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# Integrated Management Strategies for *Phytophthora sojae* Combining Host Resistance and Seed Treatments

A. E. Dorrance

*Ohio State University*, [dorrance.1@osu.edu](mailto:dorrance.1@osu.edu)

A. E. Robertson

*Iowa State University*

S. Cianzo

*Iowa State University*

L. J. Giesler

*University of Nebraska-Lincoln*, [lgiesler1@unl.edu](mailto:lgiesler1@unl.edu)

C. R. Grau

*University of Wisconsin-Madison*

*See next page for additional authors*

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**Authors**

A. E. Dorrance, A. E. Robertson, S. Cianzo, L. J. Giesler, C. R. Grau, M. A. Draper, A. U. Tenuta, and T. R. Anderson

# Integrated Management Strategies for *Phytophthora sojae*

## Combining Host Resistance and Seed Treatments

A. E. Dorrance, Department of Plant Pathology, The Ohio State University, OARDC, Wooster 44691; A. E. Robertson and S. Cianzo, Department of Plant Pathology, Iowa State University, Ames 50011-1020; L. J. Giesler, Department of Plant Pathology, University of Nebraska, Lincoln 68583; C. R. Grau, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706-1598; M. A. Draper, formerly Plant Science Department, South Dakota State University, Brookings 57007-1090; A. U. Tenuta, Ontario Ministry of Agriculture, Food and Rural Affairs, Ridgetown, Ontario, Canada, N0L 2C0; and T. R. Anderson, Agriculture and Agri-Food Canada, Harrow, Ontario, Canada, N0R 1G0

### ABSTRACT

Dorrance, A. E., Robertson, A. E., Cianzo, S., Giesler, L. J., Grau, C. R., Draper, M. A., Tenuta, A. U., and Anderson, T. R. 2009. Integrated management strategies for *Phytophthora sojae* combining host resistance and seed treatments. *Plant Dis.* 93:875-882.

*Phytophthora sojae* has re-emerged as a serious soybean pathogen in the past decade. This may be due in part to changes in resistance levels in current cultivars, adoption of *P. sojae* populations to deployed *Rps* genes, and highly favorable environments in the past decade. This multilocation study evaluated the effect of seed treatments on the incidence and severity of *Phytophthora* root and stem rot on soybeans with different combinations of *Rps* genes and levels of partial resistance. The efficacy of the seed treatments was highly variable across locations. Seed treatments (metalaxyl and mefenoxam) provided protection and increased yields across cultivars in locations where rain or irrigation occurred shortly after planting (Ohio, South Dakota, and Ontario). However, there were no significant differences in stand or yield consistently across cultivars in Iowa, Nebraska, Wisconsin, or Ohio, where heavy precipitation did not occur until later growth stages. The environment, levels of inoculum, and pathogen complex may have played a role in the different responses to the seed treatments and to the different combinations of *Rps* genes and levels of partial resistance to *P. sojae* in the cultivars. Fields that are poorly drained and have *P. sojae* populations with complex pathotypes may benefit the most from seed treatments. Individual fields where producers may see the greatest benefit to utilizing these integrated management strategies will need to be identified.

*Phytophthora sojae* (Kaufm. & Gerd.) is a limiting factor in soybean (*Glycine max* (L.) Merr.) production in more fields now than it was 50 years ago, when it was first recognized as a soybean pathogen (20,22,23). Losses from *Phytophthora* root and stem rot caused by *P. sojae* can occur anytime during the production season. Early-season infections reduce stands and necessitate replanting, while later season infections can cause plant mortality, which impacts final stands, and reductions in root health and thus yield. Disease incidence and severity are highest in fields with slow drainage or where periodic saturated soil conditions occur. Increased frequency of virulence in *P. sojae* populations to widely deployed resistance genes (*Rps*), a reduction in levels of partial resistance in recently released commercial cultivars,

shorter rotations, and highly favorable weather patterns for disease development during spring all may have contributed to an increase in incidence of *Phytophthora* root and stem rot during the past decade (1,11,14–18). Therefore, an integrated approach to reducing these losses is now required.

Several integrated management strategies were proposed by Schmitthenner (20) to manage this soilborne pathogen. The first strategy described was to combine the use of metalaxyl soil treatment (applying fungicide in-furrow) during planting with cultivars with high levels of partial resistance (tolerance). The second was to plant cultivars with high levels of partial resistance in fields that were tilled, had good tile drainage, crop rotation, and metalaxyl seed treatment. Several of these management strategies are no longer options due to changes in soybean production practices, soil conservation, and wetlands preservation demands that occurred in the past two decades since these proposals were first made. Application of metalaxyl in-furrow is limited by widespread adoption of conservation tillage or reduced tillage, narrow row-widths (17.8 to 38.1 cm versus

76.2 cm previously), and a shift to planting with grain drills, which makes in-furrow applications difficult. Furthermore, planting in narrow rows increases the amount and cost of fungicide required for each field. When soybean prices are low, as they were during the late 1990s, this type of application is cost prohibitive.

Conservation or reduced tillage production systems are widely adopted throughout the north-central region of the United States and Ontario, Canada. While this production practice conserves soil, it also favors some plant pathogens including *P. sojae*. Workneh et al. (22) were able to recover *P. sojae* more frequently from soil collected from fields in Illinois, Minnesota, Missouri, and Ohio managed with conservation tillage than from conventional tillage fields. The cropping rotation has also changed as a result of wheat and forage production declining during the late 1980s to early 1990s. Consequently, in some areas of the north-central region, a rotation of corn and soybeans with soybeans planted, at times, 2 to 5 years in a row predominates. In addition, the amount of tile that can be placed in a field to assist with drainage is restricted in some areas (M. Draper, South Dakota, *personal observation*), while in other regions antiquated tile systems need replacing (A. Dorrance, Ohio, *personal observation*). The reduced capacity to drain excess water from some fields may have also contributed to the increased prevalence of *P. sojae*. Another development in the past decade was the widespread introduction of soybean cultivars with resistance to glyphosate. Although many of these cultivars released early in this germplasm shift did have *Rps* genes (such as *Rps1a*), some were ineffective and many did not carry high levels of partial resistance to *P. sojae* (2–4). Measures of the level of partial resistance were collected throughout this germplasm shift, and dramatic differences among the entries for this trait were observed (2–4; Fig. 1).

