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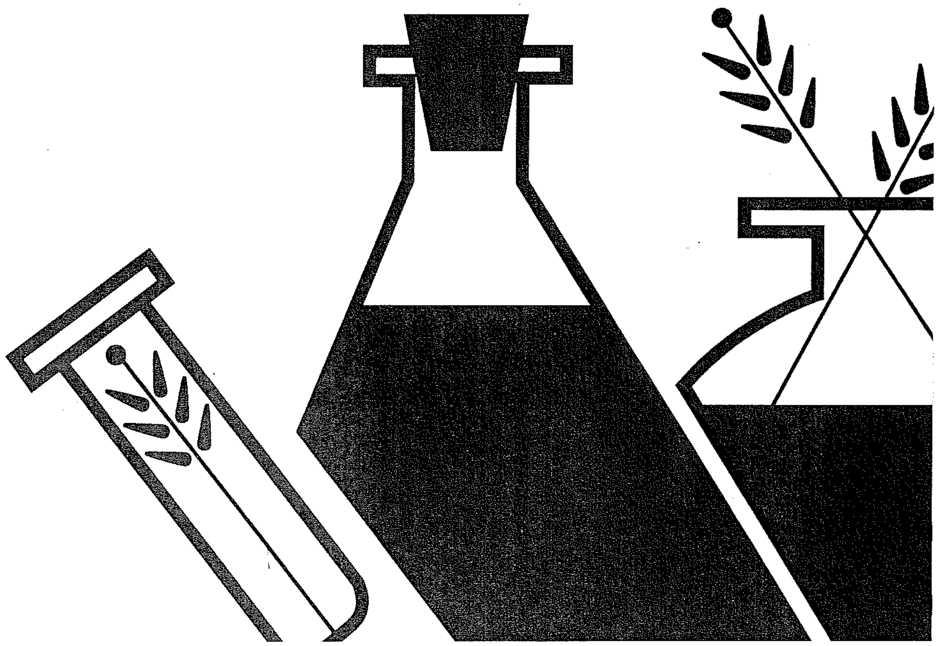

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Soil Factors Affecting Iron Chlorosis of Soybean in the Red River Valley of North Dakota and Minnesota

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ABSTRACT

Iron chlorosis tolerant soybeans exhibit chlorosis symptoms in the Red River Valley of North Dakota and Minnesota. Previous research suggests that chlorosis was generally related to high calcium (Ca) carbonate levels in the soil, so a relationship of chlorosis with the presence of Calciaquoll (carcareous at the surface, with very high levels of calcite in the subsurface) and non-Calciaquoll soil types would be expected. Six sites each in 1996, 1997, and 1998 were studied to identify soil factors correlated with the incidence and degree of chlorosis. Gradients between green soybeans and severely chlorotic plants were established, soil type was determined at the green and chlorotic gradient endpoints, and soil samples were taken at the 0-15 cm depth at each gradient location. Iron chlorosis symptoms were filmed using a video recorder. The images recorded were then analyzed using an image analyzer to give a digital chroma number. The digital values were correlated with soil factors to determine the degree of relationship of symptoms with each factor. Soil type differences were only associated with chlorosis at four of twelve sites. Both Ca carbonate equivalence and soluble salts were most often correlated with chlorosis symptoms. The relationship of iron chlorosis with soluble salts, along with soil carbonate level, appears to be a factor that should be considered in soybean chlorosis resistance breeding for the Red River Valley. A

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greenhouse experiment was conducted which was unsuccessful in duplicating field chlorosis symptoms when gypsum and Ca carbonate was added to soil obtained from non-chlorotic areas. The study was able to show a decrease in soybean nodule number with increasing Ca carbonate and gypsum levels.

INTRODUCTION

Soybean acres continue to increase in northwestern Minnesota and North Dakota in spite of severe problems with iron chlorosis in some years. Soybeans often turn yellow within a few weeks of emergence and remain yellow for up to 8 weeks before plants green up and mature. Iron chlorosis tolerant soybeans available today are somewhat effective in reducing chlorotic acreage, but are not tolerant enough to counteract the soil conditions in this area.

