EFFECT OF SOIL MOISTURE ON EXTRACTION OF SOIL AND PLANT IRON

by

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ABSTRACT OF THESIS

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A greenhouse investigation was conducted to determine the relationship of soil moisture level to extractable forms of plant and soil iron. Soybean plants (Glycine max), variety Lee, grown on Adelanto loam soil under two different soil moisture levels were used in this study.

Plants grown under 120 percent moisture equivalent level were chlorotic, while those under 75 percent moisture equivalent were normally green in appearance.

The chelating agent EDTA extracted less iron from the soil at the high moisture level.

The total plant iron concentrations were much the same in chlorotic and nonchlorotic plant tissues. The water-soluble and 1.0 N hydrochloric acid-soluble leaf iron (active iron) fractions were less in chlorotic than nonchlorotic plants. The chlorotic and nonchlorotic tissues did not differ in their water- and salt-soluble nitrogen compounds.

The presence of low amounts of "active iron" and water-soluble iron in chlorotic soybean plants grown on a calcareous soil under a high soil moisture level were explained as an indirect effect of high moisture level on the chemical forms and distribution of plant iron.

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INTRODUCTION

The prevalence of iron chlorosis on plants growing in calcareous soils has become known as lime-induced chlorosis. Chlorosis is believed to be aggravated by high levels of soil moisture. Iron deficiency symptoms are characterized by a yellowing of the entire leaf surface with the exception of the veins which tend to remain green except in severe cases. In very severe cases of iron chlorosis the yellowed leaves may abscise and die-back may result. The economic importance of this nutritional disorder is evident throughout the world in arid and semiarid climates.

While no very convincing explanation of the lime-induced chlorosis phenomenon has been advanced, it is claimed that deficiency of iron is accompanied by an excess of certain interfering ions in the soil. On the other hand, lime-induced chlorosis is believed to be iron inactivation or failure of iron metabolism within the plant itself. There is still much confusion in attempting to separate causes from effects.

In spite of the general recognition of the importance of soil moisture as a factor in high-lime chlorosis, little is known about the specific effects. Hence, it will be of importance to know the effect of changes in soil moisture content on extractable plant and soil iron.

The purpose of this research is to investigate further the relationships of soil moisture level to chlorosis and the effect of such moisture treatments on extractable forms of plant and soil iron. This relationship was studied using soybean plants (Glycine max), variety Lee, grown in a calcareous soil at 75 and 120 percent moisture equivalent levels.

LITERATURE REVIEW

The Relation of Soil Moisture to Plant Growth in Calcareous Soils

Burges and Pohlman (9) were the first investigators to show the effect of soil moisture on iron-induced chlorosis. In field studies in Arizona they found that the common practice on irrigated lands of maintaining soil moisture close to the field capacity level was one of the principal causes of chlorosis. Chapman (12) observed that iron chlorosis often develops under the influence of irrigation practice. He suggested that this may be in part the result of gradual coating of iron minerals with insoluble carbonate, the latter derived from the bicarbonate present in most irrigation water. Reuther et al. (40) found no chlorosis on any of the citrus plants during the summer regardless of moisture treatment, but during the winter and early spring chlorosis increased very markedly on plants having high soil moisture and only slightly on plants in soils of normal moisture.

Burtch et al. (10) conducted a greenhouse experiment to study the effect of light, soil moisture and soil temperature on lime-induced chlorosis. In general, they found that the higher the level of soil

moisture maintained, the greater the degree of chlorosis. The combination of high soil moisture and low temperature were the environmental conditions most conducive to the occurrence of lime chlorosis.

Lime-induced chlorosis is an important physiological disturbance of citrus on calcareous soils. Haas (16) suggested that the problem is intimately related to the soil moisture status and pH value of the soil which in turn are related to the physical status of the soil.

Water supply may influence the vegetative growth. More growth was obtained with maize (32) and citrus plants (40) grown under high moisture level than that under low moisture level. Burtch et al. (10) found that the stem length and oven-dry weight of bean plants increased by increasing the soil moisture level.

Available Soil Iron and the Interacting Factors Related to Soil Moisture

There are many different methods for extracting soil iron. Olson (34) found that ammonium acetate adjusted to pH 4.8 dissolved amounts of iron from soils that varied from 0.15 ppm. in alkaline soils to 20 ppm. in acid soils. Water extracts of iron from neutral or alkaline soils yielded negligible quantities of inorganic iron. A few parts per million of iron was extracted as soluble organic matter. Olson concluded that the soil pH value is not the only factor determining the solubility of iron in soils or the ability of soil to supply iron.

Viro (49) showed that ethylenediaminetetracetic acid (EDTA) is a more effective extracting agent for exchangeable ions than ammonium acetate in acid soils. He concluded that EDTA can dissolve water soluble carbonates that are not a part of the exchangeable complex. The chelating agent EDTA was found to extract less iron than ethylenediamine di-(o-hydroxyphenyl acetic acid), "EDDHA," from calcareous soils (50). The equilibrium of FeEDTA in the presence of excess calcium ion is shifted so that ferric hydroxide precipitated more readily than it does in the absence of the calcium ion (28). Wallace (50) suggested the use of 10^{-4} M EDDHA as an extracting agent to give approximate information on the status of soil iron. He indicated that the ability of the plant to accumulate iron is modified by other environmental factors such as pH value, phosphorus, calcium, and bicarbonate concentrations, all of which influence chelation (as a result of competition). Phosphorus may be considered as a ligand which competes with the chelating agent for iron (28).

Using available iron supply as an index to iron deficiency in plants, Olson and Carlson (35) found that ammonium acetate extractable soil iron producing chlorotic sorghum was "statistically less" than from soil producing green plants. Brown and Holmes (5) studied the response of three plant types grown on three soil types which differ in their iron supplying power. They postulated that chlorosis was related to the available soil iron supply; however, the latter could not be used

as an absolute index to the occurrence of deficiency. Lindsay and Thorne (23) found that the soil solution from chlorotic areas had a higher concentration of bicarbonate and calcium ions than that from adjacent areas producing green plants. Under certain conditions micro-organisms may also offer serious competition to higher plants for available iron (51). Kliman (21) observed a marked increase in extractable ferrous iron and an associated decrease in ferric iron when the soil was compacted or maintained at higher moisture content. Brown and Holmes (5) postulated that the difference in calcium carbonate organic matter, bicarbonate, phosphorus and the moisture content of the soil, beside the fact that plant species differ in their capacity in utilizing different forms of iron, may limit the value of data pertaining to available iron supply as an index to iron deficiency.

Soil iron may be inactivated in the soil as a result of the excess of other elements. Lindsay and Thorne (23) observed that bicarbonate ion reduced the movement of radioactive iron into the leaves and stem and accentuated its accumulation in the bean plant roots. High concentration of calcium in the soil have been thought (51) to be a major causative factor in iron chlorosis. Brown et al. (7) noticed that the incidence of iron chlorosis in soybean was related more to the phosphorus concentration than to the bicarbonate concentration in solution culture. Tiffin et al. (48) considered phosphorus as a ligand which competes with the soybean roots for iron. Brown et al. (6) reported that phosphorus is

considered to be a major contributing factor to chlorosis in calcareous soils. They postulated that calcareous soils are frequently high in phosphorus, and the variable contents could possibly account for variation observed in the severity of chlorosis which occurred on different calcareous soils.

