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R. Jay Goos<sup>a</sup>; Brian E. Johnson<sup>a</sup>

<sup>a</sup> Department of Soil Science, North Dakota State University, Fargo, North Dakota, USA

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## EVALUATION OF SOYBEAN CULTIVARS FOR RESISTANCE TO IRON DEFICIENCY CHLOROSIS IN ROWS VERSUS HILLS

**R. Jay Goos and Brian E. Johnson** □ *Department of Soil Science, North Dakota State University, Fargo, North Dakota, USA*

□ *Selection of a resistant cultivar is the most practical control measure for iron deficiency chlorosis in soybean (*Glycine max* L. Merr.). Plant breeders routinely evaluate cultivars for chlorosis resistance in hill plots, but this procedure may overestimate the chlorosis resistance of a cultivar. The objective of this research was to compare the chlorosis scores of soybean cultivars differing in chlorosis resistance, planted in conventional 76-cm rows, or with two, four, or eight plants per hill. In both 2001 and 2002, it was estimated that three plants per hill would give average chlorosis scores most similar to that observed in 76-cm rows. The highest overall precision was given with row plots, and the lowest with two plants per hill. Hill plots are more space-efficient than row plantings, but are much more easily lost due to animal predation.*

**Keywords:** soybean, iron deficiency chlorosis, cultivar screening, variety screening

### INTRODUCTION

Soybean acreage has increased dramatically in recent years in the North Central region of the U.S. (Hansen et al., 2004). A major production problem with soybean production in this region is iron deficiency chlorosis, a problem associated with alkaline, poorly-drained soils (Inskeep and Bloom, 1986). The selection of a cultivar resistant to chlorosis is the most effective control measure (Goos and Johnson, 2000, 2001), being more effective than foliar sprays, heavy seeding rates, or seed treatments. With the advent of transgenic 'Roundup-Ready' cultivars, the soybean seed industry has grown dramatically, as farmers are required to buy new seed each year. Not only has the number of companies offering soybean cultivars increased, a cultivar may only be offered for sale for three or four years before being discontinued. Thus, the need for current and reliable cultivar screening data has risen sharply in recent years.

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Address correspondence to R. Jay Goos, Department of Soil Science, North Dakota State University, Fargo, ND 58105, USA. E-mail: rj.goos@ndsu.edu

Soybean cultivars can be screened for resistance to iron deficiency chlorosis in several ways. Greenhouse methods involving soil (Fairbanks et al., 1987) or hydroponics (Byron and Lambert, 1983; Chaney et al., 1992) have been suggested. Laboratory methods involving tissue culture (Graham et al., 1992) or reductant release from roots (Stevens et al., 1993) have been proposed. Field screening trials are most common. Methodologies differ widely. We made an informal survey of agronomists and plant breeders with regards to their field screening methodologies, and found no standardization in planting method. Some workers were screening cultivars planted in short (1–2 m) single rows, but most were screening cultivars planted in hill plots. The number of seeds planted per hill ranged from 5 to 10, without thinning to a specified stand. Another worker was screening with five plants in a 15 cm-section of row (Falkner, 2002).

The severity of chlorosis can be reduced dramatically at higher seeding rates (Penas et al., 1990). Possible explanations for the “seeding rate effect” have been discussed by Goos and Johnson (2001). We became concerned about the advisability of evaluating chlorosis resistance of soybeans planted in hills, after observing dramatically reduced chlorosis and increased growth of soybeans at the intersections of 76-cm rows, cross-planted on headlands without subsequent cultivation (Figure 1). The objective of this study was to evaluate different planting methods for the screening of soybean cultivars for resistance to iron deficiency chlorosis.



**FIGURE 1** Soybean plants in a production field, showing improved growth and reduced chlorosis at the intersections of cross-planted rows on 76-cm centers. Arthur, North Dakota, 2000.