Several researchers have documented the chlorosis is associated with calcareous soils (Anderson, 1982; Clark, 1982; Vose, 1982). Loeppert and Hallmark (1985) found that the Fe oxide phase and Mg^{2++}, content of the solution was important in influencing availability of Fe. In a series of studies in western Minnesota, chlorosis was found to be associated with higher soil and plant Mg^{2+} levels, higher soil Na^+ and Cl^-, higher magnesium (Mg)/Ca ratios, over-saturation of Ca carbonate with respect to calcite, higher plant phosphorus (P), higher soil moisture, lower soil temperature and higher bicarbonate levels (Inskeep and Bloom, 1984; Bloom and Inskeep, 1986; Inskeep and Bloom, 1986). Some of these factors have been reviewed by Moraghan and Mascagni, 1991.

The concentration of bicarbonates in the soil may be influenced by the amount of carbonate minerals in the soil. In the Red River Valley of Minnesota and North Dakota, pedogenic carbonates are associated with discharge position soils in a depression focused recharge landscape (Knuteson et al., 1989). Calcium carbonate equivalent levels may reach levels of 300 g kg^{-1} or more in the discharge locations. The distance between recharge and discharge in these soil associations may be less than 30 m. Elevation difference between the recharge and discharge sites are commonly only 0.1-0.2 m. Soils developed in this association are classified as Calciaquolls and non-Calciaquolls. Salts may be more prevalent in Calciaquolls than non-Calciaquolls, but not necessarily. The appearance of salts is an ephemeral feature, while a calcic horizon is associated with much longer term water movement over centuries.

Salts may have a role in increasing chlorosis in soybeans. Dahiya and Singh (1979) showed that available soil levels decreased with increasing salt levels. Randall (1981) commented that soybean responses to foliar amendments were not effective when applied to soybeans growing in high salt soils. Saturated paste EC significantly decreased plant growth in the chlorosis study of Loeppert and Hallmark (1985), although this observation was not commented on by the authors. The relationship of Mg with Ca referred to as associated with chlorosis in previous studies may be a reflection of not only the nature of carbonate minerals in the soil, but also may have been associated with the presence of significant soluble salts in certain soils.

Iron deficiency has also been observed to decrease modulation and N-fixation in soybeans. Nodule initiation was identified as a critical stage for the deficiency effect by Tang and Robson (1992). Salinity was found to decrease nodule activity and subsequent accumulation of nitrogen (N) in the four grain legumes (Cordovilla et al., 1995).

The objective of this study was to identify soil factors that affect chlorosis symptoms in soybeans in order to better direct breeding selection efforts for reducing or eliminating severe chlorosis problems in this region.

MATERIALS AND METHODS

Field Studies

Six different locations were selected each year from 1996 through 1998 in the Red River Valley of North Dakota and Minnesota which contained chlorotic and green areas of soybeans. A gradient of six sampling sites from severely chlorotic to green soybeans was established at each site, each representing a visual category from the chlorosis rating of 1 through 6. Gradients of soybean chlorosis were selected using a visual rating of 1 to 6, with 1 being most chlorotic and 6 having a normal green color. Two gradients were established at each location in 1997 and again in 1998. Video of individual plants and soil cores at the 0-15 cm were taken at each location along the gradient. Separate 0-60 cm soil cores were obtained from the chlorotic and green ends of the gradient. The deep soil cores were measured for horizon depth in the field, then wrapped with saran wrap, folded and taken back to the lab for more thorough profile description.

At each location, soil cores from the 0-15 cm depth were taken at each of these 6 gradient sites for analysis. Soil samples were dried and analyzed for potassium (K), pH, EC (soluble salts using a 1:1 paste), Fe and zinc (Zn) by DTPA extraction, Ca, Mg, sodium (Na), and Ca carbonate equivalent (CCE). In 1998, copper (Cu) and manganese (Mn) were also analyzed. Regression analysis was used to determine relationships between the severely chlorotic and green locations and the gradient samples at each field site.

The video recording was used to assign a digital chroma value to the degree of chlorosis. The image analyzer Java (Jandel Scientific, Corte Madera, CA) was used to give a mean value to polygons representing leaf surfaces at each location in the gradient. The mean chroma value was then correlated with soil factors at each location.