The proportion of some soil elements was investigated in relation to soil moisture level. Reitemeier (39) studied the effect of soil: water ratio upon the composition of water extracts of the soil. He concluded that the water-soluble phosphorus was proportional to the soil moisture content. Haddock (17) found that low soil moisture tension increased phosphorus uptake by sugar beets. He explained this as indirect effects of soil temperature and carbonic acid concentration in the soil solution of moist soils on promoting phosphorus uptake. Metwally and Pollard (30) showed that phosphorus, nitrate and calcium contents of the soil solution (per unit dry soil) increased with the increase of water content of the soil. The pH value of the soil solution was not affected by the change in water supply.

Plant Iron and the Interaction Due to Soil Moisture

There is a disagreement as to whether there is any consistent difference in the total concentration of plant iron and the development of green or chlorotic leaves. Many investigators have shown that the total concentration of iron in chlorotic leaves is less or more than that

of nonchlorotic leaves of the same plant (51, 52). On the other hand, an equal amount of information has been presented showing there is no consistent correlation between the total concentration of leaf iron and the degree of chlorosis (22, 23, 36).

Attempts were made to find the relation between plant extracted iron and the degree of chlorosis. Linder and Harley (22) found no consistent correlation between total, water soluble and HCl (0.1 N and 1.0 N) soluble leaf iron and the degree of chlorosis. There was no difference between chlorotic and nonchlorotic leaves in the iron concentration of the cytoplasmic protein (water soluble) (36, 37, 52).

Oserkowsky (36) found that 1.0 N hydrochloric acid extracted iron,"active iron," was proportional to the chlorophyll content of the leaf. Many investigators found this correlation to be true with different plants (1, 33, 46). "Active iron" is a specific form of iron that is active in chlorophyll formation and greatly exceeds the amount of iron extracted with water (36). The level of "active iron" in three species of plants decreased to about one-half its level at the late growing stage but remained always higher the greener the leaf (1). Reuther and Crawford (40) showed that the plants grown under normal moisture level has about 40 percent more chlorophyll than the high moisture treated plants at the early stage of growth. There was only about 12 percent difference at the late stage of growth.

Lindner and Harley (22) found that over half the amount of iron in the leaves was present in a form insoluble in 1.0 N hydrochloric acid (inactive iron) presumably as iron-hematin, iron-nucleo-protein or in other complex organic compounds. Bennett (1) postulated that the development of chlorophyll depends on the amount of iron that is not absorbed by the residual protein in plant tissues.

The presence of about equal amounts of total iron concentration in both chlorotic and nonchlorotic leaves had led to the idea of inactivation of iron within the plant. Chlorosis of plants growing in alkaline calcareous soils may be a matter of iron activity within the plant rather than a failure of the plant to take up iron from the soil (33). Warnock (52) found in general that the iron content of plants grown on noncalcareous soils was greater than those plants grown on calcareous soils. Hale and Wallace (18) found that the bicarbonate and phosphate each competitively inhibit iron accumulation and translocation from the root to the leaves. In lime-induced chlorosis iron may be immobilized within the plants and tend to accumulate in the stem (27, 52).

Biddulph and Woadbridge (2) showed that as the phosphorus content of the nutrient medium is increased, root, stem and leaves continue to build up in phosphorus content. The excess of phosphorus may be responsible for immobilizing iron and other ions. They postulated that phosphate reacted with ferric iron which had already combined with immobile organic molecule. Miller et al. (31) found in

solution culture studies that the development of iron chlorosis on soybeans was more related to phosphorus concentration in solution or that absorbed by the plant than bicarbonate concentration in solution. The bicarbonate only increased the soluble phosphorus concentration in solution. Calcium was found (8, 47) to stimulate root growth, increase the absorption and translocation of phosphorus and calcium to the above ground parts. Increasing phosphorus and calcium in plant tissue allows for greater competition with iron which may inactivate iron internally (8). On the other hand, Wallace and Lunt (51) postulated that chelation with naturally occurring compounds that accumulate under abnormal conditions is a contributing factor to iron chlorosis. High soil moisture, poor aeration and cool temperature were reported by Thorne et al. (47) as a possible cause for disturbing plant metabolism which may result in fixing plant iron in forms of low biological activity.

The effect of soil moisture on the accumulation of these interfering ions in the plant has been studied. The uptake of phosphorus, nitrate and calcium by barley (30) and apple (29) plants per unit dry matter was greater with higher water supply. Phosphorus content was high in trees grown under low moisture stress; and the fluctuation in phosphorus composition of leaves was directly correlated to changes in soil moisture (4). The indirect effects of soil temperature and carbonic acid concentration in the soil solution of moist soils may promote phosphorus uptake (17).

Nitrogen Metabolism in Relation to Plant Iron

Iron chlorosis is thought to be a disturbance of nitrogen metabolism as well as iron metabolism and that the two are intimately related (1). Certain proteins are apparently synthesized only when certain trace elements such as iron become available (14). Iljin (20) reported that the nitrogen metabolism of chlorotic plants in calcareous soils is unbalanced. Such unbalance is reflected in the modification that occurs in certain nitrogenous compounds, especially in total nitrogen, soluble nitrogen, protein nitrogen and residual nitrogen. Such disturbance may effect the process of chlorophyll formation. Buslova (11) found a close relationship among iron protein and chlorophyl synthesis. Perur et al. (38) found no appreciable difference in proteins of the water soluble fraction of corn and soybean leaves. Iron chlorosis caused about 82 percent reduction in the protein content of the chloroplastic fraction of the corn leaf. Many investigators have reported an increase in the free amino acids of chlorotic leaves (15, 20, 39, 47).

MATERIALS AND METHODS

Several preliminary experiments were conducted to select the soil and the test plant that together would produce iron deficiency symptoms. Soybean plants (Glycine max), variety Lee, and an Adelanto loam soil were used for the main experiment.

Soil Analysis

Adelanto loam soil, 0 to 12 inches, from the citrus experiment station, Tempe, Arizona, was air dried and passed through a 10 mm. size plastic screen. The pH value, conductivity of the saturation extract, calcium carbonate equivalent and the moisture equivalent for the soil are shown in table 1.

Fertilizers were added to the soil at the rate of 300 pounds per acre nitrogen as NH_4NO_3 and 100 pounds per acre phosphorus as KH_2PO_4 which contained potassium at the rate of 85 pounds per acre.

For testing the effect of soil moisture level on soil iron, 500 gm. of the soil was mixed with a specific amount of the fertilizers in a plastic twin-shell blender for 15 minutes. The soil-fertilizer mixture was placed in a cardboard carton container (3 inches in height and 4

Table 1. Some Characteristics of Adelanto Loam.

Paste pH value	1:5 Soil suspension pH value	Electric conductivity	$CaCO_3$ Equivalent	Moisture Equivalent
		mmhos/cm.	%	%
7.95	8.95	1.6	3.5	14.3

inches in diameter). A perforated plastic tube was inserted in the middle of the soil as a means of maintaining equal distribution of moisture within the soil when water was added. Three containers were prepared for each of the two moisture treatments, namely, 75 percent and 120 percent moisture equivalent (M.E.). Each of the cardboard cartons was brought to its respective moisture level by adding the precalculated deionized water. The moisture treatment continued for a month through which the moisture levels were checked twice a day by weighing the soil container and adding the necessary water through the perforated plastic tube. At the end of the month the soil samples were air-dried, sieved through a 10-mesh sieve and kept in a carton container for subsequent analysis.

Iron extractions and determination

The soil was extracted with deionized water, deionized water plus anion exchange resin (Dowex 2-X8, chloride form), 10^{-4} M EDTA (sodium salt), 10^{-4} M EDDHA (sodium salt) and 1.0 M ammonium acetate. The pH values for all the extracting agents except ammonium acetate solution were adjusted to pH 6.0. The pH value of the ammonium acetate was adjusted to pH 3.0. Dilute acetic acid and ammonium hydroxide solutions were used for adjusting the pH.

