Effect of Fungicide Application and Cultivar on Soybean Green Stem Disorder

C. B. Hill, Department of Crop Sciences, University of Illinois, Urbana 61801; **C. R. Bowen,** United States Department of Agriculture–Agricultural Research Services; and **G. L. Hartman,** USDA-ARS and Department of Crop Sciences, University of Illinois, Urbana 61801

Abstract

Hill, C. B., Bowen, C. R., and Hartman, G. L. 2013. Effect of fungicide application and cultivar on soybean green stem disorder. Plant Dis. 97:1212-1220.

Green stem disorder of soybean (*Glycine max*) has increasingly become a nuisance for soybean producers. The disorder is distinguished from other manifestations of delayed plant maturity by the delayed senescence of stems only, with normal pod ripening and seed maturation. The primary objective of the first study was to determine whether green stem disorder increased with a fungicide treatment. Field cages to isolate soybean plants to prevent insect interactions were used and treatments included maturity group (MG) II insensitive and sensitive soybean cultivars with or without fungicide applications. A secondary objective was to determine fungi potentially associated with the disorder. The results indicated significant elevation of the incidence of green stem disorder when using a fungicide. Species of *Diaporthe* or *Phomopsis* and *Macrophomina phaseolina* were more frequent in stems without the disorder, whereas species of *Colletotrichum* were found mostly in stems with the disorder. In another study, field experiments were conducted without cages in replicated field plots to compare the effects of fungicides with different chemistries and timing of fungicide application on incidence of green stem disorder using green stem disorder MG II- and MG III-sensitive and insensitive soybean cultivars. There was a significant increase in percentage of green stem disorder due to fungicide application, depending on fungicide chemistry, timing of application, year, location, and cultivar sensitivity to green stem disorder. Generally, Headline and Headline-Domark applications resulted in higher incidence of green stem disorder than Domark alone or the nonsprayed control, with over 50% incidence in many cases. Higher percent green stem disorder was significantly ($P < 0.05$) associated with higher yields in 11 of the 28 trials. From the results of this research, soybean producers should be aware of the possible risk that fungicide application may have in increasing incidence of green stem disorder. In addition, producers can help manage green stem disorder by selecting soybean cultivars reported to be consistently insensitive to the disorder.

Delayed or incomplete maturation of soybean plants or plant parts can hinder soybean (*Glycine max* (L.) Merr.) grain harvesting. Immature soybean stems are more difficult to cut and combine than senesced stems. In order to harvest large areas of the field in which plants have green stem disorder, combine adjustments such as reducing ground speed while increasing engine power may be needed to optimize harvest and avoid blocking the opening between the concave and cylinder with moist plant material. This action reduces combine fuel efficiency and increases harvest time. Producers often avoid this problem by delaying harvest until a hard frost kills any remaining green stem tissue, although this can increase the potential for seed losses or increased seed decay caused by *Phomopsis longicolla* or other opportunistic organisms, resulting in reduced seed quality and market grade.

Green stem disorder of soybean has been reported to be a major field problem, with occurrences of up to 100% reported in experimental plots (11,12). The disorder is distinguished by the delayed senescence of stems with normal pod ripening and seed maturation. Occasionally, unabscised leaves or petioles remain on plants with green stem disorder. Although the disorder causes difficulty during harvest, there are no reports that it affects yield.

Corresponding author: G. L. Hartman, E-mail: ghartman@illinois.edu

Infection of plants by *Bean pod mottle virus* (BPMV) was once thought to be the primary factor causing this disorder (23); however, results of a field survey and field cage experiments provided evidence that BPMV was not a main factor in green stem disorder (12). The field survey found that green stem disorder was independent of BPMV infection. Furthermore, BPMV infection resulting from inoculations did not increase the incidence of green stem disorder compared with noninoculated controls inside field cages. Also, bean leaf beetle (which is the main insect vector transmitting BPMV to soybean), leaf hopper, or stinkbug feeding were not found to be associated with incidence of green stem disorder (12). Pod removal, however, has been shown to increase green stem incidence without delaying pod maturation (7). Although, the cause or causes of green stem disorder still have not been elucidated, soybean genetics was shown to be an important factor in determining the sensitivity of soybean cultivars toward green stem disorder because a relatively low percentage of cultivars were found to have greater than 90% green stem disorder across multiple locations and seasons, whereas most cultivars had consistently low levels of green stem disorder (11). Soybean producers in Illinois and similar geographic regions can select soybean cultivars with a low sensitivity to green stem disorder (5). Although the mode of inheritance of green stem disorder sensitivity is currently unknown, culling soybean lines in soybean breeding programs that consistently have a high incidence of the disorder may help reduce the number of sensitive cultivars in the market.

Interest in the use of fungicides to protect soybean yields has increased in the United States, primarily stimulated by the 2004 introduction of soybean rust (21). Although losses caused by soybean rust have not been as great as predicted (15), fungicides are effective in managing soybean rust (16). In addition to the use of fungicides to manage soybean rust, prophylactic fungicide application increased soybean yields in the absence of soybean rust by as much as 20% (4,24). Along with the potential positives of applying fungicides to soybean, there may be negative consequences as well, such as increased percentages of plants with green stems at

harvest (18), particularly caused by a strobilurin (Headline; BASF Corp.) fungicide (22), along with increased potential for undesirable environmental contamination (3). Strobilurin fungicides have also been implicated in producing a "greening effect" in crop plants (1,2). In soybean, this greening effect affects all plant parts (www.farmassist.com/Promo/Online_Training_Courses/Fungicides_Online_Training) (6), including seed pods, which distinguishes it from green stem disorder symptoms that have dry, ripe seed pods, as defined in this and previous publications (11,12).

The primary objective of the first experiment was to determine whether the incidence of green stem disorder in the green stem disorder-sensitive and insensitive maturity group (MG) II soybean cultivars increased with fungicide application. A secondary objective was to determine the incidence of fungi in stems with or without green stem disorder symptoms. Field cages were used to isolate soybean plants to prevent insect interactions with the treatments. Because the results indicated significant elevation of the incidence of green stem disorder when using a fungicide, follow-up field experiments were conducted without cages. The primary objective of these field experiments was to compare the effects of fungicides with different chemistries and timing of fungicide application on the incidence of green stem disorder in MG II and MG III green stem disorder-sensitive and insensitive soybean cultivars.

Materials and Methods

Two types of experiments were conducted, one in field cages and the other in field plots. The field cage experiment was repeated in three trials conducted during three summer growing seasons from 2004 to 2006 at the University of Illinois Crop Science Research and Education Center, Urbana. Each trial had identical treatments but different randomizations. Field plot experiments were conducted during three summer growing seasons from 2007 to 2009 at two locations in Illinois in 2007 and 2008 and three locations in 2009. Separate experiments for MG II and MG III soybean cultivars were conducted at each location. Fungicide treatments were modified across the years.

Soybean cultivars. Two MG II and two MG III glyphosate-resistant cultivars were used in the experiments. Stine 2463 (Stine Seed Company), MG II, and Kruger K-340 (Kruger Seeds, Inc.), MG III, were selected because of their consistent sensitivity to green stem disorder in tests at multiple locations in Illinois across multiple years (11). Hughes 441 (Hughes Hybrids), MG II, and Kruger K-341, MG III, were consistently insensitive to the disorder and reached harvest maturity, defined as 95% pods brown at growth stage R8 (8), approximately the same time as the green-stem-sensitive cultivars within each MG. Soybean seeds used in all experiments in this research were first increased in isolated field plots from subsamples of seed submitted by seed companies for testing in the Illinois Soybean Variety Testing Program (5).