Greenhouse Studies

Greenhouse experiments were conducted in 1998 and 1999 in response to field observations. Soils from the surface 0-15 cm at three sites showing no chlorosis in

TABLE 1. Greenhouse treatments, 1997 and 1998.

Calcium Carbonate	Gypsum
0	0
40g	0
80g	0
0	0.8g
0	1.6g
40g	0.8g
80g	1.6g
40g	0.8g
80g	1.6g

1997 (Fixen, Nordick, and Wang), and three sites in 1998 (Kummer, Rydell, and Gebecke), were collected and airdried. The Fixen and Nordick site soils tested 0.3 mmohs cm^{-1} in conductivity and contained 2% CCE. The Wang site soil tested 0.8 mmohs cm^{-1}, with 24% CCE. In 1998, the Kummer site soil tested 0.3 mmhos cm^{-1}, with 0.4% CCE, the Rydell site soil tested 0.5 mmohs cm^{-1} with 8.0% CCE and the Gebecke site soil tested 0.3 mmohs cm^{-1} with 0.5% CCE. Each of these soils was collected from an area within the field test showing green, normally growing soybeans.

The objective of the greenhouse study was to determine if modification of the soil with Ca carbonate and gypsum additions would transform green soybeans in the field into chlorotic soybeans in the greenhouse. The experimental design was a factorial, with three salt and three carbonate levels, and four replicates in a completely randomized design. A measured amount of soil (approximately 1 kg) was added to each pot and mixed with one of the nine treatment mixes. The amount of soil in each pot was recorded on the outside of the pots. The treatments are recorded in Table 1.

The 40 g and 80 g treatments of Ca carbonate were designed to represent a CCE of 5% and 10%, respectively. The gypsum treatments were designed to simulate a 0.5 mmoh cm^{-1} and 1.0 mmoh cm^{-1} condition with the 0.8 g and 1.6 g pot^{-1} treatments, respectively.

Treatments were thoroughly mixed with soil, then saturated with water, covered and sealed to allow the treatment materials to equilibrate in the pots for two weeks. Soils were then allowed to dry, and using weight of dried soil as a basis, kept at 50% of field capacity for the length of the experiment. Soybean seeds were soaked

TABLE 2. Comparison of soil series descriptions at chlorotic and green locations.

	Soil Series*	
Site	Green	Chlorotic
Zimmerman, 1996	Bearden sil	Bearden sil
Wang,1996	Bearden sil	Bearden sil
Nordick 1, 1996	Glyndon sil	Glyndon sil
Nordick 2, 1996	Perella sicl	Colvin sicl
Strand 1, 1996	Wyard l	Wyard l
Strand 2, 1996	Embden l	Wyndmere l
Wang east 1, 1997	Bearden sil	Colvin sicl
Wang east 2, 1997	Bearden sil	Colvin sicl
Wang west 1, 1997	Colvin sicl	Colvin sicl
Wang west 2, 1997	Colvin sicl	Colvin sicl
Fixen 1, 1997	Gardena l	Arveson l
Fixen 2, 1997	Gardena l	Arveson l
Gylland 1, 1997	Perella sicl	Colvin sicl
Gylland 2, 1997	Perella sicl	Colvin sicl
Nordick east 1, 1997	Tiffany sl	Arveson sl
Nordick east 2, 1997	Tiffany sl	Arveson sl
Nordick west 1, 1997	Embden sl	Arveson sl
Nordick west 2, 1997	Embden sl	Arveson sl
Nordick 1, 1998	Bearden sil	Bearden l
Nordick 2, 1998	Arveson l	Arveson l
Rydell 1, 1998	Colvin sicl	Colvin sicl saline
Rydell 2,1998	Colvin sicl	Colvin sicl, saline
Wang 1, 1998	Colvin sicl	Colvin sicl, saline
Wang 2, 1998	Colvin sicl	Colvin sicl, saline
Gebecke 1, 1998	Glyndon fsl	Arveson l
Gebecke 2, 1998	Glyndon fsl	Borup l
Kummer 1, 1998	Tiffany sl	Arveson sl
Kummer 2, 1998	Tiffany sl	Tiffany sl
Brodshaug 1, 1998	Perella sicl	Bearden sicl
Brodshaug 2, 1998	Bearden sicl	Bearden sicl