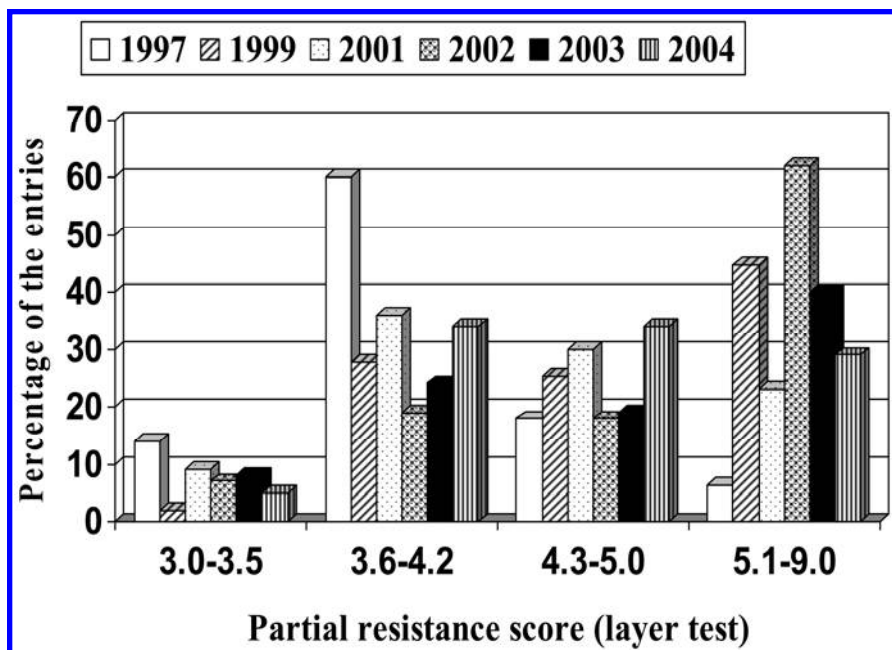
Increasing genetic diversity in endemic *P. sojae* populations has also been observed (1,11,14–18). Partial resistance to *P. sojae* in soybeans provides protection against all pathotypes. Cultivars that ex-

Corresponding author: A. E. Dorrance  
E-mail: dorrance.1@osu.edu

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**Fig. 1.** Comparison of partial resistance levels in soybean cultivars entered in the Ohio State University Performance Trials from 1997 to 2004. All entries were evaluated in a layer cup test, and 3 indicates high levels of partial of resistance with 1/3 of the roots rotted; 5 indicates most roots rotted; and 9 indicates that plants are dead. Data were summarized from annual reports (2–4).

**Table 1.** A list of soybean cultivars, *Rps* gene(s), partial resistance level, and state or province that were used to evaluate integrated management strategies for *Phytophthora sojae* in the United States and Ontario, Canada

Cultivar <sup>a</sup>	<i>Rps</i> gene(s)	Level of partial resistance <sup>b</sup>	State
Conrad	<i>rps</i>	High	Ohio, Ontario
Cult A	<i>Rps1k</i>	Low	Ohio
General	<i>Rps1k</i>	High	Ohio
Kottman	<i>Rps1k</i> plus <i>Rps3a</i>	High	Ohio
OX20-8	<i>Rps1a</i>	None	Ontario
Sandusky	<i>Rps1k</i>	Moderate	Ohio
Sloan	<i>rps</i>	Low	Ohio, Iowa
Archer	<i>Rps1k</i> plus <i>Rps6</i>	Low	Iowa
Williams	<i>rps</i>	Moderate	Iowa
Stine2402	<i>rps</i>	High (C)	Iowa
Syngenta S27-T7	<i>rps</i>	High (C)	Iowa
S31-V3	<i>rps</i>	Moderate	Iowa
2834RR	<i>Rps1k</i>	Low	Iowa
Asgrow AG2703	<i>Rps1k</i>	Low (C)	Nebraska
Garst 2903 RR	<i>Rps1k</i>	High (C)	Nebraska
Garst 3083 RR	<i>rps</i>	High (C)	Nebraska
Croplan RT2440	<i>rps</i>	Moderate	Wisconsin
Sturdy	<i>Rps1a</i>	Low	Wisconsin
Pioneer 92M40	<i>Rps1c</i>	Moderate	Wisconsin
NK S24-K4	<i>Rps1a</i>	Moderate	Wisconsin
Asgrow AG2403	<i>Rps1k</i>	Moderate	Wisconsin
Pioneer 91B91	<i>rps</i>	Low	South Dakota
Kaltenberg KB150RR	<i>rps</i>	High	South Dakota
SD1081RR	<i>Rps1a</i>	Low	South Dakota
NK S19-V2	<i>Rps1a</i>	High	South Dakota
Pioneer 91B64	<i>Rps1c</i>	Low	South Dakota
Kaltenberg KB161RR	<i>Rps1c</i>	High	South Dakota
Pioneer 92B05	<i>Rps1k</i>	Low	South Dakota
Kaltenberg KB172R	<i>Rps1k</i>	High	South Dakota
Harovinton	<i>Rps1c</i>	Moderate	Ontario
Amsoy71	<i>Rps1c</i>	Low	Ontario
Elgin87	<i>Rps1k</i>	High	Ontario

<sup>a</sup> Both public and commercial cultivars were used in these studies. Seed of public cultivars were available at the Ohio State University.

