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Field Evaluation of Green Stem Disorder in Soybean Cultivars

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ABSTRACT

Green stem is a disorder of soybean [*Glycine max* (L.) Merrill] that causes the stems to remain green, nonsenescent, and moist, although pods and seeds are fully ripe and dry. The disorder is a nuisance for producers because it complicates harvesting of soybeans by significantly increasing the difficulty in cutting the affected plants during harvest. The cause of the disorder is unknown; however, differences in relative sensitivity to the disorder have been observed. The primary objective of this research was to evaluate the relative sensitivity among commercial or near-commercial cultivars from private and public soybean breeding organizations in replicated variety tests in Illinois. In 31 tests at Dekalb, Monmouth, and Urbana, IL, during 2001 to 2004, 1187 different MG I (maturity group)-MG IV conventional and glyphosate[*N*-(phosphonomethyl)glycine]-tolerant, cultivars were visually evaluated. There were significant differences in sensitivity among cultivars in 29 of the 31 tests, indicating that genetic variability among cultivars for green stem sensitivity exists. This variability may provide a basis for breeding for low sensitivity to the green stem disorder. Total levels of green stem disorder incidence varied over years and locations. Herbicide management systems did not appear to affect the levels of green stem incidence.

GREEN STEM is a disorder of soybean that causes the stems to remain green, nonsenescent, and moist, although pods and seeds are fully mature, ripe, and dry. Sometimes petioles may persist on affected plants. Other variations of green stem disorder have been reported but these symptoms are the most commonly observed in the Midwest (Hobbs et al., 2006). Symptoms of green stem disorder may be confused with delayed maturity caused by factors such as virus infection (Sweets and Bailey, 2002), stinkbug feeding [green stink bug *Acrosternum hilare* (Say), brown stink bugs, *Euschistus* spp., and the southern green stink bug, *Nezara viridula* (L.)] (Boethel et al., 2000; Lustosa et al., 1999), fungicide treatments (Padgett et al., 2003), or possibly environmental factors (Malvick, 2001). Also, sterile plants, or plants without seed, caused by virus infection, male-sterility or haploidy, or other factors, may be confused with green stem disorder; however, these phenomena usually occur in very low frequency in a field. The main diagnostic feature of the green stem disorder is the presence of mature pods and seeds with green stems. This feature distinguishes the green stem disorder from delayed maturity and other reasons for green plants remaining at harvest time.

There is no conclusive evidence that the green stem disorder affects harvested yield of soybean, but it is a nuisance for producers because it complicates harvesting of soybeans by significantly increasing the difficulty in cutting the plants. The moist, green, tough, pliable, stems of plants with green stem disorder are difficult for the knives of the combine to cut. Combine ground speed must be slowed while keeping the engine speed high, reducing the fuel efficiency of the combining operation and increasing the fuel expenditures for producers. Combine cylinder speed must also be increased to reduce the potential for clogging the opening between the concave and cylinder with moist plant material that doesn't collapse as readily as dry material during the threshing operation. An additional potential problem caused by the disorder is that moisture from the green stems may be transferred to seed during the threshing operation, which could increase seed moisture content and reduce the grade and storability of seed. These problems encourage growers to avoid areas of fields where green stem disorder is prevalent and delay harvest until a hard frost event kills the green stem tissue.

Complaints about the disorder are on the increase in Illinois and in other states (Malvick, 2001). Recent reports indicated that it had become more common in Midwest soybean fields and incidence within fields had increased, often affecting entire fields (Hobbs et al., 2006; Sweets and Bailey, 2002; Wright, 2003). A similar but different problem called "green bean syndrome" occurs in southern states (Boethel et al., 2000; Sweets and Bailey, 2002). This problem is caused by stinkbug feeding that results in delayed maturation of soybean plants. It is different from green stem disorder because symptoms include green, unripe pods and seed, rather than brown, ripe pods and seed that are associated with green stem disorder.