The extractable iron was determined by shaking 10 gm. of the soil with 100 ml. of extracting agent in a 250 ml. Erlenmeyer flask for

6 hours on a Burrel Wrist-Action Shaker. In the case of the water plus anion exchange resin extraction, 10 gm. of chloride saturated resin were added with the water. At the completion of the 6-hour extraction the soil suspension was centrifuged* for 15 minutes at 1500 X G. The supernatant was passed through a 0.45 μ Millipore filter paper (42) to ensure the presence only of the soluble compounds in the extract. The filtrate was taken to dryness and the residue was oxidized by the use of a mixture consisting of 10 ml. deionized water, 10 ml. concentrated nitric acid and 10 ml. of 60 to 70 percent perchloric acid solutions. The residue was dissolved in 10 ml. of 0.1 N HNO_3 and filtered. The filtrate was collected in a 25 ml. volumetric flask diluted to the mark, and iron was determined colorimetrically by the modified o-phenanthroline method (25, 41).

Plant Analysis

Technique of growing the plants

Soybean plants, variety Lee, were grown in pots under greenhouse conditions. Two kilograms of the soil-fertilizer mixture prepared as mentioned previously were placed in glazed pots (7 inches in height and 5 inches in diameter). A perforated plastic tube was

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* The International refrigerated centrifuge Model PR-2 was used for all centrifugation work.

vertically placed in the middle of the soil. Twelve pots were prepared for each of the two moisture treatments.

Precalculated deionized water was added to each pot to give 100 percent moisture equivalent. Soybean seeds presoaked in water for 24 hours were planted 10 seeds to a pot in the soil at a depth of approximately 15 mm. The soil was moistened and a brown paper covering was placed over the pots to decrease moisture loss.

Seedling emergence started on the fifth day after planting. The 75- and 120-percent moisture equivalent treatments were started after complete emergence. The weight of the pots was checked twice a day and the necessary water was added through the perforated tube. An approximate value of the plant weight was taken in consideration. Two weeks after planting, the plants were thinned to six per pot.

Plants were harvested at two stages of growth. The first sampling was 37 days after planting, while the second sampling was 57 days following planting. The above ground portions of the soybean plants in two pots were harvested as one sample. Three replications were harvested for each treatment. The leaves and stems of each replication were separated, dipped in 0.0001 N hydrochloric acid for a few seconds to remove surface contamination, and rinsed twice in deionized water to remove the acid. The separated plant organs were placed in polyethylene bags and quick-frozen with dry ice.

The frozen plant materials were dried under vacuum in a refrigerated lyophilizer, and the lyophilized dry weight was recorded for each sample. The dried plant materials were ground in a Wiley mill using a 60-mesh stainless steel screen, mixed and stored in a polyethylene container at 0° F.

Determination of iron in plant tissues

Iron was determined by the o-phenanthroline method following wet oxidation with water and nitric and perchloric acids as mentioned previously (25, 41).

Total iron concentration of both stem and leaf tissues was determined by oxidizing a 200 mg.-sample.

"Active iron" was determined on a 500 mg.-sample by Oserkowsky's procedure (36).

The extraction of plant iron was accomplished as follows: cold deionized water, pH 7.0, and 0.2 M sodium chloride buffered to pH 7.0 by .02 M NaH_2PO_4 (13) were used as extracting solvents for plant iron. Each of the two solvents was used in the homogenization of a separate subsample of each tissue weighing 500 mg. for leaf and 250 mg. for stem tissues. The leaf tissues were homogenized with 30 ml. of the cold solvent in a Kontes glass homogenizer. The sample was homogenized by passing a teflon pestle through the mixture 10 times for 10 minutes at a Powerstat setting of 70. A Virtis "45" homogenizer was used

to homogenize the stem tissue in 30 ml. of cold solvent for ten minutes at a Powerstat setting of 80. Homogenization was conducted in a cold room at 36° F.

After homogenization, the homogenate was centrifuged at 24,000 X G for 10 minutes and then passed through a 0.45 μ Millipore filter paper to ensure the presence of only soluble compounds in the extract (42). The filterate was evaporated to dryness and iron determined on the residue as described previously.

Iron and nitrogen fractions in the plant tissue extracts

Svensmark (45) reported that the color developed in the protein determination by the Folin phenol method using spinal fluid sample is due to the reactions of proteins and other nitrogenous compounds such as amino acids. Hoch et al. (19), Bisset (3), and Singh (43) showed that 5 to 20 percent of trichloroacetic acid (TCA) can be used to separate protein from nonprotein nitrogen compounds without protein denaturation.

Thus, in this work the regular Folin phenol reagent method (24) was used for determining total nitrogen containing substances in plant extracts. Protein nitrogen was determined on the TCA precipitate using the same method. The nonprotein nitrogen was calculated from the difference between total nitrogen containing substances and protein nitrogen.

The previous procedure for homogenization and extraction was utilized on a larger subsample weighing 750 mg. for both stem and leaf tissues. The solution that passed through the Millipore filter paper was diluted to a final volume of 100 ml. A 5 ml. aliquot was used for determining total nitrogen containing substances and protein nitrogen. The remaining solution was used for determining total soluble iron or TCA-insoluble iron compounds.

Total cold water and 0.2 M sodium chloride soluble iron was determined on the whole extract. In another sample extract, TCA-insoluble iron compounds were separated by adding TCA to the solution to give a 10 percent final concentration (W/V). The mixture was incubated for one hour at 36° F and the precipitate was separated by passing the solution through a 0.45 μ Millipore filter paper. Iron was determined on the TCA-insoluble components. TCA-soluble iron compounds were figured from the difference between total soluble and TCA-insoluble iron.

Working curves for determining total nitrogen containing substance and protein nitrogen were prepared from crystalline bovine albumin standard solution (200 γ per ml.).

A total nitrogen containing substances working curve was prepared by adding 2 ml. of the alkaline copper solution (Reagent C) to a standard solution sample in a 10 ml. test-tube that contained protein in the range of 2 to 140 γ . After 10 minutes, 0.2 ml. of diluted Folin

reagent (Reagent E) was added and mixed very rapidly. The final volume was made to 4 ml. and the percent transmission was read at 750 mμ. In a 0.5 ml. sample of the cold water and 0.2 M sodium chloride extract the above procedure was followed to determine total nitrogen containing substances.

A protein nitrogen working curve was developed by adding 2.0 ml. of 20 percent TCA to a sample of the protein standard solution in a 25 ml. plastic tube that contained protein in the range of 2 to 140γ. The sample was mixed and incubated at 36° F for one hour. The TCA precipitate was separated by centrifugation at 10,000 X G for 10 minutes. The supernatant was discarded and the precipitate was washed with 5 ml. of 5 percent TCA and recentrifuged as above. Special care was taken not to pour off or remove protein particles in the process of precipitating and washing. To the protein precipitate in the same tube, 0.3 ml. of 1.0 N sodium hydroxide was added, mixed and left for 1/2 hour to dissolve the protein. Two ml. of the carbonate copper solution without sodium hydroxide (Reagent D) was added and mixed well. After 10 minutes, 0.2 ml. of reagent E was added and mixed rapidly. The solution was then transferred to a test-tube and the final volume was made to 4 ml. The transmittancy readings were recorded after 30 minutes. The above procedure was followed to determine protein nitrogen in a 1.0 ml. sample of the cold water and 0.2 M sodium chloride extracts.

Nonprotein nitrogen was calculated by subtracting the protein nitrogen from the total nitrogen containing substances.

Blanks were concurrently conducted for all determinations.

Transmittancy readings were read on a Bausch and Lomb Spectronic 20 Spectrophotometer.

