EFFECTS OF SOIL MOISTURE, PHOSPHORUS, AND IRON FERTILIZATION ON IRON CHLOROSIS OF SORGHUM

By

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

CHAPTER I

ABSTRACT

Iron deficiencies have been frequently observed in sorghum (Sorghum bicolor (L.) Moench) grown in western Oklahoma where the soils are generally calcareous. Iron chlorosis in soybeans grown in calcareous soils contributed by the increases in soil moisture, pCO_2, and HCO_3^- (Inskeep and Bloom, 1986) has not been established in sorghum. Although it is clear that application of $FeSO_4$ with fluid ammonium polysposphate (APP) may help alleviate Fe deficiencies in sorghum (Datin and Westerman, 1982), the extent of this effect and the mechanism by which polyphosphates influence the deficiency have not been established. Experiments with potted soils have been used to screen sorghum genotypes for Fe-efficient varieties, however, the sorghum genotypes have responded inconsistently with different soils (Clark, et al., 1982).

The objective of this research was to evaluate the effects of soil moisture, phosphorus sources and rates on iron chlorosis of sorghum grown in pot experiments with two calcareous soils obtained from western Oklahoma, and to determine whether these soils are suitable for screening Fe-efficient sorghum genotypes. The soils used were Quinlan clay loam (Typic Ustocrept) and Spur silt loam (Fluventic Haplustoll). Sorghum (RTx428) was grown in both soils at four soil matric potentials (-0.5, -2.69, -14.1, -75 kPa) to study the influence

of soil moisture on the severity of Fe chlorosis. The same cultivar was grown with three different P sources (ammonium polyphosphate, APP; monoammonium phosphate, MAP; and monobasic potassium phosphate, KH_2PO_4) at two P rates, with and without a soil application of Fe as $FeSO_4$. Nine cultivars were grown to determine their tolerance to Fe stress in these soils.

Increases in soil moisture resulted in high concentration of HCO_3^- and soil pCO_2 in soil solution; however, severity of Fe chlorosis associated with increasing soil moisture was not observed in grain sorghum. Total chlorophyll (Chl_T) and dry matter were higher when APP was applied to the Quinlan soil relative to the MAP and KH_2PO_4 treatments. Ammonium polyphosphate appeared to play a role in stimulating uptake of Fe in Fe deficient soils. The range of Fe efficiency shown by the nine cultivars indicated that these calcareous soils from western Oklahoma can be used to effectively screen sorghum cultivars in controlled pot experiments. Application of APP with Fe may be used to correct the Fe chlorosis of grain sorghum grown in western Oklahoma.

CHAPTER II

LITERATURE REVIEW

A green plant deprived of Fe soon becomes chlorotic in its new growth, while its older tissue remains green. The new growth of plants cannot depend on the export of Fe from older tissue but must depend on a continuous supply via the xylem or receive external applications of Fe (Tiffin, 1972).

According to Brown et al. (1972) any factor that interferes with absorption, translocation, or metabolism of Fe may cause Fe chlorosis. These factors include soil conditions such as low organic matter content, low soil Fe, free $CaCO_3$, high HCO_3^-, soil moisture extremes, high concentrations of heavy metals, high soil P, poor aeration, excess CO_2, and high soil pH. In addition, genetic differences among plants and within the same species can influence the susceptibility of plants to Fe deficiency.

The most wide spread occurrence of iron deficiency in plants is found on calcareous soils that cover over 30 percent of the earth's land surface (Chen and Barat, 1982). Over 90 percent of the United States' sorghum production is grown in the Great Plains, where the soils are generally neutral or alkaline and usually have underlying calcareous deposits (Clark, 1982). Thus, Fe deficiency of sorghum in this area is a common problem.

Soil moisture content has a strong influence on many chemical reactions in the soil. Excessive moisture content resulting in near saturated conditions cause a reduction in oxygen content, and an increase in Fe^{2+}. The change in Fe^{2+} as related to pH was illustrated by Lucas and Knezek (1972), where Fe values exceeded 1,000 ppm at pH 6.0, 352 ppm at pH 6.5, 35 ppm at pH 7.0, and only 3.5 ppm at pH 7.5. In calcareous soil where the pH is generally alkaline, an increase in Fe^{2+} with excessive moisture may not be significant to the plant.

When soil moisture increases, microbial respiration normally increases, gas exchange decreases, and pCO_2 contents increase. Soil solution HCO_3^- concentrations then rise according to the equilibrium: $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$ (Lindsay, 1979). Lindsay and Thorne (1954) presented evidence that the increase of plant chlorosis associated with poorly aerated conditions was not attributed solely to a reduced oxygen level, but to the increasing levels of CO_2 and HCO_3^- at the root surface of plants. Porter and Thorne (1955) reported that at constant pH the leaf chlorophyll of beans and tomatoes decreased as HCO_3^- concentration increased. Coulombe et al. (1984a and 1984b) and Fleming et al. (1984) found that HCO_3^- is a necessary factor to induce chlorosis in susceptible soybean cultivars, and that HCO_3^- inhibits the translocation of Fe.

The interrelationship among soil moisture, soil pCO_2, HCO_3^-, and the incidence of Fe chlorosis of soybeans was studied by Inskeep and Bloom (1986). They found that decreases in total chlorophyll content (Chl_T) were associated with increases in soil moisture, soil pCO_2, and soil solution HCO_3^- when Anoka soybeans were grown on several calcareous soils. They suggested that the high amount of clay sized $CaCO_3$ in the calcareous soils may be associated with HCO_3^- induced chlorosis in soybeans. Changes in soil pH in a calcareous soil associated with changes in pCO_2 concentration were very well buffered by the exchange reaction of H^+ with Ca^{2+} (Inskeep and Bloom, 1986).

Römheld and Marschner (1986) provided evidence that the strategy for the acquisition of Fe by graminaceous species (monocotyledonous plants) was different from that of the nongraminaceous species (dicotyledonous plants). The strategy of grasses is characterized by the enhanced release of phytosiderophores and by a highly specific uptake system for Fe^{3+} phytosiderophores, whereas the strategy of the dicots is characterized by an inducible plasma membrane-bound reductase and enhancement of H^+ release. Consequently, there may be differences in how HCO_3^- influences Fe uptake in grasses compared to dicots. Certainly, the principle differences in the two strategies have to be taken into account when developing screening methods for resistance to 'lime induced chlorosis.'

Bernardo et al. (1984a and 1984b) conducted experiments to determine the effects of nitrogen source on Fe nutrition of sorghum. The form of N in the nutrient solutions greatly influenced the solution pH: pH rose to near 7 with NO_3^- as the sole source of N and decreased to near or below 4 with NH_4^+ added to the solution. They observed that sorghum plants growing in NO_3^- solution began to develop severe Fe chlorosis in the leaves whereas plants growing in a mixture of NO_3^- and NH_4^+ solution were still normal and did not develop Fe chlorosis. They also observed that the dry matter yield was generally higher for plants grown with some NH_4^+ compared to plants grown with NO_3^- alone. They proposed that the cause of Fe deficiency in the plants supplied with only NO_3^- was the result of Fe, supplied as FeHEDTA in the nutrient solution, not being translocated to the leaves, rather than the lack of Fe uptake by the roots. High P concentrations have also been known to induce Fe deficiency, particularly in high pH soil where Fe availability is usually low (Murphy et al., 1981). According to Adams (1980), when P is absorbed in excess of the plant tissue needs, the metabolic use of Fe and other ions is disturbed. Ajakaiye (1979) observed that a high P concentration in the growth medium (nutrient solution) inhibited ^{55}Fe absorption and translocation in sorghum. Plants showing Fe chlorosis induced by high levels of P generally showed a normal concentration of Fe in the tissues, but the ratio of phosphorus to iron (P/Fe) was larger in chlorotic plants (Olsen, 1972). Brown et al. (1959a and 1959b) observed a lower Fe concentration and higher P, Ca, and K concentrations in the top of chlorotic soybean plants. They also noted that the P/Fe ratios were higher in the chlorotic than the nonchlorotic plants and suggested that Fe was inactivated internally by a combined effect of the high P and Ca concentrations in the plant tissues. DeKock (1955) demonstrated that P/Fe ratio provided an accurate assessment of the Fe status of the plants.

Growth chamber experiments with sunflower in nutrient solution were performed by Kolesch et al. (1984) to investigate the effect of P and HCO_3^- in inducing Fe chlorosis. They observed that Fe chlorosis was induced by HCO_3^- alone and was more pronounced by a combination of HCO_3^- and P, but not by P alone. Therefore, they provided evidence that P enhanced the effect of HCO_3^- on Fe chlorosis in dicots. Coulombe et al. (1984b) also showed that higher P in the presence of high HCO_3^- (10 mM) resulted in an increase in soybean chlorosis score from 2.2 to 3.9.

The correction of Fe deficiency has often been accomplished by using inorganic Fe materials and chelated Fe sources applied both in the soil and by foliar methods (Mortvedt and Giordano, 1971; and Mortvedt, 1982). Mortvedt (1982) reported that $FeSO_4$ (the most economical source of Fe) was the least efficient source of Fe when compared to FeEDDHA, FeHEDTA, Hamp-Iron [a ferric-sodium salt of o-hydroxybenzyl polycarboxylic aminoacid (5.0% Fe)], and Fe-lignosulfonate when applied alone. However, he observed that the effectiveness of $FeSO_4$ in correcting Fe deficiency in sorghum was enhanced by band applications of $FeSO_4$ with fluid fertilizers like ammonium polyphosphate, 12-18-0 (12-40-0 oxide basis) especially at Fe rates greater than 8 mg per pot, which is approximately equal to 5 kg/ha.

Mortvedt and Giordano (1973) reported that forage yields and Fe uptake by grain sorghum were doubled from a band application of a $FeSO_4$-ammonium polyphosphate suspension compared to polyphosphate alone when sorghum was grown in potted calcareous soil in the greenhouse. Datin and Westerman (1982) also observed a positive yield response with grain sorghum when ammonium polyphosphate was applied with $FeSO_4$ and suggested that a synergistic effect existed between P and Fe in calcareous soils.

Greater solubilization of soil organic matter was observed when ammonium polyphosphates were applied to soils (Mortvedt and Osborn, 1977; and Sample et al., 1980). Giordano et al. (1971) reported that the Zn concentration in the soil solution increased when the soil was incubated with triammonium pyrophosphate (TPP) and proposed that the increase was due to the sequestering of metal ions by TPP or the solubilization of soil organic matter. Giordano et al. (1971) also found that up to 10 percent of the soil organic matter was solubilized by TPP, and that TPP dissolved about twice as much organic C as did monoammonium polyphosphate (MAP). It would be expected that such a large increase in soluble C would influence the amount of Fe complexation and the amount of Fe_{TS} in the soil solution. However, Mortvedt and Osborn (1977) reported that soluble micronutrient concentrations in calcareous soil were not affected by P applications at least as determined by DTPA extraction. They suggested that solubilization of soil micronutrients by polyphosphates did not appear to play an important role in micronutrient nutrition of crops, especially in calcareous soil. Although it is clear that polyphosphate applications may help alleviate Fe deficiencies in sorghum, the extent of this effect and the mechanism by which polyphosphates influence the deficiency is not established.

Although sorghum is fairly susceptible to Fe chlorosis relative to barley, wheat, and other grasses (Römheld and Marschner, 1986), sorghum cultivars differ in their tolerance to Fe stress. Leaf chlorophyll content and visual ratings assigned to Fe chlorotic plants have been used extensively to evaluate the severity of Fe chlorosis, and are also used as screening tools for Fe-efficient varieties (Clark et al., 1982; Williams et al., 1982; and McKenzie et al., 1984). McKenzie et al. (1984) visually assessed sorghum plants for Fe chlorosis and assigned a score to each plant based on the overall appearance: 0 = green normal leaves; 1 = green area equal to 3 times the chlorotic area; 2 = green area equal to chlorotic areas; 3 = green area equal to 1/3 the chlorotic area; and 4 = completely chlorotic. They also demonstrated a high correlation between the visual rating system and the leaf chlorophyll content in growth chamber-grown plants.

A screen to differentiate sorghum genotypes to Fe deficiency was conducted by Clark et al. (1982) using both a $CaCO_3$ buffered nutrient solution to simulate the high level of HCO_3^- produced in wet calcareous soils, and potted calcareous soil maintained at field capacity. They observed that the sorghum genotypes grown in low available Fe soils under greenhouse conditions responded differently to different soils with varying Fe deficiency conditions, and these responses were not necessarily the same as those noted in nutrient solutions. Nevertheless, they concluded that sorghum genotypes differed in their response to Fe deficiency conditions regardless of the screening method.

