Effect of Fungicide Seed Treatments on Stand Establishment, Seedling Disease, and Yield of Soybean in North Dakota

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ABSTRACT

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Seedling diseases of soybean (*Glycine max*) can be common under cool and moist soil conditions and may be caused by a complex of pathogens in North Dakota. Managing these diseases can be difficult due to wide host ranges of the pathogens and lack of resistant cultivars. Field trials were conducted to evaluate the effects of three different fungicide seed treatments and an untreated control on soybean at six locations in 2003 and eight locations in 2004 in North Dakota, for a total of 14 environments. The fungicides evaluated were fludioxonil + mefenoxam (Warden RTA), azoxystrobin + metalaxyl (SoyGard), and *Bacillus pumilus* GB34 (Yield Shield). Significant ($P \leq 0.05$) environment–seed treatment interactions were observed, indicating that environment played a role in when benefits from seed treatments were observed. At least one of the fungicide seed treatments provided significant protection against plant stand and yield losses compared with the untreated control in 4 of the 12 environments where plant stand was measured and 4 of the 14 environments where yield was measured. Root lesions were reduced significantly by at least one of the fungicide seed treatments compared with the untreated control in 5 of the 11 environments where root lesions were evaluated. Yield and economic benefits with fungicide seed treatments were observed more often in environments that had low soil temperatures at planting (<15°C) and moist soil conditions. Based on this research, fungicide seed treatments may be a viable option for soybean growers in North Dakota when planting into cool and moist soil conditions.

Additional keywords: *Fusarium* spp., *Phytophthora sojae*, *Pythium* spp., *Rhizoctonia solani*, root rot

Soybean (*Glycine max*) production in North Dakota has increased dramatically in the past few years, with the planted area increasing from 267,000 ha in 1995 to 1.5 million ha in 2004 (United States Department of Agriculture, National Agricultural Statistics Service, North Dakota Field Office, Fargo). The increased production has occurred in new areas as well as in existing soybean production areas in the state. In the latter, some of the increased production has resulted from shorter crop rotations or continuous soybean production. With the changes in crop rotation practices, soilborne seedling diseases caused by pathogens such as *Rhizoctonia solani*, *Fusarium solani* f. sp. *phaseoli*, *Phytophthora sojae*, and *Pythium* spp. are becoming more common (*personal observation*). This is especially true in eastern North Dakota, which is the major soybean production region of the state, where cool and wet spring seasons can occur frequently.

Excellent genetic resistance to *P. sojae* is available in cultivars adapted to North Dakota but is not available to other seedling disease pathogens. Two methods that can be used to help manage these diseases are crop rotation and fungicide seed treatments. The wide host ranges of *R. solani* and *Pythium* spp. limit the effectiveness of crop rotation (32,33). Although not always consistently, fungicide seed treatments have been shown to provide protection to soybean against stand or yield reductions caused by seedling diseases in certain instances (2,6,7,10–12,17,24,29); however, their effectiveness has not been evaluated extensively under North Dakota growing conditions. Traditionally, North Dakota soybean growers concerned with losses due to seedling diseases may have increased seeding rates to compensate for stand losses. With the cost of soybean seed on the increase and the development of new fungicides for seed treatment, the viability of fungicide seed treatments for North Dakota soybean growers needs to be examined. The objective of this study was to determine the effect of fungicide seed treatments on soybean growing under field conditions in North Dakota.