<sup>b</sup> Partial resistance levels that were verified were determined through a greenhouse layer test in which none indicates 90 to 100% kill of all seedlings; low indicates that roots are all colonized and 50 to 89% of the seedlings are killed; moderate indicates that all seedlings survived but roots are 40 to 70% colonized; and high indicates that all seedlings survive and 10 to 39% of roots are colonized. Values followed by (C) indicate the seed companies rating for this trait.

press high levels of partial resistance have the most consistent yield across locations (12) and do not select for *P. sojae* pathotypes that can adapt to single-gene resistance. However, a caveat of partial resistance is that it is not expressed in seeds and germinating seedlings (10). Thus, cultivars with this type of resistance are still highly susceptible to infection by *P. sojae* at least until the unifoliate leaves are visible (5 to 14 days after planting depending on soil temperatures).

Incidence and severity of Phytophthora root and stem rot have increased in the region; thus, we evaluated several management strategies that producers could implement in the changing production landscape. While metalaxyl and mefenoxam are not economically viable as in-furrow treatments, seed treatment applications may provide sufficient protection against early-season damping-off. Dorrance and McClure (10) proposed that high rates of these active ingredients were required to protect seedlings from infection based on greenhouse studies. However, a widespread assessment of these higher rates has not been completed in the field nor with the different resistance “packages” currently available in soybean cultivars. Therefore, the objective of this study was to evaluate the effect of the addition of a seed treatment on soybean cultivars with *Rps* genes with or without high levels of partial resistance. The development of Phytophthora root and stem rot is very dependent on soil moisture, thus this study was evaluated across the north-central region in six states in the United States and Ontario, Canada during 2003 to 2006.

## MATERIALS AND METHODS

**Plant material.** A total of 32 soybean cultivars from both public and private seed companies were selected based on their maturity group, adaptation to local environments, and resistance to *P. sojae* (Table 1). Cultivars had different resistance combinations of *Rps* genes plus low or high levels of partial resistance. In some cases, seed company data were used for the resistance descriptions, while for others the presence of *Rps* genes was verified through a series of inoculations with known pathotypes (races) of *P. sojae* using the hypocotyl technique (8,21). Partial resistance levels from seed companies was also used, while a few were verified with the layer test (21) in which soybeans are planted in cups 2.5 cm above an agar layer colonized by *P. sojae*. The plants were scored 3 weeks after planting, and a 3 indicates high levels of partial of resistance with 1/3 of the roots rotted, 5 is most roots rotted, and 9 indicates that the plants are dead.

**Seed treatments.** The effect of seed treatment on the incidence and severity of Phytophthora root rot was studied on the test cultivars. Seed treatments included

metalaxyl (Allegiance; Bayer Crop Science, Research Triangle Park, NC) at 0.94 g a.i./kg seed; mefenoxam (Apron XL, Syngenta Crop Protection, Inc., Greensboro, NC) at 15.0 g a.i./100 kg seed; mefenoxam at 3.75 g a.i./100 kg plus fludioxonil at 2.5 g a.i./100 kg seed (Apron Maxx, Syngenta); or the combination of mefenoxam at 3.75 g a.i./100 kg plus fludioxonil at 2.5 g a.i./100 kg plus mefenoxam at 11.25 g a.i./100 kg seed (Apron Maxx plus Apron XL, Syngenta). Seed treatments were applied as a slurry at a rate of 237 ml/45.4 kg.

**Study locations.** Treatments were evaluated in fields with long-term histories of *Phytophthora* root rot in Iowa during 2005 and 2006, Ohio during 2005 and 2006, Nebraska during 2004 and 2005, South Dakota during 2003, Wisconsin

during 2006, and Ontario, Canada during 2004 (Table 2). Specifics for each location are listed for each state below.

**Iowa.** Field studies were planted on 19 May 2005 on commercial farms near Eddyville, and on 26 and 29 May 2006 at the Albion and Albert City fields, respectively. Each location has numerous pathotypes of *P. sojae* and is poorly drained with greater than 22% clay. The study was arranged in a split-plot design with cultivar as the whole plot and the seed treatments as the subplots. Each experimental unit was 6.1 m long and 3 m wide with four rows spaced 76 cm apart. Approximately 33 seeds were planted per meter length of row. There were six cultivars (Table 1) and two seed treatments: a combination of mefenoxam, 11.25 g a.i./100 kg of seed (Apron XL), plus mefenoxam, 3.75 g a.i., and

fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA); or mefenoxam, 15.0 g a.i./100 kg of seed (Apron XL). Nontreated seed of each cultivar was planted as a control. The number of soybean plants was recorded in a 2-m section within the center two rows of each plot approximately 3 weeks after planting and immediately prior to harvest. *Phytophthora* stem rot incidence was assessed throughout the growing season 7 to 10 days after a heavy rain (>2.5 cm) for a total of three evaluations. Disease incidence was calculated as the percentage of plants showing stem rot symptoms from the initial stand count. Yield data were collected at maturity by harvesting 4 m from each of the center two rows of each plot and adjusted to 13% moisture.