Pertinent data were analyzed statistically by the "t test value" (44).

RESULTS

Results of Soil Analysis

An experiment was conducted with Adelanto loam soil to determine the effect of different soil moisture levels on chemical forms of soil iron. Concentrations of iron extracted from the soils held at two moisture levels, by 1.0 M ammonium acetate, water, water plus anion exchange resins, 10^{-4} M ethylenediaminetetracetic acid (EDTA) and 10^{-4} M ethylenediamine di (o-hydroxyphenyl acetic acid) are shown in table 2. The pH values for all soil extracts except ammonium acetate ranged from 7.6 to 8.0. The pH value of ammonium acetate soil extract was 3.5. The data for extracted soil iron in relation to soil moisture level are represented graphically in figure 1. The data show a significant difference at the 5 percent level for EDTA extractable soil iron as a function of soil moisture level. Iron soluble in EDTA was significantly less in the soil with the high moisture level. Soil iron extracted with ammonium acetate, water, water plus anion exchange resin or EDDHA showed no significant difference as a function of moisture treatment.

Table 2. Concentration of Soil Iron Extracted by Different Extracting Agents as Affected by Soil Moisture Level.

Extracting agents	Soil moisture level	Iron concentration			
		Replicates			Mean
		1	2	3	
	% of M.E.	ppm.			
1.0 M Ammonium acetate	75	0.40	0.05	0.15	0.20
	120	0.0	0.0	0.0	0.0
Water	75	1.73	1.98	2.01	1.90
	120	1.93	2.13	1.93	2.00
Water and Anion exch. resin	75	0.38	0.43	0.48	0.43
	120	0.68	0.48	0.33	0.49
10^{-4} M EDTA	75	3.20	2.70	2.70	2.86
	120	1.20	1.60	1.20	1.33
10^{-4} M EDDHA	75	6.25	8.15	9.25	7.88
	120	6.55	6.55	6.85	6.65

The data are expressed on basis of air-dry soil.

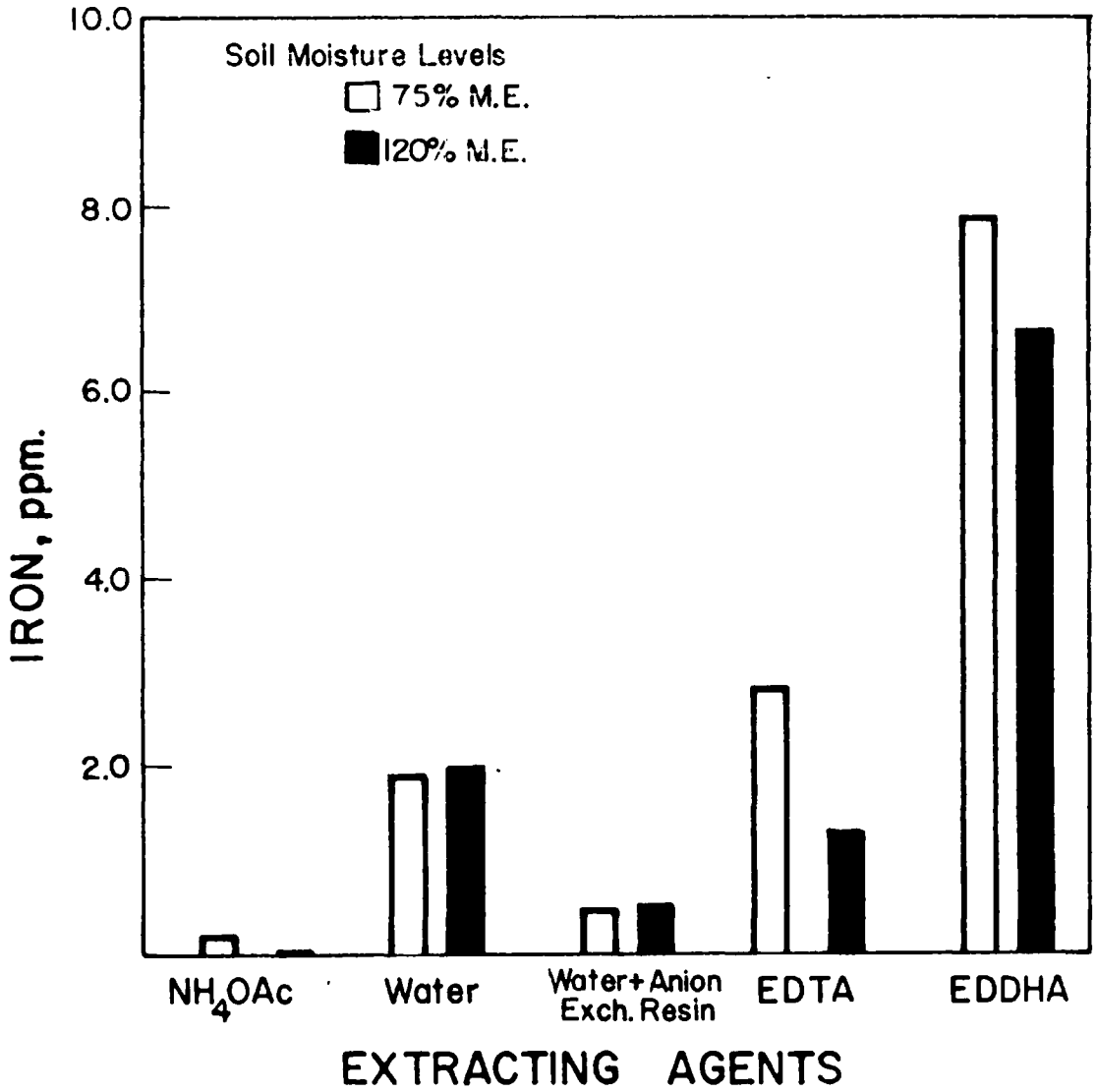

Fig. 1–Average concentration of extracted soil iron by different extracting agents as affected by soil moisture level.

In general, iron extracted with either ammonium acetate or water plus anion exchange resin was less than that extracted with water. On the other hand, chelating agents EDTA and EDDHA extracted more iron than water. Soil iron extracted with EDDHA was significantly greater than that extracted with EDTA.

An important difference was observed in the color of the water and water plus anion exchange resin extracts. The water extract had a yellowish color, while the water plus anion exchange resin was completely colorless.

Results of Plant Analysis

Vegetative growth and yield

There was a striking interaction of moisture treatments on the amount of vegetative growth and the incidence of chlorosis. Plants grown under 120 percent moisture equivalent level were chlorotic while those with 75 percent moisture equivalent were green in appearance. On the other hand, more vegetative growth was produced by the plants grown under high moisture level than with those grown under low moisture level. There was a significant difference in the oven dry-weights of the plants with respect to the moisture treatment level as shown in table 3 and figure 2. The oven dry-weight of the plants grown in the soil in which the high moisture level had been maintained were greater

Table 3. Oven-dry Weight of Soybean Plants at Two Stages of Growth as Affected by Soil Moisture Level.