Field cage experiments. Field cages were used to isolate soybean plants from insects, as previously reported (12). They were set on top of Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquolls) and Flannagan silty loam (fine, montmorillonitic, mesic Aquic Argiudolls). There were six cages (Redwood Empire Awning and Furniture Co.), two in each of the three replications, with one used for the caged control and the other used for the caged fungicide treatment. The cages were 2.5 by 3 by 2 m (height, width, and length) and constructed as previously described (12).

The field cage experiment was a factorial with three factors: year, treatment, and soybean cultivar. Treatments and cultivars were arranged in a split-plot design with three replications. Treatments were whole plots and cultivars were subplots. There were three treatments: noncaged control, in which plots were left out in the open; caged control; and caged with fungicide application. The fungicide Pristine was used at a higher-than-recommended rate to fully maximize the fungicide effect of its broad-spectrum activity against fungal pathogens. Pristine (boscalid and pyraclostrobin) was mixed at the rate of 5.1 g per 600 ml of water, approximately 0.3% a.i., and applied biweekly, starting at plant emergence and

ending when the plants reached growth stage R7 (8). Two MG II soybean cultivars, Hughes 441 and Stine 2463, were used for this experiment. Each of the three trials (years) had identical treatments with different randomizations.

Two-row plots of each of the two cultivars were planted within each treatment and were the experimental units. Rows were 1.8 m long, spaced 0.3 m apart, and centered inside the cage. The seeding rate was 30 seeds/m of row. Prior to planting, macronutrients were applied to the soil based on soil fertility analyses, and standard soybean field cultivation practices were used. A 0.76-m-tall 20-gauge mesh poultry netting wire fence was placed around each noncaged control plot to exclude small animals. Glyphosate was applied as needed to control weeds and was not considered as a factor in these experiments because it has been demonstrated that neither glyphosate application nor glyphosate resistance had an effect on incidence of green stem disorder (12).

Percent green stem disorder in each experimental unit was visually rated at soybean growth stage R8, when pods appeared to be ripe and crispy dry, using a 0-to-5 pretransformed scale, with 0 = no green stem disorder present and 1 = 1 to 10, 2 = 11 to 35, 3 = 36 to 65, 4 = 66 to 90%, and 5 = 91 to 100% green stem disorder (11,12). The steps of the scale represented increments of percentages that were pretransformed by the arcsine-square root transformation method (14). The use of this scale obviated the requirement to transform percentage data to normalize variance.

Stem samples from plants in the field cages were collected to identify and enumerate commonly isolated fungi from inside the stems. In 2004, at growth stage R8, 10 stems were randomly collected from each of the two rows of each experimental unit. Stems were cut from the ground line, just above the plant crown region, up to approximately 15 cm above the soil. The condition of each stem, whether it had green stem disorder or not, was noted. Prior to surface disinfestation, the stems were cut into four equal sections by length. All four sections of each stem were soaked in 20% household bleach (5.25% NaOCl) for 2 min, rinsed twice in sterile distilled water to remove the bleach solution, and then transferred to a petri dish containing 20 ml of 1.5% water agar (15 g of Bacto agar per liter of distilled water). The four sections of each stem were placed equidistantly in the same petri dish. The plates were incubated on a laboratory bench, under intermittent fluorescent illumination provided by ceiling fixtures, at $23 \pm 2°C$.

In 2005 and 2006, up to 10 stems showing green stem disorder symptoms and up to 10 stems not showing green stem disorder were collected from each plot for each experimental unit at growth stage R8 (8). Stems were cut, surface disinfested, plated, and incubated as previously described.

Beginning 1 day and up to 14 days after plating, each stem section was examined under a stereo microscope at different magnifications to observe diagnostic signs (acervuli, microsclerotia, perithecia, pycnidia, stroma, or other structures) of potential soybean stem pathogens (10). Presence of a pathogen on each stem section was recorded. Detailed mycological work to identify species was not done. The number of positive identifications of each pathogen on all of the stem sections with and without green stem disorder in each experimental unit were summed, which was divided by the total number of stem sections in the sample that were plated, to calculate the percentage of stem sections that were infected. Examination of the stem sections continued until the plates were overgrown.

Field experiments. Separate field plot experiments for MG II and MG III cultivars were conducted during the 2007, 2008, and 2009 growing seasons. Trials of each experiment were conducted at two University of Illinois Department of Crop Sciences Research and Education Centers, Urbana and Monmouth, in 2007 and 2008. Planting dates at Urbana were 11 May 2007 and 22 April 2008. Monmouth planting dates were 9 May 2007 and 15 May 2008. In 2009, three trials of each experiment were conducted, two at two different fields in the Urbana location (Urbana 1 and 2) and one at Monmouth. Planting dates in 2009 were 30 May at Urbana 1, 23 May at Urbana 2, and 8 May at Monmouth.

The experimental design of each MG II and MG III experiment was a factorial with the factors location, cultivars, and fungicide spray treatments. In 2007, there were four spray treatments, with a fungicide mixture sprayed at three different times during the season and a nonfungicide control. In 2008 and 2009, there were 10 spray treatments in total, consisting of two fungicides and their mixture sprayed at three times during the season plus a nonfungicide control. Fungicide and spray time were not separate factors (referred to as fungicide spray treatments). Factor combinations were arranged in a randomized complete block design with four blocks.

For the 2007 field experiment, a mixture of Domark 230ME (Isagro S.P.A.; Valent U.S.A. Corporation), 21% tetraconazole, at 219 ml/ha and Headline (BASF Corporation), 24% pyraclostrobin, at 438 ml/ha was used at three different times during the season. Spray treatments were timed to represent fungicide applications during the reproductive period. An early (growth stage R2), mid-season (growth stage R3 to R4), and late-season (growth stage R5 to R6) application was applied. This mixture was prepared to provide broad-spectrum protection against soybean fungal pathogens. In 2008 and 2009, Domark and Headline were applied separately along with the Domark-Headline mixture at three different times during the season. The rates of the fungicides used in 2008 were the same as in 2007 whereas, in 2009, the rate of Domark was increased to 292 ml/ha and Headline to 584 ml/ha. Fungicides were applied at a volume of 187.1 liters/ha at a speed of 4.5 kph using a hand sprayer (Bellspray Inc., Opelousas, LA) equipped with Tee-Jet 1002 Turbo T nozzles (TeeJet Technologies, Wheaton, IL) and compressed CO_2 regulated at a pressure of 193 kPa.

Field plots of each cultivar were the experimental units planted in four rows that were 6.1 m long and spaced 0.8 m apart. Plots were trimmed to approximately 5.3 m. Seeding rate was approximately 40 viable seed/m of row. Macronutrients were applied to the soil based on soil fertility analyses prior to planting. The Monmouth plots were grown on a field consisting of 50% Muscatune silt loam and 50% Sable silty-clay loam, whereas the Urbana locations varied from a Flanagan silt loam (2007) to a Drummer silty-clay loam (2008 and 2009) or an Elburn silt loam (2008 and 2009). Glyphosate was applied to the experiments as needed to control weeds.

Data were collected on green stem disorder, number of days to harvest maturity, and yield. The percent green stem disorder for each experimental unit was visually rated at harvest maturity or soybean growth stage R8 (8), when pods appeared to be ripe and crispy dry, using the scale previously described.

The number of days to harvest maturity was recorded as the number of days after 31 August when 95% of the pods reached maturity. The center two rows of each plot in all field experiments were harvested. Yields were adjusted to 13.5% seed moisture.