**Nebraska.** Field studies were planted on 6 May near Herman in 2004 and 27 May

**Table 2.** Characteristics of study locations used for evaluation of integrated pest management strategies for *Phytophthora* seedling blight and root and stem rot of soybean caused by *Phytophthora sojae*

Characteristic	Iowa		Nebraska		Ohio			South Dakota	Ontario, Canada	Wisconsin	
Location/year	Eddyville 2005	Albert City 2006	Albion 2006	Tekamah 2005	Herman 2004	Custar 2005	Custar 2005	Custar 2006	Groton 2003	Harrow 2004	Racine 2006
Soil type	Pershing silt loam/Douds loam	Canisteo clay loam/Nicollet loam	Muscatine silty clay loam/Garwin silty clay loam	Luton silty clay	Butler silt loam	Hoyteville clay			Silt loam	Brookston silty clay loam	
Percent clay	23-24	22-29	28-32	60	50	35			18-45	30	30
Drainage	Poor to moderate	Poor	Poor	Poor	Poor	Poor			Poor	Poor	Moderate
Rainfall 10 DAP <sup>a</sup> (cm)	0.6	0	3.6	6.63	3.33	2.5	1.6	8.0	9.9 <sup>b</sup>	6.7	3.8
Irrigation 10 DAP <sup>a</sup> (cm)	0	0	0	0	0	7.5		5.3	0		0
Mean temp 10 DAP <sup>a</sup> (C)	16.8	23.5	22.4	18.2	17.6	14.4	24.2	21.6	14.4-31.7	23.9	13.0
Total rainfall/season (cm)	31.6	40.9	45.7	21.4	29.6	42.7		51.1	53.3	34.7	35.8
<i>P. sojae</i> race	Many	Many	Many	25 (1a, 1b, 1c, 1k, 7)	28 (1a, 1b, 1k, 7)		Many		1,3,4,25 (1a, 1b, 1c, 1k, 7)	Many	3 (1a, 7)

<sup>a</sup> DAP = days after planting.

<sup>b</sup> 9.4 cm of rain was received within 20 DAP in South Dakota during 2003. Plants were just at the VC stage.

**Table 3.** Comparison of stand counts and yield of soybean cultivars with different resistance combinations to *Phytophthora sojae* and seed treated with mefenoxam, 11.25 g a.i./100 kg (Apron XL), or nontreated in Ohio during 2005 and 2006

Cultivar (C)	Seed treatment (T)	Irrigated at V1 2005		Irrigated at planting 2005		Irrigated at planting 2006	
		Final stand <sup>a</sup>	Yield <sup>b</sup>	Final stand	Yield	Final stand	Yield
Sloan	Nontreated	9.8	2,419.2	5.0	1,223.0	6.4	1,868.2
	ApronXL	11.5	2,298.2	6.2	1,357.4	8.0	2,466.2
Conrad	Nontreated	13.7	2,856.0	10.3	2,076.5	6.8	2,197.4
	ApronXL	14.0	3,010.6	9.2	2,016.0	9.7	2,748.5
Cult A	Nontreated	12.8	2,493.1	5.9	1,014.7	7.3	2,278.1
	ApronXL	12.6	2,667.8	8.6	1,532.2	14.5	2,936.6
General	Nontreated	15.6	3,313.0	8.6	2,573.8	12.3	3,373.4
	ApronXL	14.1	3,386.9	12.1	2,956.8	15.6	3,487.7
Sandusky	Nontreated	13.0	3,259.2	6.6	2,137.0	13.2	3,138.2
	ApronXL	14.1	3,259.3	8.3	2,607.4	11.2	3,218.9
Kottman	Nontreated	14.9	3,595.2	11.4	2,802.2	10.7	3,010.6
	ApronXL	14.1	3,702.7	10.3	2,600.6	10.9	3,400.3
Means	Nontreated	13.2	3,003.8	7.9	1,975.7	9.5	2,627.5
	Apron XL	13.4	3,057.6	9.0	2,177.3	11.6	3,050.9
LSD <sup>c</sup> ( $P \leq 0.05$ ) C		2.1**	2.1**	NS	3.9**	3.1**	3.6*
LSD ( $P < 0.05$ ) T		NS	NS	1.0*	3.1**	1.8*	7.5**
LSD ( $P \leq 0.05$ ) C × T		NS	NS	1.7*	3.7*	NS	NS

<sup>a</sup> Plants per meter.

<sup>b</sup> kg/ha. Adjusted to 13% moisture.

<sup>c</sup> Fisher's protected least significant difference, \*\* indicates  $P < 0.01$ , and \* indicates  $P < 0.05$ .

near Tekamah in 2005 on commercial farms. Both fields had a history of Phytophthora root rot with *P. sojae* race 25 (vir 1a, 1b, 1c, 1k, 7), which was recovered the

year prior to the study. The soil type for each field was a Luton silty clay and a Butler silt loam for Tekamah and Herman, respectively. In 2004, the plots were 401 m

long with 12 rows spaced 76 cm apart. In 2005, the field was pivot irrigated and the plots were 800 m long with six rows spaced 76 cm apart. Three cultivars were selected and each was treated with a combination of mefenoxam, 11.25 g a.i./100 kg of seed (Apron XL), plus mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA). The plots were arranged in a randomized complete block design with four replications of each treatment. Stand counts were collected from six 3-m lengths of row in the center two rows of each plot at the third trifoliate growth stage on 9 June and postharvest on 14 October in 2004 and at the R1 (beginning flowering) growth stage on 12 July and postharvest on 31 October in 2005. Plots were harvested 4 October 2004 and 22 October 2005 with a combine and weigh wagon, and yield data were adjusted to 13% moisture.