The cause of green stem disorder is unknown. There is little information on the disorder in the scientific literature. An early report implicated *Bean pod mottle virus* (BPMV) as the main cause (Schwenk and Nickell, 1980). Recent work indicated there was no direct association between the incidence of green stem disorder and BPMV infection (Hobbs et al., 2006). Not all BPMV-infected plants developed the green stem disorder, and conversely, not all plants that developed green stem were infected with BPMV. Many other possible causes of the green stem disorder have been put forward, including infection by other viruses, insect feeding damage, low soil

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Abbreviations: MG, maturity group; UISVT, University of Illinois Soybean Variety Testing program; VIPS, Variety Information Program for Soybean.

moisture, potassium deficiency, phytoplasmas, soybean population density, and genetic mutations in soybean plants (Hobbs et al., 2006; Malvick, 2001).

Although the cause of the green stem disorder is not known, there is evidence of variability among soybean cultivars for green stem disorder incidence (Hobbs et al., 2006; Hill et al., 2003). This variability may be due to differences in cultivar genetics. Soybean breeders could exploit genetic variability for sensitivity to the green stem disorder to develop cultivars less sensitive to the disorder.

Information on differences in green stem disorder sensitivity among soybean cultivars would help producers choose soybean cultivars that are less likely to develop the green stem disorder. Since 1998, public and private developed soybean cultivars have been tested at several locations in Illinois through a cooperative effort by the University of Illinois Soybean Variety Testing Program (UISVT) and the Variety Information Program for Soybeans (VIPS, 2004), for the purpose of providing information on the agronomic performance of soybean cultivars to Illinois soybean producers. The UISVT/VIPS tests provided a unique opportunity to study green stem disorder sensitivity among soybean cultivars. Three of the UISVT/VIPS locations, Dekalb, Monmouth, and Urbana, IL, were chosen as the focus for evaluations of green stem disorder sensitivity in this study.

The primary objective of this research was to evaluate the relative sensitivity to green stem disorder among commercial or near-commercial cultivars from private and public soybean breeding organizations in replicated variety tests in Illinois to determine if cultivar genetics is a factor in the disorder.

MATERIALS AND METHODS

Field Plots

Although green stem disorder evaluations were done at several locations in Illinois over the 2001–2004 period, the focus of evaluations was at three locations: Dekalb, Monmouth, and Urbana. Soil types at each location were DeKalb — Drummer silt loam, Monmouth — Sable and Muscatine silt loam, and Urbana — Flanagan silt loam.

Several hundred cultivars from public and private soybean breeding organizations were tested by UISVT/VIPS each year and included nontransgenic conventional and transgenic glyphosate-tolerant types. Cultivars in different MGs and with different herbicide management requirements were tested separately at each location. Each test had cultivars belonging to the same MG and of the same type, with separate herbicide management systems applied in tests for glyphosate-tolerant and conventional types.

Plots in UISVT/VIPS trials were the experimental units in the green stem disorder evaluations. The plots were planted in four rows, 6 m long and spaced 0.76 m apart. Test plots were randomized in an α incomplete block design if there were more than 20 entries in a test. If there were fewer than 20 entries, plots were arranged in a randomized complete design. There were three replications in each test.

Green Stem Disorder Evaluation

In most of the green stem disorder evaluations, data were recorded a day or two after the inner two rows of each plot were harvested, leaving the outer two rows for evaluation, to minimize differences caused by variation in maturity among the cultivars. A few tests were evaluated a day or two before plots were harvested, when pods and seed appeared to be fully ripe. If the plots did not appear to be ready for harvest with ripe pods and seed, no estimate of green stem disorder incidence was recorded. The outer two rows were examined in harvested plots and all four rows were examined in unharvested plots. Standing plants were examined for green stem disorder symptoms. Green stem disorder symptoms disappear within hours when soybean stem tissue is killed following exposure to subfreezing temperatures during hard frost events and when these events occurred, no stem disorder data was collected.