Sorghum cultivars that have been used frequently in the studies of Fe chlorosis are BTx378, BTx623, Redlan, RTx430, RTx2536, SC33-9-8-E4, SC118-15E, and Wheatland. According to studies conducted by Williams et al. (1982), SC118-15E, Redlan, and Wheatland developed Fe deficiency more rapidly and did not tolerate low Fe whereas

SC33-9-8-E4, BTx623 and BTx378 remained green and tolerated Fe deficiency conditions for a longer period of time.

McKenzie et al. (1984) reported that the average chlorosis symptoms of grain sorghum induced by a high $CaCO_3$ pH buffered nutrient solution were more severe for BTx378 than for RTx2536 and RTx430. Clark and Gross (1986) also categorized RTx2536, RTx430, and SC33-9-8-E4 as tolerant, and BTx378, Redlan, and SC118-15E as susceptible to Fe deficiency chlorosis. The cultivar Redlan, developed by the Sorghum Program at Oklahoma State University, is the same as BTx378.

In an effort to continue work on the influence of soil moisture on HCO_3^- induced chlorosis (Inskeep and Bloom, 1986; and Coulombe et al., 1985) and the influence of ammonium polyphosphate on Fe chlorosis of sorghum (Datin and Westerman, 1982), three pot experiments were performed under controlled conditions using calcareous soils from western Oklahoma. The first objective of this study was to determine the relationship among soil moisture content, soil HCO_3^-, soil pCO_2, and the extent of Fe chlorosis of susceptible sorghum cultivars grown in calcareous soils from western Oklahoma. The second objective was to determine the effects of two P sources on the expression of Fe deficiency in sorghum grown on calcareous soils. The third objective was to determine whether the visual rating system and the determination of chlorophyll content of sorghum plants grown in a controlled environment with potted calcareous soil can be used to screen Fe-efficient from Fe-inefficient sorghum cultivars.

CHAPTER III

MATERIALS AND METHODS

Two calcareous soils from western Oklahoma were used to induce Fe chlorosis in sorghum plants. Surface soil samples of the Quinlan clay loam (Typic Ustocrept) and Spur silt loam (Fluventic Haplustoll) series were taken from western Oklahoma where Fe deficiency in sorghum was very severe. Each sample was sieved through a 2 mm screen, homogenously mixed, and stored in plastic bags at room temperature to retain the moisture content at field condition. Basic soil characterization was carried out before the experiments were established (Table 1). Total $CaCO_3$ was determined by HCl dissolution (Allison and Moodie, 1965). Plant available P was determined with the Olsen method by extraction with $NaHCO_3$ (Olsen and Sommers, 1982). Iron, Zn, and Cu were determined by the DTPA extraction method (Lindsay and Norvell, 1978). Cation exchange capacity and pH (1:1 H_2O) were determined by methods outlined by Rhoades (1982) and McLean (1982), respectively. The method used to determine organic matter content were outlined by Nelson and Sommers (1982). The Pipette method (Day, 1965) was used to determine the particle size analysis of the soils.

Brass rings (volume = 68.7 cm^3) were packed with moist soil to yield a bulk density of 1.3 g/cm^3. The volumetric water content (θ_v) was determined at matric potential of 0, -10, -30, -90, and -1500 kPa and a soil moisture curve was prepared for each soil (Hillel,

Table 1. Selected characteristics of Quinlan and Spur soils used in the pot experiments.

Soil Classification	Sand	Silt	Clay	pH	Organic Matter	$CaCO_3$	DTPA-Fe*	Olsen-P
	------ % ------				--- % ---		(mg/kg soil)	
Quinlan clay loam (Typic Ustocrept)	18.57	66.68	14.80	8.3	1.2	15.74	0.58	2.62
Spur silt loam (Fluentic Haplustoll)	13.29	65.28	21.32	7.7	0.9	7.98	0.72	12.51

*Values were obtained with moist soils.

1982). Experiments were later performed at constant matric potential by utilizing the soil moisture curves to determine θ_v at a desired matric potential.

Pot Experiments

The soils were fertilized based on the soil test results at the recommended rate for sorghum cultivation with the exception of P and Fe. Phosphorus was added at the recommended P rate or greater to obtain the desired phosphate treatments. Iron was added at the recommended rate (11.2 kg/ha) with treatments containing the recommended P rate (29.3 kg/ha). The calculated amount of soil for each pot was weighed, then packed to a bulk density of 1.3 g/cm^3 into 2.2 L plastic pots with no drainage holes. Ten sorghum seeds were planted 0.5 cm deep directly in the pots, and the pots were placed randomly into a growth chamber. The growth chamber was programmed with a daylength of 16 h at a daytime temperature of 24^oC and a night time temperature of 18^oC. The light intensity of the growth chamber was set at 380 umol photon m^{-2} s^{-1}. Each pot was watered with deionized-distilled water to keep the soil surface moist and to avoid surface crusting. The pots were covered with plastic sheets to reduce surface evaporation. Seven days after planting, the seedlings were thinned to 6 plants per pot. On the same day, 25 g of perlite was added to the pot to prevent surface drying. Moisture treatments were initiated 10 days after planting. Deionized-distilled water was added daily to bring the weight up to the corresponding moisture content to replace the water lost from each pot by evapotranspiration. Visual ratings of Fe chlorosis severity were obtained once weekly, using a scale of 1 to 5 where 1 was assigned to sorghum plants that were not chlorotic and 5 to those that expressed severe chlorosis (McKenzie, et al. 1984). The experiments were terminated when the plants were six weeks old and final visual ratings were taken at that time.

After termination, plants were harvested at the soil surface, and fresh weight was determined. After leaf discs were taken for chlorophyll analysis, all the plants from each pot were consolidated and dried at 65^{o}C for 48 h. The dry weight yield for each pot was determined and an average weight per plant was calculated. The dried plants were ground to pass through a 20 mesh sieve (850 um) for plant tissue analysis.

Chlorophyll Analysis

Five leaf discs (diameter = 0.7 cm) were obtained from the most recently expanded leaves of each plant. Each disc was taken half way from the top of the leaf blade, avoiding the mid rib. If the plant had less than 5 leaves that were not more than 0.7 cm wide, then 2 discs were taken from the largest leaf blade. Five discs were placed into light-proof test tubes wrapped with aluminum foil. Six ml of DMF (N,N,-Dimethylformamide) was added to each tube, then placed upright on a horizontal shaker and shook for 24 hours. Absorbance was measured at 664 and 647 nm with a Bausch and Lomb model spectronic 710 spectophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll (Chl_T) were calculated from the absorbance readings based on the equations presented by Inskeep and Bloom (1985).

Plant Tissue Analysis

A known amount of dry, finely ground plant tissue from each pot was digested by the nitric-perchloric digestion method. After dilution, the matrix of the plant tissue digests were adjusted to 2 M HCl and were analyzed for P, K, Ca, Mg, Al, Fe, Na, Mn, Zn, Cu, and B with inductively coupled plasma optical spectrometry (ICP) (Munter et al., 1984).

Specific details pertaining to each experiment are described in the following.

Experiment 1: Effects of Soil Moisture

The amount of moist Quinlan and Spur soils required to yield a bulk density of 1.3 g/cm^3 was packed into 2.2 liter plastic containers. While packing each plastic pot with a calculated amount of moist fertilized soil, a copper gas well was inserted into the pot. The opened end of the gas well was placed in the middle of the pot with the closed end 3 cm above the soil surface. The gas wells were 12 cm long, made of 0.63 cm (diameter) copper tubings, brass compression unions, rubber-silicone septum, and had a total volume of approximately 2.5 cm^3 similar to the construction used by Tackett (1968) and Inskeep and Bloom (1986).

A susceptible sorghum variety (RTx428) was planted in soil adjusted to four soil matric potential levels: -0.5, -2.69, -14.1, and -75 kPa. Phosphorus rates and sources used in Quinlan soil were 14.7 kg P/ha as KH_2PO_4, 29.3 kg P/ha as APP, and 29.3 kg P/ha as APP plus 11.2 kg Fe/ha as $FeSO_4.7H_2O$. For the Quinlan soil, 56 kg N/ha were added based on the fertilizer recommendations (Oklahoma State University Extension Fact Sheet No. 2225) for sorghum production in Oklahoma. For the Spur soil, fertilization was not necessary and no P variables were examined. Each treatment was replicated with 3 pots. Plant growth was terminated by harvest six weeks after emergence.

Within 24 h after termination, the partial pressure of soil CO_2 (pCO_2) of each pot was analyzed. After measuring CO_2, approximately 1000 cm^3 of soil was removed from the center portion of each pot for soil solution analysis and stored in plastic bags at 5^oC. At the same time, the moisture content ($_v$) of each pot was determined gravimetrically. DTPA extractable Fe, Cu, and Zn were determined on moist soils using 0.005 M DTPA as an extractant (Lindsay, and Norvell, 1978).

Soil CO_2 and Soil Solution Analysis

Samples of soil atmosphere were withdrawn from the gas wells through the rubber-silicone septum with a 1 ml Hamilton model #1001 gas-tight syringe. Then, the air samples were injected into a Hariba Pir-2000 infrared gas analyzer and the concentration of CO_2 was determined from a standard curve.

The amount of moist soil and distilled water necessary to obtain 0.15 kg of oven dried soil with a 1:0.5 soil:water mix was calculated using $_v$ for each sample. The soil:water mix was stirred until homogenous with a spatula, and the slurry was transferred to a 250 ml centrifuge bottle and centrifuged at 8,000 rpm for 40 m. The supernatants were decanted and further filtered through 0.45 um Metricel membrane filters and stored in 6 ml plastic bottles. The pH was determined immediately after filtration. The HCO_3^- concentration was determined by HCl titration (Stumm and Morgan, 1981), within 1 to 2 h after the soil solution was collected.

Experiment 2: Effects of Phosphorus Treatments

The same sorghum variety, RTx428, was planted in plastic pots packed with either Quinlan and Spur soils. The moisture content chosen for this experiment was determined at a matric potential of -10 kPa. The volumetric water content (θ_v), at -10 kPa of Quinlan and Spur soils were 27 and 30.5 percent, respectively. Sources and rates of P were varied so that the influence of polyphosphate on Fe nutrition of sorghum could be determined. Sources of P fertilizers were KH_2PO_4; monoammonium phosphate ($NH_4H_2PO_4$), MAP; and ammonium polyphosphate [$(NH_4)_3HP_2O_7$], APP. The high and low rates of P fertilizers used in Quinlan were 44.0 and 29.3 kg P/ha, respectively. For the Spur soil, the high and low rates used were 24.5 and 14.7 kg P/ha, respectively. Nitrogen levels provided from different P sources to Quinlan soil were adjusted to supply 56 kg N/ha with NH_4NO_3 fertilization. In addition to these treatments, 11.2 kg Fe/ha as $FeSO_4.7H_2O$ was added to the low rates of KH_2PO_4, MAP, and APP for a total of 8 different P or P + Fe treatments. The appropriate amount of fertilizers needed for each treatment was dissolved in 100 ml of deionized-distilled water in a plastic bottle, and mixed with the soil uniformly prior to packing the pots. All treatments were replicated three times and placed at random into the growth chamber.

Experiment 3: Screening Sorghum Cultivars

Nine cultivars were chosen for this experiment to examine the difference of representative sorghum cultivars to Fe stress in potted calcareous soil. Cultivars chosen were RTx428, BTx623, Wheatland, Redlan, SC33-9-8-E4, SC118-15E, RTx430, RTx2536, and BTx378. The Sorghum Research Breeding Program of the Texas Agricultural Experiment Station at Texas A&M University contributed the seeds for the RTx2536, RTx430, and BTx378 cultivars. Seeds for cultivars of SC118-15E and SC33-9-8-E4 were supplied by the University of Nebraska. Seeds of cultivars RTx428, BTx628, Wheatland, and Redlan were provided by the Sorghum Program at Oklahoma State University. Moisture content in this experiment was kept constant at -10 KPa. Quinlan soil was fertilized with 29.3 kg P/ha as KH_2PO_4 and 56 kg N/ha as NH_4NO_3. Spur soil was not fertilized. Three replications of each cultivar were planted and the pots were placed into the growth chamber at random.