MATERIALS AND METHODS

Field trials were established at 6 locations in 2003 and 8 locations in 2004 to give a total of 14 different North Dakota environments. In 2003, the environments were Casselton, Grandin, Great Bend, LaMoure, Northwood, and Wyndmere. In 2004, the environments were Arthur, Casselton, Fargo, Grandin, Great Bend, LaMoure, Northwood, and Wyndmere. Trials at Casselton and Fargo were conducted on North Dakota State University research sites; all other trials were conducted on producers' fields. Either dent corn (*Zea mays*) or hard red spring wheat (*Triticum aestivum*) was the previous crop at each environment, and conventional tillage (chisel plow–field cultivator or moldboard plow–field cultivator) was used prior to planting. Cv. Walsh (maturity group 0) (13) was planted at all of the environments; cv. Walsh carries the *Rps* 6 gene for resistance to *P. sojae*, which is effective against the most predominant races of this pathogen present in North Dakota (20). Fungicide seed treatments consisted of an untreated control, azoxystrobin + metalaxyl (SoyGard; Bayer CropScience, Research Triangle Park, NC) at 4.1 + 5.4 g a.i./100 kg of seed, fludioxonil + mefenoxam (Warden RTA; Agriliance LLC, St. Paul, MN) at 2.5 + 7.5 g a.i./100 kg of seed, and *Bacillus pumilus* GB34 (Yield Shield; Bayer CropScience) at 1.74×10^4 CFU/100 kg of seed. Slurries of the seed treatments were applied to the seed with a batch lab seed treater (Seedburo Equipment Company, Chicago) approximately 2 weeks prior to planting.

In 2003, plots were planted 25 May at Casselton, 29 May at Grandin, 22 May at Great Bend, 27 May at LaMoure, 15 May at Northwood, and 25 May at Wyndmere. In 2004, plots were planted 16 May at Arthur, 19 May at Casselton, 18 May at Fargo, 14 May at Grandin, 10 May at Great Bend, 15 May at LaMoure, 14 May at Northwood, and 13 May at Wyndmere. Plots were planted with a four-row tractor-mounted planter fitted with cone units (Allan Machine Company, Nevada, IA) at a seeding rate of 54 seeds/m^2. Plots were four rows wide (76-cm row spacing) and 6.7 m long, and later trimmed to 5.2 m long. The experimental design at each environment was a randomized complete block with four replications. Plant stand was measured at the R1 developmental stage (9; approximately 50 to 60 days after planting) by counting the number of plants in a 1-m

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section in each of the two middle rows in each plot, and converted to number of plants per square meter. Due to time and travel constraints, plant stand was not evaluated at the Northwood locations in 2003 or 2004.

At the R1 developmental stage, 10 consecutive plants collected from one of the outside rows were dug with a shovel and brought to the laboratory, where they were washed with water and lesions on the roots were measured lengthwise. Lesions were considered to be areas on the root that were discolored (tan, brown, black, or dark red in color). The sum total length of all the root lesions was calculated for all of the 10 plants per plot and was divided by the number of plants to calculate the mean of the plot, which was used for analysis. To determine what pathogens were present, a few root lesions (approximately 5 to 10) from the untreated control plots within each environment were excised, soaked in a 0.5% NaOCl solution for 30 s, rinsed with sterile distilled water, and placed on potato dextrose agar (PDA; Becton, Dickinson, and Company, Franklin Lakes, NJ) amended with streptomycin sulfate (200 mg/liter). Root lesion length was not determined at the LaMoure location in 2004 or at the Northwood locations in either year. At all environments, the two middle rows of each plot were harvested with a small plot combine and yields were adjusted to 13% seed moisture.

Economic net return (dollars per hectare) was calculated for each plot. At the time this article was written, the approximate costs of fludioxonil + mefenoxam, azoxystrobin + metalaxyl, and *B. pumilus* GB34 were \$0.15, \$0.11, and \$0.04/kg of seed, respectively (G. Dahl, Agriliance LLC, *personal communication* and R. Knake, Bayer CropScience, *personal communication*). Based on a seeding rate of 54 seeds/m^2 and 5,500 soybean seeds/kg, fludioxonil + mefenoxam, azoxystrobin + metalaxyl, and *B. pumilus* GB34 would cost a soybean grower \$14.72, \$10.80, and \$3.93/ha, respectively. The economic net return was calculated for each plot by the formula (yield × market price) – cost of treatment. The market price used was \$0.20/kg, which is based on the 10-year (1995 to 2004) average soybean marketing price for North Dakota (United States Department of Agriculture, National Agricultural Statistics Service, North Dakota Field Office, Fargo).