In this research, plants defined with symptoms of the green stem disorder had green, yellow-green, or yellow, moist, and nonsenescent stems with brown, ripe, pods, containing fully mature, dry seeds. Sometimes some petioles remained attached to the stem. Normal ripe soybean plants had dry, brown or gray, and senescent stems along with ripe, dry, brown pods containing mature, dry seeds; therefore, the main difference between green stem affected and normal plants was the condition of the stems. Occasionally, plants were observed that were completely green with immature pods and others that were sterile, having no pods or seeds. These plants were ignored during the green stem disorder evaluations because those symptoms were considered caused by factors not related to the cause of green stem disorder, such as late germination and plant emergence, systemic virus or mycoplasma-like organism infection, male-sterility, haploidy, or other causes.

Random samples of plants with symptoms of green stem disorder and plants that were completely green were collected from plots at Urbana in 2001 that were mature and ready for harvest to test for the presence of BPMV and other viruses by ELISA. Leaf tissue is generally sampled for detection of virus infection in soybean plants; however, because green stem disorder was evaluated after full maturity, leaves were not available for sampling. As alternative tissue samples, strips approximately 3×20 mm were peeled from the outer layers of stems with forceps and processed in the same manner as leaf tissue. Preliminary testing of late season-sampled stem strips from BPMV-infected plants, identified by prior leaf testing, produced a BPMV ELISA detection rate of over 80% in the stem strips (H.A. Hobbs, person. comm.).

Incidence of green stem disorder, the percentage of plants with green stem disorder symptoms in each test plot, was visually rated by a 0-to-5 pretransformed scale, with 0 = no green stem disorder present, 1 = 1 to 10%, 2 = 11 to 35%, 3 = 36 to 65%, 4 = 66 to 90%, and 5 = 91 to 100% green stem disorder. The steps of the scale represented equal increments of percentages that were pretransformed by the arcsine-square root transformation method (Little and Hills, 1978). The use of this scale increased the efficiency of data collection from thousands of plots at multiple locations and obviated the requirement to transform percentage data to meet the assumptions of the analysis of variance, in particular, the assumption of homogeneity of variances.

Statistical Analyses

For analysis of green stem disorder incidence ratings, the tests were assumed to be in randomized complete blocks with three replications. Analyses of variance were performed with the aid of JMP version 5.1 (SAS Institute Inc., 2004). Least square estimates of mean green stem disorder ratings were detransformed for presentation of green stem disorder incidence in the accompanying tables and figures.

RESULTS

A total of 31 tests were evaluated for green stem disorder (Table 1). Green stem disorder evaluations were initiated in 2001 at Urbana with four tests evaluated. In 2002, 13 tests were evaluated, five at Dekalb, four at Monmouth, and four at Urbana. There was an earlier than normal hard freeze event in Illinois in 2003 that limited the number of evaluations done. The number of tests that were evaluated in 2004 was also limited by early frost events.

In the 31 tests evaluated for green stem disorder incidence, a total of 1187 different cultivars were evaluated during the 2001 through 2004 period at the three locations. Of those, seven were MG I glyphosate-tolerant, 89 MG II conventional, 439 MG II glyphosate-tolerant, 109 MG III conventional, 537 MG III glyphosate-tolerant, and six MG IV glyphosate-tolerant. Nearly all cultivars in the tests were entered by private breeding organizations. Private cultivars were rarely repeated in tests each year. Newer cultivars often replaced older cultivars. Therefore, the total number of tests that individual cultivars were entered into varied. There were four MG II and seven MG III public conventional cultivars that were tested together at each location each year.

Mean green stem disorder incidence ranged from a high of 71% in a test of MG III conventional cultivars at Monmouth in 2002 to a low of less than 1% in a test of MG II glyphosate-tolerant cultivars at Urbana in 2003 (Table 1). Different tests at a location in some years often had different mean levels of green stem disorder incidence.

There were significant differences among cultivars in 29 of the 31 tests evaluated for green stem disorder (Table 1), indicating significant variability for sensitivity to the green stem disorder among the cultivars. Differences among cultivars were nonsignificant in two tests at Urbana in 2003, possibly because overall green stem disorder levels were too low to accurately detect differences in incidence of green stem disorder there.

The vast majority of cultivars evaluated had low levels of green stem disorder incidence (Fig. 1). About 7% of the cultivars had mean green stem disorder incidence above 50%, with 1% of them having 100% green stem disorder incidence. Nearly 4% of the cultivars averaged 0% green stem disorder incidence. The largest proportion of cultivars had about 10% mean green stem disorder incidence.