Statistical Analysis

The experimental design of the three pot experiments was a completely randomized design with three replications (Steel and Torrie, 1980). The data collected from these experiments was analyzed through the University Computer Center with the Statistical Analysis

System (SAS, 1982) to generate ANOVA to determine if treatment effects were significant at the 0.05 level of probability. Data from the first experiment were analyzed statistically as a 3 x 4 factorial arrangement of treatments in a completely randomized design and LSD ($P \leq 0.05$) were used to identify differences among treatments. The differences among treatments in the second and third experiments were identified by LSD at $P \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment 1: Effects of Soil Moisture

The Chl_T of plants grown in near saturated soil (-0.5 kPa) was significantly higher than those grown in drier (-2.69, -14.1, and -75 kPa) Quinlan soil (Table 2). There were no significant differences in Chl_T in plants when KH_2PO_4 was added to the soil and adjusted to matric potentials of -2.69, -14.1, and -75 kPa. For soils amended with APP, the Chl_T content in plants was lowest (4.79 ug/cm^2) at a matric potential of -2.69 kPa whereas the Chl_T content in plants at matric potential of -14.1 and -75 kPa were slightly higher (14.79 and 11.91 ug/cm^2, respectively) but differences were not significant at $P \leq 0.05$. Plants growing in Quinlan soil amended with APP and 11.2 kg Fe/ha at a matric potential of -75 kPa had higher Chl_T (37.91 ug/cm^2) than plants grown at -0.05 kPa (23.63 ug/cm^2). There were no differences in Chl_T in plants grown at -0.05 to -14.1 kPa or between -2.69 to -75 kPa. These plants were all visually very healthy and had an average cumulative chlorosis score of near 7 to 8 (Table 2). The chlorosis score was the sum of 5 weekly ratings assigned to each pot during the span of the experiment. Increasing soil moisture level decreased the Chl_T content of the plants grown in Quinlan soil amended with APP and 11.2 kg Fe/ha, however, there were no significant

Table 2. Influence of P sources and soil matric potential in Quinlan soil on soil solution parameters, DTPA-Fe, and dry matter, total chlorophyll content, and chlorosis score of grain sorghum; experiment 1.

P Source	Matric Potential	θ_v	HCO_3^-	pCO_2	pH	DTPA-Fe*	Dry Matter	Chl_T	Chlorosis[+] Score
	kPa	%	(mM)	kPa		(ug/g)	mg/plant	ug/cm^2	
KH_2PO_4	-75.00	15	1.00	0.09	7.65	0.57	119	5.02	18.33
	-14.10	19	0.97	0.16	7.64	0.83	109	5.89	17.00
	- 2.69	26	1.93	0.21	7.72	0.47	154	6.57	19.27
	- 0.50	32	3.36	0.59	7.84	0.80	215	25.72	15.67
APP	-75.00	15	1.62	0.31	7.88	0.63	279	11.91	12.33
	-14.10	19	1.80	0.55	7.95	0.63	391	14.79	12.33
	- 2.69	26	2.37	0.34	7.88	0.60	235	4.79	19.10
	- 0.50	32	3.96	0.81	7.97	1.20	486	16.01	15.23
APP+Fe	-75.00	15	2.21	0.48	7.87	1.20	501	37.91	6.67
	-14.10	19	2.39	0.77	7.89	1.37	506	31.25	6.67
	- 2.69	26	2.26	1.27	7.83	1.37	648	30.24	6.67
	- 0.50	32	3.10	1.04	7.87	1.23	611	23.63	8.17
$LSD(0.05)_{sources}$			0.43	0.22	0.06	0.19	107	4.64	1.47
$LSD(0.05)_{matric\ potential}$			0.50	0.25	0.07	0.22	124	5.36	1.70
$LSD(0.05)_{sources\ x\ mat.pot.}$			0.86	0.43	0.12	0.39	214	9.29	2.91

+Chlorosis score represents the sum of 5 individual visual ratings ranging from 1 to 5 (1=healthy, 5=severely chlorotic). Possible range = 5 to 25.

*Values were obtained with moist soils.

differences in the sum of visual ratings. Dry matter yield of plants grown in the APP + Fe amended soil were not significantly affected by increasing moisture content. Plants grown in Quinlan soil amended with APP + Fe performed better than soil amended with APP and KH_2PO_4 sources. Plants grown at the highest matric potential in soil amended with KH_2PO_4 and APP grew better and healthier than those grown in drier soil amended with the same P sources. Plants grown in Quinlan soil fertilized with APP had higher dry matter production, especially at matric potential of -0.5 kPa, than when KH_2PO_4 was the P sources (Table 2).

The amount of Chl_T of the sorghum plants grown in the Spur soil did not decrease by the increasing soil matric potential (Table 3). There was a slight increase in Chl_T for plants grown in the Spur soil at a matric potential of -0.5 kPa (Table 3), which is consistent with increases in Chl_T observed in the Quinlan soil when KH_2PO_4 or APP applied to the soil at near saturation. However, the chlorosis score of the plants grown in Spur soil at -0.5 kPa was not significantly different from those grown in drier soil conditions (Table 3).

Plants grown in Quinlan soil amended with APP at a matric potential of -14.1 kPa had higher dry matter production and Chl_T than the KH_2PO_4 was added as the P source (Table 2). Quinlan soil was a very Fe deficient soil with a DTPA-Fe level of 0.58 ug/g soil (Table 1). The application of 11.2 kg Fe/ha as $FeSO_4$ with 29.3 kg P/ha as APP in Quinlan soil, resulted in significant increases in dry matter and Chl_T at all matric potentials, when compared to those grown in the APP and KH_2PO_4 amended Quinlan soil (Table 2). This

Table 3. Influence of soil matric potential in Spur soil on soil solution parameters, DTPA-Fe, and dry matter, total chlorophyll content, and chlorosis score of grain sorghum; experiment 1.

Matric Potential	θ_v	HCO_3^-	pCO_2	pH	DTPA-Fe[=]	Dry Matter	Chl_T	Chlorosis[+] Score
kPa	%	(mM)	kPa		(ug/g)	mg/plant	ug/cm^2	
-75.00	18	0.62	0.09	7.11	0.97	69	9.55	17.50
-14.10	22	0.66	0.28	7.20	0.63	137	9.01	17.67
- 2.69	28	0.81	0.37	7.05	0.57	129	8.01	17.67
- 0.50	35	3.00	0.81	7.63	0.60	285	13.24	16.77
*LSD(0.05)		0.30	0.20	0.09	0.42	130	3.81	1.65

+Chlorosis score represents the sum of 5 individual visual ratings ranging from 1 to 5 (1=healthy, 5=severely chlorotic). Possible range = 5 to 25.

=Values were obtained with moist soils.

*LSD for comparison of any two treatment means.

was consistent with the findings of Datin and Westerman (1982) that an application of APP with Fe to calcareous soils reduced Fe chlorosis, and improved plant growth and dry matter production of sorghum. The fact that these plants remained green and healthy for six weeks after an application of Fe shows that the cause of Fe chlorosis in Quinlan soil was lack of Fe. Levels of DTPA extractable Fe were significantly higher in the Quinlan soil at matric potentials of -2.69, -14.1, and -75 kPa when fertilized with APP + Fe than when amended with KH_2PO_4 or APP as the P source at the same matric potentials (Table 2). Datin and Westerman (1982) also observed an increase in DTPA extractable Fe at the termination of their experiments from 1.4 to approximately 2.25 (ug/g of soil) with added P plus Fe treatments and suggested that APP + Fe appeared to be very effective at alleviating chlorosis of grain sorghum in Fe-P deficient soils.

The soil pCO_2 and soil solution HCO_3^- concentration tended to increase with increasing soil moisture (θ_v) in the Quinlan soil amended with all the three P sources and the Spur soil (Fig. 1 and Fig. 2). This was consistent with the relationship among HCO_3^-, pCO_2, and soil matric potential observed in Calciaquolls of western Minnesota (Inskeep and Bloom, 1986). Increases in HCO_3^- and pCO_2 did not appear to result in regular increases in chlorosis as has been observed in dicots. The evidence that HCO_3^- is a necessary factor to induce chlorosis in dicots was shown by Chaney and his colleagues (Coulombe et al., 1984a and 1984b; and Fleming et al., 1984). Inskeep and Bloom (1986) provided further evidence that the Chl_T of Anoka soybeans grown in calcareous soils decreased with increased soil moisture, soil pCO_2, and soil solution HCO_3^-.

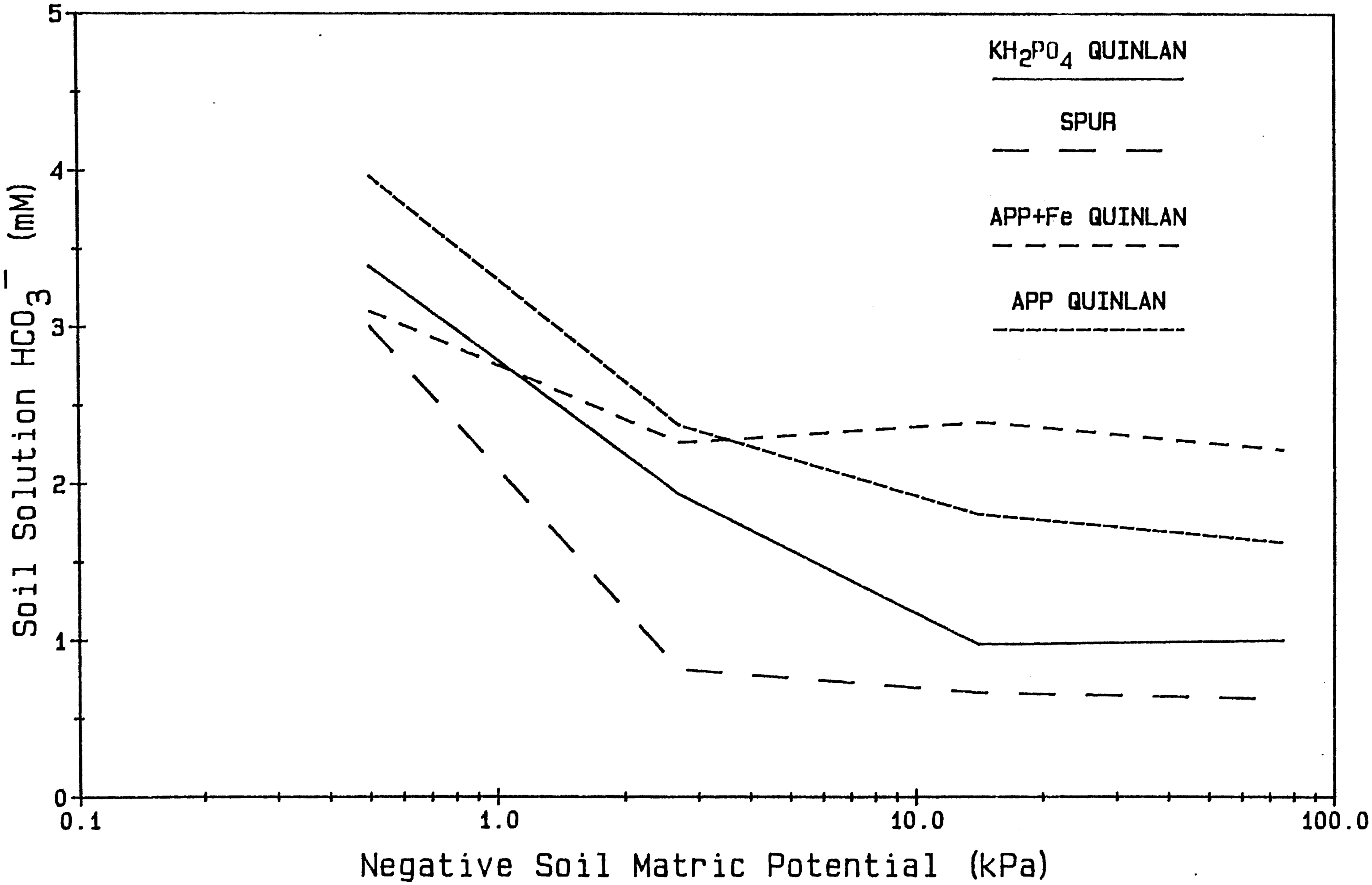

Fig. 1. The effect of soil matric potential on pCO_2 in Spur soil and Quinlan soil amended with three P sources that were cropped with grain sorghum.