## MATERIALS AND METHODS

Four field trials were conducted during the 2001 growing season, and four field trials were conducted during the 2002 growing season, on alkaline soils with a history of producing chlorosis in soybean. Selected soil characteristics are shown in Table 1. The experimental treatments consisted of a factorial combination of cultivars (four in 2001, seven in 2002) by four planting methods. The planting methods were: (1) thirty seeds planted in a 1.5 m section of row; (2) five seeds in a hill, thinned to two plants after emergence; (3) eight seeds in a hill, thinned to four plants after emergence; and (4) fifteen seeds in a hill, thinned to eight plants after emergence. A jab planter with a planting length of 6.4 cm was used to plant the hills, and a single-row cone seeder used to plant the rows. The hill plots were planted in a 1.5 m section of row, with about 40 cm separating the three hills of each cultivar. The cultivars used in 2001, in expected order from most to least resistant to chlorosis, were: 'Traill' (Helms and Nelson, 1998), 'Council' (Helms and Halvorson, 1996), 'Glacier' (Orf and Denny, 1997), and 'Mycogen 5072' (MY5072). The cultivars used in 2002 were 'ISU A11' (Jessen et al., 1988), Traill, Council, 'Barnes' (Helms et al., 2001), Glacier, MY5072, and 'Stine 0480' (ST0480). The treatments were randomly placed within a larger cultivar screening trial. There were four replicates in a randomized complete block design.

Chlorosis severity was determined by visual ratings of the uppermost leaves. At most sites, ratings were made at three stages of growth, at the

**TABLE 1** Selected site characteristics, 2001–2002

Measurement†	Units	Site			
		1	2	3	4
2001					
Nearest town		Amenia	Argusville	Ayr	Galesburg
Soil series		Glyndon	Glyndon	Hamerly	Glyndon
pH		8.2	8.1	8.0	8.0
CaCO <sub>3</sub>	g kg <sup>-1</sup>	111	57	119	50
Conductivity	dS m <sup>-1</sup>	0.6	0.4	1.0	2.0
Avail. Fe	mg kg <sup>-1</sup>	3	4	5	5
2002					
Nearest town		Argusville	Ayr	Galesburg	Leonard
Soil series		Glyndon	Hamerly	Glyndon	Ulen
pH		8.1	8.2	8.1	8.5
CaCO <sub>3</sub>	g kg <sup>-1</sup>	57	84	165	45
Conductivity	dS m <sup>-1</sup>	0.3	0.5	0.4	0.2
Avail. Fe	mg kg <sup>-1</sup>	3	3	4	7

† All soil series are Aeric calciaquolls. Soil analyses performed on 0–15 cm samples. pH and conductivity were determined on a 1:1 soil:water suspension, CaCO<sub>3</sub> by manometry, and available Fe by the DTPA method.

2–3 trifoliolate stage, the 5–6 trifoliolate stage, and two weeks after the 5–6 trifoliolate stage. Only the first two ratings were made at site 4 in 2001, and sites 2 and 4 in 2002. A third rating was not taken at these sites due to uneven recovery from chlorosis across the experimental site after the second rating. A 1–5 rating scale was used, where: 1 = no chlorosis, 2 = slight chlorosis of the upper leaves, with no color difference between the veins and interveinal leaf tissues, 3 = interveinal chlorosis of the upper leaves, with no stunting of overall growth and necrosis apparent, 4 = interveinal chlorosis of the upper leaves, with stunting of growth, or necrosis beginning to appear, and 5 = interveinal chlorosis and necrosis, growing point damaged, plants severely stunted, or entire plants dead. Chlorosis scores were recorded within 0.5 unit.