Weather data (soil temperature and rainfall) were collected from the nearest weather station (North Dakota Agricultural Weather Network) to each research site in 2003 and 2004. These data included the average soil temperature at a 10-cm depth on the day of planting and the total rainfall measured from 1 week prior to planting to 3 weeks after planting.

Data were statistically analyzed using the general linear model procedure (PROC GLM) in SAS (version 9.1; SAS Institute Inc., Cary, NC) with block and environment as random factors and seed treatment as a fixed factor. Least-square mean *t* tests were used to compare treatments using the PDIFF option in SAS (version 9.1). Single-degree-of-freedom contrasts were conducted for 2003 environments versus 2004 environments, net return of untreated versus net return of chemical fungicides, net return of untreated versus net return of *B. pumilus* GB34-treated seed, and net return of untreated versus treated seed using SAS (version 9.1). Correlations between stand and yield and between lesion length and yield were determined using the Pearson correlation procedure (PROC CORR) in SAS (version 9.1).

RESULTS

All environments in 2003 had soil temperatures >13°C on the day of planting, whereas all but two environments in 2004 (Fargo and Great Bend) had soil temperatures <13°C (Table 1). Total rainfall measured from 1 week prior to planting to 3 weeks after planting was lower in 2003 versus 2004, and ranged from 34 to 75 mm at the locations in 2003 and from 99 to 168 mm at the locations in 2004.

An analysis of variance for all of the dependent variables indicated that some main and interactive effects were significant ($P \leq 0.05$; Table 2). The environment effect was significant for all of the dependent variables and the seed treatment effect was significant for lesion length, yield, and net return. The environment–seed treatment effect was significant for plant stand, yield, and net return. Coefficients of variation and R^2 values were 16.9% and 0.81, 37.2% and 0.80, 11% and 0.95, and 11.2% and 0.95 for plant stand, lesion length, yield, and net return, respectively. Due to significant environment and environment–seed treatment effects, means are presented by environment (Table 3).

Significant ($P \leq 0.05$) differences among the seed treatments for plant stand occurred in three of the five environments where plant stand was measured in 2003 (Table 3). In 2003, plant stand at Casselton from *B. pumilus* GB34-treated seed was significantly less than plant stand from untreated and fludioxonil + mefenoxam-treated seed but not significantly different from plant stand from azoxystrobin + metalaxyl-treated seed. Plant stand at Grandin from azoxystrobin + metalaxyl-treated seed was significantly less than plant stand from untreated, fludioxonil + mefenoxam-treated, and *B. pumilus* GB34-treated seed. Plant stand at Wyndmere from fludioxonil + mefenoxam-treated seed was significantly less than plant stand

Table 1. Soil temperature and rainfall for the different environments in which soybean fungicide seed treatment trials were conducted in North Dakota[x]

Year, location	Soil temperature (°C)[y]	Rainfall (mm)[z]
2003		
Casselton	13.9	75
Grandin	18.3	63
Great Bend	16.1	45
LaMoure	17.2	69
Northwood	14.4	50
Wyndmere	14.4	34
2004		
Arthur	10.0	132
Casselton	10.0	99
Fargo	14.4	153
Grandin	7.8	144
Great Bend	14.4	112
LaMoure	8.9	115
Northwood	7.8	168
Wyndmere	7.2	164

[x] Weather data were obtained from the nearest North Dakota Agricultural Weather Network weather station to each location.

[y] Average temperature measured at a 10-cm depth on day of planting.

[z] Total rainfall measured 1 week prior to planting to 3 weeks after planting.