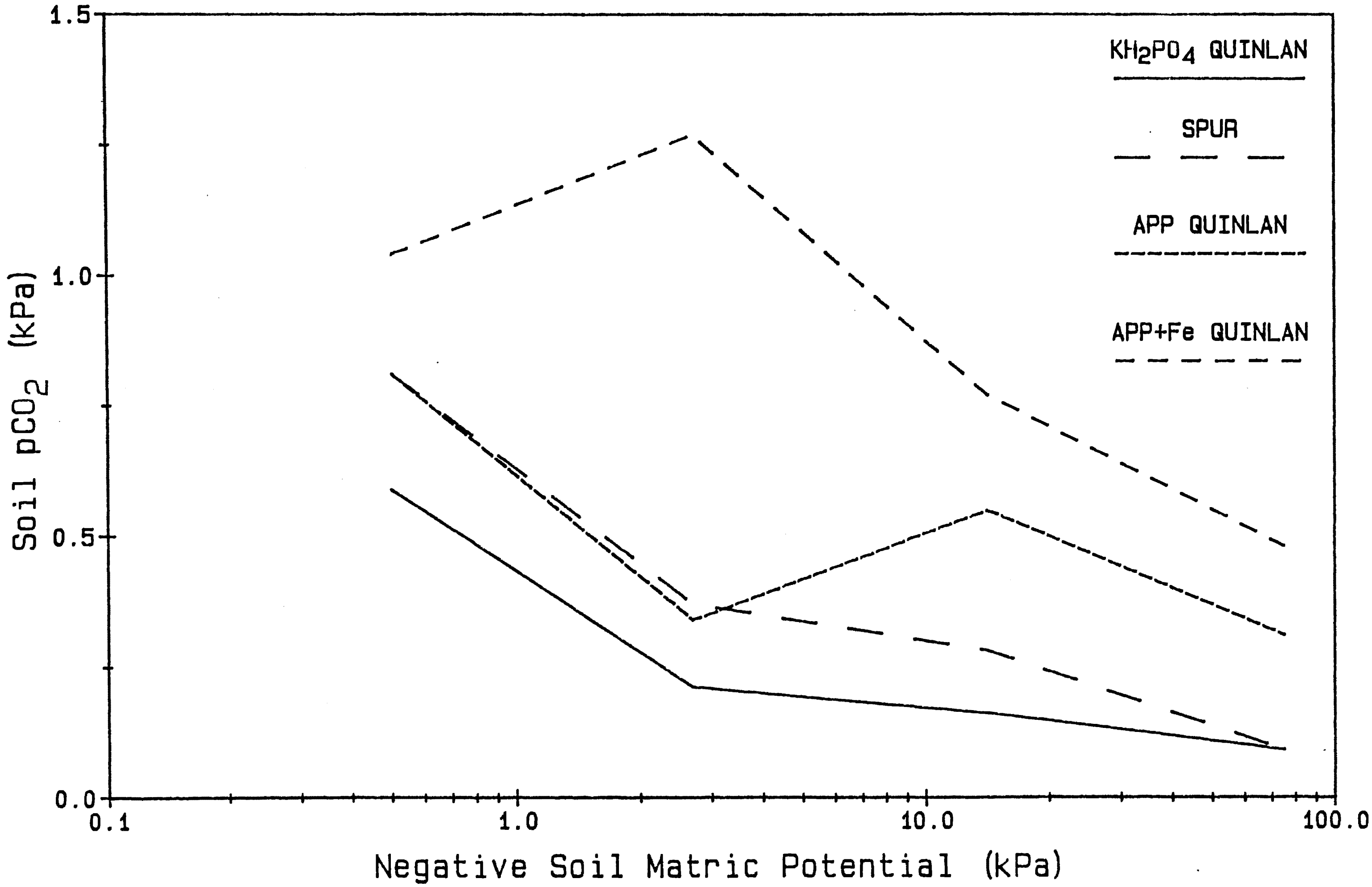

Fig. 2. The effect of soil matric potential on soil solution HCO_3^- in Spur soil and Quinlan soil amended with three P sources that were cropped with grain sorghum.

Soils used in their experiments were not considered Fe deficient as determined by DTPA extraction. The pH remained relatively constant when HCO_3^- and pCO_2 increased with increasing matric potential of the soil, probably as a result of H^+ buffering by cation exchange sites (Inskeep and Bloom, 1986). Consequently, Fe chlorosis in those conditions was associated with HCO_3^- induced chlorosis. Sorghum plants in this experiment did not develop chlorosis that was associated with increasing HCO_3^- or pCO_2 which supports the idea that the strategy to absorb Fe in monocots is different from that found in dicots (Römheld and Marschner, 1986). Chlorosis in both the Quinlan and Spur soils was a result of lack of available Fe and not contributed by the increased HCO_3^- concentration associated with increasing soil moisture content.

During the span of the experiment, sorghum plants in the wettest Quinlan soil (-0.5 kPa) with APP and KH_2PO_4 as the sources of P were more chlorotic during the first 3 weeks. However, greening up of the initially chlorotic plants in these treatments was observed after the third week (Table 4). The near saturated condition of these soils may have provided a favorable environment for the reduction of Fe^{3+} to Fe^{2+}. The higher solubility of the Fe^{2+} compounds in near saturated soils (-0.5 kPa) may have contributed to an increase in the supply of available Fe for the plants uptake (Tisdale and Nelson, 1975).

Experiment 2: Effects of Phosphorus Fertilizers

Ammonium polyphosphate (APP), MAP, and KH_2PO_4 were used to determine the effects of P fertilizers on the deficiency of Fe in

Table 4. Influence of P sources and soil matric potential on Fe chlorosis of grain sorghum grown in Quinlan soil.

Treatments P Source	Matric Potential	θ_v	Days 16	22	30	36	41	Chlorosis Scores[+]
	kPa	%	Visual Ratings*					
KH_2PO_4	-75.00	15	2.83	2.67	4.17	4.17	4.50	18.33
	-14.10	19	2.83	2.33	3.67	3.83	4.33	17.00
	- 2.69	26	3.17	3.33	4.33	4.17	4.27	19.27
	- 0.50	32	4.17	4.33	2.83	2.33	2.00	15.67
APP	-75.00	15	1.67	2.00	2.67	3.00	3.00	12.33
	-14.10	19	1.67	2.00	3.00	2.83	2.83	12.33
	- 2.69	26	3.17	3.33	4.17	4.00	4.43	19.10
	- 0.50	32	3.50	3.50	2.83	2.73	2.67	15.23
APP+Fe	-75.00	15	1.00	1.33	1.67	1.33	1.33	6.67
	-14.10	19	1.00	1.50	1.50	1.33	1.33	6.67
	- 2.69	26	1.17	1.50	1.33	1.33	1.33	6.67
	- 0.50	32	1.33	1.50	1.67	1.83	1.83	8.17
$LSD(0.05)_{source}$			0.24	0.27	0.59	0.46	0.40	1.47
$LSD(0.05)_{matric\ potential}$			0.28	0.31	0.68	0.53	0.46	1.70
$LSD(0.05)_{source\ x\ mat.pot.}$			0.49	0.54	1.17	0.91	0.80	2.91

*Visual ratings range from 1 to 5 (1 = healthly, 5 = severely chlorotic).
+Chlorosis score represents the sum of 5 individual visual ratings.

sorghum. Plants grown in Quinlan soil fertilized with 29.3 kg P/ha as APP had higher dry matter yield than those grown in soil with applications of the same rate of P as MAP (Table 5 and Fig. 3). Quinlan soil amended with APP produced plants that were significantly less chlorotic than the soil amended with MAP or KH_2PO_4 (Table 5 and Fig. 4). The chlorosis score (a sum of 5 individual scores) assigned to the plants was significantly lower in APP fertilized pots than in the MAP and KH_2PO_4 treated pots (Table 5). The correlation between Chl_T and the sum of the weekly chlorosis ratings was -0.93. Similarly, the correlation between Chl_T and the final visual ratings assigned to the plants during the termination of the experiment was -0.94.

The higher rate (44.0 kg P/ha) of APP applied in Quinlan soil promoted a significant increase in dry matter production as well as a significant increase in Chl_T compared to the lower rate (29.3 kg/ha) (Table 4, Fig. 3 and Fig. 4). However, the dry matter yield and the Chl_T were not significantly increased by the higher rate of MAP application (Table 4, Fig. 3 and Fig. 4). The increase in dry matter production and Chl_T of plants grown in Quinlan soil amended with APP over those grown with MAP as a P source may be attributed to the lower NO_3^-/NH_4^+ ratio in the soil. Quinlan soil contained a supply of NO_3^- from NH_4NO_3 which was used to provide the plants with the balance amount of the recommended 56 kg N/ha. Bernardo et al., (1984a and 1984b) found that a lower NO_3^-/NH_4^+ ratio enhanced forage yield and decreased chlorosis in sorghum.

DTPA extractable Fe was significantly lower in Quinlan soil with an application of 44.0 than with 29.3 kg P/ha as APP (Table 5).

Table 5. Influence of P sources and rates in Quinlan soil on soil solution parameters, DTPA-Fe, and dry matter, total chlorophyll content, chlorosis score, and P/Fe ratio of grain sorghum; experiment 2.

P Source	Rate	HCO_3^-	pH	DTPA-Fe=	Dry Matter	Chl_T	Chlorosis+ Score	P/Fe Ratio
	kg P/ha	(mM)		(ug/g)	mg/plant	ug/cm^2		
KH_2PO_4	29.3	0.95	7.53	0.93	174	3.44	20.67	126
KH_2PO_4+Fe	29.3	1.90	7.80	2.13	689	24.83	6.16	18
APP	29.3	1.50	7.66	1.40	391	11.16	13.40	71
APP	44.0	1.55	7.82	0.87	842	15.05	11.30	28
APP+Fe	29.3	1.57	7.81	1.80	699	29.30	5.50	13
MAP	29.3	0.93	7.38	1.33	104	5.58	17.50	84
MAP	44.0	0.93	7.43	1.53	124	5.69	18.50	122
MAP+Fe	29.3	1.22	7.55	1.53	188	32.04	8.26	25
* LSD(0.05)		0.50	0.20	0.39	169	3.56	1.37	54
**LSD(0.05)$_{sources}$		0.59	0.24	0.44	185	4.12	1.29	55

+Chlorosis score represents the sum of 5 individual visual ratings ranging from 1 to 5 (1=healthy, 5=severely chlorotic). Possible range = 5 to 25.

=Values were obtained with moist soils.

*LSD for comparison of any two treatment means.

**LSD for comparison of P sources means at 29.3 kg P/ha with and without Fe.

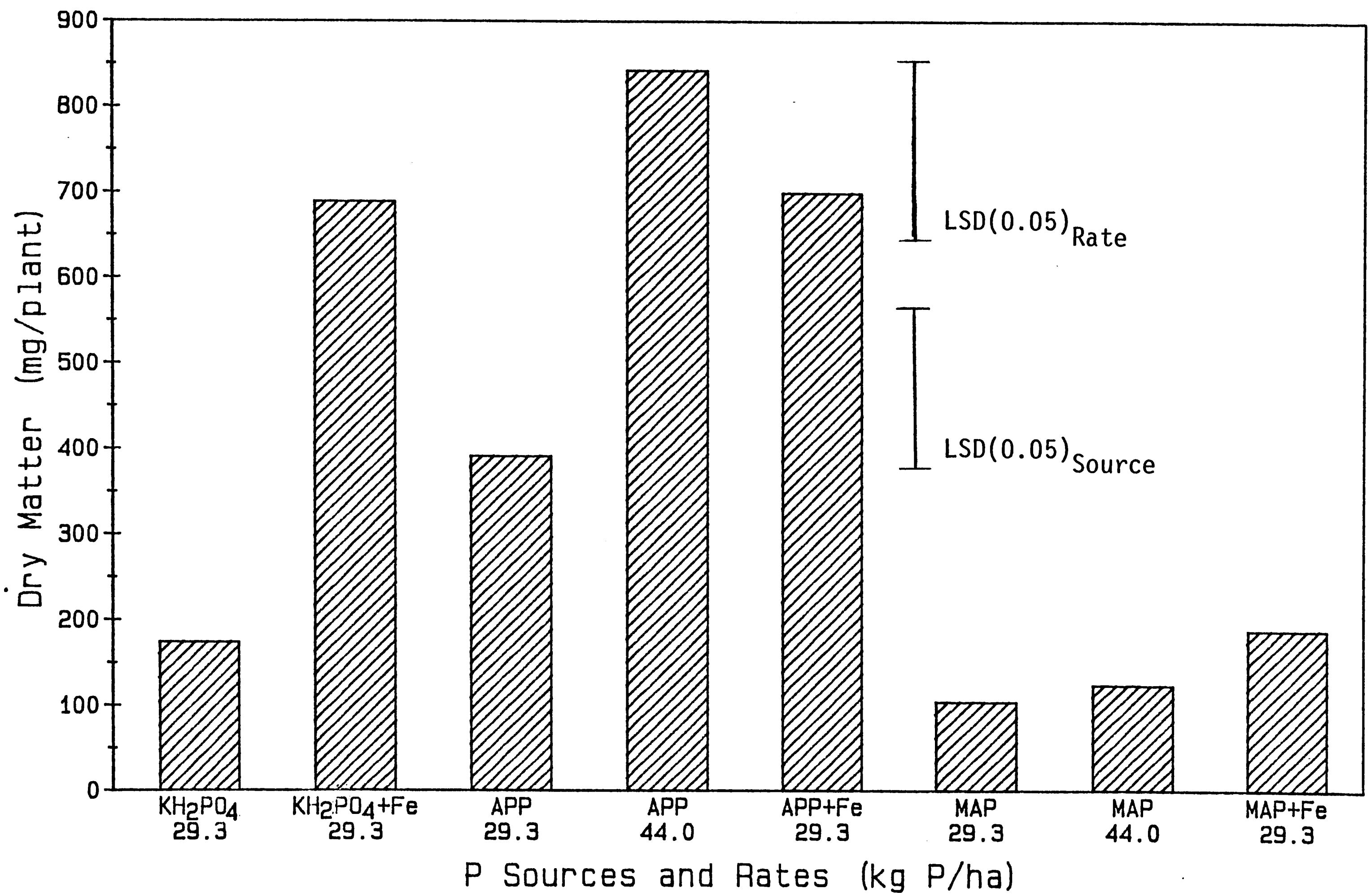

Fig. 3. Dry matter production of grain sorghum grown in Quinlan soil with different P sources and rates with and without Fe.