A higher percentage of cultivars with moderate to high green stem disorder incidence were found in 2002 and 2004 than in 2001 and 2003 (Fig. 1). Green stem disorder incidence was lowest in 2003 tests; however, early hard frost events limited the number of evaluations done. Evaluations were only performed at Urbana in 2001 and not at Dekalb or Monmouth that year.

Table 1. Analyses of variance among soybean cultivars for green stem disorder incidence in 31 experiments at three locations in Illinois during 2001–2004.

Year	Location	MG†	Type	Number of cultivars tested	Significance of differences among cultivars	Mean green stem disorder incidence‡ (%)	CV§ (%)
2001	Urbana	II	conventional	31	***	8	77
2001	Urbana	II	glyphosate tolerant	24	*	16	84
2001	Urbana	III	conventional	40	***	8	60
2001	Urbana	III	glyphosate tolerant	141	***	6	73
2002	Dekalb	I	glyphosate tolerant	7	***	19	69
2002	Dekalb	II	conventional	29	***	20	56
2002	Dekalb	II	glyphosate tolerant	141	***	20	73
2002	Dekalb	III	conventional	11	***	27	60
2002	Dekalb	III	glyphosate tolerant	7	*	7	56
2002	Monmouth	II	conventional	25	***	17	89
2002	Monmouth	II	glyphosate tolerant	87	***	27	72
2002	Monmouth	III	conventional	19	**	71	34
2002	Monmouth	III	glyphosate tolerant	80	***	30	64
2002	Urbana	II	glyphosate tolerant	35	***	7	50
2002	Urbana	III	conventional	25	***	14	61
2002	Urbana	III	glyphosate tolerant	144	***	9	74
2002	Urbana	IV	glyphosate tolerant	6	**	19	59
2003	Monmouth	II	conventional	36	***	22	59
2003	Monmouth	II	glyphosate tolerant	102	***	7	85
2003	Urbana	II	conventional	15	NS	<1	258
2003	Urbana	II	glyphosate tolerant	37	NS	<1	343
2003	Urbana	III	conventional	40	***	5	81
2003	Urbana	III	glyphosate tolerant	196	***	4	106
2004	Dekalb	II	conventional	35	***	16	80
2004	Dekalb	II	glyphosate tolerant	187	***	12	90
2004	Dekalb	III	glyphosate tolerant	34	***	49	52
2004	Monmouth	II	glyphosate tolerant	123	***	39	53
2004	Monmouth	II	conventional	32	***	67	46
2004	Monmouth	III	glyphosate tolerant	143	***	21	61
2004	Urbana	III	conventional	40	***	6	78
2004	Urbana	III	glyphosate tolerant	210	***	2	133

* Significant at the 0.05 level.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.
NS, nonsignificant at the 0.05 level.
† MG = maturity group.
‡ Green stem disorder incidence = percent of plants in the experimental unit (plot) with green stem disorder symptoms.
§ CV = coefficient of variation.

Monmouth had the highest proportion of cultivars with moderate to high green stem disorder incidence over 2001 through 2004, followed by Dekalb, with the lowest proportion at Urbana (Fig. 1). Over 36% of the cultivars evaluated at Monmouth had green stem disorder incidence equal to or higher than 50%. Less than 2% of cultivars had green stem disorder incidence 50% or higher at Urbana. The order of the locations, from highest to lowest mean green stem disorder incidence, Monmouth, Dekalb, Urbana, appeared to be consistent across the years.

A larger proportion of MG II cultivars had moderate to high green stem disorder incidence compared with MG III cultivars (Fig. 1). Over 18% of MG II cultivars had at least 50% green stem disorder incidence, whereas less than 4% of MG III cultivars had 50% or greater green stem disorder incidence.