Table 2. Analysis of variance for the dependent variables soybean plant stand, root lesion length, and seed yield for four fungicide seed treatments evaluated over 14 environments in North Dakota in 2003 and 2004

Dependent variable, source of variation	df	MS	$P > F$
Plant stand			
Environment	11	1,208	0.0001
Block (environment)	36	41	0.5700
Seed treatment	3	162	0.3756
Environment–seed treatment	33	151	0.0001
Lesion length			
Environment	10	1,539	0.0001
Block (environment)	33	82	0.0946
Seed treatment	3	805	0.0002
Environment–seed treatment	30	88	0.0668
Yield			
Environment	13	6,404,039	0.0001
Block (environment)	42	116,214	0.0001
Seed treatment	3	1,108,119	0.0003
Environment–seed treatment	39	5,384,820	0.0001
Net return			
Environment	13	256,177	0.0001
Block (environment)	42	4,649	0.0001
Seed treatment	3	26,974	0.0001
Environment–seed treatment	39	5,522	0.0001

from untreated seed but not significantly different from plant stand from azoxystrobin + metalaxyl- and *B. pumilus* GB34-treated seed.

Significant ($P \leq 0.05$) differences among the seed treatments for plant stand occurred in five of the seven environments where plant stand was measured in 2004 (Table 3). In 2004, plant stand at Arthur from untreated seed was significantly less than plant stand from azoxystrobin + metalaxyl-treated seed but not significantly different from plant stand from fludioxonil + mefenoxam- and *B. pumilus* GB34-treated seed. Plant stand at Casselton from fludioxonil + mefenoxam-treated seed was significantly less than plant stand from azoxystrobin + metalaxyl-treated seed but not significantly different from plant stand from untreated and *B. pumilus* GB34-treated seed. Plant stand at Fargo from untreated seed was significantly lower than plant stand from all of the other seed treatments. At this Fargo environment, plant stand from fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed was significantly greater than plant stand from other treatments. Plant stand at Great Bend from untreated seed was significantly less than plant stand from fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed but was not significantly different from plant stand from *B. pumilus* GB34-treated seed. Plant stand at Grandin from untreated seed was significantly lower than plant stand from all other treatments. Overall, plant stand was greater ($P = 0.0001$) in the 2003 environments compared with the 2004 environments according to a single-degree-of-freedom contrast.

Fungal cultures isolated from root lesions included *R. solani*, *F. solani* f. sp. *phaseoli*, *F. graminearum*, and other *Fusarium* spp. Significant ($P \leq 0.05$) differences among the seed treatments occurred in one of the five environments where root lesion length was measured in 2003 (Table 3). At Grandin in 2003, lesion length was significantly smaller on the roots from fludioxonil + mefenoxam-treated seed than on the roots from *B. pumilus* GB34-treated seed but was not significantly different from the roots from untreated and azoxystrobin + metalaxyl-treated seed.

Significant ($P \leq 0.05$) differences among the seed treatments occurred in five of the six environments where root lesion length was measured in 2004 (Table 3). In 2004, lesion length at Arthur and Fargo was significantly smaller on the roots from azoxystrobin + metalaxyl-treated seed than on the roots from untreated and *B. pumilus* GB34-treated seed but was not significantly different from the roots from fludioxonil + mefenoxam-treated seed. Lesion length at Great Bend was significantly smaller on the roots from azoxystrobin + metalaxyl-treated seed than on the roots of all other treatments. Lesion length at Grandin was significantly smaller on the roots from azoxystrobin + metalaxyl- and *B. pumilus* GB34-treated seed than on the roots of untreated seed but was not significantly different from the roots from fludioxonil + mefenoxam-treated seed. At Wyndmere, lesion length was significantly smaller on the roots from azoxystrobin + metalaxyl-treated seed than on the roots of untreated and fludioxonil + mefenoxam-treated seed but was not significantly different from the roots from *B. pumilus* GB34-treated seed. Overall, lesion length was smaller ($P = 0.0001$) in the 2003 environments compared with the 2004 environments according to a single-degree-of-freedom contrast.