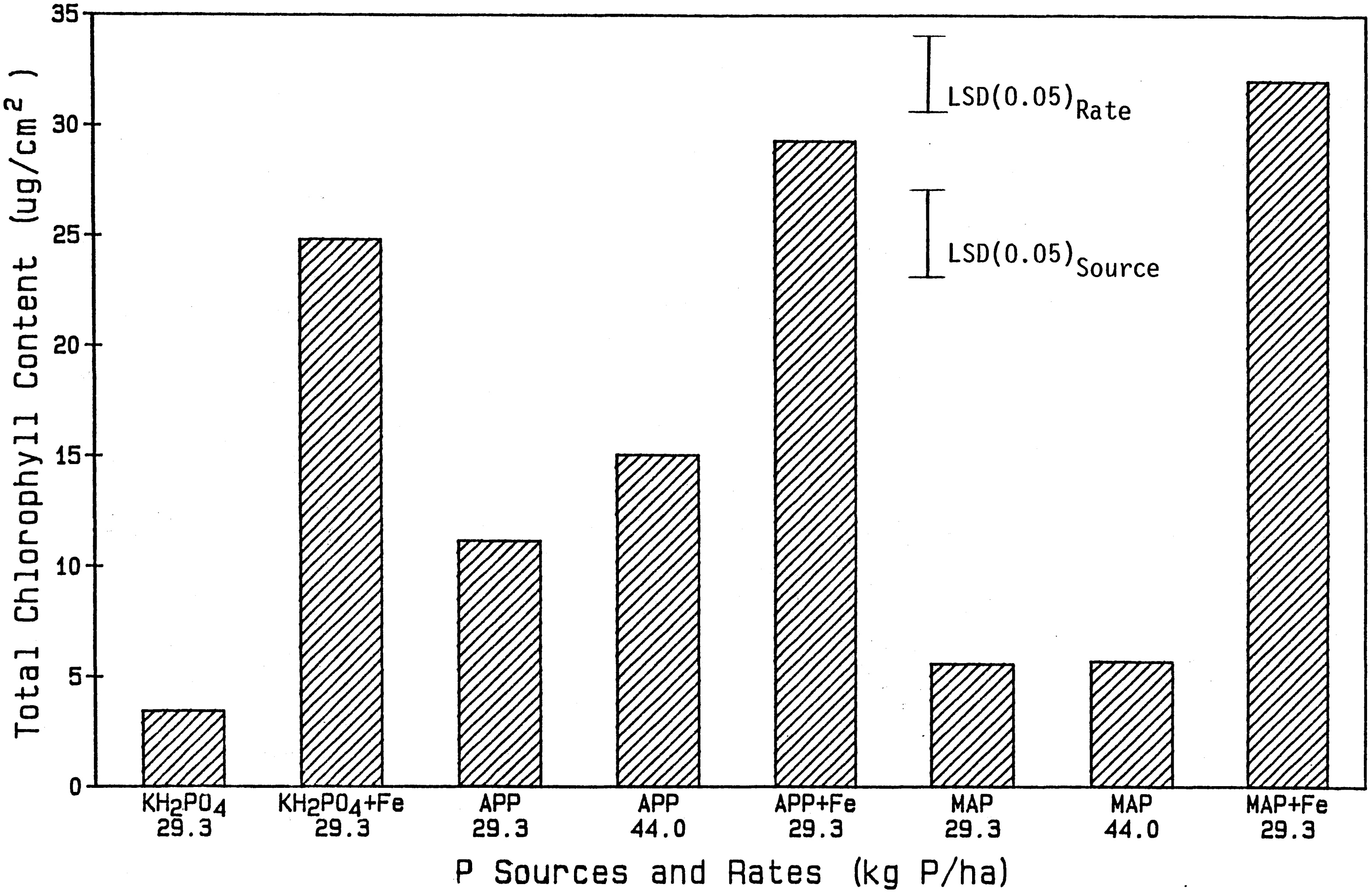

Fig. 4. Total chlorophyll content of grain sorghum grown in Quinlan soil with different P sources and rates with and without Fe.

However, there was a significantly greater amount of Fe taken up by plants that were grown in soils fertilized by the higher rate of APP (Table 5). Conversely, DTPA extractable Fe and uptake of Fe by plants grown in Quinlan soil amended with both 44.0 and 29.3 kg P/ha as MAP were not significantly different (Table 5 and Table 6). The addition of Fe with KH_2PO_4, MAP, or APP to the Quinlan soil resulted in an increase in Chl_T (Fig. 4) indicating that plants were able to take up the supplied Fe. These results supported the conclusion from Experiment 1 that Fe chlorosis of sorghum in these soils was a result of Fe deficiency and not attributed by HCO_3^- induced chlorosis associated with increasing soil moisture content. The addition of APP appears to enhance the uptake of Fe and plant growth significantly. This was consistent with the results discussed by Datin and Westerman (1982) and Mortvedt (1982).

The influence of APP over MAP or KH_2PO_4 on Fe chlorosis was not observed in the Spur soil (Table 7). The Chl_T of plants that were grown in the soil with both low and high rates of APP was not significantly different from those grown with MAP or KH_2PO_4 as the source of P (Table 7 and Fig. 5). Plants grown at the high rate of APP produced slightly more dry matter, but not significantly more than plants grown at the low rate of APP (Table 7 and Fig. 6). Characterization of the Quinlan and Spur soils (Table 1) showed that the Spur soil had a sufficient amount of extractable P for the production of sorghum whereas Quinlan was very P deficient. The high amount of P in the Spur soil may have inhibited the absorption and translocation of Fe in sorghum and thus overwhelmed the enhancing effect of APP on Fe chlorosis (Ajakaiye, 1979). The combined effect

Table 6. Influence of P sources and rates in Quinlan soil on the uptake of nutrients by grain sorghum; experiment 2.

Source	Rate	Uptake P	K	Ca	Mg	Mn	Al	Na	Fe	Zn	Cu	B
	kg P/ha	mg/plant				ug/plant						
KH_2PO_4	29.3	1.20	7.89	3.06	0.92	57.34	72.48	44.51	9.22	25.78	8.16	3.05
KH_2PO_4+Fe	29.3	1.07	6.94	14.73	3.66	118.15	162.17	112.80	62.85	49.59	11.25	16.72
APP	29.3	1.91	7.31	9.82	2.92	167.45	89.39	64.46	28.58	69.29	33.69	10.23
APP	44.0	1.79	7.03	23.44	9.93	285.16	292.74	216.79	64.09	76.25	22.91	28.69
APP+Fe	29.3	1.23	7.90	14.36	3.85	92.65	117.01	100.82	88.74	48.01	44.91	12.78
MAP	29.3	0.44	4.03	2.08	0.62	26.41	29.99	21.77	4.58	27.07	4.82	2.01
MAP	44.0	0.66	5.19	2.47	0.72	36.78	46.46	29.79	5.62	18.12	4.11	2.17
MAP+Fe	29.3	0.39	4.63	2.96	0.92	22.56	25.82	29.39	15.53	19.71	8.90	2.47
* LSD(0.05)		0.64	2.65	3.70	2.68	46.44	77.64	56.59	43.63	32.93	18.00	7.30
**LSD(0.05)$_{source}$		0.50	2.56	3.87	1.02	52.18	58.38	33.97	47.44	33.96	17.93	5.61

*LSD for comparison of any two treatment means.

**LSD for comparison of P sources means at 29.3 kg P/ha with and without Fe.

Table 7. Influence of P sources and rates in Spur soil on soil solution parameters, DTPA-Fe, and dry matter, total chlorophyll content, chlorosis score, and P/Fe ratio of grain sorghum; experiment 2.

P Source	Rate	HCO_3^-	pH	DTPA-Fe=	Dry Matter	Chl_T	Chlorosis+ Score	P/Fe Ratio
	kg P/ha	(mM)		(ug/g)	mg/plant	ug/cm^2		
KH_2PO_4	14.7	0.53	6.97	0.87	106	7.04	17.80	99
KH_2PO_4+Fe	14.7	0.61	6.99	0.93	289	34.23	8.00	28
APP	14.7	0.50	7.04	1.13	157	5.96	19.90	170
APP	24.5	0.74	6.99	0.87	238	7.40	17.80	159
APP+Fe	14.7	1.16	7.24	1.47	910	42.15	6.90	24
MAP	14.7	0.54	7.01	0.80	136	7.30	18.70	135
MAP	24.5	0.51	7.06	1.07	148	6.65	19.30	120
MAP+Fe	14.7	1.07	7.38	1.53	556	42.60	7.00	20
* LSD(0.05)		0.65	0.35	0.39	337	3.51	1.66	33
**$LSD(0.05)_{source}$		0.69	0.33	0.35	395	4.13	1.62	37

+Chlorosis score represents the sum of 5 individual visual ratings ranging from 1 to 5 (1=healthy, 5=severely chlorotic). Possible range = 5 to 25.

=Values were obtained with moist soils.

*LSD for comparison of any two treatment means.