Age of plants from planting	Soil moisture level	Oven-dry weight			
		Replicates			Mean
		1	2	3	
days	% of M.E.	gm.			
37 (early stage)	75	5.67	7.66	6.22	6.51
	120	9.25	9.83	8.28	9.12
57 (late stage)	75	7.15	7.89	8.84	7.96
	120	13.42	11.21	13.86	12.83

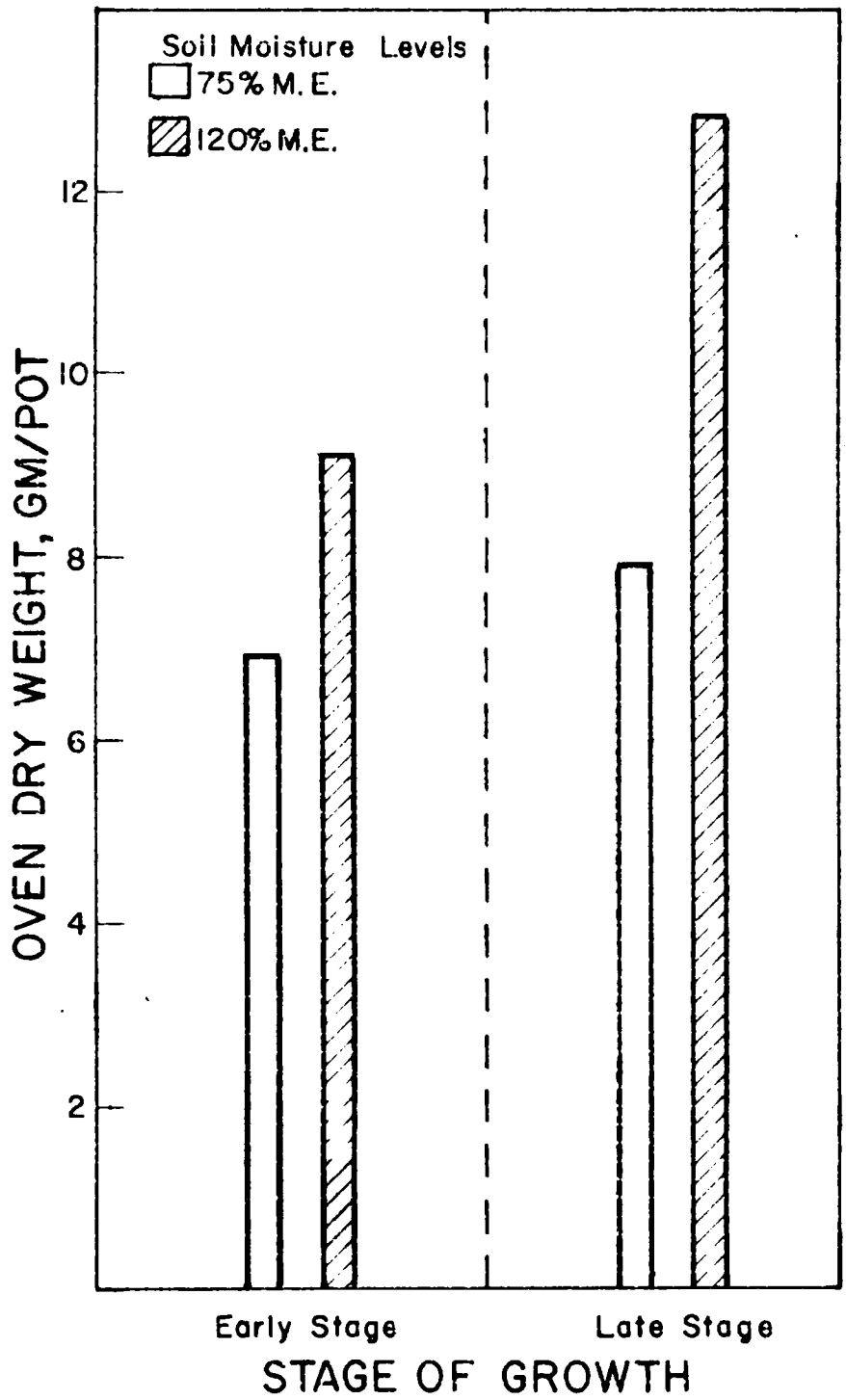

Fig. 2—Average oven dry-weight of the above-ground portion of soybean plants at two stages of growth as affected by soil moisture level.

at each stage of growth as compared to those plants grown with the low moisture level.

Concentration of total iron in stem and leaf tissues

There was no significant difference in the concentration of iron in stem tissues of chlorotic and nonchlorotic soybean plants at the two stages of growth. These data are presented in table 4 and figure 3.

Leaf tissues of chlorotic and nonchlorotic plants also did not differ significantly in their concentration of total iron at the two stages of growth. The total iron concentrations of leaf tissues are shown in table 5 and figure 4.

Previous investigators (27, 52) have shown that in some plants the production of iron deficiency chlorosis symptoms in the leaves is related to the immobilization of iron in the stems. The data in table 6 show the ratio of total leaf-iron to total stem-iron per pot of chlorotic (120% M.E.) and nonchlorotic (75% M.E.) plants. The data show that the leaf-stem iron ratio did not differ significantly with respect to soil moisture level or presence of chlorosis. The data are represented graphically in figure 5.

Water soluble iron of stem and leaf tissues

There was no significant difference in the concentration of water soluble iron of stem tissues in comparisons between chlorotic and nonchlorotic plants. The concentration of water soluble iron

decreased significantly only in stem tissues from chlorotic plants in the late stage of growth (table 4 and figure 3). In order to evaluate water-soluble iron concentrations in relation to the total concentration of iron in the stem, table 7 and figure 6 show the data for the percent water-soluble iron in proportion to the total iron in stem tissues. The data show that the percent water-soluble iron was significantly less in chlorotic plants than nonchlorotic plants at the late stage of growth. Also, the percent water-soluble iron significantly decreased only in chlorotic plants with increasing plant age.

The concentration of water-soluble iron in the leaf tissues at the two stages of growth are shown in table 5 and figure 4. The water-soluble iron concentration was significantly less in chlorotic leaves than nonchlorotic leaves at the early stage of growth. No significant difference was detected at the late stage. The percent of the total iron which was water-soluble was significantly less for chlorotic leaves as compared to nonchlorotic leaves at the early stage (table 8 and figure 7). No significant difference was detected for plants harvested at late stage of growth.

Salt-soluble iron in stem and leaf tissues

Two-tenth molar sodium chloride solution was used in extracting plant tissues in an attempt to find a difference between chlorotic and nonchlorotic plants with respect to other forms of iron. The data

in table 4 and figure 3 show the concentration of salt-soluble iron of stem tissues. The concentration of salt-soluble iron was the same in chlorotic and nonchlorotic stem tissues. The percent of the total iron represented by the salt-soluble iron fraction also did not differ significantly in chlorotic and nonchlorotic stem tissues at the two stages of growth (table 7 and figure 6).

The nonchlorotic leaves did not show a significant difference in the concentration of salt-soluble iron as compared to chlorotic leaves at the two stages of growth. However, this iron fraction decreased significantly in both chlorotic and nonchlorotic leaves with increasing plant age. These data are presented in table 5 and figure 4. The percent values of the salt-soluble iron did not differ significantly between chlorotic and nonchlorotic leaves but the values generally decreased significantly in the second stage of growth (table 8 and figure 7).

Hydrochloric acid-soluble leaf iron

Leaf-iron soluble in 1.0 N hydrochloric acid termed "active iron" by Oserkowsky (36), was determined in both chlorotic and nonchlorotic leaf tissues. The data are presented in table 9 and figure 8. The active iron concentration was significantly higher in nonchlorotic leaves than chlorotic leaves in the early stage of growth. The level of active iron decreased significantly in nonchlorotic leaves at the late growing stage; however, it remained higher than that in chlorotic

Table 4. Concentration of Total, Water-soluble and Salt-soluble Iron of Soybean Stem Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Concentration of iron							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	ppm.							
Total	75	75.8	75.9	62.5	71.4	64.9	55.8	79.9	66.8
	120	52.6	97.9	64.9	71.8	77.2	77.6	100.1	85.0
Water soluble	75	29.1	18.9	15.3	21.1	12.2	11.1	12.2	11.8
	120	12.0	18.8	22.3	17.7	9.7	6.5	10.9	9.0
Salt soluble	75	17.8	25.5	17.5	20.3	22.1	22.2	24.5	22.9
	120	19.6	19.9	17.8	19.1	23.7	25.1	10.9	19.9

The data are expressed on oven-dry weight basis.