## RESULTS AND DISCUSSION

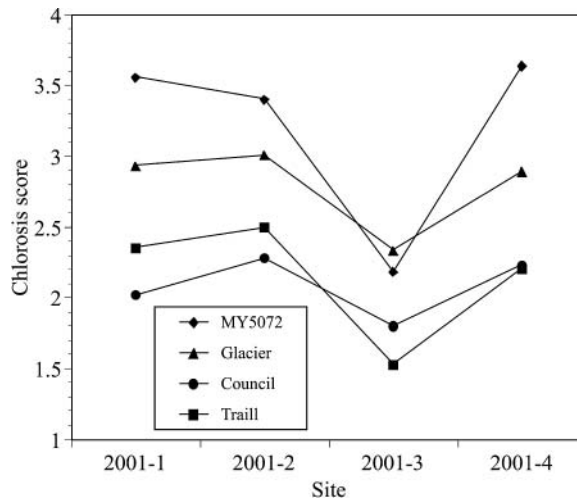
The data for the ratings at different stages of growth were averaged for each plot before statistical analysis. For each year, a combined analysis of variance was performed (Table 2). For both years, the main effects of cultivar and planting method were significant ( $P \leq 0.05$ ), and the cultivar  $\times$  site and cultivar  $\times$  planting method interactions were also significant. The overall effect of cultivar and the cultivar  $\times$  site interaction for 2001 can be seen in Figure 2. At sites 2001-1, 2001-2, and 2001-4, the cultivars gave chlorosis scores generally as expected, with chlorosis scores of 'Trail' and 'Council' being lower than for 'Glacier' and 'MY5072'. We usually observe that 'Trail' has somewhat lower chlorosis scores than 'Council' (Goos and Johnson, 2000, 2001), but the order was reversed at sites 2001-1 and 2001-2.

**TABLE 2** Analysis of variance for effects of cultivar, planting method, and interactions on severity of iron deficiency chlorosis in soybean

Source†	2001		2002	
	df	MS	df	MS
Site	3	10.056	3	9.059
Rep (site)	12	0.848	12	3.052
Cultivar	3	18.330**	6	39.455**
Cultivar $\times$ Site	9	0.871**	18	0.540**
Method	3	1.443**	3	5.444**
Site $\times$ Method	9	0.036 NS	9	0.291 NS
Cultivar $\times$ Method	9	0.575**	18	0.266*
Site $\times$ Cult. $\times$ Meth.	27	0.087 NS	54	0.133 NS
Residual	177	0.284	324	0.188

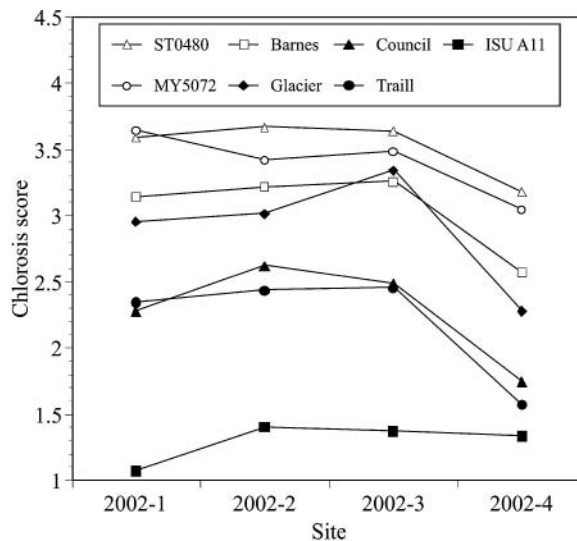
† Cultivar was tested with Cultivar  $\times$  Site as the error term, Method was tested with Method  $\times$  Site as the error term, Cultivar  $\times$  Method was tested with Site  $\times$  Cultivar  $\times$  Method as the error term. Other effects were tested with Residual as the error term.

NS, \*, \*\*, not significant, and significant at the 0.05 and 0.01 levels, respectively.