**LSD for comparison of P sources means at 29.3 kg P/ha with and without Fe.

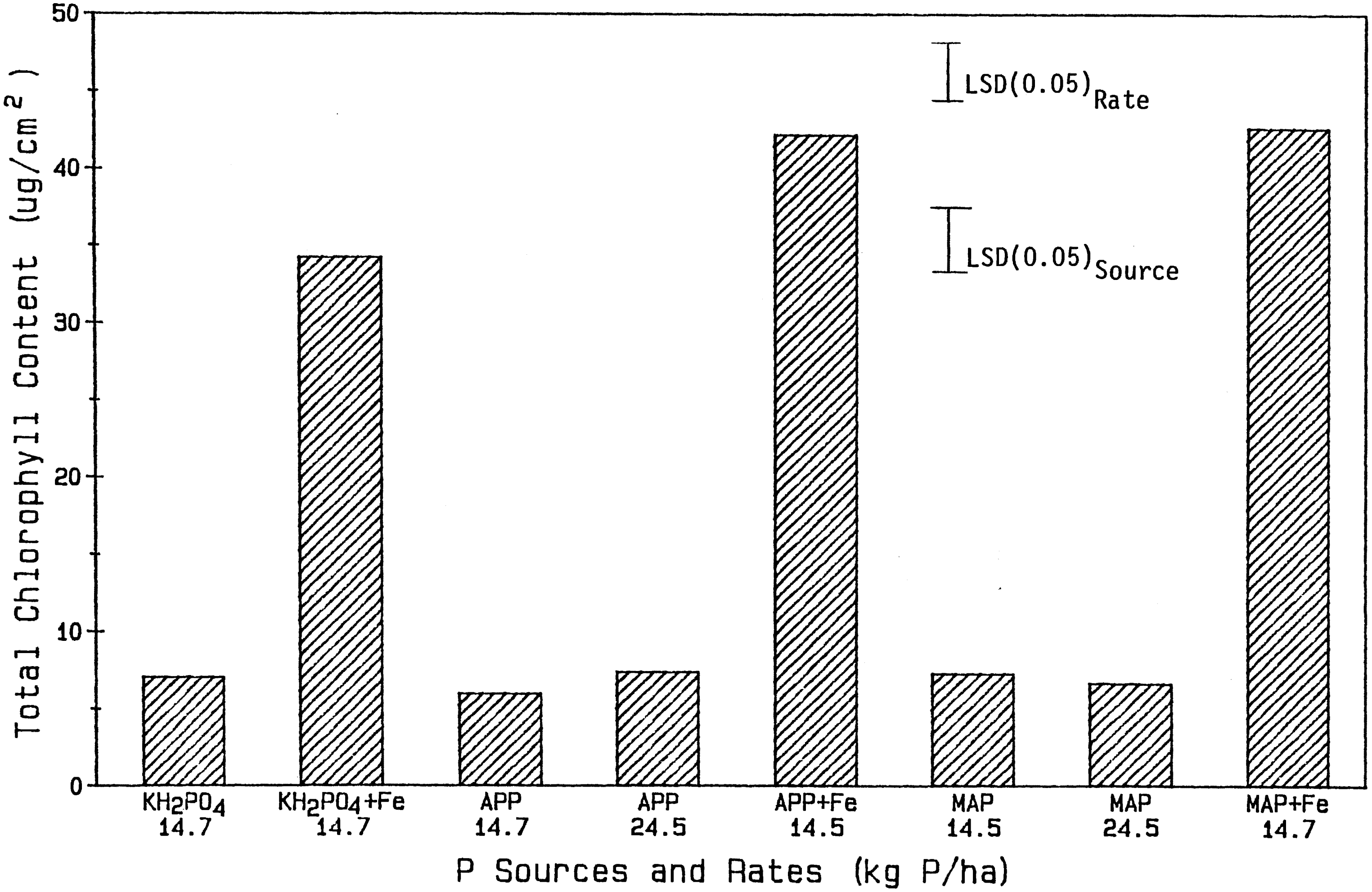

Fig. 5. Total chlorophyll content of grain sorghum grown in Spur soil with different P sources and rates with and without Fe.

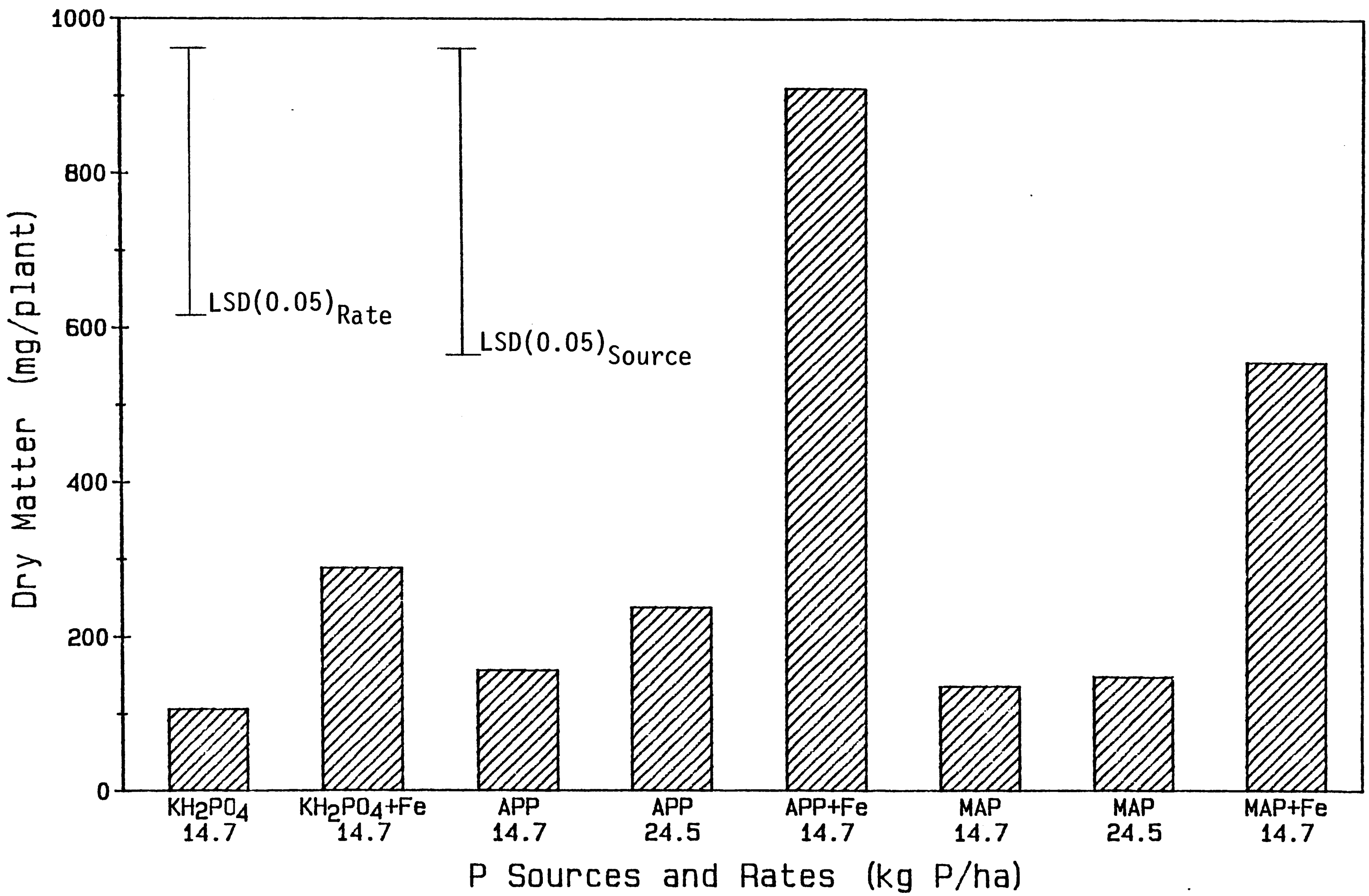

Fig. 6. Dry matter production of grain sorghum grown in Spur soil with different P sources and rates with and without Fe.

of HCO_3^- and P in the Spur soil may have resulted in P induced Fe chlorosis, as has been shown by Adams (1980) and Kolesch et al. (1984). High P in the presence of HCO_3^- has resulted in an increase in Fe deficiency of soybeans (Coulombe et al., 1984a and 1984b). Like the Quinlan soil, the addition of Fe to APP and MAP promoted significantly larger plants that were not chlorotic (Table 7).

Nutrient analysis of the plant tissue showed that plants grown in Quinlan soil fertilized with APP at the rate of 29.3 kg P/ha contained significantly higher concentrations of Ca, Fe, Mn, and B per g of dry matter than those plants that had MAP and KH_2PO_4 fertilization (Table 8). The increased Fe concentration and Fe uptake in the plants that were grown in Quinlan soil with APP application may have promoted the bigger and less chlorotic plants. The mechanism associated with the increased plant uptake of Fe in the presence of APP may be due to the solubilization of organic matter by polyphosphate, which could have complexed more Fe and thus increased the amount of total soluble Fe in the soil (Mortvedt and Osborn, 1977; Sample et al., 1980). Alternatively, APP may stimulate root growth enough so that the total uptake of most nutrients increases, hence, improving the growth of the plants. The concentration of P in the plants grown in Quinlan soil, to which 29.3 kg P/ha of APP or MAP was applied, were not significantly different (Table 7). However, P concentration in the plants grown at 44.0 kg P/ha of APP was significantly lower than that of the same rate of MAP (Table 7).

Plant P, K, and Ca concentration were significantly greater in chlorotic plants regardless of P source applied to Quinlan soil compared to those grown with the same P sources in presence of Fe (Table 8). The lower concentration of P, K, and Ca in the tissues of the nonchlorotic plants may be due to the dilution effect caused by their significantly higher dry matter yield. The higher concentration of P, K, and Ca in the chlorotic plants were consistent with the findings presented by Brown, et al. (1959a and 1959b) who suggested that the development of chlorosis appeared to be related to the P concentration in the plant tissues. The concentration of Mg, Mn, Al, Na, Zn, Cu, and B did not seem to follow the trend shown by the concentration of P, K, and Ca.

The total uptake of plant nutrients was dependent upon the amount of dry matter. Uptake of nutrients by plants grown in Quinlan soil amended with APP was higher than when grown with MAP as the P source (Table 6). This increase in nutrient uptake was directly related to the higher amount of the dry matter produced by the plants that were grown in soil with APP fertilization. Plants grew faster and were able to absorb more nutrients when APP was used to fertilize the P deficiency soil.

The addition of Fe as $FeSO_4$ with the P sources significantly increased the concentration (Fig. 7) and uptake of Fe (Fig. 8), and decreased the concentration of plant P (Fig. 9, Table 7). The P/Fe ratios of the chlorotic plants were higher than the nonchlorotic plants (Tables 5 and 7) which supported the proposal of DeKock (1955) that Fe deficiency in plants could be assessed by the P/Fe ratio. Although, additions of Fe as $FeSO_4$ were successful at correcting the Fe deficiency of the sorghum plants, the concentration of the P in the plant tissue was significantly decreased (Fig. 9) and plant Fe was

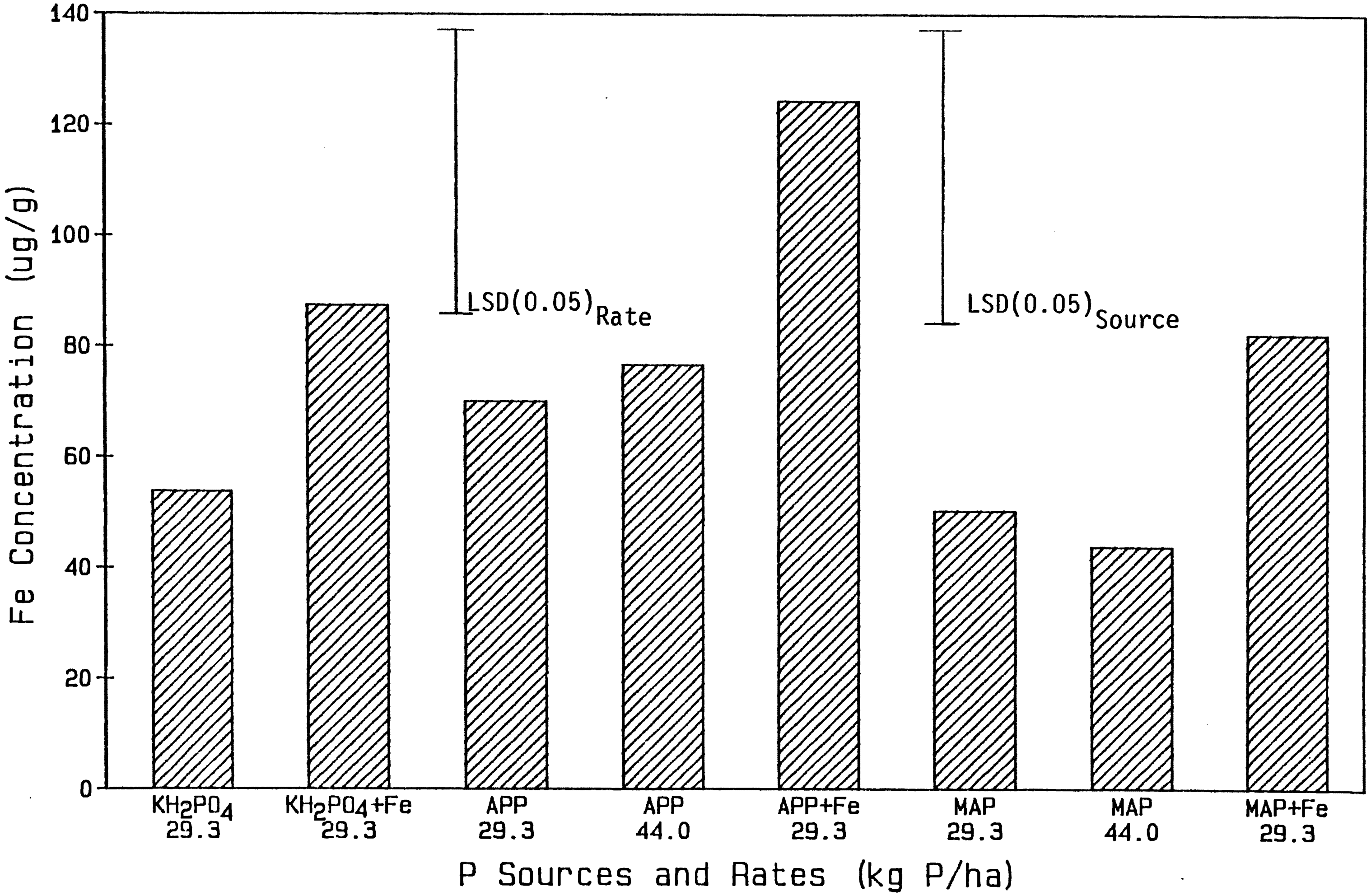

Fig. 7. Iron concentration in the tissue of grain sorghum grown in Quinlan soil with different P sources and rates with and without Fe.