No significant ($P \leq 0.05$) differences among seed treatments for yield occurred in 2003; however, significant differences did occur among seed treatments in six of the eight environments in 2004 (Table 3). In 2004, yield at Casselton from fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed was significantly greater than yield from *B. pumilus* GB34-treated seed but was not significantly different from yield from untreated seed. At Fargo and LaMoure, yield from fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed was significantly greater than yield from untreated and *B. pumilus* GB34-treated seed. Yield at Great Bend and Northwood from fludioxonil + mefenoxam-, azoxystrobin + metalaxyl-, and *B. pumilus* treated-seed was significantly greater than yield from untreated seed. At Wyndmere, yield from fludioxonil + mefenoxam-treated seed was significantly greater than yield from *B. pumilus* GB34-treated seed but was not significantly different from yield from the untreated and azoxystrobin + metalaxyl-treated seed. Overall, yield was greater ($P = 0.0001$) in the 2003 environments compared with the 2004 environments according to a single-degree-of-freedom contrast.

No significant ($P \leq 0.05$) differences among seed treatments for net return occurred in 2003; however, significant differences did occur among seed treatments in six of the eight environments in 2004 (Table 3). In 2004, net return at Casselton from fludioxonil + mefenoxam-treated

Table 3. Effect of seed treatments on stand, root lesion length, yield, and net return of soybean grown across several North Dakota environments[x]

Environment, treatment[y]	Stand (plants/m^2)	Lesion length (mm)	Yield (kg/ha)	Net return ($/ha)[z]
2003				
Casselton				
Untreated	37 a	9 a	1,935 a	387 a
Fludioxonil + mefenoxam	37 a	10 a	2,115 a	408 a
Azoxystrobin + metalaxyl	31 ab	6 a	2,051 a	399 a
Bacillus pumilus GB34	28 b	11 a	2,030 a	402 a
Grandin				
Untreated	43 a	14 ab	2,355 a	471 a
Fludioxonil + mefenoxam	45 a	10 b	2,511 a	488 a
Azoxystrobin + metalaxyl	33 b	15 ab	2,363 a	462 a
Bacillus pumilus GB34	43 a	23 a	2,587 a	513 a
Great Bend				
Untreated	46 a	13 a	2,375 a	475 a
Fludioxonil + mefenoxam	47 a	15 a	2,463 a	478 a
Azoxystrobin + metalaxyl	49 a	11 a	2,433 a	476 a
Bacillus pumilus GB34	49 a	11 a	2,416 a	479 a
LaMoure				
Untreated	46 a	15 a	2,302 a	460 a
Fludioxonil + mefenoxam	50 a	13 a	2,353 a	456 a
Azoxystrobin + metalaxyl	44 a	9 a	2,373 a	464 a
Bacillus pumilus GB34	46 a	13 a	2,318 a	460 a
Northwood				
Untreated	ND	ND	1,686 a	337 a
Fludioxonil + mefenoxam	ND	ND	1,736 a	332 a
Azoxystrobin + metalaxyl	ND	ND	1,736 a	336 a
Bacillus pumilus GB34	ND	ND	1,643 a	325 a
Wyndmere				
Untreated	53 a	17 a	2,629 a	526 a
Fludioxonil + mefenoxam	42 b	18 a	2,571 a	499 a
Azoxystrobin + metalaxyl	44 ab	10 a	2,771 a	543 a
Bacillus pumilus GB34	44 ab	13 a	2,666 a	503 a

(*continued on next page*)

[x] For each environment (location and year) within a column, means followed by a common letter are not significantly different according to least-square means *t* tests ($P \leq 0.05$); ND = not determined.

[y] Fludioxonil + mefenoxam was applied at 2.5 + 7.5 g a.i./100 kg of seed as Warden RTA (Agriliance LLC, St. Paul, MN), azoxystrobin + metalaxyl was applied at 4.1 + 5.4 g a.i./100 kg of seed as Soy-Gard (Bayer CropScience, Research Triangle Park, NC), and *Bacillus pumilus* GB34 was applied at 1.74×10^4 CFU/100 kg of seed as Yield Shield (Bayer CropScience).