*Arveson, Bearden, Borup, Colvin, Embden, Gardena, Glyndon, Perella, Tiffany, Wyard, and Wyndmer.

over night and planted at a seeding rate of 10 pot^{-1} and later thinned to 6 plants pot^{-1}. The first experiment in 1997 used Pioneer 9091 as a variety and the second experiment used Glacier. The 1998 experiment used Pioneer 9091 as a variety. Pioneer 9091 was rated as very tolerant of chlorosis, while Glacier was rated as only somewhat tolerant. In the second experiment, the water content was maintained at

TABLE 3. Correlation (r) of 1996 soil fertility factors within gradients of chlorotic to green soybeans at each location.

Site	pH	EC	Fe	Na	Mg/Ca	CCE
Zimmerman	0.46	0.28	0.16	0.88‡	0.96‡	0.95‡
Wang	0.39	0.84‡	0.17	0.97‡	0.92‡	0.87‡
Nordick 1	0.24	0.53	0.25	0.44	0.58	0.33
Nordick 2	0.43	0.62	0.34	0.47	0.52	0.55
Strand 1	0.90‡	0.68	0.88‡	0.61	0.18	0.60
Strand 2	0.21	0.28	0.00	0.01	0.27	0.71†

†Significant at $P>0.10$.
‡Significant at $P>0.05$.

70% field capacity. Water content was maintained in each experiment by twice daily watering to weight. Video was taken of each pot at the end of the experiment for image analysis. When nodule numbers were recorded, soil was gently removed from the roots by washing.

RESULTS AND DISCUSSION

Field Studies

In 1996, rainfall was higher than normal, as it has been in the Red River Valley since 1993. In 1997, there was a dry period for about three weeks after planting. This was followed by a wet period of about two weeks. Soybean fields with one to two true leaves that had been green during the dry period turned chlorotic during this two week period. It was toward the end of the two-week wet period that the 1997 sampling was conducted. In 1998, an unusually dry planting season was followed by an unusually wet and cold period that extended from early May until early June. During this period, most of the soybeans were planted. The Rydell and Nordick fields were planted first, followed by the Wang and Gebecke fields, and the Kummer and Brodshaug fields were planted last. About two weeks in planting and maturity separated the first fields from the last.

Soil taxonomic type and soil series phase was not entirely consistent in separating green and chlorotic soybeans in this study (Table 2). In the three years of the study, soil type was not different between green and chlorotic sites in eleven of thirty comparisons, or 37% of the time. Calciaquolls were chlorotic and non-Calciaquolls were green in twelve of thirty comparisons (40%). Internal drainage

TABLE 4. Correlation (r) of 1997 soil fertility factors within gradients of chlorotic to green soybeans at each location.

Site	pH	EC	Fe	Na	Mg/Ca	CCE
Wang east 1	0.62	0.02	0.55	0.22	0.52	0.02
Wang east 2	0.95‡	0.68	0.15	0.40	0.11	0.12
Wang west 1	0.71	0.75†	0.62	0.50	0.82‡	0.94‡
Wang west 2	0.69	0.53	0.93‡	0.16	0.68	0.55
Fixen 1	0.67	0.93‡	0.73†	0.92‡	0.63	0.43
Fixen 2	0.43	0.55	0.85‡	0.49	0.76‡	0.86‡
Gylland 1	0.72†	0.43	0.66	0.20	0.93‡	0.91‡
Gylland 2	0.32	0.85‡	0.91‡	0.65	0.97‡	0.73†
Nordick East 1	0.68	0.88‡	0.53	0.39	0.94‡	0.91‡
Nordick East 2	0.56	0.75†	0.68	0.69	0.95‡	0.82‡
Nordick West 1	0.20	0.73†	0.43	0.82‡	0.36	0.88‡
Nordick West 2	0.00	0.87‡	0.43	0.42	0.51	0.25

†Significant at P>0.10.
‡Significant at P>0.05.

as evidenced by soil morphology accounted for differences in three sites (10%) and saline phases accounted for four sites (13%). Undoubtedly, chlorosis was more complex than soil type. There may be several interacting causes at work simultaneously. Some relationship between evaporates and dissolved constituents seems likely, however.