Statistical analysis. For the field cage experiment, homogeneity of variance among the 3 years for green stem rating was tested using Bartlett's test performed with the aid of JMP (version 9.03; SAS Institute Inc.) using the 0-to-5 pretransformed scale data as recommended by Little and Hills (14) and not the percentages detransformed from the scale. Restricted or residual maximum likelihood (REML) analysis of the green stem ratings from the two-row-plot experimental units of each cultivar in each treatment

in the field cage experiment was also performed with JMP. Years and treatments were fixed effects. Cultivars were nested within treatments. Replications were random effects.

Percentages of positive incidence of organisms identified from stem sections in each experimental unit with or without green stem disorder in the cage experiments were transformed using the arcsine square root (14) prior to statistical analysis to correct for nonconstant variance among samples. REML analysis was performed as above for the green stem disorder ratings on the data using the same statistical model plus the additional sub-subplot factor of stem condition. The 2004 experiment was analyzed separately because the sampling method used was different from the method used in 2005 and 2006. Homogeneity of variance between the 2005 and 2006 experiments was analyzed prior to performing REML analysis.

For the field experiments, REML analyses were performed with SAS using the MIXED procedure (SAS version 9.2; SAS Institute Inc.). MG II and MG III experiments for each year were analyzed separately. Locations, cultivars, and spray treatments were fixed effects. Blocks were random effects.

When significant differences were indicated in the REML analyses, least square means were separated using least significant difference at $\alpha = 0.05$. Means of the green stem disorder ratings were detransformed to percentages by multiplying the mean ratings with the angular increment of $18°$ (14) before reporting and displaying in tables or figures. Means of the transformed percentage of positive identifications of pathogens in the stem samples was also detransformed for presentation.

Pairwise correlation coefficients were calculated with the CORR procedure, and general linear models (Proc GLM, SAS version 9.2) were used to compare variables and develop regression models of percent green stem disorder on yield, respectively [$Y_{ijk} = b_0 + b_1$ (green stem disorder$_{ij}$) + replication$_k$ + error]. The effect of green stem on yield was estimated using spray treatment plot values from each cultivar at each location.

Results

Field cage experiments. The distributions of levels of percent green stem disorder in each of the 3 years were non-normal and skewed toward lower levels; however, results of Bartlett's test indicated that the variance among the years was homogeneous ($F = 0.7$, $P = 0.5$); therefore, REML analysis was performed on the combined data across years (Table 1). There was a highly significant ($P < 0.001$) year–cultivar (treatment) interaction for percent green stem disorder, indicating that percent green stem disorder was dependent on a combination of all three factors. The mean
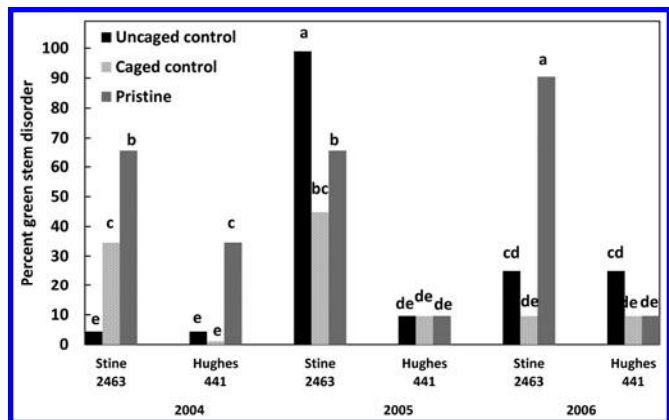
Table 1. Restricted or maximum likelihood analysis of field cage experiments using two soybean cultivars (maturity group II) in Urbana, IL during the 2004, 2005, and 2006 growing seasons

Source	df	F ratio[z]
Year	2	6.9*
Treatment	2	16.5**
Year × treatment	4	8.5***
Cultivar (treatment)	3	28.0***
Year × cultivar (treatment)	2	8.5***

[z] Asterisks: *, **, and *** indicate significant differences at $P < 0.05$, 0.01, and 0.001, respectively.

Fig. 1. Percent green stem disorder in two maturity group II soybean cultivars (Stine and Hughes) in noncaged control, caged control, and caged with fungicide applications in Urbana, IL during the 2004, 2005, and 2006 growing seasons. Bars with the same letters are not significantly different by the least significant difference test ($P = 0.05$). Pristine fungicide was mixed at the rate of 5.1g per 600 ml of water, approximately 0.3% a.i., and applied biweekly starting at plant emergence and ending when the plants reached growth stage R7.

percent green stem disorder was 28% (1.8 using a 0-to-5 pretransformed rating scale) over all 3 years and the coefficient of variation was 20%. The lowest percent green stem disorder was 1% in Hughes 441 in the caged control in 2004 (Fig. 1). Stine 2463 in the noncaged control in 2005 had the highest percent green stem disorder at nearly 99%.

Pristine application significantly ($P < 0.05$) increased percent green stem disorder in the sensitive Stine 2463 in 2004 and 2006 over the caged and noncaged control treatments but not in 2005, when the noncaged control had a significantly ($P < 0.05$) higher percent green stem than the Pristine and caged control treatments, which had percent green stem disorder not significantly different from each other (Fig. 1). Pristine application also significantly increased percent green stem disorder in the insensitive Hughes 441 in 2004 but not in 2005 and 2006.

Stine 2463 had significantly ($P < 0.05$) higher percent green stem disorder than Hughes 441 within the caged control and the Pristine treatments in 2004, in all three treatments in 2005 (Fig. 1), and only in the Pristine treatment in 2006. The percent green stem disorder in the caged and noncaged controls was not significantly ($P > 0.05$) different for Hughes 441 in all 3 years (Fig. 1). Stine 2463 had a significantly ($P < 0.05$) higher percent green stem disorder in the caged control than in the noncaged control in 2004 but this was reversed in 2005.

Four organisms were most frequently identified growing out of plated stem sections collected in the field cage experiments. Fungi resembling *Diaporthe* or *Phomopsis* spp. were recognized by the presence of pycnidia or perithecia embedded in stroma. *Colletotrichum* spp. were identified by the presence of dark acervuli with long, protruding setae. *Macrophomina phaseolina* was identified by the presence of numerous microsclerotia on charcoal-gray stem lesions. Less frequently, *Alternaria* spp. were identified by the presence of characteristic pear-shaped, muriform, multiseptate conidia borne in simple chains. Species of each fungus, except *M. phaseolina*, were not determined. Several other organisms were observed, including bacteria, but the four fungi previously mentioned were most frequently observed and, therefore, used for the analyses.

Results of plating stem sections indicated a higher frequency of *Diaporthe* or *Phomopsis* spp. and *M. phaseolina* in stems without green stem disorder than stems with the disorder, which had a higher occurrence of *Colletotrichum* spp. In 2004, the samples of 20 stems randomly selected from each plot varied in percent green stem disorder from 1 to 20%. There were highly significant ($P < 0.01$) differences between stems with green stem disorder and stems without the disorder in the percentage of *Diaporthe* or *Phomopsis* spp., *Colletotrichum* spp., and *M. phaseolina*. The percentage of stems with green stem disorder that had *Diaporthe* or *Phomopsis* spp. was 19% compared with 74% of stems without the disorder. *Colletotrichum* spp. were present in 23% of the stems with green stem disorder whereas only 2% of the stems without the disorder had the organism. *M. phaseolina* was found in 6% of the stems with green stem disorder and 24% of the stems without the disorder. There were no differences in the percentage of *Alternaria* spp. found in stems with or without green stem disorder. The effects of cultivar, treatment, and their interaction on the percentage of stems with each of the four organisms were nonsignificant.