[z] Net return was calculated by (yield × market price) – cost of treatment, where the market price was \$0.20/kg and the cost of treatment was \$0, \$14.72, \$10.80, and \$3.93/ha for untreated, fludioxonil + mefenoxam, azoxystrobin + metalaxyl, and *Bacillus pumilus* GB34, respectively.

seed was significantly greater than net return from *B. pumilus* GB34-treated seed but was not significantly different from net returns from untreated and azoxystrobin + metalaxyl-treated seed. At Fargo, net returns from fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed were significantly greater than net returns from untreated seed, and net return from *B. pumilus* GB34-treated seed was greater than untreated seed but less than fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed. Net returns at Great Bend and Northwood from fludioxonil + mefenoxam-, azoxystrobin + metalaxyl-, and *B. pumilus* GB34-treated seed were significantly greater than net return from untreated seed. At Wyndmere, net return from untreated seed was significantly greater than net return from *B. pumilus* GB34-treated seed but was not significantly different from net returns from fludioxonil + mefenoxam- and azoxystrobin + metalaxyl-treated seed. Overall, net return was greater ($P = 0.0001$) in the 2003 environments compared with the 2004 environments according to a single-degree-of-freedom contrast. Net return from the use of the chemical fungicide seed treatments ($394/ha), azoxystrobin + metalaxyl and fludioxonil + mefenoxam, was significantly greater ($P = 0.0001$) than net return from the use of untreated seed ($350/ha). Net return from the use of the biological seed treatment ($372/ha), *B. pumilus* GB34, was significantly greater ($P = 0.0013$) than net return from the use of untreated seed ($350/ha). Net return from the use of treated seed ($383/ha), azoxystrobin + metalaxyl, fludioxonil + mefenoxam, and *B. pumilus* GB34, was significantly greater ($P = 0.0001$) than net return from the use of untreated seed ($350/ha).

Lesion length and yield were negatively correlated ($P = 0.0001$, $R = –0.59$). The correlation between stand and yield was not significant ($P = 0.0945$, $R = 0.24$).

DISCUSSION

As observed by the significant environment–seed treatment interactions, environment was one of the factors in determining when seed treated with a fungicide produced a greater yield than untreated seed in our trials. The four environments where seed treated with either fludioxonil + mefenoxam or azoxystrobin + metalaxyl produced significantly higher yields than untreated seed were all in 2004 environments (Fargo, Great Bend, LaMoure, and Northwood). At these specific 2004 environments, the soil temperature at planting was <15°C and the total rainfall from 1 week prior to planting to 3 weeks after planting was >111 mm (Table 1). These soil temperature and moisture conditions also were present at other 2004 environments, but fungicide seed treatments did not significantly increase yield over the untreated control at these environments. Therefore, factors other than rainfall and soil temperature likely played a role as well. Differences in soilborne pathogens present and their inoculum densities at the different environments were factors that also may have been involved, although the pathogen isolations from roots do not necessarily support this. Guy et al. (11) reported that beneficial yield responses to metalaxyl soybean seed treatment occurred only in environments where *P. sojae* was present and conditions were favorable for infection.

Table 3. (*continued from preceding page*)