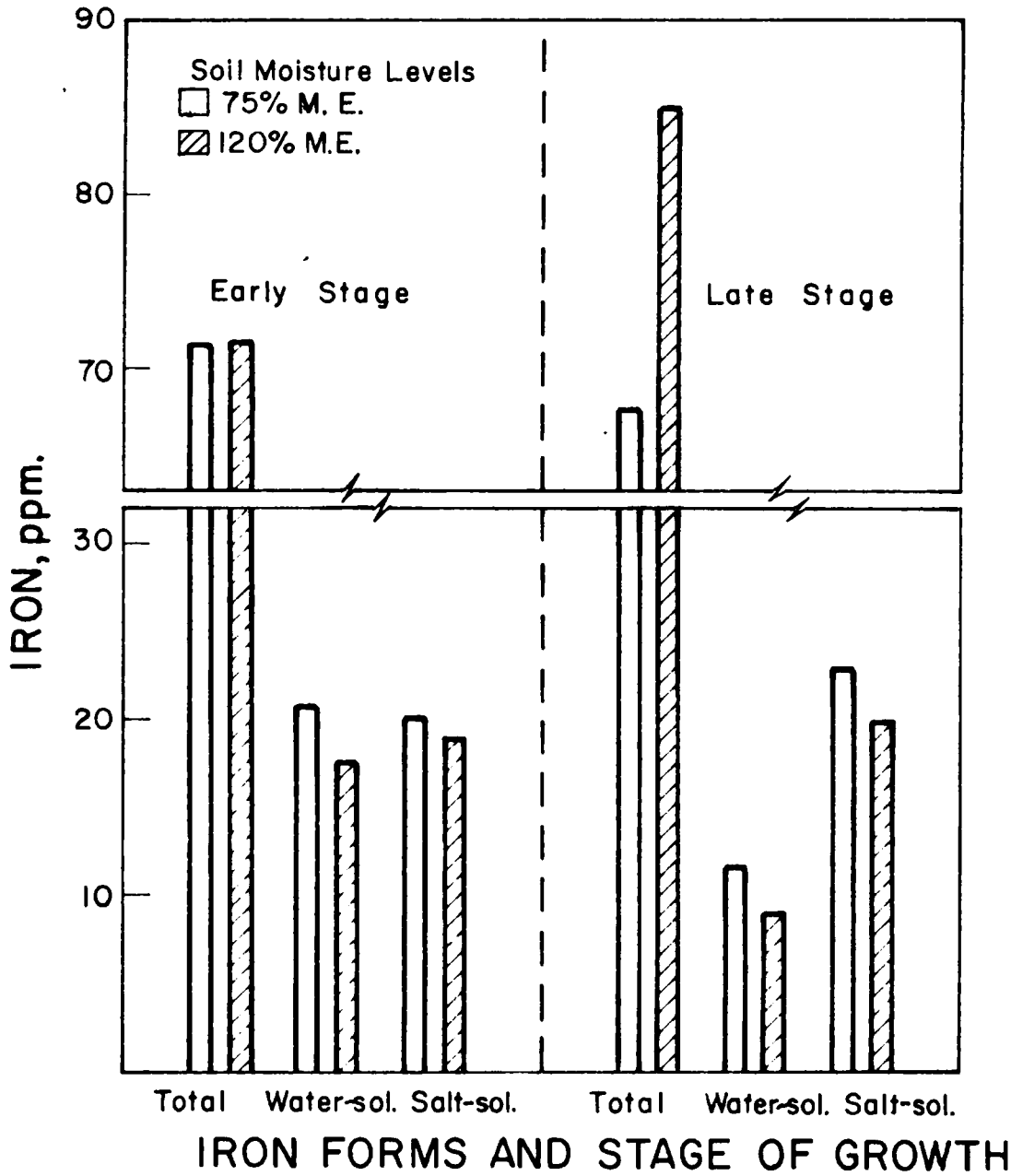

Fig. 3—Average concentration of total, water-soluble, and salt-soluble iron of soybean stem tissues at two stages of growth as affected by soil moisture level.

Table 5. Concentration of Total, Water-soluble, and Salt-soluble Iron of Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Concentration of iron							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	ppm.							
Total	75	144.3	127.1	134.2	135.2	107.8	110.1	118.2	112.0
	120	130.2	126.7	99.4	118.8	151.1	97.2	114.7	121.0
Water soluble	75	36.6	34.7	33.9	35.1	30.7	24.4	38.7	31.3
	120	22.6	24.2	27.2	24.7	23.7	33.5	23.7	27.0
Salt soluble	75	41.5	36.0	38.0	38.5	20.5	22.3	21.1	21.3
	120	35.0	33.2	29.3	32.5	21.7	19.2	18.9	21.0

The data are expressed on oven-dry weight basis.