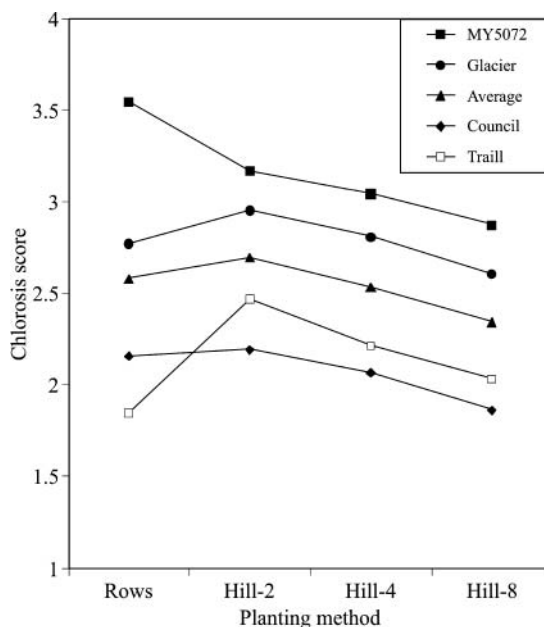


**FIGURE 2** Chlorosis scores of the four cultivars, averaged across planting method, North Dakota, 2001.

'Glacier' gave lower chlorosis scores than 'MY5072' at sites 2001-1, 2001-2, and 2001-4, but not at site 2001-3. The overall effect of cultivar and the cultivar  $\times$  site interaction for 2002 can be seen in Figure 3. The data for the seven cultivars separated into four groups, with chlorosis scores for 'ISU A11' < 'Traill' and 'Council' < 'Glacier' and 'Barnes' < 'ST0480' and 'MY5072'. The cultivar  $\times$  site interaction was significant, but not as dramatic as in 2001.



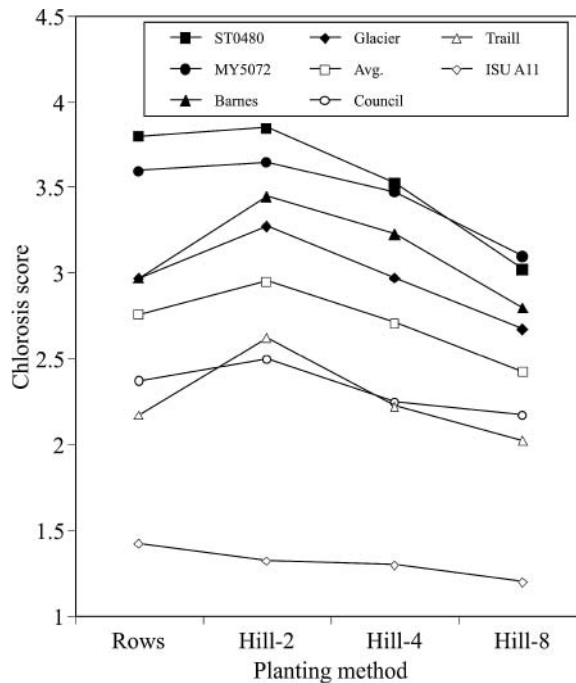
**FIGURE 3** Chlorosis scores, averaged across planting method, North Dakota, 2002.



**FIGURE 4** Chlorosis scores, averaged across site and rating date, for four soybean cultivars, as influenced by planting method. North Dakota, 2001. Row, Hill-2, Hill-4, and Hill-8 refer to planting in 76-cm rows, and two, four, or eight plants per hill, respectively.

The overall effect of cultivar, planting method, and the cultivar  $\times$  planting method interaction, for 2001, is shown in Figure 4. In rows, the cultivars separated themselves as expected, with 'Traill' exhibiting the least chlorosis, and with 'MY5072' showing the most. The cultivar  $\times$  planting method interaction was observed most dramatically with 'Traill', which had less chlorosis than 'Council' in row plots, but the opposite was true with hill plots. For all cultivars except 'Traill', the chlorosis score was the lowest with 8 plants per hill. Averaged across cultivar, the chlorosis score for row plantings was intermediate between that observed for two or four plants per hill. Thus, it was estimated that three plants per hill would give a chlorosis score most similar to plants seeded in 76-cm rows.