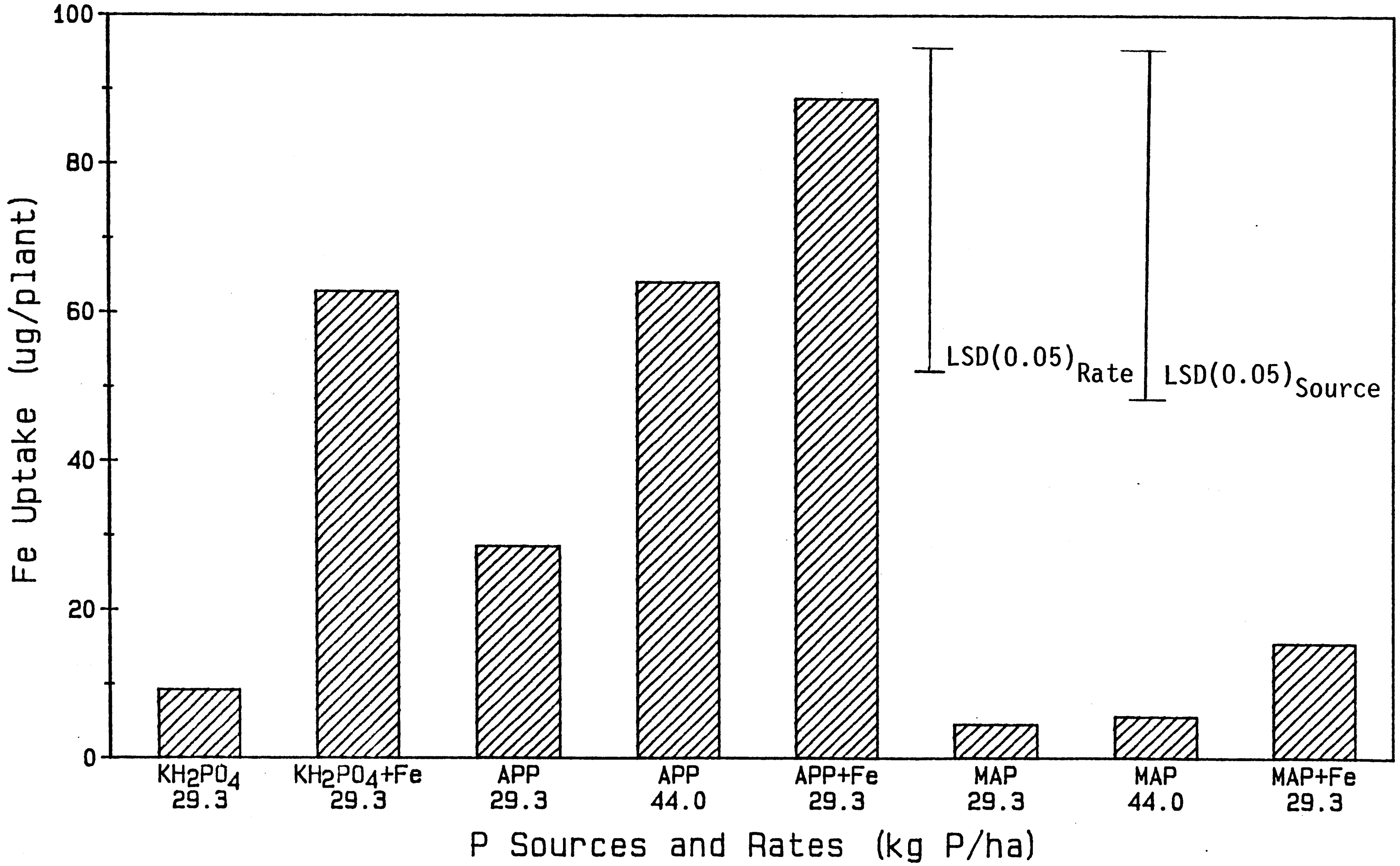

Fig. 8. Iron uptake by grain sorghum grown in Quinlan soil with different P sources and rates with and without Fe.

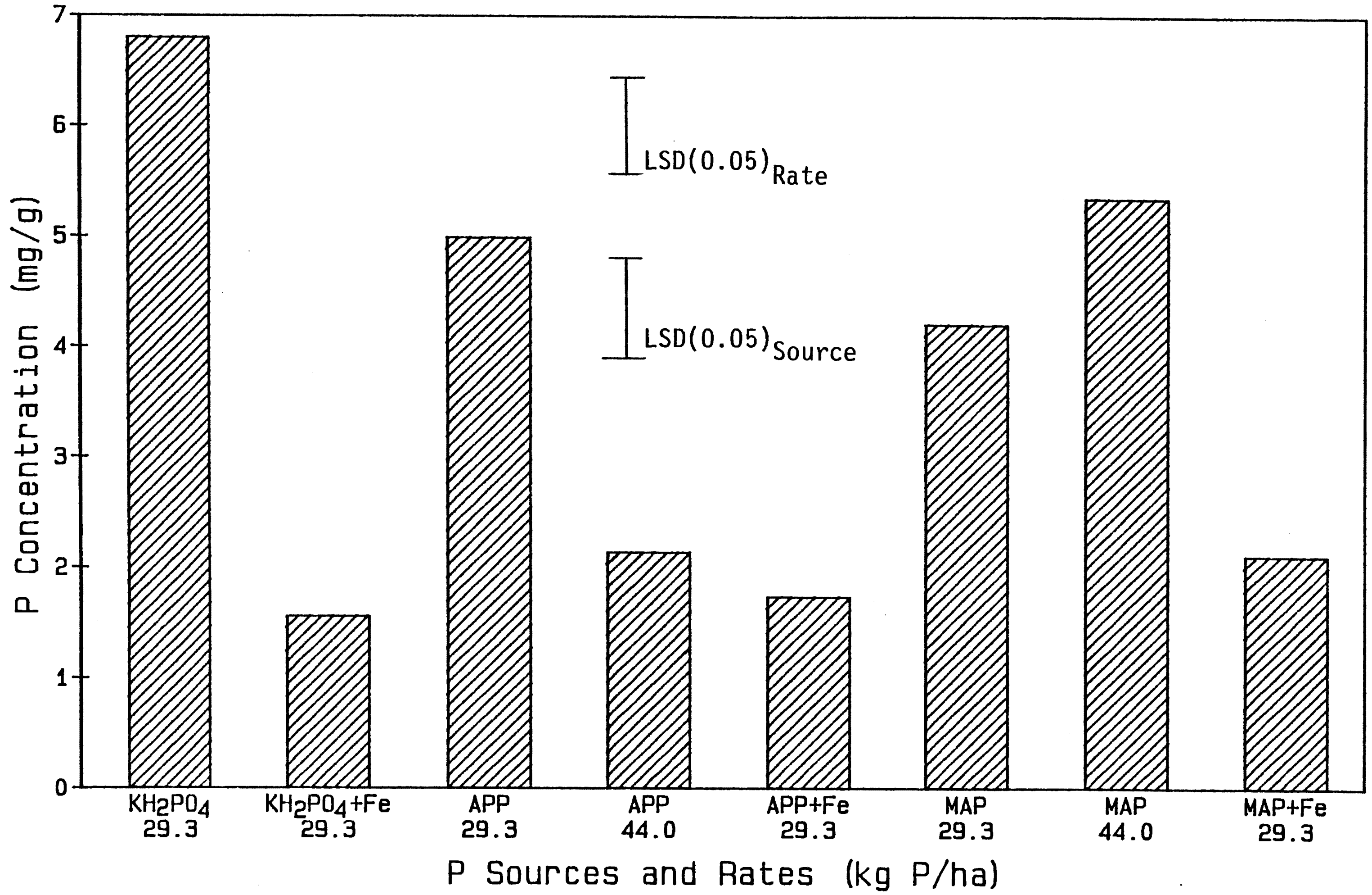

Fig. 9. Phosphorus concentration in the tissue of grain sorghum grown in Quinlan soil grown with different P sources and rates with and without Fe.

significantly increased (Fig. 7). Phosphorus deficiency symptoms, red coloration along the margin of the leaves, were observed in the plants that were not chlorotic. There was an inverse relationship between the concentration of Fe and P in the plants. Higher Fe concentration and uptake that was observed in plants grown in the soils that were the fertilized with APP + Fe than with MAP + Fe or KH_2PO_4 + Fe indicating that APP + Fe was the most effective treatment for alleviating Fe deficiency.

The influence of APP on the plant nutrient concentrations that was observed in the Quinlan soil were not observed in the Spur soil (Table 8). The concentration and uptake of P by the plants grown in KH_2PO_4 treatment were significantly lower than those grown in APP and MAP (Table 8). Like the Quinlan soil, the addition of Fe as $FeSO_4$ with KH_2PO_4, APP, and MAP to the Spur soil increased the Fe concentration in the plant tissue, the uptake of Fe, and decreased the concentration and uptake of P (Tables 9 and 10).

Experiment 3: Screening Sorghum Cultivars

The Chl_T and chlorosis scores of the nine cultivars grown in the Quinlan soil ranged from 6.54 to 2.15 ug/cm^2 and 13.0 to 20.0, respectively (Table 11). The Chl_T of cultivars grown in the Spur soil, ranged from 11.27 to 4.25 ug/cm^2 and the chlorosis scores ranged from 10.08 to 15.83 (Table 11 and Fig. 10). The correlation between the Chl_T and the chlorosis score was -0.80. Although the nine sorghum cultivars grown on both soils expressed a range in Chl_T and chlorosis scores, they were all chlorotic. Iron chlorosis of each cultivar was more severe in the Quinlan soil than in the Spur soil.

Table 8. Influence of P sources and rates in Quinlan soil on the concentration of nutrients in grain sorghum; experiment 2.

Source	Rate	Concentration										
		P	K	Ca	Mg	Mn	Al	Na	Fe	Zn	Cu	B
	kg P/ha	mg/g							ug/g			
KH_2PO_4	29.3	6.80	45.19	17.48	5.26	0.32	0.46	0.28	53.76	147.30	42.56	17.36
KH_2PO_4+Fe	29.3	1.56	10.65	21.14	5.27	0.17	0.24	0.17	87.42	71.00	17.45	23.78
APP	29.3	4.99	19.00	25.07	7.46	0.43	0.23	0.16	70.00	169.10	82.99	26.32
APP	44.0	2.14	8.29	27.85	11.70	0.34	0.35	0.26	76.61	67.20	28.96	33.77
APP+Fe	29.3	1.74	11.04	20.89	5.57	0.13	0.17	0.15	124.32	95.92	73.08	18.48
MAP	29.3	4.21	38.66	20.01	5.98	0.25	0.28	0.20	50.33	260.30	46.33	19.28
MAP	44.0	5.35	41.37	20.11	5.82	0.30	0.39	0.25	43.85	146.50	31.92	17.77
MAP+Fe	29.3	2.11	24.97	15.63	5.04	0.12	0.14	0.15	82.32	103.60	44.80	12.88
* LSD(0.05)		0.90	4.54	2.44	2.85	0.05	0.35	0.17	51.29	99.95	41.42	7.29
**$LSD(0.05)_{source}$		1.02	4.87	2.22	0.46	0.06	0.37	0.17	53.03	118.59	45.34	5.30

*LSD for comparison of any two treatment means.

**LSD for comparison of P sources means at 29.3 kg P/ha with and without Fe.

Table 9. Influence of P sources and rates in Spur soil on the concentration of nutrients in grain sorghum; experiment 2.