**Ohio.** The study was planted at the Ohio Agricultural Research and Development Center North West Branch Station near Custar on 18 May and 2 June 2005 and 13 June 2006 in a Hoytville clay soil. This field, which has 35% clay soil, has numerous pathotypes of *P. sojae*. Six cultivars were selected and one seed treatment, 15.0 g a.i./100 kg of seed (Apron XL), was evaluated. The study was planted in a split-plot design with the cultivars as the whole

**Table 4.** Comparison of soybean cultivars with different resistance combinations to *Phytophthora sojae* and seed nontreated and treated with metalaxyl (Allegiance 0.94 g/kg [1.5 fl oz/cwt] in Groton, SD during 2003

Cultivar (C)	Seed treatment (T)	Stand (plants/m) <sup>a</sup>	Yield (kg/ha) <sup>b</sup>
Pioneer 91B91	Nontreated	18.1	2,997.1
	Allegiance	21.0	3,436.6
Kaltenberg KB153RR	Nontreated	22.5	3,470.9
	Allegiance	22.9	3,555.6
SD1081RR	Nontreated	10.4	975.7
	Allegiance	21.7	2,427.9
NK S19-V2	Nontreated	21.2	2,326.5
	Allegiance	24.6	2,173.9
Pioneer 91B64	Nontreated	21.5	3,240.4
	Allegiance	25.2	3,214.2
Kaltenberg KB161RR	Nontreated	18.8	2,645.7
	Allegiance	24.4	3,210.1
Pioneer 92B05	Nontreated	30.4	3,567.0
	Allegiance	27.5	3,565.6
Kaltenberg KB172RR	Nontreated	26.9	3,775.3
	Allegiance	27.9	3,824.4
Nontreated		21.2	2,874.8
Allegiance		24.4	3,176.0
Fisher's protected LSD <sup>c</sup> ( $P \leq 0.05$ ) T		1.9**	161.3**
Fisher's protected LSD ( $P \leq 0.05$ ) C		4.0**	598.1**
Fisher's protected LSD ( $P \leq 0.05$ ) C × T		3.2*	262.1**

<sup>a</sup> Stand counts were recorded at the R3 growth stage when a pod was 5 mm long at one of the four uppermost nodes on the main stem.

<sup>b</sup> Yield adjusted to 13% moisture.

<sup>c</sup> Fisher's protected LSD, \* indicates  $P < 0.05$ , and \*\* indicates  $P < 0.01$ .

**Table 5.** Effect of fungicide seed treatments mefenoxam 15.0 g a.i./100 kg of seed (Apron XL), mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA); and mefenoxam, 11.25 g a.i./100 kg of seed (Apron XL) plus mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA) on emergence, final stand, plant loss, and yield of five soybean cultivars from fields heavily infested with *Phytophthora sojae* in Woodslee, Ontario, in 2004

Cultivar (C)	Seed treatment (T)	Woodslee field A			Woodslee field B		
		Emergence <sup>a</sup>	Final stand <sup>a</sup>	Yield (kg/ha) <sup>b</sup>	Emergence	Final stand	Yield (kg/ha)
OX 20-8	Nontreated Check	16.8	0.9	8.6	18.1	0.7	66.0
	Apron Maxx RTA	17.6	1.5	13.1	17.4	3.2	90.9
	Apron XL	18.5	2.9	67.4	18.7	3.3	291.0
	Apron Maxx + Apron XL	18.8	3.7	67.10	17.6	4.4	284.7
Amsoy 71	Nontreated Check	16.2	6.4	222.9	15.3	7.0	726.2
	Apron Maxx RTA	16.6	8.4	697.3	17.5	10.2	1,341.6
	Apron XL	17.3	11	695.8	18.1	11.5	1,296.5
	Apron Maxx RTA + Apron XL	18.0	8.2	1,357.0	16.8	13.2	1,599.7
Conrad	Nontreated Check	14.6	11.4	1,664.1	17.2	15.2	2,429.8
	Apron Maxx RTA	16.9	16.1	1,582.4	18.5	18.3	2,241.8
	Apron XL	17.2	17.0	2,100.0	17.2	16.9	2,968.5
	Apron Maxx RTA + Apron XL	17.3	16.2	2,030.5	18.5	17.4	2,879.4
Harovinton	Nontreated Check	17.3	13.0	828.3	17.0	12.5	1,700.5
	Apron Maxx RTA	18.1	16.1	1,341.2	18.6	17.4	1,561.1
	Apron XL	18.2	17.7	1,846.3	19.4	19.1	1,562.3
	Apron Maxx RTA + Apron XL	18.6	17.5	1,407.7	18.9	18.5	2,086.5
Elgin 87	Nontreated Check	17.5	15.9	1,547.5	17.9	17.3	2,613.2
	Apron Maxx RTA	18.2	17.9	1,710.0	19.3	18.7	2,658.7
	Apron XL	19.0	18.5	1,598.3	18.7	18.4	2,666.3
	Apron Maxx RTA + Apron XL	17.8	16.7	1,739.9	19.0	18.4	2,190.6
Means	Nontreated Check	16.5	9.5	854.3	17.2	10.5	1,507.1
	Apron Maxx RTA	17.5	12.0	1,068.8	18.3	13.5	1,578.8
	Apron XL	18	13.4	1,261.6	18.3	13.7	1,756.9
	Apron Maxx RTA + Apron XL	18.1	12.5	1,320.4	18.2	14.4	1,808.2
LSD <sup>c</sup> ( $P \leq 0.05$ ) C		1.04**	1.52**	363.15*	0.90**	1.34**	NS
LSD ( $P \leq 0.05$ ) T		0.92**	1.35**	322.8**	0.80**	1.20**	361.63**
LSD ( $P \leq 0.05$ ) C × T		NS	NS	NS	NS	NS	NS

<sup>a</sup> Number of plants per meter at VC growth stage (plant emergence) and R7 growth stage (when all leaves are gone and one pod on main stem has mature pod color).

<sup>b</sup> Adjusted to 13% moisture.