Environment, treatment[y]	Stand (plants/m^2)	Lesion length (mm)	Yield (kg/ha)	Net return ($/ha)[z]
2004				
Arthur				
Untreated	28 b	38 a	902 a	180 a
Fludioxonil + mefenoxam	34 ab	27 bc	993 a	184 a
Azoxystrobin + metalaxyl	41 a	20 c	973 a	184 a
Bacillus pumilus GB34	37 ab	36 ab	1,021 a	200 a
Casselton				
Untreated	42 ab	14 a	1,326 ab	265 ab
Fludioxonil + mefenoxam	38 b	13 a	1,589 a	303 a
Azoxystrobin + metalaxyl	48 a	13 a	1,454 a	280 ab
Bacillus pumilus GB34	45 ab	17 a	1,127 b	221 b
Fargo				
Untreated	14 c	44 a	299 c	60 c
Fludioxonil + mefenoxam	47 a	26 bc	1,388 a	263 a
Azoxystrobin + metalaxyl	45 a	18 c	1,280 a	245 a
Bacillus pumilus GB34	26 b	33 b	850 b	166 b
Grandin				
Untreated	39 b	48 a	1,031 a	206 a
Fludioxonil + mefenoxam	49 a	39 ab	1,319 a	249 a
Azoxystrobin + metalaxyl	50 a	37 b	1,316 a	252 a
Bacillus pumilus GB34	53 a	37 b	1,102 a	216 a
Great Bend				
Untreated	12 b	35 a	1,760 b	352 b
Fludioxonil + mefenoxam	24 a	29 a	2,615 a	508 a
Azoxystrobin + metalaxyl	22 a	12 b	2,794 a	548 a
Bacillus pumilus GB34	20 ab	28 a	2,603 a	517 a
LaMoure				
Untreated	32 a	ND	1,125 b	225 c
Fludioxonil + mefenoxam	32 a	ND	1,599 a	305 ab
Azoxystrobin + metalaxyl	33 a	ND	1,629 a	315 a
Bacillus pumilus GB34	32 a	ND	1,289 b	254 bc
Northwood				
Untreated	ND	ND	1,796 b	359 b
Fludioxonil + mefenoxam	ND	ND	2,453 a	476 a
Azoxystrobin + metalaxyl	ND	ND	2,524 a	494 a
Bacillus pumilus GB34	ND	ND	2,294 a	455 a
Wyndmere				
Untreated	49 a	27 a	2,810 ab	562 a
Fludioxonil + mefenoxam	46 a	24 a	2,834 a	552 ab
Azoxystrobin + metalaxyl	43 a	12 b	2,776 ab	544 ab
Bacillus pumilus GB34	45 a	22 ab	2,534 b	503 b

Pearson correlation analysis indicated a significant negative relationship between root lesion length and yield but no significant relationship between plant stand and yield. Although no significant correlation was found between plant stand and yield, some yield losses likely were due at least partially to plant stand losses. Plant stands from the untreated controls at Fargo and Great Bend in 2004 were reduced up to 70 and 50%, respectively, compared with the plant stands from the chemical fungicide seed treatments, and yields were reduced up to 78 and 37%. This lack of significant correlation probably was due to the ability of soybean to maintain adequate yields despite reductions in plant stand (26). The significant negative correlation between root lesion length and yield indicates the importance of root lesions in reducing yield. At least one of the chemical fungicide seed treatments was able to reduce lesion size on soybean roots in five of the six 2004 environments. Root lesions were evaluated at the R1 developmental stage, which would be at a time when the chemical fungicide seed treatments most likely were no longer active. In these instances, the fungicide may have protected the root from early infection, thus slowing the development of root lesions.

Only *Fusarium* and *Rhizoctonia* spp. were isolated from diseased roots in our trials. Metalaxyl and mefenoxam have specific activity against oomycete pathogens only (14,28). Azoxystrobin has been reported to have activity on *Fusarium*, *Rhizoctonia*, *Phytophthora*, and *Pythium* spp. (1,3,4,14,15,18,25,27,31). Fludioxonil has been reported to have activity on *Rhizoctonia* and *Fusarium* spp. (4,14,18,19,28) but not *Phytophthora* or

Pythium spp. (14). Root rot diseases caused by *P. sojae*, *R. solani*, and *F. solani* f. sp. *phaseoli* have been well-documented in North Dakota (20–22). Additional pathogens such as *Pythium* spp., and other *Fusarium* spp. such as *F. graminearum*, also may be involved in seedling blights and root rots of soybean in North Dakota. In Ohio, multiple *Pythium* spp. were found to be pathogenic to soybean and caused seedling establishment problems (3,5). *F. graminearum* isolates in Ohio and Argentina have been reported to be pathogenic to soybean (4,23). Although *F. graminearum* was isolated from soybean roots in the North Dakota trial, no pathogenicity tests were conducted to determine their pathogenicity on soybean. *P. sojae* or *Pythium* spp. were not isolated from diseased roots in this trial. This may be due to the high level of resistance to *P. sojae* in cv. Walsh, and due to when the isolations were made. At the time isolations were made (R1 soybean developmental stage), *Pythium* spp. may not have been active due to higher soil temperatures; this is especially true for *Pythium debaryanum*, *P. torulosum*, and *P. ultimum* (32). Although more research is needed to definitively identify all of the pathogens responsible for soybean seedling establishment problems in North Dakota, the problems likely are caused by a complex of pathogens.