In 2005, there were significant ($P < 0.01$) differences in percent *Diaporthe* or *Phomopsis* spp. between stems with or without green stem disorder. No (0%) *Diaporthe* or *Phomopsis* spp. were found in stems with green stem disorder, whereas 75% of the stems without the disorder had these fungi. A significant ($P < 0.01$) interaction between stem condition and cultivar was found for the percentage of stems with *Colletotrichum* and *Alternaria* spp. Stine 2463 stems with green stem disorder had significantly ($P < 0.05$) more *Colletotrichum* spp. (50%) than Stine 2463 without the disorder (6%) and Hughes 441 with (7%) or without (12%) the disorder. Similarly, Stine 2463 stems with (33%) the disorder had significantly ($P < 0.05$) more *Alternaria* spp. than without (6%) and Hughes 441 stems with (4%) or without (12%) the disorder. There was a significant ($P < 0.05$) interaction between stem condition and treatment for percentage of stems with *M. phaseolina*. Sampled stem sections without the disorder had significantly ($P < 0.05$) greater percentages of *M. phaseolina* in both the caged (67%) and noncaged (86%) samples compared with stems without the disorder in the Pristine treatment (17%), and compared with stem sec-

Table 2. Restricted or maximum likelihood analysis of soybean cultivars in maturity group (MG) II and III for green stem disorder, yield, and days to harvest maturity for the 2007, 2008, and 2009 growing seasons in Illinois

| | | F ratio[y] | | | | | | |
| | | Green stem disorder | | Yield | | Days to maturity | | |
Year, source[z]	df	MG II	MG III	MG II	MG III	df	MG II	MG III
2007								
Loc	1	NS	***	**	**	–	–	–
Cv	1	***	***	NS	NS	1	***	***
Loc × cv	1	***	***	*	NS	–	–	–
Fungic	3	***	***	NS	*	3	*	NS
Loc × fungic	3	NS	NS	NS	NS	–	–	–
Cv × fungic	3	NS	NS	NS	NS	3	NS	NS
Loc × cv × fungic	3	NS	NS	NS	NS	–	–	–
2008								
Location (loc)	1	***	**	***	***	–	–	–
Cultivar (cv)	1	***	**	***	NS	1	NS	*
Loc × cv	1	***	***	NS	NS	–	–	–
Fungic	9	*	***	NS	*	9	*	**
Loc × fungic	9	NS	**	NS	NS	–	–	–
Cv × fungic	9	NS	**	NS	NS	9	NS	NS
Loc × cv × fungic	9	NS	NS	NS	NS	–	–	–
2009								
Location (loc)	2	***	***	***	***	1	***	***
Cultivar (cv)	1	***	***	***	***	1	**	***
Loc × cv	2	***	***	**	***	1	NS	***
Fungic	9	***	***	NS	***	9	***	NS
Loc × fungic	18	NS	NS	NS	NS	9	NS	NS
Cv × fungic	9	**	NS	NS	NS	9	NS	NS
Loc × cv × fungic	18	NS	NS	NS	NS	9	NS	NS

[y] NS = not significant; *, **, and *** indicate significant differences were found at $P < 0.05$, 0.01, and 0.001, respectively; and – = no data.
[z] Loc = location, Cv = cultivar, and Fungic = fungicide spray treatments.

tions with the disorder, which had 1, 4, and 0% *M. phaseolina* in the caged, noncaged, and Pristine treatments, respectively.

In 2006, there were highly significant ($P < 0.01$) differences between stems with (77%) or without (1%) green stem disorder for percentage of *Diaporthe* or *Phomopsis* spp. There were also highly significant ($P < 0.01$) differences between stems with (0.2%) or without (44%) the disorder for percent *M. phaseolina*. Treatments significantly affected the percent *Colletotrichum* spp. found in the stems, with significantly ($P < 0.05$) more in the noncaged treatment (16%) than the caged (2%) or Pristine (0.1%) treatments. Significant ($P < 0.05$) differences between stem condition and treatments were found for the percentage of *Alternaria* spp. in the stems. More *Alternaria* spp. were found in stems with (7%) than without (0%) the disorder. Stems from the Pristine treatment (0.1%) had significantly ($P < 0.05$) less *Alternaria* spp. than stems from the noncaged treatment (5%) but not the caged treatment (3%).

Field experiments, 2007. For percent green stem disorder, interactions were not significant except for a significant ($P < 0.001$) location–cultivar interaction found in both MG II and MG III experiments (Table 2). There were significant ($P < 0.001$) differences among fungicide spray treatments in both experiments. For the MG II experiment, Stine 2463 had significantly ($P < 0.05$) higher percent green stem disorder than Hughes 441 at both locations (Fig. 2). For the MG III experiment, Kruger K-340 had significantly ($P < 0.05$) higher percent green stem disorder than Kruger K-341 at both locations (Fig. 2). Fungicide spray treatments for the MG II and MG III experiments were a significant ($P < 0.05$) source of variation (Table 2) and, in each experiment, the nonsprayed control had significantly ($P < 0.05$) less percent green stem disorder than the fungicide treatments (Table 3).
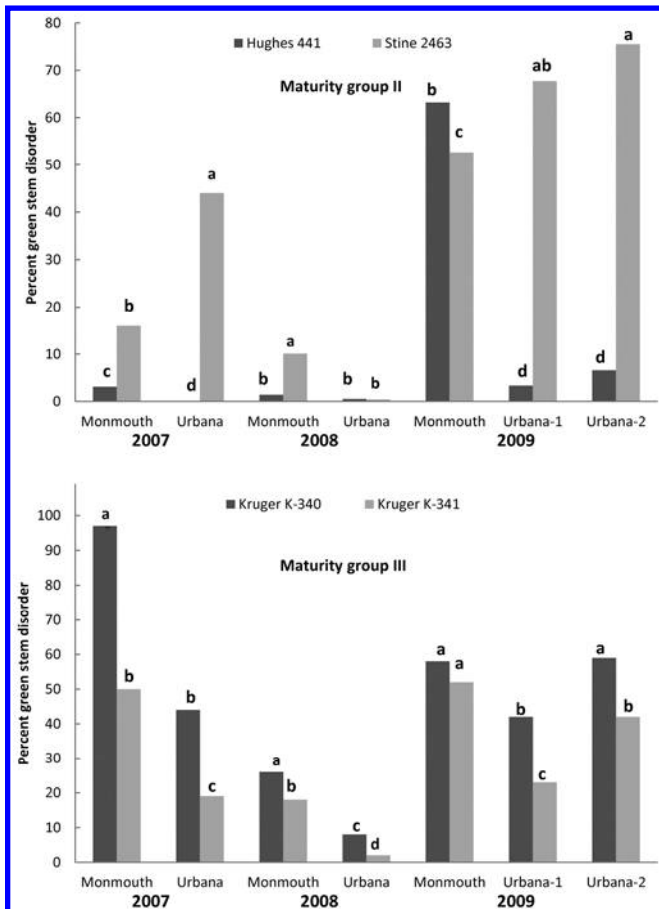

Fig. 2. Mean percent green stem disorder for two soybean cultivars in each of two maturity groups (MG II, Stine and Hughes, and MG III, K-340 and K-341) over fungicide spray treatments at three locations in the 2007, 2008, and 2009 growing seasons. Bars within the same year with the same letters are not significantly different by the least significant difference test ($P = 0.05$).

For days to maturity, there were significant ($P < 0.05$) differences between cultivars in both experiments and among fungicide spray treatments for the MG II experiment (Table 2). For the MG II experiment, on average, Hughes 441 matured 3 days earlier than Stine 2463. For the MG III experiment, on average, Kruger K340 matured less than 1 day earlier than Kruger K341.