<sup>c</sup> Fisher's protected least significant difference, \*\* indicates  $P < 0.01$ , and \* indicates  $P < 0.05$ .



plot and seed treatment as the subplot. Each experimental unit consisted of four rows spaced 38 cm apart, 9.1 m long with 3-m alleyways between plots. Alleyways were planted with the susceptible cultivar Sloan to serve as an indicator of the total disease level. During 2005, the field was irrigated with a total of 7.3 cm of water from 6 to 12 June, which coincided with the first trifoliolate fully expanded (V1 growth stage, 19) for the 18 May and preemergence for the 2 June plantings. Stand counts were recorded 21 June, 29 June, 12 July, 26 July, 11 August, 23 August, and 21 September 2005 from a 0.9-m section in each row in each plot. Yields were recorded at harvest on 18 October. During 2006, the study was irrigated with 5.3 cm of water from 14 to 16 June, and stand counts were recorded on 29 June, 17 August, and 10 October. The plots were harvested on 13 October.

**South Dakota.** The study was planted on 6 June 2003 in Brown County near Groton, SD in a commercial production field with a history of *Phytophthora* root and stem rot even when cultivars with *Rps1a*, *Rps1c*, or *Rps1k* were planted. The soil was a Brookston silty clay loam with 30% clay content. The study was arranged in a randomized split-plot design with the eight cultivars (Table 1) as the whole plot and seed treatment as the subplot with three replications. The subplots were 4.6 m long with two rows of each cultivar treated with metalaxyl at 0.94 g/kg (Allegiance FL) and two rows nontreated. Rows were spaced 76 cm apart. Stand counts were recorded from 1 m of row near the center of the plot on 31 July 2003 when a pod is 5 mm long on one of the four uppermost nodes of the main stem with a fully developed trifoliolate (R3 growth stage, 19). Yields were recorded on 9 October 2003 and adjusted to 13% moisture.

**Wisconsin.** The field study was planted on 23 May 2006 at a commercial farm in Racine County. The field is comprised of 30% clay and has a history of disease caused by *P. sojae* race 3 (vir 1a, 7). Five soybean cultivars were selected and treated with mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA) or left nontreated. Treatments were planted in a complete randomized block design with four replications. Individual plots consisted of six rows spaced 38 cm apart, 7.7 m long with 3-m alleyways between blocks. Stand counts were recorded on 15 June from a 1-m section of one row in each plot at the V1 growth stage. Plant mortality was recorded on 15 June as a percentage of dead seedlings per meter of row and as a percentage of dead seedlings per plot on 28 June. Plots were harvested on 10 October and yield adjusted to 13% grain moisture.

**Ontario, Canada.** The study was planted at the Agriculture and Agri-Food Canada's Greenhouse and Processing

Crops Research Centre Woodslee Research Farm in Woodslee, Ontario, Canada on 7 June 2004. The Woodslee fields A and B are classified as a Brookston clay-loam soil and were naturally infested with *P. sojae*. The fields were adjacent to the Ontario Soybean Performance "Phytophthora Nursery" which has been used annually in Ontario since 1978 to evaluate the partial resistance of commercial soybean varieties

and candidate lines to root rot caused by *P. sojae*. *P. sojae* isolates from the field study locations contained a complex virulence formula as demonstrated by their ability to infect soybeans with *Rps1a*, 1k, 6, or 8 as well as additional differentials. In 2004, three seed treatments including mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA); mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g



**Fig. 2.** Soybean seedlings with symptoms of damping-off caused by *Phytophthora sojae* on both treated and nontreated seed. Note the presence of seed coat above soil surface following germination of seedlings.

a.i./100 kg seed (Apron Maxx RTA) plus mefenoxam, 11.25 g a.i./100 kg (Apron XL) and mefenoxam, 15 g a.i./100 kg (Apron XL) were evaluated on five cultivars (Table 1) in a randomized complete block design with four replications. Individual plots consisted of four rows spaced 50 cm apart and 2.5 m in length with 50 seeds per row. Emergence and final stand counts were recorded from the center two rows on 30 June and 9 August. The center two rows were harvested on 27 October 2004 and yields were adjusted to 13% moisture.

**Statistical analysis.** Due to the different cultivars, planting dates, and overall level of disease, each location-year was analyzed separately. For all locations, a factorial or split-plot analysis of variance using PROC GLM (SAS Institute, Cary, NC) was completed. When significant differences were detected for treatment effects, Fishers protected least significant difference (LSD,  $P \leq 0.05$ ) was used for mean separations.

## RESULTS

Disease, as measured by the number of seedlings damped-off in the study and reduced germination of nontreated controls, was highest in this study in Ohio (Table 3), South Dakota (Table 4), and Ontario (Table 5). In addition, Albert City,

IA had moderate levels of late-season Phytophthora stem rot based on the overall reduction in plant stand during 2006 (Table 6). Disease was low in the two remaining locations in Iowa (Table 6), as well as locations in Nebraska (Table 7) and Wisconsin (Table 8).

Stands were significantly higher in plots with a seed treatment and when irrigation or rainfall occurred shortly after planting, in Ohio during 2005 and 2006 (Table 3), South Dakota (Table 4), and Ontario (Table 5). In Ohio, when fields were irrigated following planting, yields were 215.0 and 416.6 kg/ha higher across the study during 2005 and 2006, respectively (Table 3). A similar trend also occurred in South Dakota, where there was a 289.0 kg/ha average increase in yield resulting from the addition of the fungicide seed treatment over nontreated across all cultivars (Table 4). In Ontario, seed treatments increased emergence and final stands in two 2004 trials. Yields were increased significantly by 216.4 to 471.0 kg/ha in one trial (Woodslee A) but not in another trial (Woodslee B) during 2004 (Table 5).

In contrast, there were no significant increases in stand or yield consistently across cultivars when seed treatments were applied in Iowa, Nebraska, Wisconsin, or Ohio when the study was irrigated at the V1 growth stage (2005). In Wisconsin,

there was an average of 11% seedling mortality across all fungicide seed treatments and cultivars (Table 8). In Iowa, for some cultivars, final plant stand was either significantly increased or decreased with the addition of the seed treatments. At these three locations in Iowa, as well as Nebraska, there was little seedling damping-off (data not shown).