There were significant differences among MG I glyphosate-tolerant cultivars in a test evaluated in 2002 at Dekalb. The cultivar with the highest mean green stem disorder incidence had 55% and the lowest had 4% in that test. Between MG IV glyphosate-tolerant cultivars evaluated in a 2002 Urbana test, one cultivar had significantly higher ($P > 0.05$) green stem disorder incidence compared with five other cultivars. That cultivar had a mean green stem disorder incidence of 65% and the next highest was 25%.

Distributions of different types of cultivars for green stem disorder incidence were similar (Fig. 1), indicating

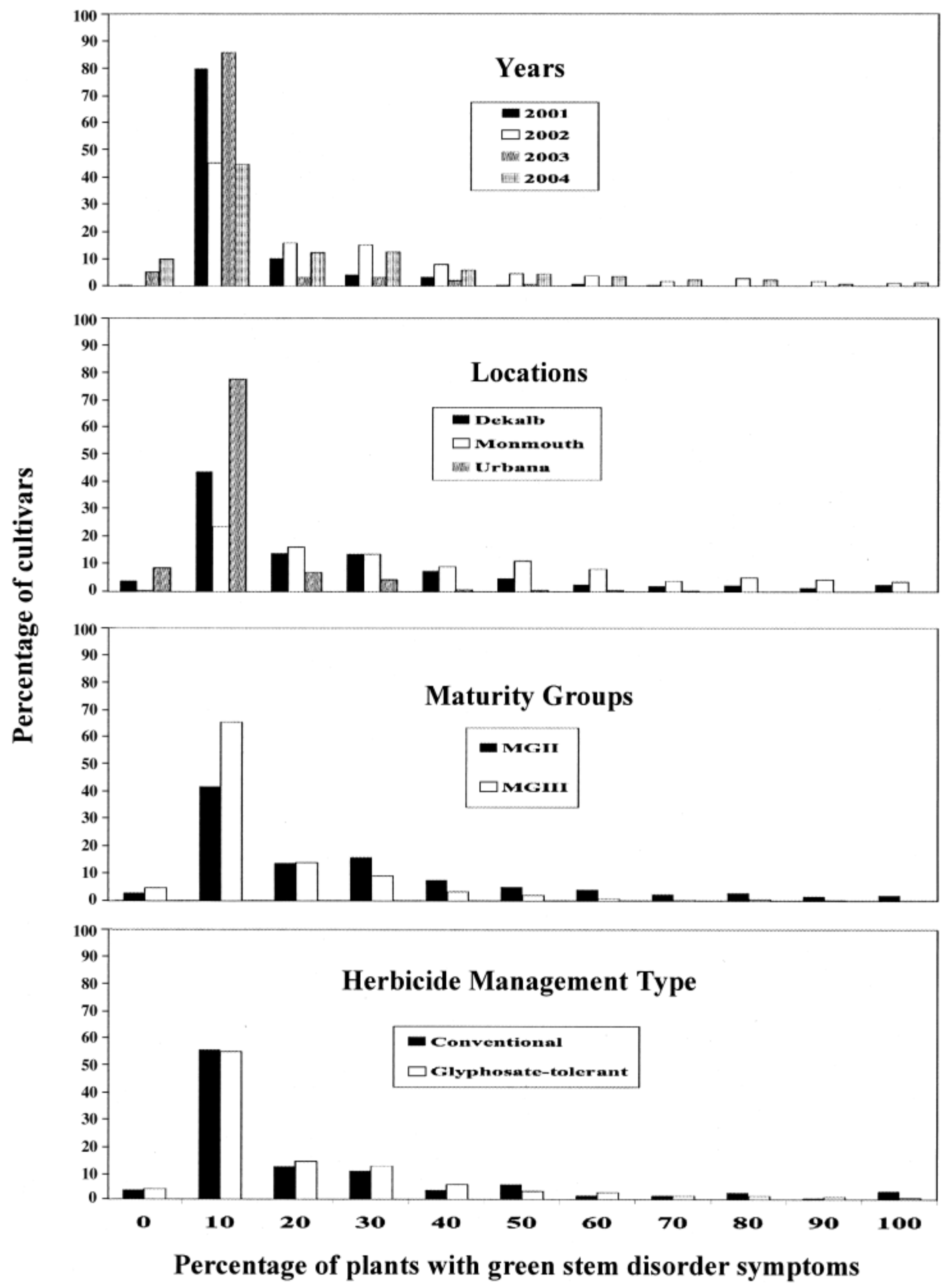

Fig. 1. Green stem disorder incidence by year, location, maturity group, and herbicide management type at three locations in Illinois during 2001–2004.

that herbicide management or cultivar type did not appear to affect green stem incidence. There were over 14% of glyphosate-tolerant cultivars and less than 10% of conventional cultivars with 50% or greater green stem disorder incidence.