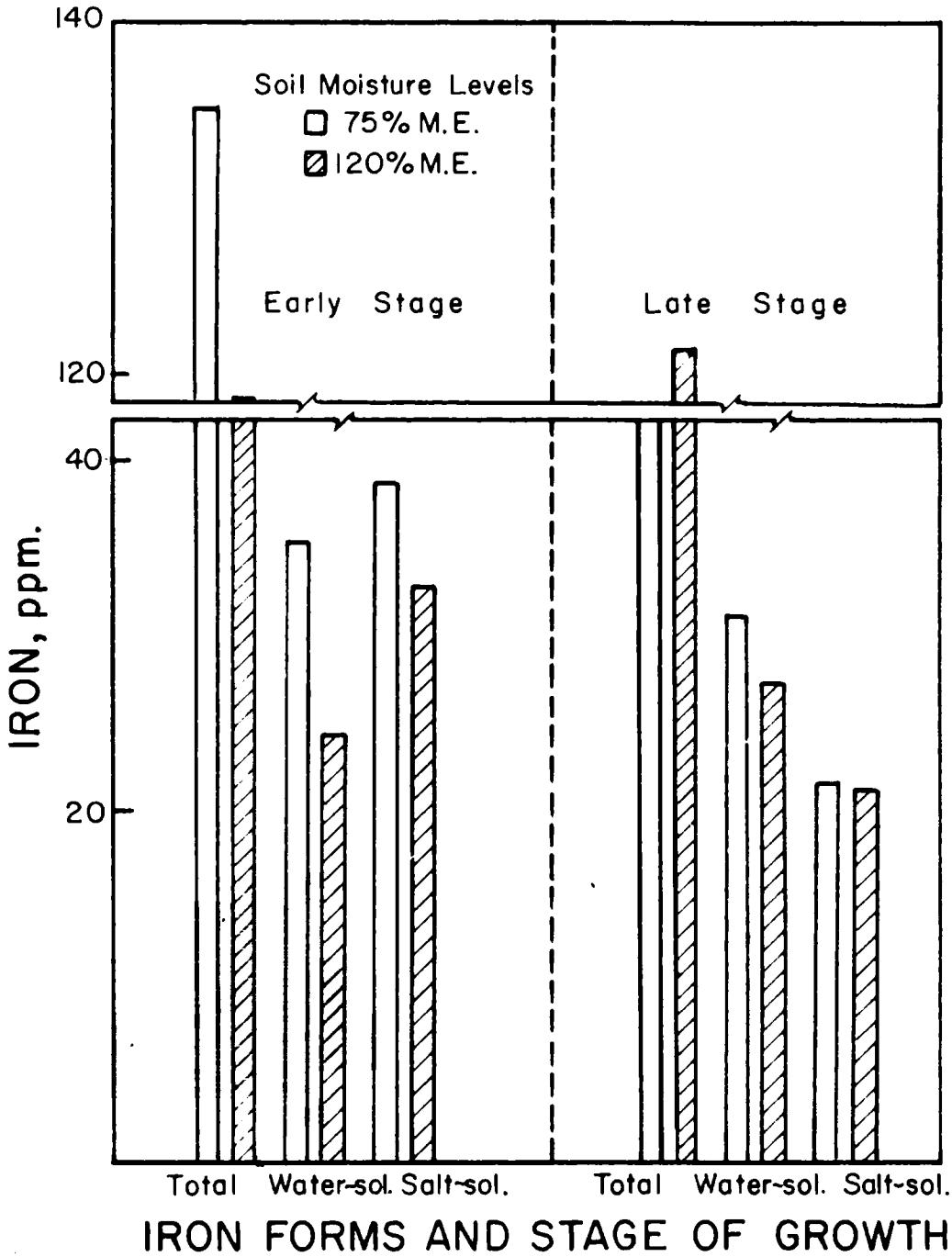

Fig. 4—Average concentration of total, water-soluble and salt-soluble iron in soybean leaf tissues at two stages of growth as affected by soil moisture level.

Table 6. Average Ratio of Total Leaf Iron to Total Stem Iron of Soybean Plants per Pot at Two Stages of Growth as Affected by Soil Moisture Level.

Stage of growth	Soil moisture level	Iron ratio (leaf/stem)			
		Replicates			Mean
		1	2	3	
	% of M.E.				
Early stage	75	2.75	2.63	3.28	2.88
	120	3.71	1.93	2.20	2.61
Late stage	75	2.73	2.94	2.56	2.74
	120	2.96	1.81	1.74	2.17

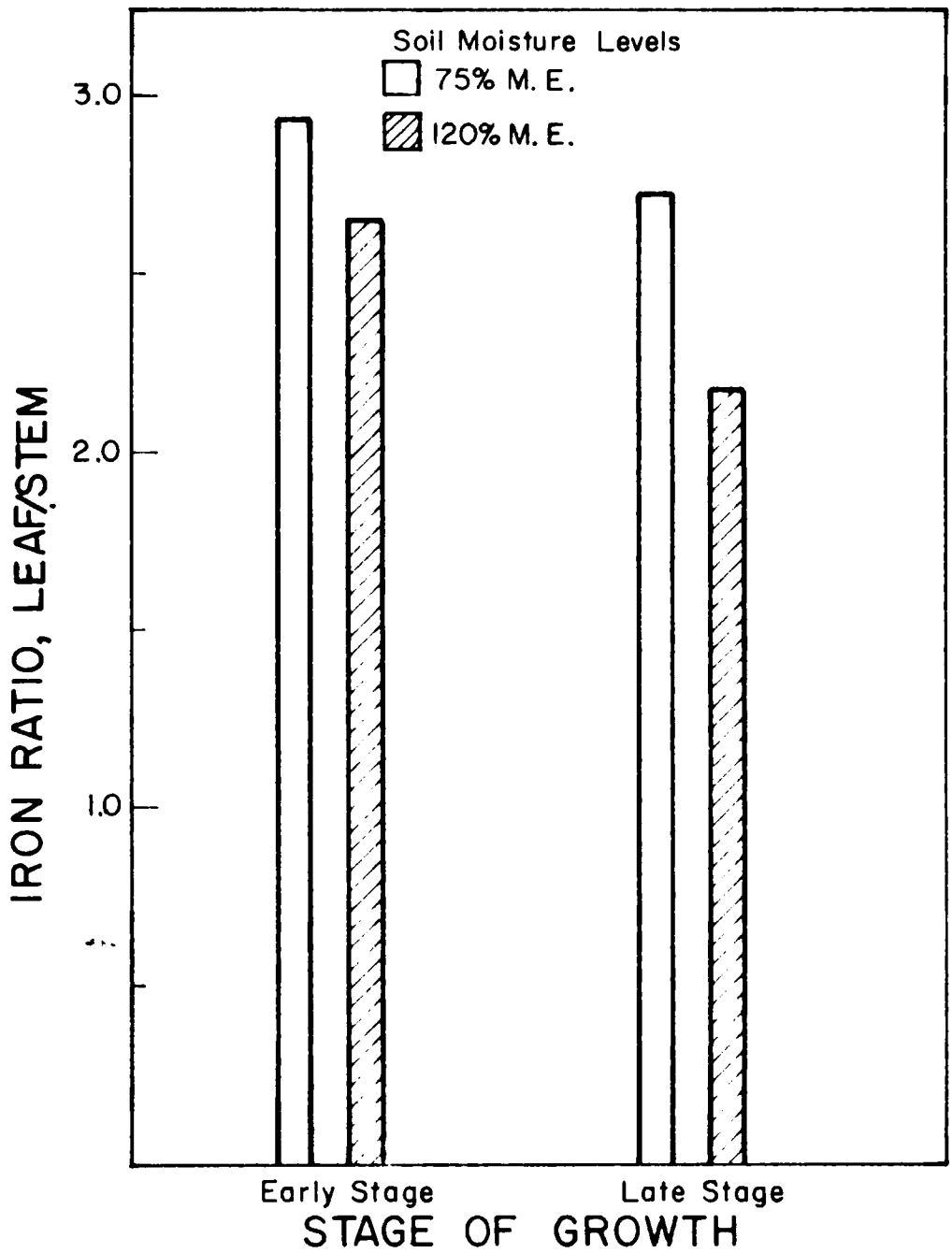

Fig. 5—Average ratio of total leaf-iron to total stem-iron of soybean plants per pot at two stages of growth as affected by soil moisture level.

Table 7. Water-and-salt-soluble Iron in the Stem Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Iron as percent of the total							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	percent							
Water soluble	75	38.35	24.87	24.55	29.25	18.84	19.99	15.33	18.05
	120	22.75	19.24	34.27	25.42	12.54	8.42	10.85	10.60
Salt soluble	75	23.41	33.65	28.05	28.37	34.28	40.01	30.70	35.00
	120	37.25	20.30	27.42	28.35	30.70	32.31	10.84	24.61