For Iowa, Ohio, and Ontario, the yields of cultivars with high partial resistance combined with or without an *Rps* gene were significantly higher than cultivars with low partial resistance whether a seed treatment was used or not. In Wisconsin, cultivars with no *Rps* gene (Croplan RT 2440) or *Rps1c* (Pioneer 92M40) had significantly higher yields than cultivars with either *Rps1a* or *Rps1k*. Damping-off did occur across all cultivars, indicating that the *P. sojae* population that was present in Wisconsin had virulence to all of the *Rps* genes in the cultivars planted at this location.

## DISCUSSION

Environment plays a key role in the development of Phytophthora seedling blight and root and stem rot in soybeans. *P. sojae* is a water mold, and for the infective zoospores to develop, saturated soils are necessary. Therefore, the length of time for an infection period is the time that the soils

**Table 6.** Stand and yield of soybeans with different levels of resistance to *Phytophthora sojae* treated with mefenoxam, 15.0 g a.i./100 kg of seed (Apron XL), mefenoxam, 11.25 g a.i./100 kg of seed (Apron XL) plus mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed (Apron Maxx RTA) or nontreated in three locations in Iowa in 2005 at Eddyville and 2006 at Albert City and Albion

Cultivar (C)	Seed treatment (T)	Eddyville			Albert City			Albion		
		Stand V2 <sup>a</sup>	Final stand <sup>a</sup>	Yield (kg/ha) <sup>b</sup>	Stand V2	Final stand	Yield (kg/ha)	Stand V2	Final stand	Yield (kg/ha)
Sloan	None	50.0	36.4	4,019.9	23.8	21.5	2,983.7	55.0	56.2	2,731.7
	Apron XL	52.8	34.4	3,901.6	24.4	23.4	3,303.6	62.3	60.3	2,958.1
	Apron Maxx + Apron XL	47.5	32.4	3,886.8	23.5	21.5	3,396.3	56.8	57.3	2,770
Williams	None	-	-	-	29.3	23.8	2,823.1	49.0	47.0	2,744.4
	Apron XL	-	-	-	27.6	25.7	2,915.1	44.5	45.9	2,696.7
	Apron Maxx + Apron XL	-	-	-	27.0	20.4	2,339.1	54.5	48.4	2,628.9
S31-V3	None	52.4	46.4	5,079.6	-	-	-	-	-	-
	Apron XL	46.8	36.4	5,097.1	-	-	-	-	-	-
	Apron Maxx + Apron XL	47.5	37.3	5,230.2	-	-	-	-	-	-
Stine 2402	None	49.8	38.2	4,145.6	16.7	16.2	4,230.2	57.2	57.4	4,116
	Apron XL	48.3	42.4	4,277.3	18.0	16.9	4,140.2	57.4	50.5	4,118
	Apron Maxx + Apron XL	40.3	32.2	3,829.1	27.8	19.9	4,302.8	57.7	57.5	4,192.6
S27-T7	None	51.8	40.0	4,949.3	24.3	20.4	4,437.2	59.2	57.3	3,888.9
	Apron XL	46.0	38.5	4,892.8	24.8	17.7	4,364.6	52.7	52.3	3,781.3
	Apron Maxx + Apron XL	44.8	36.0	4,676.4	26.3	22.2	4,453.3	58.0	60.0	3,837.1
2834RR	None	49.3	35.5	5,228.2	31.0	28.7	4,497	53.0	47.3	3,966.1
	Apron XL	50.0	40.0	5,245.0	25.3	22.5	4,590	53.3	46.7	4,013.2
	Apron Maxx + Apron XL	48.0	39.5	5,191.9	26.8	23.4	4,278.6	52.5	43.5	4,182.5
Archer	None	46.0	32.4	3,171.4	30.5	26.8	3,480.4	40.5	46.4	3,299.0
	Apron XL	51.8	40.8	3,225.1	25.0	29.2	3,258.7	34.2	37.4	3,332.6
	Apron Maxx + Apron XL	51.3	34.5	2,855.6	27.0	24.9	3,292.3	40.8	35.2	3,352.8
Means	None	49.9	38.2	4,432.3	25.9	22.9	3,741.9	52.3	51.9	3,457.7
	Apron XL	49.3	38.7	4,439.8	24.2	22.6	3,762.0	50.7	48.9	3,483.3
	Apron Maxx + Apron XL	46.6	35.3	4,278.3	26.4	22.1	3,677.1	53.4	50.3	3,494.0
LSD <sup>c</sup> ( $P < 0.05$ ) C	4.6*	6.6*	264.6**	4.6*	3.1*	285.6**	6.3**	5.9**	581.7**	
LSD ( $P < 0.05$ ) T	3.3*	4.0*	187.1*	NS	NS	NS	NS	NS	NS	
LSD ( $P < 0.05$ ) C × T	8.0*	9.7*	458.2**	8.0*	5.3*	494.6*	11.0*	10.2*	1,007.6*	

<sup>a</sup> Number of plants in 1 m of row with counts made in each of two center rows. V2 = two fully developed trifoliate leaves that have unrolled.

<sup>b</sup> Adjusted to 13% moisture.

<sup>c</sup> Fisher's protected least significant difference, \*\* indicates  $P < 0.01$ , and \* indicates  $P < 0.05$ .



are saturated. Seed treatment compounds have limited effective period. When a soybean seed germinates, the seed coat is pushed out of the ground and remains on the cotyledons (Fig. 2); thus, this period of seed protection is further restricted in soybean. This study provides some of the first substantiated evidence where the seed treatment failed to provide any protection for damping-off (Wisconsin).