The overall effect of cultivar, planting method and the cultivar  $\times$  planting method interaction, for 2002, can be seen in Figure 5. Overall levels of chlorosis for 2002 were higher than for 2001. The germplasm 'ISU A11' exhibited dramatically more growth and less chlorosis than the other cultivars, suggesting that the potential remains for significant improvement of the chlorosis resistance of cultivars. In general, the cultivars separated themselves as expected. Chlorosis severity was, for most cultivars, higher with two plants per hill than with row planting, with the lowest chlorosis scores observed with eight plants per hill. As in 2001, averaged across cultivar, the chlorosis score for rows was intermediate between that observed for two and



**FIGURE 5** Chlorosis scores, averaged across site and rating date, for seven soybean cultivars, as influenced by planting method. North Dakota, 2002. Row, Hill-2, Hill-4, and Hill-8 refer to planting in 76-cm rows, and two, four, or eight plants per hill, respectively.

four plants per hill, meaning that three plants per hill should give a reasonable approximation of the chlorosis score observed in rows. The cultivar  $\times$  planting method interaction was significant in 2002 (Table 2), but was not as dramatic as in 2001. There was some minor reordering of the cultivar rankings with different planting methods. For example, 'Trail' exhibited less chlorosis than 'Council' when planted in rows, but not with two plants per hill.

An indication of the precision of the results given by each planting method is shown in Table 3. The data for each site and planting method were analyzed by individual analyses of variance, and the percent coefficients of variation associated with each site and planting method shown. In general, the highest precision (lowest coefficient of variability) was associated with row plots, and the lowest precision associated with hill plots with two plants per hill. The highest variability was observed with site 2001-3, which may explain why the cultivars were not properly ranked at this site (Figure 2).

## PRACTICAL CONSIDERATIONS

Standard methods of the visual rating of chlorosis severity rely primarily on the degree of yellowing of the uppermost trifoliolates. While usually



**TABLE 3** Coefficients of variation associated with each planting method at each site. Row, Hill-2, Hill-4, and Hill-8 refer to planting in 76-cm rows, and 2, 4, or 8 plants per hill, respectively

Site	Planting method			
	Rows	Hill-2	Hill-4	Hill-8
			%	
2001-1	3.2	5.4	5.2	5.6
2001-2	8.4	16.1	18.4	18.4
2001-3	20.7	31.5	19.5	27.1
2001-4	7.3	13.6	9.7	8.6
2002-1	3.6	7.8	7.3	5.8
2002-2	4.3	4.0	3.5	4.5
2002-3	3.4	8.8	4.1	3.2
2002-4	9.2	19.7	16.3	14.1
Average	7.5	13.4	10.5	10.9

adequate for separation of susceptible vs. resistant cultivars, such a rating system usually underestimated the stimulative effect of the heaviest planting rate in hills in this study. Figure 6 shows a typical effect of having two, four, and eight plants per hill on the overall growth of the plants in individual hill plots. As a general observation, hill plots with two plants per hill gave plants with more stunted growth than plants in 76-cm rows, and hill plots with eight plants per hill gave plants with considerably taller growth than plants in rows. If a seed company only evaluates chlorosis in heavily-seeded hill plots, the ability of the cultivar to resist chlorosis in a production field will



**FIGURE 6** Glacier soybean, at a stand of two, four, or eight plants per hill, Argusville, North Dakota, 2001.

likely be overestimated. This is possibly one reason for the common farmer complaint that the chlorosis resistance of cultivars in production fields is less than predicted by seed companies.

If hill plots will be used to screen cultivars, it is recommended to thin the plots shortly after emergence, to three plants per hill, if the results are going to be related to production in 76-cm rows. Thinning to two plants per hill is reasonable if the results are going to be related to “solid seeded” production. It is generally observed that the degree of chlorosis observed in narrow (15-cm) rows is more severe than observed in 76-cm rows, at similar seeding rates per hectare.

There are practical advantages and disadvantages to evaluating cultivars in hill plots. Hill plots are more space-efficient than row plots. This is desirable given the high degree of soil variability inherent to this disorder, and the large numbers of entries common in such trials. The major disadvantages of hill plots over row plots are the extra labor needed to thin the hills to a desired stand, and significant loss due to predation by animals, such as deer or rabbits. In our experience, seldom does animal predation take out an entire row plot, but individual hill plots are easily destroyed at early stages of growth by deer and rabbits. Fencing and repellants are often required for hill plots.

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