For yield, the location–cultivar interaction in the MG II experiment, the fungicide spray treatments in the MG III experiment, and the location in both experiments were significant ($P < 0.05$) (Table 2). The mid-season spray increased ($P < 0.05$) overall yield of the two cultivars by 423 kg/ha compared with the nonsprayed control. Mean yields over cultivars were higher ($P < 0.05$) at Monmouth than Urbana by 289 and 276 kg/ha in the MG II and III experiments, respectively.

There was a significant ($P < 0.05$) positive correlation ($r = 0.72$) between days to maturity and percent green stem disorder for Stine 2463, between yield and percent green stem disorder for Hughes 441 at Monmouth ($r = 0.54$) and Stine 2463 at both Monmouth ($r = 0.69$) and Urbana ($r = 0.52$) in the MG II experiment, and between yield and percent green stem disorder for Kruger K341 at Monmouth ($r = 0.54$).

Field experiments, 2008. For percent green stem disorder, the location–cultivar interaction was significant ($P < 0.001$) as well as the fungicide spray treatments ($P < 0.05$) in the MG II experiment (Table 2). For the MG III experiment, all interactions were significant ($P < 0.01$), except the location–cultivar–fungicide spray treatments (Table 2). For the MG II experiment, the percent green stem disorder for Stine 2463 was significantly ($P < 0.05$) greater than Hughes 441 at only the Monmouth location (Fig. 2). For the MG III experiment, Kruger K-340 was significantly ($P < 0.05$) greater than Kruger K-341 at both locations (Fig. 2). Within the fungicide spray treatments for the MG II experiments, the Domark-Headline mixture sprayed at mid-season produced significantly ($P < 0.05$) higher percent green stem disorder than all other treatments except for Domark sprayed late, Domark sprayed mid-season, and Headline sprayed early (Fig. 3). Only the mixture sprayed at mid-season and Domark sprayed at late-season produced significantly ($P < 0.05$) higher percent green stem disorder than the nonfungicide control. Within the fungicide spray treatments in the MG III experiments, Headline and the Domark-Headline mixture produced significantly ($P < 0.05$) higher percent green stem disorder across the two cultivars, whereas no Domark sprays produced significant differences in percent green stem disorder from the nonfungicide control at Monmouth (Fig. 4). No fungicide treatment produced significantly higher percent green stem disorder than the nonfungicide control at Urbana. For the cultivars–fungicide spray treatment interaction in the MG III experiments, only Headline at mid- and late-season produced significantly ($P < 0.05$) higher percent green stem disorder than the nonfungicide control for Kruger K-340, whereas the Domark-Headline mixture at all spray times significantly ($P < 0.05$) increased percent green stem disorder in Kruger

Table 3. Mean percent green stem disorder of soybean cultivars in maturity group (MG) II and MG III based on fungicide spray treatments in field experiments at Monmouth and Urbana, IL in 2007

	Green stem disorder (%)[y]	
Fungicide[z]	**MG II**	**MG III**
Mid	16.1 a	70.9 a
Early	13.3 a	67.3 a
Late	12.0 a	67.3 a
Nonsprayed	3.8 b	40.2 b

[y] Means with the same letters are not significantly different by the least significant difference test ($P = 0.05$).

[z] Fungicide spray treatments. Fungicide applications were completed during the reproductive period at early season (growth stage R2), mid-season (growth stages R3 to R4), and late season (growth stages R5 to R6). A mixture of Domark 230ME (Isagro S.P.A.; Valent U.S.A. Corporation), 21% tetraconazole, at 219 ml/ha, and Headline (BASF Corporation), 24% pyraclostrobin at 438 ml/ha, was used.

K-341 (Fig 5). Headline sprayed at early and mid-season significantly increased ($P < 0.05$) percent green stem disorder, whereas Domark sprayed alone at any time during the season did not significantly ($P > 0.05$) increase percent green stem disorder over the nonfungicide control for Kruger K-341.

The number of days to maturity was significant ($P < 0.05$) for fungicide application treatments in both experiments and for cultivar in the MG III experiment. The mid-season spray of the fungicide mixture significantly ($P < 0.05$) delayed maturity by almost a full day, on average, compared with the nonfungicide control. Kruger K340 matured 0.6 days later ($P < 0.05$) than Kruger K431 across both locations.

Differences in overall yields were significant among locations in both experiments, cultivars in the MG II experiment, and fungicide application treatments in the MG III experiment (Table 2). All of the spray treatments, except the early-season Domark application, significantly ($P < 0.01$) increased yields of the MG III cultivars from 74 to 350 kg/ha over the nonfungicide control. Hughes 441 (3,268 kg/ha) overall produced a significantly ($P < 0.05$) higher yield than Stine 2463 (3,053 kg/ha) across both locations. Yields at Monmouth were significantly higher ($P < 0.05$) than yields at Urbana in both MG II trials, 3,410 versus 2,912 kg/hectare, respectively, and MG III trials, 4,116 versus 3,779 kg/hectare, respectively.

There were significant ($P < 0.05$) positive correlations between days to maturity and percent green stem disorder for MG III Kruger K340 ($r = 0.47$) and K341 ($r = 0.52$) at the Urbana location and between yield and percent green stem disorder for all four cultivars, ranging from $r = 0.36$ for Stine 2463 to $r = 0.71$ for Kruger K340 at Monmouth and for Kruger K340 ($r = 0.46$) at Urbana. There was a significant ($P < 0.05$) negative correlation between yield and percent green stem disorder for Stine 2463 ($r = –0.32$) at Urbana.

Field experiments, 2009. For percent green stem disorder in the MG II experiment, there were highly significant location–cultivar ($P < 0.001$) and cultivar–spray treatment ($P < 0.01$) interactions (Table 2). In the MG III experiment, there was also a highly significant ($P < 0.001$) location–cultivar interaction. There was no significant interaction between spray treatment and location or cultivar. Differences among spray treatments in the MG III experiment were highly significant ($P < 0.001$). For the MG II and MG III experiments, the mean green stem disorder incidence was 42%.

For the MG II experiment, Stine 2463 had significantly ($P < 0.05$) higher percent green stem disorder than Hughes 441 at the Urbana 1 and 2 locations but not at Monmouth (Fig. 2). For the MG III experiment, Kruger K-340 had significantly ($P < 0.05$) higher percent green stem disorder than Kruger K-341 at the Urbana 1 and 2 locations but not at Monmouth (Fig. 2).

Only the Domark-Headline mixture sprayed at early season and Headline sprayed at mid- and late season produced significantly ($P < 0.05$) higher percent green stem disorder than the nonfungicide control treatment in Hughes 441 in the 2009 MG II experiment (Fig. 6). For the relatively sensitive Stine 2463, all of the spray treatments except Domark sprayed at mid-season produced significantly ($P < 0.05$) higher percent green stem disorder than the nonfungicide control treatment.

All fungicide spray treatments except Domark sprayed alone at early and mid-season produced percent green stem disorder significantly higher ($P < 0.05$) than the nonfungicide control treatment in the MG III experiment across all three locations (Fig. 7). Headline sprayed alone at mid-season produced the highest green stem disorder at 51%.

For days to maturity in the MG II experiment, all fungicide treatments significantly ($P < 0.05$) delayed harvest maturity compared with the nonfungicide control treatment, from 1 day for the mid-season Domark application to 1.9 days for early-season Headline and fungicide mixture applications. There was a highly significant ($P < 0.01$) interaction between locations and genotypes in the MG III experiment for harvest maturity, with a reversal between K340 and K341, depending on the location. In the MG II experiment, Hughes 441 was later to mature, overall, than Stine

2463 by 0.4 days, and overall maturity at Urbana 1 was later than at Urbana 2 by 2.1 days.