In 1996, calcium carbonate equivalent was correlated with chlorosis rating at r>0.4 at five of six locations, while soluble salts (EC) had a correlation r>0.4 at four of five locations (Table 3). At the two sites not highly correlated with salt levels, CCE was very highly correlated. At the site with lower CCE correlation, salt levels were highly correlated. Soil pH was correlated higher than 0.4 at three of six locations, Fe at one of six, with sodium highly correlated at five of six locations.

In 1997 (Table 4), both EC and CCE were significantly correlated (P>0.1) at seven of twelve comparisons. EC had a correlation r>0.4 at eleven of twelve locations,

TABLE 5. Correlation (r) of 1998 soil fertility factors within gradients of chlorotic to green soybeans at each location.

Site	pH	EC	Fe	Na	Mg/Ca	CCE
Nordick 1	0.90‡	0.71	0.89‡	0.43	0.94‡	0.88‡
Nordick 2	0.00	0.70	0.26	0.52	0.03	0.81†
Rydell 1	0.25	0.01	0.00	0.01	0.58	0.38
Rydell 2	0.25	0.03	0.36	0.08	0.24	0.74
Wang 1	0.96‡	0.89‡	0.91‡	0.98‡	0.95‡	0.41
Wang 2	0.57	0.64	0.62	0.65	0.24	0.65
Gebecke 1	0.61	0.20	0.15	0.11	0.64	0.86†
Gebecke 2	0.18	0.53	0.00	0.41	0.41	0.32
Kummer 1	0.72	0.71	0.81†	0.10	0.75	0.73
Kummer 2	0.74	0.97‡	0.89‡	0.69	0.96‡	0.34
Brodshaug 1	0.87†	0.23	0.85†	0.20	0.09	0.58
Brodshaug 2	0.61	0.40	0.66	0.20	0.21	0.42

†Significant at P>0.10.
‡Significant at P>0.05.

TABLE 6. Effect of salt and calcium carbonate additions on degree of chlorosis in three soils (% significance).

	With Pioneer 9091		
Factor	**Nordick**	**Wang**	**Fixen**
CCE	NS	NS	1%
EC	NS	NS	5%
Interaction	5%	NS	5%
	With Glacier		
Factor	**Nordick**	**Wang**	**Fixen**
CCE	5%	5%	5%
EC	5%	NS	NS
Interaction	5%	NS	NS

TABLE 7. Effect of salt and calcium carbonate additions on nodule number per plant in three soils (% significance), 1998.

	With Pioneer 9091		
Factor	**Nordick**	**Wang**	**Fixen**
CCE	1%	5%	Not
EC	5%	NS	recorded
Interaction	5%	NS	
	With Glacier		
Factor	**Nordick**	**Wang**	**Fixen**
CCE	1%	1%	5%
EC	NS	5%	NS
Interaction	NS	NS	NS

while CCE has a correlation >0.4 at nine of twelve locations. At Wang east, 1997, chlorotic soybeans associated with the Colvin soil type, compared to Bearden in the green soybean area. Although both soils were classified as a calciaquoll, the Colvin soil had poor internal drainage (Typic Calciaquoll compared to Aerie Calciaquoll in the Bearden), so the difference may have been the increased moisture status of the Colvin soil. In the other two comparisons not correlated with EC or CCE, soil pH and extractable were correlated with chlorosis.

In 1998, EC was significantly correlated with chlorosis at two of twelve locations and r>0.4 at eight of twelve locations (Table 5). Calcium carbonate equivalent was significantly correlated with chlorosis at three of twelve locations and r>0.4 at nine of twelve locations. Over all years, EC was significantly and positively correlated with chlorosis at ten of thirty locations, and r>0.4 at twenty-four of thirty locations. Calcium carbonate equivalent was significantly correlated with chlorosis at twelve of thirty locations and r>0.4 at twenty-three locations. Soil pH was significantly correlated only six times, with r>0.4 at twenty locations. Soil Fe was significantly correlated ten times, with r>0.4 at nineteen locations.

