci underlying field resistance to soybean sudden death syndrome (SDS). Crop. Sci. 36: 393-400

Jackson, E.W., Fenn, P., Chen, P. 2005. Inheritance to resistance to Phomopsis seed decay in soybean PI 80837 and MO/PSD-0259 (PI 562694).Crop Sci. 45:2400-2404.

Keeling, B.L 1974. Soybean seed rot and the relation of seed exudates to host susceptibility. Phytopathology 64:1445-1447

Kirkpatrick, M.T., Rothrock, C.S., and Rupe, J.C. 2006a. The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Dis. 90: 597-602

Kirkpatrick, M.T., Rupe, J.C., and Rothrock, C.S.2006b. Soybean response to flooded soil conditions and the association with soilborne plant pathogenic genera. Plant Dis. 90: 592-597

Koning, G., TeKrony, D.M., Ghabrial S.A., and Pfeiffer, T.W. 2002. *Soybean mosaic virus* (SMV) and the SMV resistance gene (*Rsv*1): Influence on *Phomopsis* spp. seed infection in an aphid free environment. Crop Sci. 42: 178-185

Kulkarni, R.N., and Baskaran, K. 2003. Inheritance of resistance to *Pythium* dieback in the medicinal plant periwinkle. Plant Breeding 122:184-187

Lark. K. 2001. Identification of a QTL Associated with tolerance of soybean to soil waterlogging. Crop Sci. 41:1247-1252

Lifshitz, R., Windham, M.T., and Baker, R. 1986. Mechanism of biological control of preemergence damping-off of pea by seed treatment with *Trichoderma* spp. Phytopathology 76:720-725

Littrell, R.H., and McCarter, S.M. 1970. Effect of soil temperature on virulence of *Pythium aphanidermatum* and *Pythium myriotylum* to rye and tomato. Phytopathology 60: 704

Lockwood, J.L. 1977. Fungistasis in soils. Biol. Rev. 52: 1-43

Mahuku, G.S., Buruchara, R.A., and Navia, M. 2005. A gene that confers resistance to *Pythium* root rot in common bean: Genetic characterization and development of molecular markers. Phytopathology 95:S64

Mathew, S., and Bradnock, W.T. 1968. Relationship between seed exudation and field emergence in peas and French beans. Hortic.Res. 8:89-93

McCarter, S.M., and Littrell, R.H. 1970 Comparative pathogenicity of *Pythium aphanidermatum* and *Pythium myriotylum* to twelve plant species and intraespecific variation in virulence. Phytopathology 60: 264-268

McGee, D.C. 1992. Soybean Diseases: A reference source for seed technologists. APS Press, St. Paul, Minnesota

Mitchell, J.E. 1976. The effect of roots on the activity of soilborne pathogens. In Encyclopedia of Plant Physiology, New Series, Vol.4, Physiological Plant Pathlogy

Nanayakara, R. 2001. Influence of soybean cultivars, seed quality, and temperature on seed exudation and *Pythium* disease development. Ph.D. Dissertation. University of Arkansas

Nelson, E.B., 1990. Exudate molecules initiating fungal responses to seeds and roots. Plant and Soil 129:61-73

Otsyula, R., Rubaihayo, P., and Buruchara, R.A. 2003. Inheritance of Resistance to *Pythium* Root Rot in Beans (*Phaseolus vulgaris*) genotypes. African Crop Science Conference Proceeding 6: 295-298.

Poag, P.S., Popp, M., Rupe, J., Dixon, B., Rothrock, C., and Boger, C. 2005. Economic evaluation of soybean fungicide seed treatments. Agron. J. 97:1647-1657.

Rose, T.L., da Silva Conceição,A., Xavier-Filho, J., Okorokov, L.A., Fernandes, K.V.S., Marty, F., Marty-Mazars, D., Carvalho, A.O., and Gomes, V.M. 2006. Defense proteins from *Vigna unguiculata* seed exudates: characterization and inhibitory activity against *Fusarium oxysporum.* Plant Soil 286:181-191

Rosso, M.L., Rupe, J.C., and Rothrock, C. 2005. Resistance of soybean cultivars to *Pythium* damping-off and root rot based on *Rps*1k gene. Phytopathology 95:S90