For yield, no interactions were significant except for the location–cultivar for both MG II and MG III experiments (Table 2). All single sources of variation were significant except for the application treatments for the MG II experiment. The mid-season applications of Headline and the mixture and all late-season applications significantly ($P < 0.05$) boosted overall yields of the MG III cultivars compared with the unsprayed control, up to 175 kg/ha for the late-season Headline and mixture spray applications.

There were significant ($P < 0.05$) positive correlations between days to harvest maturity and percent green stem disorder for
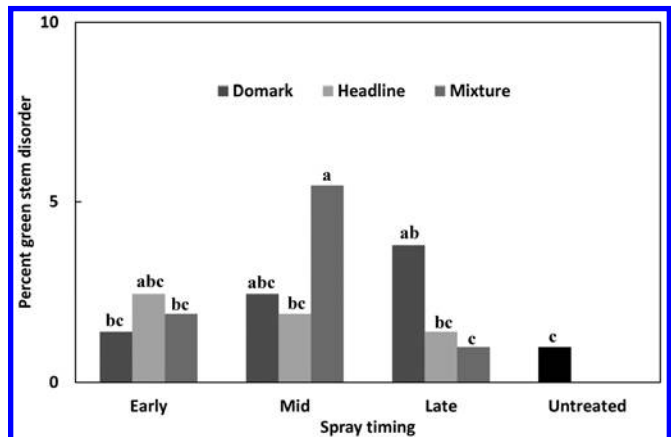

Fig. 3. Mean percent green stem disorder of fungicide spray treatments at three applications periods over two maturity group II soybean cultivars at two locations in 2008. Bars with the same letters are not significantly different by the least significant difference test ($P = 0.05$). Domark, Headline, and a Domark-Headline mixture were applied separately at the following rates: Domark 230ME, 21% tetraconazole, at 219 ml/ha and Headline, 24% pyraclostrobin, at 438 ml/ha. Spray treatments were timed to represent fungicide applications during the reproductive period and are referred to as early season (growth stage R2), mid-season (growth stage R3 to R4), and late season (growth stage R5 to R6). Fungicides were applied at a volume of 187.1 liters/ha at a speed of 4.5 kph using a hand sprayer (Bellspray Inc., Opelousas, LA) equipped with Tee-Jet 1002 Turbo T nozzles (TeeJet Technologies, Wheaton, IL) and compressed CO_2 regulated at a pressure of 193 kPa.
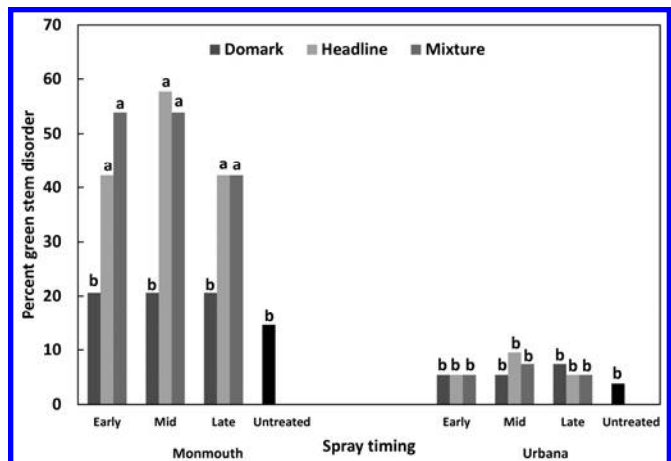

Fig. 4. Mean percent green stem disorder between locations with fungicide spray treatments over two maturity group III soybean cultivars and two locations in 2008. Bars with the same letters are not significantly different by the least significant difference test ($P = 0.05$). Domark, Headline, and a Domark-Headline mixture were applied separately at the following rates: Domark 230ME, 21% tetraconazole, at 219 ml/ha and Headline, 24% pyraclostrobin, at 438 ml/ha. Spray treatments were timed to represent fungicide applications during the reproductive period and are referred to as early season (growth stage R2), mid-season (growth stage R3 to R4), and late season (growth stage R5 to R6). Fungicides were applied at a volume of 187.1 liters/ha at a speed of 4.5 kph using a hand sprayer (Bellspray Inc., Opelousas, LA) equipped with Tee-Jet 1002 Turbo T nozzles (TeeJet Technologies, Wheaton, IL) and compressed CO_2 regulated at a pressure of 193 kPa.

Hughes 441 ($r = 0.57$) and Stine 2463 ($r = 0.39$) at the Urbana 1 location. There were significant ($P < 0.05$) positive correlations between yield and percent green stem disorder for Stine 2463 ($r = 0.34$), Kruger K340 ($r = 0.63$), and Kruger K341 ($r = 0.52$) at Monmouth; for Kruger K340 ($r = 0.4$) and Kruger K341 ($r = 0.41$) at the Urbana 1 location; and for Hughes 441 ($r = 0.57$) at the Urbana 2 location.

Regression models of yield on percent green stem disorder. Of the 28 linear regression analyses performed, 11 were significant ($P < 0.05$), with positive regression coefficients or slopes for 3, 3, and 4 in 2007, 2008, and 2009 experiments, respectively (Table 4).
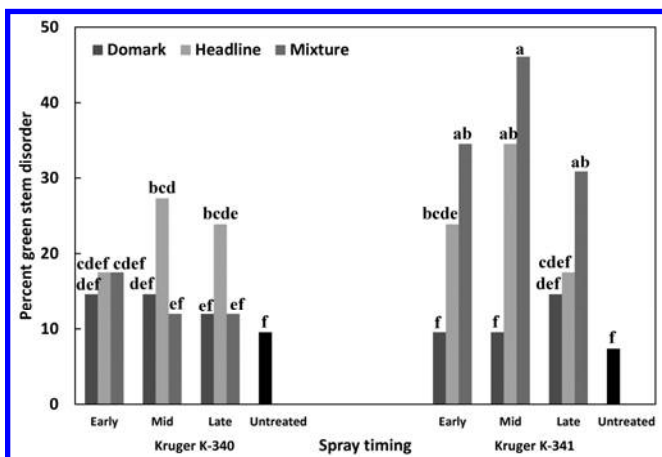

Fig. 5. Mean percent green stem disorder between two maturity group III cultivars (K-340 and K-341) and among fungicide spray treatments and application periods over two locations in 2008. Bars with the same letters are not significantly different by the least significant difference test ($P = 0.05$). Domark, Headline, and a Domark-Headline mixture were applied separately at the following rates: Domark 230ME, 21% tetraconazole, at 219 ml/ha and Headline, 24% pyraclostrobin, at 438 ml/ha. Spray treatments were timed to represent fungicide applications during the reproductive period and are referred to as early season (growth stage R2), mid-season (growth stage R3 to R4), and late season (growth stage R5 to R6). Fungicides were applied at a volume of 187.1 liters/ha at a speed of 4.5 kph using a hand sprayer (Bellspray Inc., Opelousas, LA) equipped with Tee-Jet 1002 Turbo T nozzles (TeeJet Technologies, Wheaton, IL) and compressed CO_2 regulated at a pressure of 193 kPa.
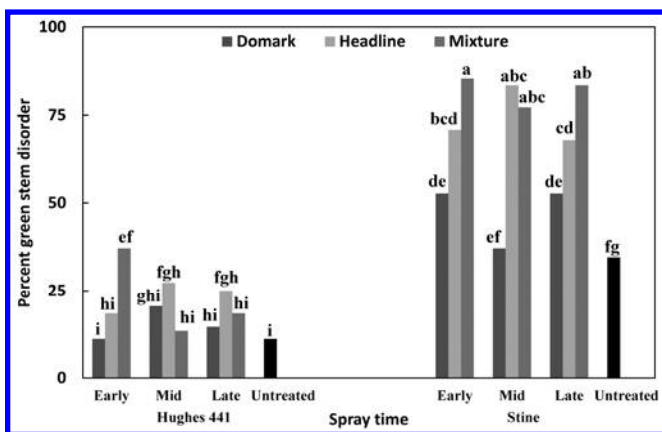