Source	Rate	P	K	Ca	Mg	Mn	Al	Na	Fe	Zn	Cu	B
		Concentration										
	kg P/ha	mg/g							ug/g			
KH_2PO_4	14.7	4.20	27.20	20.64	5.30	0.35	0.45	0.28	42.57	85.96	61.75	34.98
KH_2PO_4+Fe	14.7	1.50	14.22	23.34	4.61	0.19	0.25	0.17	53.20	67.76	70.67	34.72
APP	14.7	6.49	34.33	19.64	5.44	0.37	0.52	0.31	38.15	71.02	40.70	34.91
APP	24.5	6.33	21.47	28.08	6.75	0.57	0.32	0.19	39.76	62.72	45.36	51.52
APP+Fe	14.7	1.99	15.40	19.44	4.90	0.20	0.63	0.30	83.44	47.04	17.92	45.36
MAP	14.7	5.25	33.60	18.25	5.30	0.33	0.39	0.29	38.64	76.72	33.60	31.36
MAP	24.5	5.47	22.90	24.70	5.61	0.51	0.28	0.20	45.08	82.01	69.82	44.71
MAP+Fe	14.7	1.60	11.02	26.55	4.71	0.27	0.23	0.13	81.76	63.28	15.12	52.64
* LSD(0.05)		0.86	5.19	4.56	0.44	0.07	0.45	0.15	15.25	19.41	36.27	11.45
**$LSD(0.05)_{source}$		0.85	3.92	3.81	0.39	0.06	0.53	0.17	16.12	20.89	39.44	13.23

*LSD for comparison of any two treatment means.
**LSD for comparison of P sources means at 29.3 kg P/ha with and without Fe.

Table 10. Influence of P sources and rates in Spur soil on the uptake of nutrients by grain sorghum; experiment 2.

Source	Rate	Uptake P	K	Ca	Mg	Mn	Al	Na	Fe	Zn	Cu	B
	kg P/ha	mg/plant				ug/plant						
KH_2PO_4	14.7	0.46	2.90	2.26	0.56	38.89	48.28	29.41	4.81	9.64	7.17	3.73
KH_2PO_4+Fe	14.7	0.43	4.03	6.75	1.33	55.25	70.23	49.42	15.17	18.82	18.49	10.20
APP	14.7	1.00	5.35	3.14	0.87	57.79	99.04	56.16	6.42	10.83	6.07	5.51
APP	24.5	1.48	4.90	6.89	1.63	137.98	74.67	42.48	9.30	14.68	10.04	12.47
APP+Fe	14.7	1.81	13.85	17.28	4.41	190.26	398.00	286.82	80.13	42.69	16.70	45.40
MAP	14.7	0.73	4.59	2.47	0.72	44.61	54.04	42.08	5.37	10.56	4.76	4.30
MAP	24.5	0.81	3.26	3.75	0.83	77.33	43.50	31.06	6.81	11.85	10.00	6.58
MAP+Fe	14.7	0.82	5.63	15.18	2.64	157.67	130.15	71.35	44.48	34.17	8.66	30.93
* LSD(0.05)		0.73	4.64	7.38	1.62	97.73	117.26	127.06	31.99	16.16	9.85	24.36
**LSD(0.05)$_{source}$		0.79	5.45	8.42	1.87	103.02	137.20	150.05	37.84	18.88	11.43	28.68

*LSD for comparison of any two treatment means.
**LSD for comparison of P sources means at 29.3 kg P/ha with and without Fe.

Table 11. Performance of nine grain sorghum cultivars in Quinlan and Spur soils.

Cultivars	Tolerant[a] or Susceptible	Quinlan Chl_T	Quinlan Chlorosis+ Score	Quinlan Dry Matter	Spur Chl_T	Spur Chlorosis+ Score	Spur Dry Matter
		ug/cm^2		mg/plant	ug/cm^2	mg/plant	
RTx430	T	6.54	13.00	139	11.27	10.83	182
RTx2536	T	6.18	13.20	164	10.35	10.50	205
SC33-9-8-E4	T	4.90	15.00	243	9.17	12.00	271
Redlan	S	4.58	17.00	115	6.16	13.67	176
BTx378	S	4.57	18.00	157	9.49	13.17	216
Wheatland		3.95	19.30	130	4.73	17.00	208
BTx623	S	3.24	18.30	113	5.39	15.33	167
SC118-15E		3.24	19.30	170	6.30	14.30	219
RTx428		2.15	20.00	118	4.25	15.83	125
*LSD (0.05)		1.84	1.16	60	2.07	1.69	117

[a]Source: McKenzie et al., 1984 and Williams et al., 1982.
+Chlorosis score represents the sum of 5 individual visual ratings ranging from 1 to 5 (1=healthy, 5=severely chlorotic). Possible range = 5 to 25.
*LSD for comparison of any two treatment means.

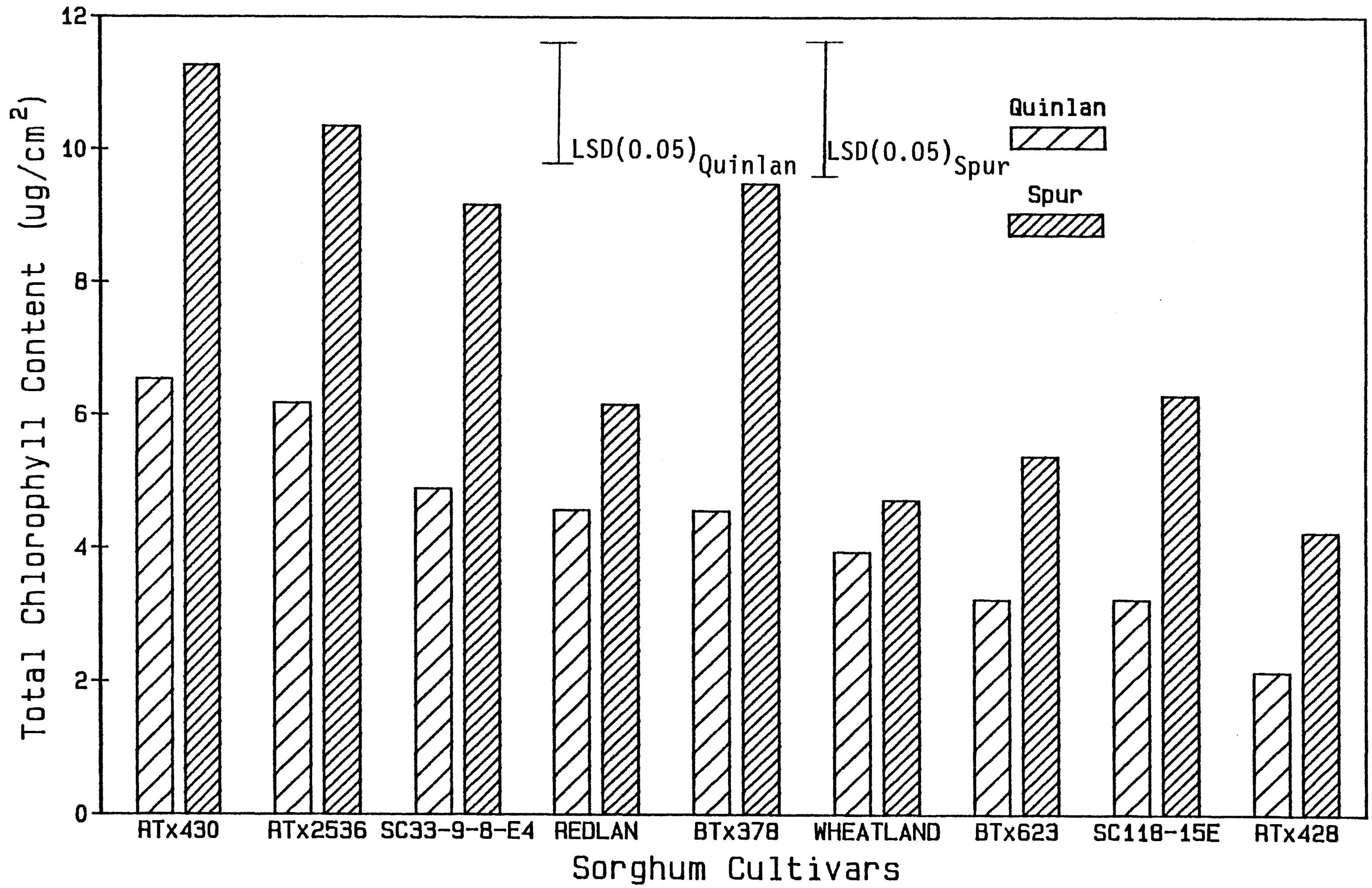

Fig. 10. Total chlorophyll content of nine sorghum cultivars grown in Quinlan and Spur soils.

The lower dry matter production and increased chlorosis of sorghum cultivars grown in the Quinlan soil suggested that soil parameters of the Quinlan soil such as a pH = 8.3, a CCE = 15.74 percent, and DTPA-Fe of 0.58 ug/g of soil provided an environment conducive to Fe deficiency.

The cultivars RTx430, RTx2536, and SC33-9-8-E4 which were categorized by Clark and Gross (1986) as tolerant to Fe deficiency, had the lowest chlorosis scores and the most Chl_T when grown in the Quinlan soil (Table 11). The cultivars (BTx378, Redlan, and SC33-9-8-E4) which were classified as susceptible to Fe deficiency by Clark and Gross (1986), had expressed more Fe chlorosis in the Quinlan soil than those cultivars categorized as tolerant to Fe stress. The average chlorosis score of the three tolerant cultivars grown in Quinlan soil was 13.7 and the average chlorosis score of the three susceptible cultivars was 18.1 (Table 11). In Spur soil, RTx430, RTx2536, and SS33-9-8-E4 were also less chlorotic with an average chlorosis score of 10.9 when compared with the rest of the cultivars. Similarly, the three susceptible cultivars that were classified by Clark and Gross (1986) were more chlorotic, with an average chlorosis score of 13.7, than those three tolerant cultivars in Spur soil (Table 11). In the screening experiment conducted by McKenzie et al. (1984), the mean visual scores assigned to RTx2536, RTx430, and BTx378 were 1.2, 1.2, and 1.8 out of the highest possible score of 4 which denoted severe chlorosis. Williams et al. (1982) conducted similar screening experiments and assigned visual ratings (0 = no Fe deficiency and 4 = severe Fe deficiency) of 1.8, 3.0, 3.0 and 3.8 to the cultivars, SC33-9-8-E4, Wheatland, SC118-15E, and Redlan, respectively. The average chlorosis scores of the present study (chlorosis sources in Table 11 divided by 5; where 1 equals no Fe chlorosis and 5 equals severe Fe chlorosis) were compared to the visual ratings assigned to the cultivars by Williams et al. (1982). The average chlorosis scores assigned to SC33-9-8-E4, Wheatland, SC118-15E, and Redlan were 3, 3.9, 3.9, and 3.4, respectively for the Quinlan soil, and 2.4, 3.4, 2.9, and 2.7, respectively for the Spur soil. Seed provided by the Sorghum Program at Oklahoma State University (RTx428 and BTx623) were among the most chlorotic cultivars tested with an average chlorosis score of 3.8 and 3.1 in the Quinlan and Spur soils, respectively.

Calcareous soils in western Oklahoma can be used for screening Fe-efficient sorghum from Fe-inefficient lines. Further work in the Oklahoma State University Sorghum Program is continuing on the selection of sorghum lines which are more Fe efficient.

CHAPTER V

CONCLUSION

The increase in soil pCO_2 and soil solution HCO_3^- with increasing soil moisture (θ_v) in Quinlan and Spur soils did not appear to result in regular increases in chlorosis in sorghum as has been observed in dicots. This supports the idea proposed by Römheld and Marschner (1986) that the strategy to absorb Fe in monocots are different from dicots.

Increased dry matter and Chl_T of sorghum plants grown in soil amended with APP may be due to the lower NO_3^-/NH_4^+ ratio, increased total soluble Fe due to the solubilization of organic matter by polyphosphates, and stimulated root growth that enhanced total uptake of nutrients. The addition of Fe as $FeSO_4$ to the KH_2PO_4, APP, and MAP fertilizations was successful at correcting the Fe deficiency of the sorghum plants. Therefore, chlorosis of sorghum grown in Quinlan and Spur soils was a result of a lack of Fe and may not be attributed by HCO_3^- induced chlorosis associated with increasing soil moisture content. Addition of Fe as $FeSO_4$ with APP to the soils from western Oklahoma was the most effective method to alleviate Fe deficiency of sorghum.

The nine cultivars which were screened with both soils showed a range in Fe efficiency consistent with other investigators. The calcareous soils in western Oklahoma can be used effectively to screen Fe-efficient sorghum from Fe-inefficient lines.

Research to determine how polyphosphates influence the soil environment, the chemistry of Fe in the soil, and the uptake and translocation of Fe in sorghum plants is necessary to provide a better understanding of the beneficial effects of polyphosphate on Fe chlorosis. Studies on the effect of soil moisture on pCO_2 and HCO_3^- concentration should include a set of pots containing the same soils without the plants and treated with the same moisture levels. Further work in the Oklahoma State University Sorghum Program is required to continue selecting for Fe efficient sorghum lines.

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