Rosso, M.L., Rupe, J.C., Chen, P., Mozzoni, L.A. 2008. Inheritance and Genetic Mapping of Resistance to *Pythium* Damping-off Caused by *Pythium aphanidermatum* in 'Archer' Soybean. Crop Sc. 48 : 2215-2222

Sandhu, D., Schallock, K.G., Rivera-velez, N., Lundeen, P., Cianzio, S., and Bhattacharyya, M.K. 2005. Soybean *Phytophthora* resistance gene *Rps*8 maps closely to the *Rps*3 region. J. Hered. 96: 536-541

Schmitthenner, A.F.1999. *Phytophthora* rot. Pages 39-42 in: Compendium of soybean diseases. G.L. Hartman, J.B. Sinclair, and J.C. Rupe, ed. 4th edition. APS Press. St. Paul. MN 55121.

Stanghellini, M.E., and Burr, T.J. 1973. Germination *in vivo* of *Pythium aphanidermatum* oospores and sporangia. Phytopathology 63:1493-1496

Stanghellini, M.E., and Hancock, J.G. 1971a. The sporangium of *Pythium ultimum* as a survival structure in soil. Phytopathology 61:157-164

Stanghellini, M.E., and Hancock, J.G. 1971b. Radial extent of the bean spermosphere and its relation to the behavior of *Pythium ultimum.* Phytopathology 61:165-168

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A., and Boerma, H.R. 1997a. DNA markers associated with resistance to Javanese root-knot nematode in soybean. Crop Sci. 37: 783-788

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A., and Boerma, H.R. 1997b. DNA marker analysis of loci conferring resistance to peanut root-knot nematode in soybean. Theor. Appl. Genet. 95: 664-670

Terras, F.G.R., Eggermont, K., Kovaleva, V., Raikhel, N.V., Osborn, R.W., Kester, A., Rees,B., Torrekens, S., Van Leuven, F., Vanderleyden, J., Cammue B.P.A., and Broekaert, W.F. 1995. Small cysteine-rich antifungal proteins from radish: their role in host defense. Plant Cell 7:573-588

Thomson, T.B., Athow, K.L. and Laviolette, F.A. 1971. The effect of temperature on the pathogenicity of *Pythium aphanidermatum*, *P. debaryanum*, and *P. ultimum* on soybean. Phytopathology 61: 933-935

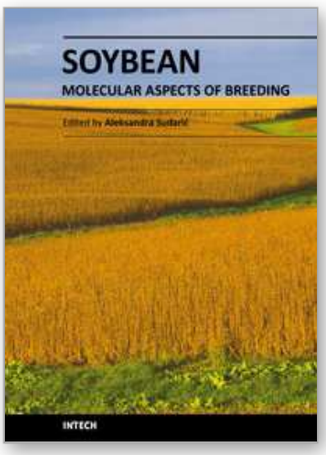
VanToai, T., St. Martin, S., Chase, K., Boru, G., Schnipke, V., Schmitthenner, A. F., and Lark. K. 2001. Identification of a QTL Associated with tolerance of soybean to soil waterlogging. Crop Sci. 41:1247-1252

Webb, D.M., Baltazar, B.M. Rao-Arelli, A.P., Schupp, J., Clayton, K. Keim, P., and Beavis, W.D. 1995. Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.695. Theor. Appl. Genet. 91: 574-581

Yang, D.E., Jin, D.M., Wang, B., Zhang, D.S., Nguyen, H.T., Zhang, C.L., and Chen, S.J. 2005. Characterization and mapping of Rpi1, a gene that confers

dominant resistance to stalk rot in maize. Molecular Genetics and Genomics 274 (3): 229-234

Yang, X.B. 1999. *Pythium* damping-off and root rot. Pages 42-44 in: Compendium of Soybean Diseases. G.L. Hartman, J.B. Sinclair, and J.C. Rupe, ed. 4th edition. APS Press. St. Paul. MN 55121



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The book Soybean: Molecular Aspects of Breeding focuses on recent progress in our understanding of the genetics and molecular biology of soybean and provides a broad review of the subject, from genome diversity to transformation and integration of desired genes using current technologies. This book is divided into four parts (Molecular Biology and Biotechnology, Breeding for Abiotic Stress, Breeding for Biotic Stress, Recent Technology) and contains 22 chapters.

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