Fig. 6. Mean percent green stem disorder between two maturity group II soybean cultivars (Stine and Hughes) and among fungicide spray treatments over two locations in 2009. Bars with the same letters are not significantly different by the least significant difference test ($P = 0.05$). Domark, Headline, and a Domark-Headline mixture were applied separately at the following rates: Domark 230ME, 21% tetraconazole, at 219 ml/ha and Headline, 24% pyraclostrobin, at 438 ml/ha. Spray treatments were timed to represent fungicide applications during the reproductive period and are referred to as early season (growth stage R2), mid-season (growth stage R3 to R4), and late season (growth stage R5 to R6). Fungicides were applied at a volume of 187.1 liters/ha at a speed of 4.5 kph using a hand sprayer (Bellspray Inc., Opelousas, LA) equipped with Tee-Jet 1002 Turbo T nozzles (TeeJet Technologies, Wheaton, IL) and compressed CO_2 regulated at a pressure of 193 kPa.

Experiments conducted at the Monmouth location (8 of 12 trials) had more significant regressions than experiments at the Urbana location (3 of 16 trials). Seven regressions of the yields of green-stem-sensitive Stine 2463 and Kruger K340 on percent green stem disorder were significant ($P < 0.05$) with positive slopes, whereas four regressions of yields of the insensitive Hughes 441 and Kruger K341 on percent green stem disorder were significant and had positive slopes. Yield gains per each incremental increase in green stem disorder rating (0-to-5 scale) ranged from 70 kg/ha for Kruger K340 in the Urbana 1 location in 2009 to 598 kg/ha for Stine 2463 at the Monmouth location in 2008.

Discussion

Increased incidence of soybean green stem disorder promoted by fungicide application was clearly demonstrated, although the level of increase in the incidence of the disorder was influenced by soybean cultivar, year, location, and fungicide treatment, especially by the timing of applications. The importance of soybean genetics as a factor influencing green stem disorder incidence was in agreement with a previous study (11). In addition, results from a Louisiana study (22) also found a strong interaction between Headline and sensitive soybean cultivars for green stem disorder. Year and location variability in overall incidence of green stem disorder had also previously been observed (11). The differences could be due to soil texture, biota, or other edaphic factors among field locations or, possibly, also differences in local weather conditions. The results of the current study are in agreement with an earlier study (11) that showed that the Monmouth location had higher overall green stem disorder incidence than the Urbana location. There is no convincing explanation for this difference.

Comparison of stems of plants with or without symptoms of the disorder has often revealed less fungal colonization on stems with green stem disorder symptoms (11,12). Results of the current study indicated that there were strong associations between green stem disorder and the presence of specific fungi in soybean stems at harvest. It is possible that fungal pathogens or opportunistic fungi colonize soybean plants, especially the stems, when the stress of reproduction and seed ripening weaken innate plant defenses, and ultimately kill the stems before harvest. Fungicide application may slow fungal colonization in the stems, resulting in noninfected or symptomless green stem tissue resembling green stem disorder
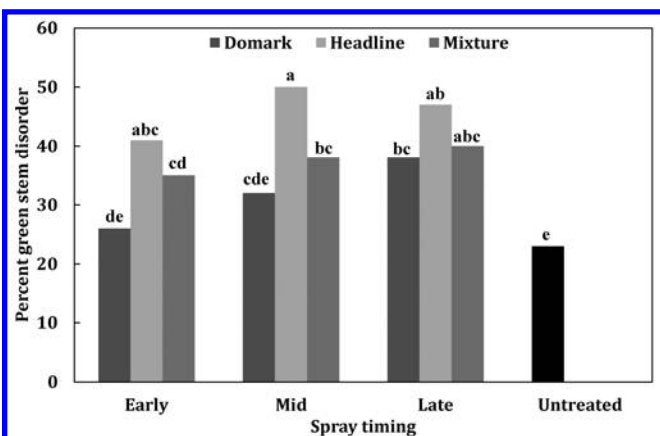

Fig. 7. Mean percent green stem disorder among fungicide spray treatments applied at three periods over two maturity group III soybean cultivars at three locations in 2009. Bars with the same letters are not significantly different by the least significant difference test ($P = 0.05$). Domark, Headline, and a Domark-Headline mixture were applied separately at the following rates: Domark 230ME, 21% tetraconazole, at 219 ml/ha and Headline, 24% pyraclostrobin, at 438 ml/ha. Spray treatments were timed to represent fungicide applications during the reproductive period and are referred to as early season (growth stage R2), mid-season (growth stage R3 to R4), and late season (growth stage R5 to R6). Fungicides were applied at a volume of 187.1 liters/ha at a speed of 4.5 kph using a hand sprayer (Bellspray Inc., Opelousas, LA) equipped with Tee-Jet 1002 Turbo T nozzles (TeeJet Technologies, Wheaton, IL) and compressed CO_2 regulated at a pressure of 193 kPa.

symptoms. In fact, the disorder may simply be a lack of fungal colonization by certain fungi. In the current study, fungicide application reduced the presence of *M. phaseolina* in one year but not the others and did not reduce the presence of other fungi in the stems. Further investigations are needed to determine the basis for increased green stem disorder with fungicide applications and to determine, through inoculation experiments, how fungal pathogens may or may not play a role in green stem disorder. In addition, use of improved pathogen detection methods, such as quantitative PCR of pathogen-specific primer pairs, would give more precise information on relative fungal colonization levels in each stem.

Colletotrichum truncatum has been reported to use a hemibiotrophic infection strategy when it attacks plants (13,17). During the early development of *C. truncatum* infection in soybean, there is a biotrophic stage in which the organism can live as an endophyte within soybean stems without producing signs or disease symptoms (9,20). Endophytes in soybean may play a role in protecting soybean plants against infection of pathogens and other invaders (24). They may also increase the ability of soybean plants to tolerate abiotic stresses such as drought. There are no reports on the association of anthracnose and green stem disorder but it is possible that *C. truncatum* growing as an endophyte within soybean stems may play a role in the development of the disorder by inhibiting the infection of stems by *M. phaseolina, Diaporthe* or *Phomopsis* spp., or other organisms, resulting in fewer stems with stem disease and more stems remaining immature. The endophytic phase of *C. truncatum* infection inhibiting colonization by *M. phaseolina* and *Diaporthe* or *Phomopsis* spp. could explain why a high proportion of soybean stems with green stem disorder were infected with *Colletotrichum* spp., with a lower proportion stems having *M. phaseolina* and *Diaporthe* or *Phomopsis* spp. compared with normal stems in this study. Further work, including inoculation treatments with specific fungal species in controlled conditions, would help determine the potential relationships between soybean stem pathogens and green stem disorder.