Currently, the majority of North Dakota soybean growers have not adopted the use of fungicide seed treatments, and soybean seed purchased from seed dealers generally do not contain a seed treatment (36). A North Dakota grower considering a soybean fungicide seed treatment should use a combination of either mefenoxam or metalaxyl and fludioxonil and azoxystrobin, which should provide protection against the widest range of pathogens. Broders et al. (3) reported that mefenoxam and azoxystrobin, when used individually, may not inhibit all *Pythium* spp. found in Ohio soils. Additionally, due to the different fungicide classes involved, this also may provide management against the development of fungicide-resistant pathogens. In Ohio, isolates of both *Pythium* spp. and *F. graminearum* had differing levels of sensitivity to metalaxyl and fludioxonil, respectively (4,5). This indicates that fungicide resistance in seedling disease pathogens potentially could be a problem, and provides additional justification that a mixture of fungicide classes should be used as a seed treatment.

Although not always as effective at protecting against plant stand and yield losses as the chemical fungicide treatments, *B. pumilus* GB34 did protect against plant stand and yield losses in two of the seven 2004 environments and three of the eight 2004 environments, respectively, where plant stand and yield were measured. *B. pumilus* GB34 (also known as strain INR7) has been reported to have both seedling growth promotion and induced systemic resistance properties (16). *B. pumilus* INR7 also has been shown to reduce several diseases of cucumber (*Cucumis sativus*) such as cucurbit wilt disease caused by *Erwinia tracheiphila*, angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), and anthracnose (*Colletotrichum orbiculare*) (30,34,35). Fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) of loblolly pine (*Pinus taeda*) also was reduced by *B. pumilus* INR7 (8). *B. pumilus* GB34 was tested only as an individual seed treatment in these trials in North Dakota; however, the addition of chemical fungicides as a seed treatment should be studied, because the spectrum or efficacy of diseases controlled potentially could be enhanced with such a mixture. Kiewnick et al. (15) found that the combination of azoxystrobin and *Bacillus* spp. had the best Rhizoctonia crown rot control in sugar beet (*Beta vulgaris*) compared with azoxystrobin alone and *Bacillus* spp. alone. Organic soybean growers could consider using *Bacillus pumilus* GB34, because the product Yield Shield is listed by the Organic Materials Review Institute (OMRI, Eugene, OR) for use in organic production. Soybean growers concerned with excess seed at the end of the planting season also could consider *B. pumilus*, because seed treated with the product Yield Shield can be used for feed, food, or oil purposes, unlike seed treated with chemical fungicide treatments.

Averaged over all environments, the economic net return achieved from the use of seed treatments was $33/ha more than the net return achieved from the use of untreated seed. The net return achieved from the use of the chemical fungicide seed treatments or the *B. pumilus* biological seed treatment was $44 or $22/ha more than the net return achieved from untreated seed, respectively. These economic returns achieved with seed treatments are similar results from Poag et al. (24), who reported that fungicide seed treatments could enhance profitability to soybean growers by an average of $43.71/ha across multiple conditions in Arkansas.

From this research, it was shown that fungicide seed treatments on soybean could prevent stand and yield losses, especially under cool and moist soil conditions. It should be noted that the fungicide seed treatments were evaluated on one cultivar only in these trials, and some cultivars may not respond to seed treatments in the same manner. These trials also were conducted in sites where conventional tillage practices were used, and benefits of fungicide seed treatments could have been observed more often under conservational tillage systems. Guy and Oplinger (10) reported that metalaxyl seed treatment was beneficial under no-tillage systems but not under conventional tillage systems in Wisconsin.

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