There were differences in green stem disorder incidence among 11 public conventional cultivars that were entered together in multiple tests at the three locations over 2001 through 2004 (Fig. 2). Ranking of the cultivars for sensitivity to the green stem disorder was consistent across locations and years. At Monmouth in 2002, for example, the MG II public cultivars Dwight (1% incidence), Loda (1%), and Savoy (9%) had green stem disorder incidence significantly different ($P < 0.05$) from Jack (44%), whereas differences among them were not significant, as indicated by single degree of freedom comparisons. Also, at Dekalb in 2002, green stem disorder incidence in Dwight (9%), Loda (0%), and Savoy (16%) was significantly different from Jack (55%), but differences among them were also significant in this test ($P < 0.05$). Similarly, significant differences ($P < 0.05$) in green stem disorder incidence among MG III public conventional cultivars were also found (Fig. 2). For example, at Monmouth in 2002, incidences in the cultivars

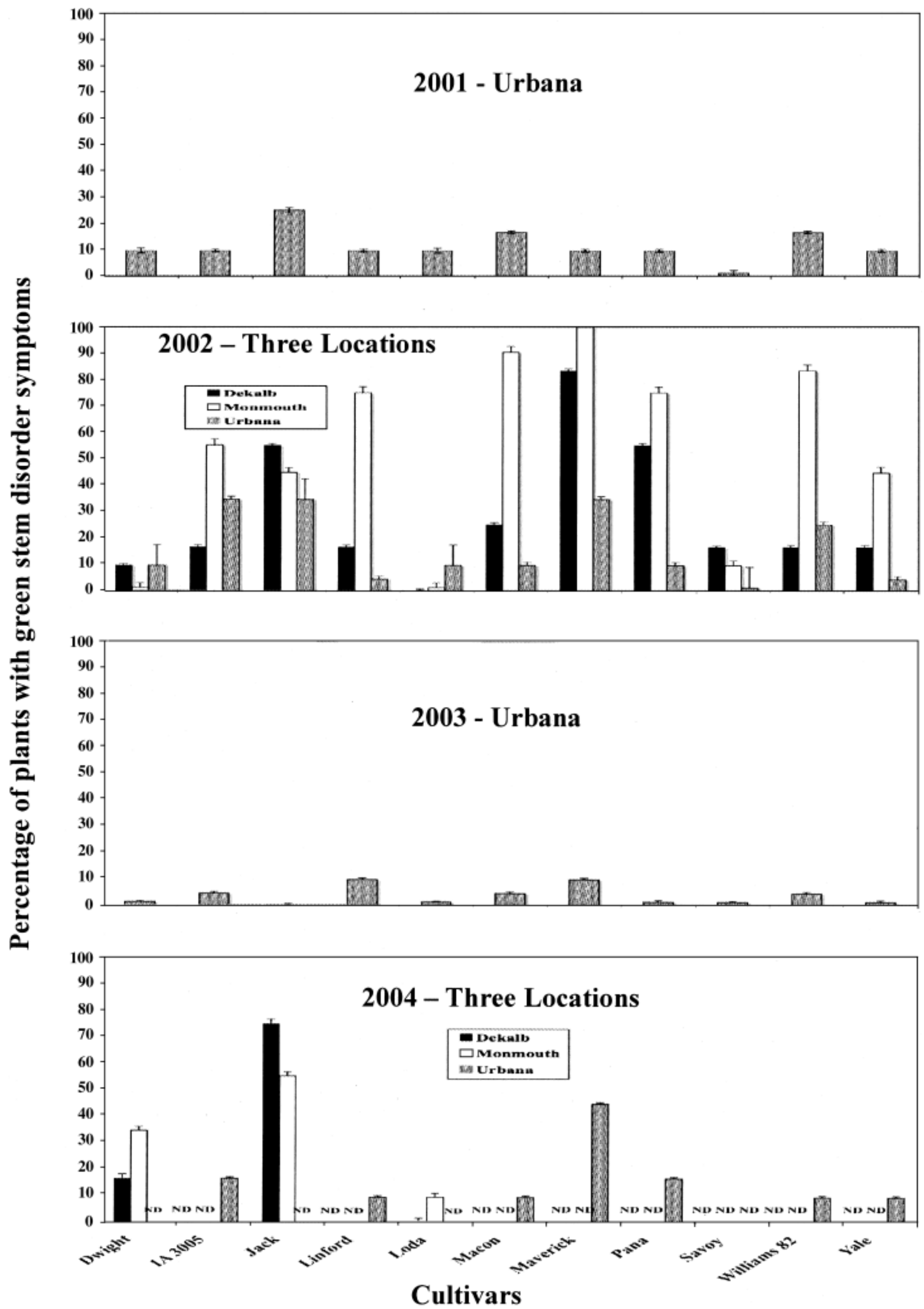

Fig. 2. Green stem disorder incidence in 11 public soybean cultivars evaluated at three locations in Illinois during 2001–2004. Error bars above the bars represent standard error of the mean. ND means that no data was collected because the cultivar was not tested or evaluated.

IA 3005 (55%), Linford (75%), Pana (75%), and Yale (45%) were significantly lower than in Maverick (100%). Incidence in Yale (45%) was significantly lower than Williams 82 (83%) and in Macon (90%). At Dekalb in 2002, incidences in IA 3005 (16%), Linford (16%), Macon (25%), Yale (16%), and Williams 82 (16%) were significantly lower than in Pana (55%). Incidence in Pana (55%) was significantly lower than in Maverick (83%).