Fungicides have been reported to delay maturity of soybean leaves, pods, and stems (19); and strobilurins, in particular, can produce a "greening" effect, in which plant parts such as leaves and pods stay green longer than normal (1,2,6). Results of delayed harvest maturity date in fungicide treatments found in this study were consistent with these other reports. The green stem disorder may be the result of fungicides having some association with the greening effect; however, green stem disorder ratings were taken after pods and seed had dried and ripened. Reduction in fungal colonization on plants due to fungicide application could explain why stems remained green longer and why pods and stems may also mature longer. Use of techniques to quantify fungal colonization, as previously mentioned, could help elucidate the role that fungal colonization plays in green stem disorder.

It has been suggested that green stem disorder might be managed by adjusting planting date (www.pubs.ext.vt.edu/2912/2912-1430/2912-1430.html) but yield reductions associated with delayed planting are an additional risk. With results demonstrating strong year, location, and soybean cultivar effects on green stem disorder incidence, planting date effect may have been masked in the current study by the stronger effects of the other factors. Indeed, the effect of planting date is likely influenced by interactions with the other factors. Experiments to study planting date effect in many soybean production locations may be limited by short planting and harvesting windows due to restricted growing seasons and seasonal weather variability.

Results from an Iowa study (25) found that fungicide applications applied on soybean at mid-season generally increased yields but also observed decreased fungal disease, especially during seasons when fungal disease incidence and severity was high. Higher green stem disorder incidence was significantly associated with higher yields in 7 of 14 environments for the two green stem disorder-sensitive cultivars and 4 of 14 environments for the two insensitive cultivars in the current study. These results suggested that there was no negative impact of higher incidence of green stem disorder on soybean yield whether the disorder was increased by fungicide application or not.

To avoid green stem disorder, soybean producers should be aware of the possible risk that fungicide application can have in increasing incidence of green stem disorder. In addition, producers can help manage green stem disorder by selecting soybean cultivars that have been found to be consistently insensitive to the disorder (5,11).

Literature Cited

1. Balba, H. 2007. Review of strobilurin fungicide chemicals. J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes 42:441-451.
2. Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest. Manage. Sci. 58:649-662.
3. Battaglin, W. A., Sandstrom, M. W., Kuivila, K. M., Kolpin, D. W., and Meyer, M. T. 2011. Occurrence of azoxystrobin, propiconazole, and selected other fungicides in US streams, 2005-2006. Water Air Soil Pollut. 218:307-322.
4. Bradley, K. W., and Sweets, L. E. 2008. Influence of glyphosate and fungicide coapplications on weed control, spray penetration, soybean response, and yield in glyphosate-resistant soybean. Argon. J. 100:1360-1365.
5. Chawla, S., Bowen, C. R., Slaminko, T. L., Hobbs, H. A., and Hartman, G. L. A public program to evaluate commercial soybean cultivars for pathogen and pest resistance. Plant Dis. 97:568-578.
6. Delaney, D. P., Sikora, E. J., Lawrence, K. S., Dawkins, T., and Ducar, J. 2009. Strobilurin fungicide greening effects on soybeans in north Alabama (Crossville), 2009. In: 2009 AU Crops Soybean Research Report. www.aaes.auburn.edu/comm/pubs/researchreports/09soybeanrr.pdf
7. Egli, D. B., and Bruening, W. P. 2006. Depodding causes green-stem syndrome in soybean. Crop Manage. Online publication. doi:10.1094/CM-2006-0104-01-RS
8. Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. Crop Sci. 11:929-931.
9. Hartman, G. L., Manandhar, J. B., and Sinclair, J. B. 1986. Incidence of *Colletotrichum* spp. on soybeans and weeds in Illinois and pathogenicity of *Colletotrichum truncatum*. Plant Dis. 70:780-782.
10. Hartman, G. L., Sinclair, J. B., and Rupe, J. C. 1999. Compendium of Soy-

Table 4. Slope estimates for linear regressions predicting soybean yield with percent incidence of green stem disorder for four soybean cultivars from two maturity groups grown in seven field experiments in Illinois[y]

Year	Location	Maturity group II		Maturity group III	
		Hughes 441	Stine 2463	K341	K340
2007	Urbana	−260	101	131	144*
2007	Monmouth	355	299*	276	241*
2008	Urbana	−33[z]	−60[z]	145	177
2008	Monmouth	265**	598**	143**	192**
2009	Urbana-1	93	62	95**	70**
2009	Urbana-2	156	18	94	27
2009	Monmouth	12[z]	62[z]	179**	160**

[y] $Y_{iik} = b_0 + b_1$ (green stem disorder$_{ii}$) + replication$_k$ + error; * and ** indicate significant differences were found at $P < 0.05$ and 0.01, respectively.
[z] Environments where cultivars did not differ significantly ($P < 0.05$) in the percentage incidence of green stem disorder.

bean Diseases. American Phytopathological Society, St. Paul, MN.

11. Hill, C. B., Hartman, G. L., Esgar, R., and Hobbs, H. A. 2006. Field evaluation of green stem disorder in soybean cultivars. Crop Sci. 46:879-885.

12. Hobbs, H. A., Hill, C. B., Grau, C. R., Koval, N. C., Wang, Y., Pedersen, W. L., Domier, L. L., and Hartman, G. L. 2006. Green stem disorder of soybean. Plant Dis. 90:513-518.

13. Latunde-Dada, A., and Lucas, J. 2007. Localized hemibiotrophy in *Colletotrichum*: Cytological and molecular taxonomic similarities among *C. destructivum*, *C. linicola* and *C. truncatum*. Plant Pathol. 56:437-447.

14. Little, T. M., and Hills, F. J. 1978. Agricultural Experimentation: Design and Analysis. John Wiley and Sons, New York.

15. Miles, M. R., Frederick, R. D., and Hartman, G. L. 2003. Soybean rust: Is the U.S. soybean crop at risk? APSnet Feature APS Net Plant Pathology. 10.1094/APSnetFeature-2003-0603

16. Mueller, T. A., Miles, M. R., Morel, W., Marios, J. J., Wright, D. L., Kemerait, R. C., Levy, C., and Hartman, G. L. 2009. Effect of fungicide and timing of application on soybean rust severity and yield. Plant Dis. 93:243-248.

17. O'Connell, R., Uronu, A., Waksman, G., Nash, C., Keon, J., and Bailey, J. 2007. Hemibiotrophic infection of *Pisum sativum* by *Colletotrichum truncatum*. Plant Pathol. 42:774-783.

18. Padgett, B., Schneider, R., and Whitman, K. 2003. Foliar-applied fungicides in soybean disease management. La. Agric. 46:7-9.

19. Phillips, D. 1984. Performance of foliar fungicides on soybeans in Georgia. Plant Dis. 68:558-560.

20. Pimentel, I. C., Glienke-Blanko, C., Fabardo, J., Stuart, R. M., and Azevedo, J. L. 2006. Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.) Merril) under different environmental conditions. Braz. Arch. Biol. Technol. 49:705-711.

21. Schneider, R., Hollier, C., Whitam, H., Palm, M., McKemy, J., Hernandez, J., Levy, L., and DeVries-Paterson, R. 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis. 89:774-774.

22. Schneider, R., Padgett, G., Boquet, D., and Valverde, R. 2010. Factors affecting the development of the green stem malady in soybean. (Abstr.) Phytopathology 100:S115.

23. Schwenk, F. W., and Nickell, C. D. 1980. Soybean green stem caused by bean pod mottle virus. Plant Dis. 64:863-865.

24. Sinclair, J. B. 1991. Latent infection of soybean plants and seeds by fungi. Plant Dis. 75:220-224.

25. Yang, X., Navi, S., Shriver, J., and Pecinovsky, K. 2008. Use of fungicide to control soybean foliar diseases: A 6-year summary. Integrated Crop Management News Iowa State University. www.extension.iastate.edu/CropNews/2008/1117yang.htm