The benefit of seed treatment was highly variable across all locations. The efficacy of seed treatments was realized primarily when heavy rains occurred shortly after planting (a week or two). This was demonstrated in Ontario during the 2004 season, when 6.7 cm of rain fell within 10 days of planting; in South Dakota during 2003, when 14.4 cm of rain fell within 20 days of planting; and in Ohio, where the fields were irrigated with 7.3 and 5.3 cm of water shortly after planting. There were no negative effects of the seed treatments on yield, as in locations where there was no net gain in yield with seed treatments, no significant reductions in yield occurred. Environment by seed treatment differences in soybeans have been reported previously (5,6,13). Bradley (5) reported that 4 out of 12 environments had higher yields with seed treatment than without. This difference in effect was proposed to be related to low soil temperatures (<15°C) and high soil moisture conditions at planting, as well as a different pathogen complex present in any particular field (5). Guy et al. (13) found the highest benefit with metalaxyl applications was achieved when there was a high proportion of *P. sojae* in the field. In addition, in fields in Ohio where stand establishment was a concern even with metalaxyl or mefenoxam treated seed, *Pythium* spp., which were insensitive to these compounds, were identified (7,9). More surveys are needed across the north-central region to identify the soilborne pathogen complex that may be affecting soybeans.

In locations where substantial disease occurred, there were large differences in yield among the cultivars with different levels of partial resistance. Cultivars with low partial resistance had significantly lower yields than those with higher levels of partial resistance regardless of the presence of an *Rps* gene. These results support an earlier finding by Dorrance et al. (12) in which cultivars with high partial resistance had consistently higher yields across locations. As the diversity and expansion of *P. sojae* continues to increase across the region, the partial resistance component of the resistance package will become increasingly more important.

In this study, both environment and pathogen inoculum density may have played a role in achieving a positive yield response when a seed treatment was used. Fields where soybeans may see the greatest benefit of utilizing these integrated management strategies, namely partial resistance and fungicide seed treatment,

will need to be identified on an individual or region-wide basis. Furthermore, characterizing the soilborne pathogen complex that is limiting soybean production would greatly benefit appropriate disease management choices. This was emphasized with the development of damping-off of seedlings from treated seed in Wisconsin.

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**Table 8.** Comparison of soybean cultivars that differ in resistance combinations to *Phytophthora sojae* and seed treated with mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg (ApronMaxx) or nontreated in Wisconsin during 2005

Cultivar (C)	Seed treatment (T)	Plant population (plants/m) <sup>a</sup>	Percent damping-off	Yield (kg/ha)
Croplan RT 2440	Nontreated	18	12	5,476.8
	ApronMaxx	14	8	5,745.6
Sturdy	Nontreated	10	6	NA <sup>b</sup>
	ApronMaxx	13	21	NA
Pioneer 92M40	Nontreated	11	3	5,006.4
	ApronMaxx	14	9	5,597.8
NK S24-K4	Nontreated	14	2	4,912.3
	ApronMaxx	17	4	4,132.8
Asgrow AG2403	Nontreated	12	21	4,576.3
	ApronMaxx	13	23	4,368.0
Means	Nontreated	13	9	5,006.4
	ApronMaxx	14	12	4,993.0
LSD <sup>c</sup> ( $P \leq 0.05$ ) C		NS	NS	564.5*
LSD ( $P \leq 0.05$ ) T		NS	NS	NS
LSD ( $P \leq 0.05$ ) C × T		NS	NS	NS

<sup>a</sup> Stand counts were recorded at the VI growth stage.

<sup>b</sup> Yields of cultivar Sturdy (conventional cultivar) were not included due to application of glyphosate.

<sup>c</sup> Fisher's protected least significant difference. \* indicates  $P = 0.05$ .

**Table 7.** Comparison of soybean cultivars with different resistance combinations to *Phytophthora sojae* and seed treated with mefenoxam, 3.75 g a.i. and fludioxonil, 2.5 g a.i./100 kg seed plus mefenoxam, 11.25 g a.i./100 kg seed (ApronMaxx plus Apron XL) or nontreated in Nebraska during 2004 and 2005

Cultivar (C)	Seed treatment (T)	2004			2005		
		Stand V2 <sup>a</sup>	Harvest stand <sup>a</sup>	Yield (kg/ha) <sup>b</sup>	Stand R1	Final stand	Yield (kg/ha)
Asgrow AG2703	Nontreated	27.2	22.0	3,064.3	25.3	14.8	2,473.0
	ApronMaxx plus Apron XL	28.6	21.1	3,131.5	25.6	13.1	2,499.8
Garst 2903 RR	Nontreated	29.5	21.4	3,151.7	25.3	15.7	2,681.3
	ApronMaxx plus Apron XL	27.5	23.2	3,138.2	24.9	14.9	2,540.2
Garst 3083 RR	Nontreated	29.8	22.5	3,192.0	24.5	18.8	2,607.4
	ApronMaxx plus Apron XL	28.6	21.2	3,097.9	22.9	14.8	2,620.8
Means	Nontreated	28.8	22.0	3,136.0	25.0	16.4	2,587.2
	ApronMaxx plus Apron XL	28.2	21.8	3,122.5	24.5	14.3	2,553.6
LSD <sup>c</sup> ( $P \leq 0.05$ ) C		NS	NS	NS	NS	NS	NS
LSD ( $P \leq 0.05$ ) T		NS	NS	NS	NS	NS	NS
LSD ( $P \leq 0.05$ ) C × T		NS	NS	NS	NS	NS	NS

<sup>a</sup> Number of plants per meter of row with counts made in each of two center rows six times for each plot at the V2 growth stage (when plants have two fully developed trifoliate leaves).

<sup>b</sup> Adjusted to 13% moisture.

<sup>c</sup> Fisher's protected least significant difference.

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