BPMV was detected by ELISA in only 8% of stem strip samples collected from plants with green stem disorder symptoms in 2001 versus 100% of stem strip samples from plants that were still completely green and immature at harvest time (H.A. Hobbs, person. comm.). As indicated earlier, the stem strip sampling procedure had a high BPMV detection rate in BPMV-infected plants. Because relatively few plants with green stem disorder symptoms were infected with BPMV, there did not appear to be an association between BPMV infection and green stem disorder symptoms in the samples collected in 2001. The independence of BPMV infection with the green stem disorder was also found in another study when green stem disorder developed in soybean plants that were isolated in insect-proof screen houses in the field and were not exposed to BPMV or its beetle vectors and where no BPMV infection was detected (H.A. Hobbs, person. comm.). No other viruses were detected in the samples.

DISCUSSION

Significant differences among cultivars for incidence of green stem disorder were found in 29 of the 31 field tests evaluated. Although combined analyses across locations and years were not performed because most of the cultivars were not repeated in different locations and years, differences appeared to be consistent for conventional-type public cultivars that were tested together in multiple tests across locations and years. For instance, the MG II cultivars Dwight, Loda, and Savoy generally had lower incidences of green stem disorder than Jack in all of the tests where they were tested together. Hobbs et al. (2006) also observed consistent differences in green stem disorder incidence among lines tested in two locations in Wisconsin. These results indicated that variability among cultivars for green stem disorder sensitivity exists. If this variability is heritable, soybean breeders may be able to exploit the genetic variability to develop new cultivars that are less sensitive to the disorder. Further work is necessary to determine the effect of genotype $\times$ environment (location) interaction on green stem disorder sensitivity.