Greenhouse Studies

In each of the 1998 studies with both Pioneer 9091 and Glacier, and in the 1999 study using Pioneer 9091, none of the treatments resulted in severely chlorotic soybeans. However, there were differences in degree of chlorosis in Pioneer 9091 with CCE and EC factors with the soil from the Fixen site (Table 6). Chlorosis decreased with Ca carbonate additions, and increased with the addition of salt. Interactions were significant in both the Fixen and Nordick soils. In the Nordick soil using Glacier soybeans, chlorosis increased with both EC and CCE increases. Interactions were also significant in the Nordick soil. In the Wang soil, which was

TABLE 8. Effect of salt and calcium carbonate additions on nodule number per plant in three soils (% significance), 1999.

Factor	Kummer	Rydell	Gebecke
CCE	5%	5%	1%
EC	NS	NS	1%
Interaction	NS	NS	1%

relatively high in CCE to begin the experiment, increased CCE reduced chlorosis.

During dismantling of the first experiment with Pioneer 9091 in the soils from the Fixen site, it was observed that plant roots from untreated pots tended to have more nodules present than those with rates of Ca carbonate or gypsum. Therefore, nodule numbers were recorded on the remaining two soils from the first experiment and the three soils from the Glacier experiment. Calcium carbonate equivalent additions most consistently reduced nodule number, although decreasing in nodule number were seen in the Nordick soil and the Wang soil (Table 7).

In 1999, nodule number was reduced in all three soils with increasing CCE. Increasing gypsum levels significantly lowered nodule numbers in only the Gebecke soil (Table 8). There was also a significant interaction effect in the Gebecke soil (Figure 1). These results are consistent with previous studies that show a response of soybeans to modest levels of preplant N additions in the Red River Valley (Lamb et al., 1990).

The results of these greenhouse experiments suggest that the use of soils that

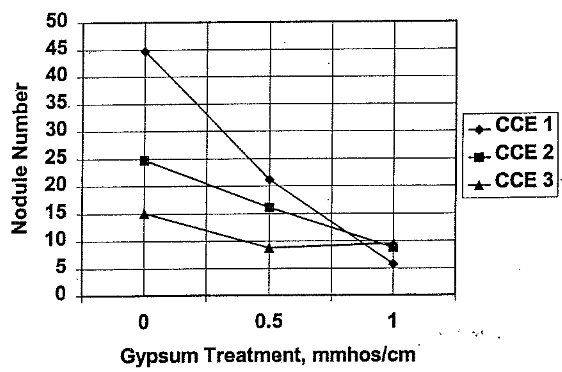

FIGURE 1. Nodule number in the Gebecke soil as influenced by calcium carbonate and gypsum additions.

do not normally produce chlorotic symptoms in the field would be poor soils to serve in greenhouse variety screening studies. These soils do not respond readily to treatments of Ca carbonate additions, nor do they respond with consistency to gypsum additions. Perhaps if a combination of gypsum and a more soluble salt, such a Mg sulfate was added to increase the EC even more, the effect would be more evident. It would appear that the best soil to use for screening varieties in the greenhouse for chlorosis might be soil from severely chlorotic areas of fields.

CONCLUSIONS

Soil type was related to the degree of chlorosis at twelve of thirty sites from 1996 through 1998, however, both green and chlorotic plants could be found in the same soil type at eleven of thirty sites. Over all years, EC was significantly correlated with chlorosis at ten of thirty locations, and $r>0.4$ at twenty-four of thirty locations. Calcium carbonate equivalent was significantly correlated with chlorosis at twelve of thirty locations and $r>0.4$ at twenty-three locations. Soil pH was significantly correlated only six times, with $r>0.4$ at twenty locations. Soil Fe was significantly correlated ten times, with $r>0.4$ at nineteen locations. Greenhouse studies confirmed the influence of both gypsum and CCE on degree of chlorosis, although the chlorosis produced was not severe and was inconsistent. The greenhouse study also showed that nodule numbers are decreased with the addition of gypsum and Ca carbonate. Decrease in nodule numbers with addition of gypsum and Ca carbonate may explain responses to low rate early season N applications in soybeans in this region.

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