Level of sensitivity may directly or indirectly involve resistance or susceptibility to a biotic or abiotic agent that causes the green stem disorder. It is improbable that the variability among cultivars for incidence of green stem disorder was a response to BPMV infection because there is no known resistance to BPMV in soybean (Wang et al., 2005; Zheng et al., 2005). No BPMV resistance was found in over 700 cultivars entered into UISVT/VIPS tests in 2002 (VIPS, 2004). Furthermore, Hill et al. (pers. comm.) found no BPMV resistance in over 3000 plant introduction accessions after screening them as part of a larger disease resistance screening project. Results of experiments involving monitoring soybean plants in several fields over 3 yr and field cage experiments involving BPMV inoculations indicated that the green stem disorder was independent of BPMV infection (Hobbs et al., 2006). BPMV inoculations with different strains of the virus did not increase the incidence of green stem disorder in any of the cultivars tested. At present, no biotic or abiotic agent is known to be directly associated with the green stem disorder.

Since the cause of the disorder has not been identified, breeders will need to screen segregating populations in the field and rely on natural development of green stem disorder. Results in this study indicated that green stem disorder incidence varied among locations and years. The efficiency of selection for lines less sensitive to the green stem disorder may be reduced by the inability to accurately identify lines with low sensitivity because of escapes caused by variable green stem disorder development in field nurseries in different locations and years. However, green stem disorder-sensitive germplasm could be identified for culling from breeding programs in nurseries with a high incidence of green stem disorder.

Ranking of the three locations for mean green stem disorder incidence appeared to be consistent across years in this study; however, reasons for this were not clear. It is possible that differences in local climatic patterns, soil characteristics, or biota may be responsible for the differences. Monmouth is located in northwestern Illinois, Dekalb is in north central, and Urbana is in east central Illinois.

Green stem disorder incidence can be overestimated if evaluations are done before plants have reached harvest maturity. Although cultivars in soybean variety tests are grouped by MGs to aid harvesting, maturity dates among cultivars within a test can vary by a few days. In this study, performing evaluations only on plots with plants that had fully ripe pods minimized the effect of variation in maturity among the cultivars.

There did not appear to be an association between the soybean herbicide management type, conventional or glyphosate-tolerant, and green stem disorder incidence. Herbicide management practices also did not appear to affect green stem disorder incidence.

Variability among plots, indicated by coefficients of variation (CV) (Table 1), ranged from 34 to 343% and appeared to be inversely correlated with mean green stem disorder incidence. The use of the 0-to-5 pretransformed percentage scale may not have been as effective as a scale with more steps in correcting heterogeneity of variances, especially when green stem disorder incidence was very low and near 0%. In those experiments where the green stem disorder was a rare event, the application of the square root transformation to equalize the variances may be more effective (Little and Hills, 1978). For example, when data from the two MG II tests at Urbana in 2003 with the highest CVs was transformed to their square roots, the CV for the conventional test was reduced from 258 to 23% and from 343 to 18% for the glyphosate-tolerant test; however, the effect of cultivars in the ANOVA remained nonsignificant for both tests. High variation among plots may have been caused by uneven distribution of the disorder in the tests. Consistent differences in disease responses among cultivars can be difficult to detect because of increased variability among experimental units when disease levels are low. For example, Yang et al. (1999) found that incidence of Sclerotinia stem rot [caused by *Sclerotinia sclerotiorum* (Lib.) deBary] among soybean cultivars was inconsistent when disease incidence was very low. In this study, a mean incidence of green stem disorder as low as 2% was high enough to detect highly significant differences among cultivars (Table 1), despite high variability among experimental units.

The apparent differences in green stem disorder incidence between MG II and MG III cultivars may have been skewed because more MG III cultivars were evaluated than MG II cultivars. Also, higher proportions of MG II cultivars were evaluated at Monmouth and Dekalb, where there were higher total levels of green stem disorder incidence, compared with the higher proportions of MG III cultivars evaluated at Urbana, where there were lower levels of green stem disorder.

The results of this research demonstrated that significant variability in sensitivity to the green stem disorder exists among soybean cultivars. To design efficient breeding programs for developing cultivars less sensitive to the disorder, additional studies are needed to determine the heritability of sensitivity to the disorder and the effect of environment on its expression. Knowledge of the genetic basis of green stem disorder sensitivity may also aid in determining the exact cause or causes of the disorder. Detailed comparisons made between cultivars with highly different sensitivity to the disorder may help rule out potential causes or provide new clues on other possible causes.

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