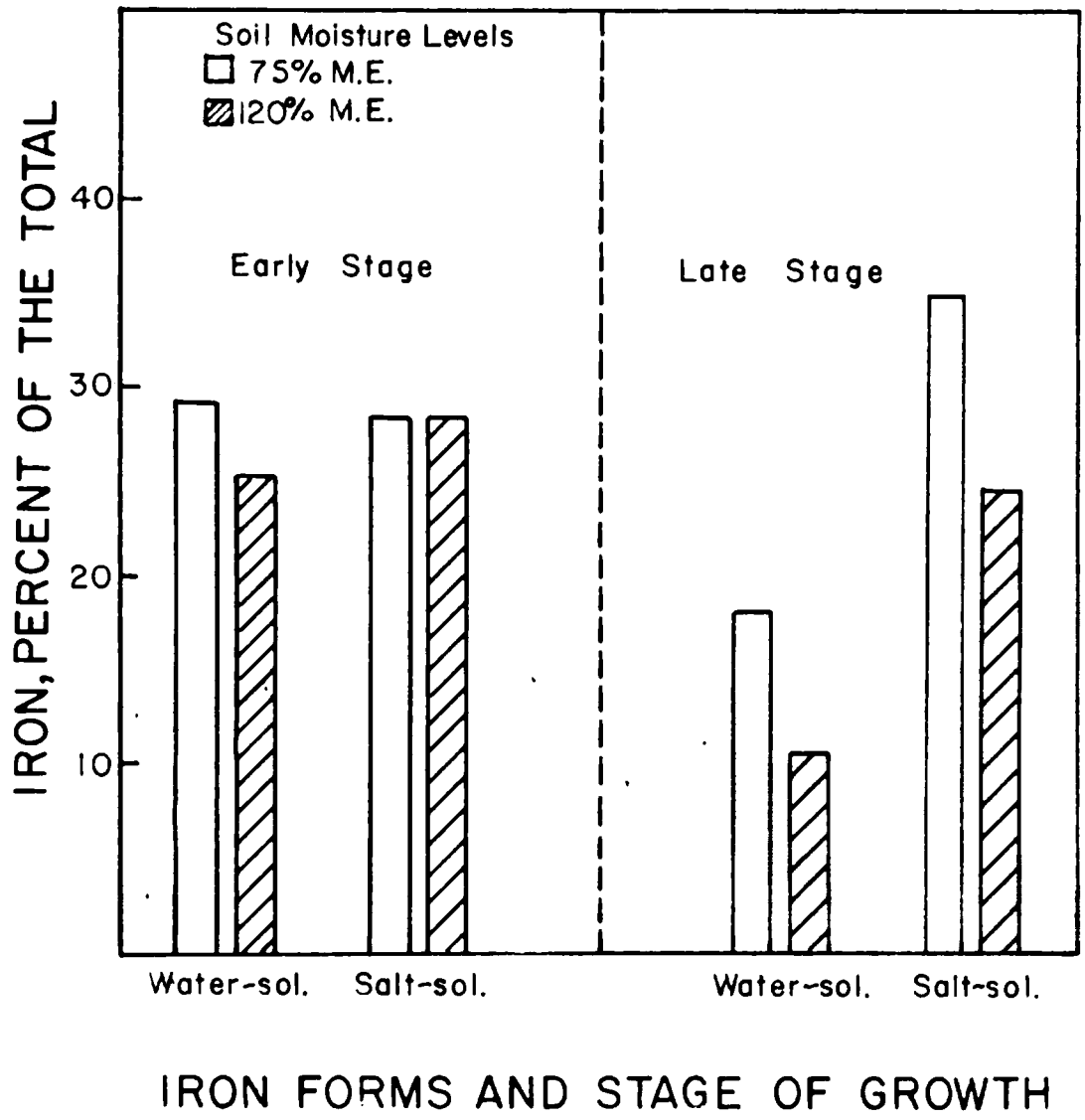

Fig. 6 – Average percentage of water- and salt-soluble iron in soybean stem tissues at two stages of growth as affected by soil moisture level.

Table 8. Water-and-salt-soluble Iron in Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Iron as percent of the total							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	percent							
Water soluble	75	25.34	27.31	25.25	25.96	28.48	22.14	32.75	27.75
	120	17.35	19.23	28.44	21.67	15.68	34.50	20.66	23.61
Salt soluble	75	28.73	28.29	28.34	28.45	18.98	20.24	17.82	19.01
	120	26.84	26.37	29.45	27.55	14.33	19.72	16.47	16.84

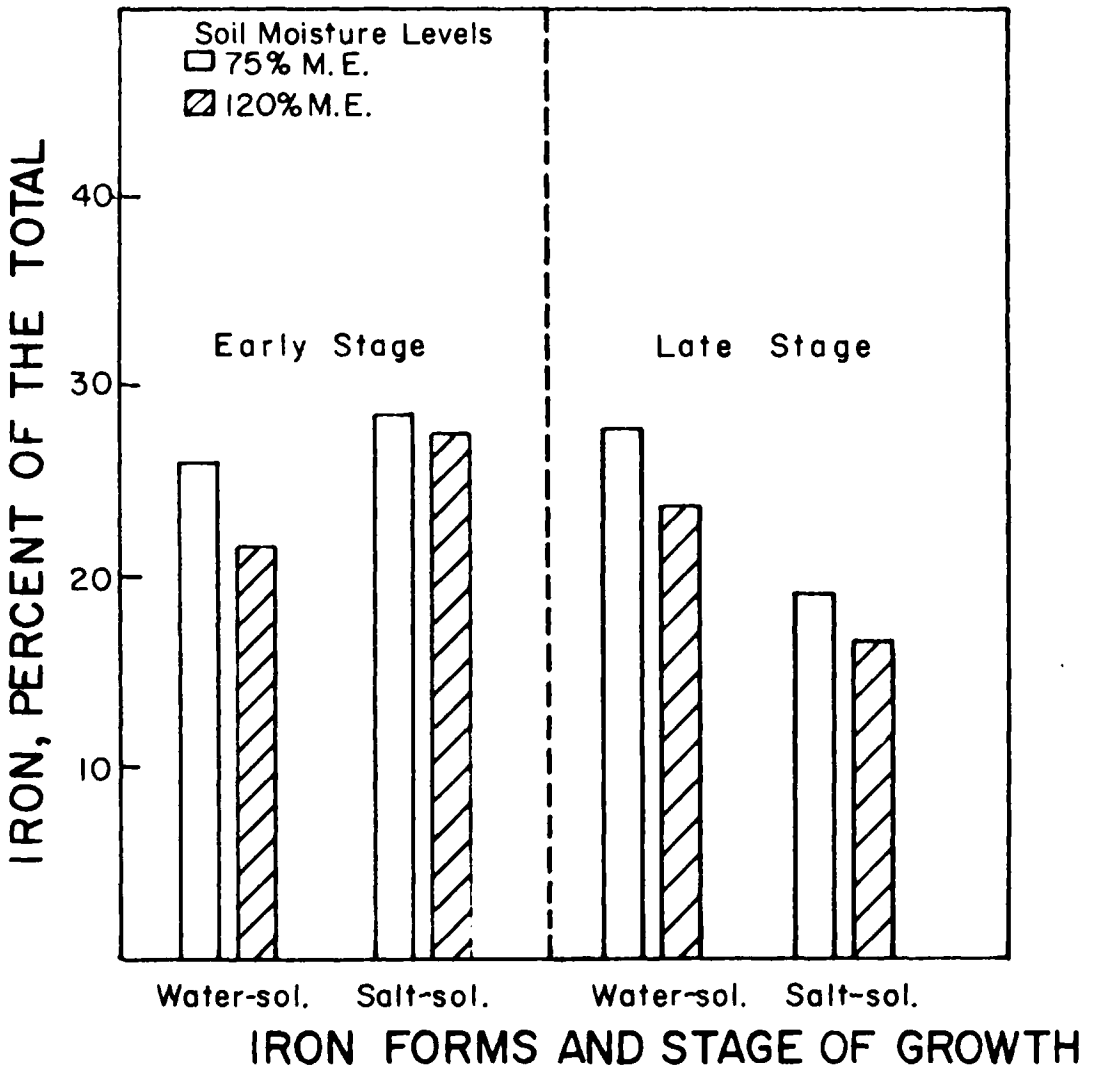

Fig. 7—Average percentage of water- and salt-soluble iron in soybean leaf tissues at two stages of growth as affected by soil moisture level.

Table 9. Concentration of "Active Iron" in Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Stage of growth	Soil moisture level	Concentration of active iron			
		Replicates			Mean
		1	2	3	
	% of M.E.	ppm.			
Early stage	75	57.9	58.0	66.9	60.9
	120	39.9	45.8	41.8	42.5
Late stage	75	50.7	46.2	45.0	47.3
	120	39.4	47.1	32.8	39.8

The data are expressed on oven-dry weight basis.

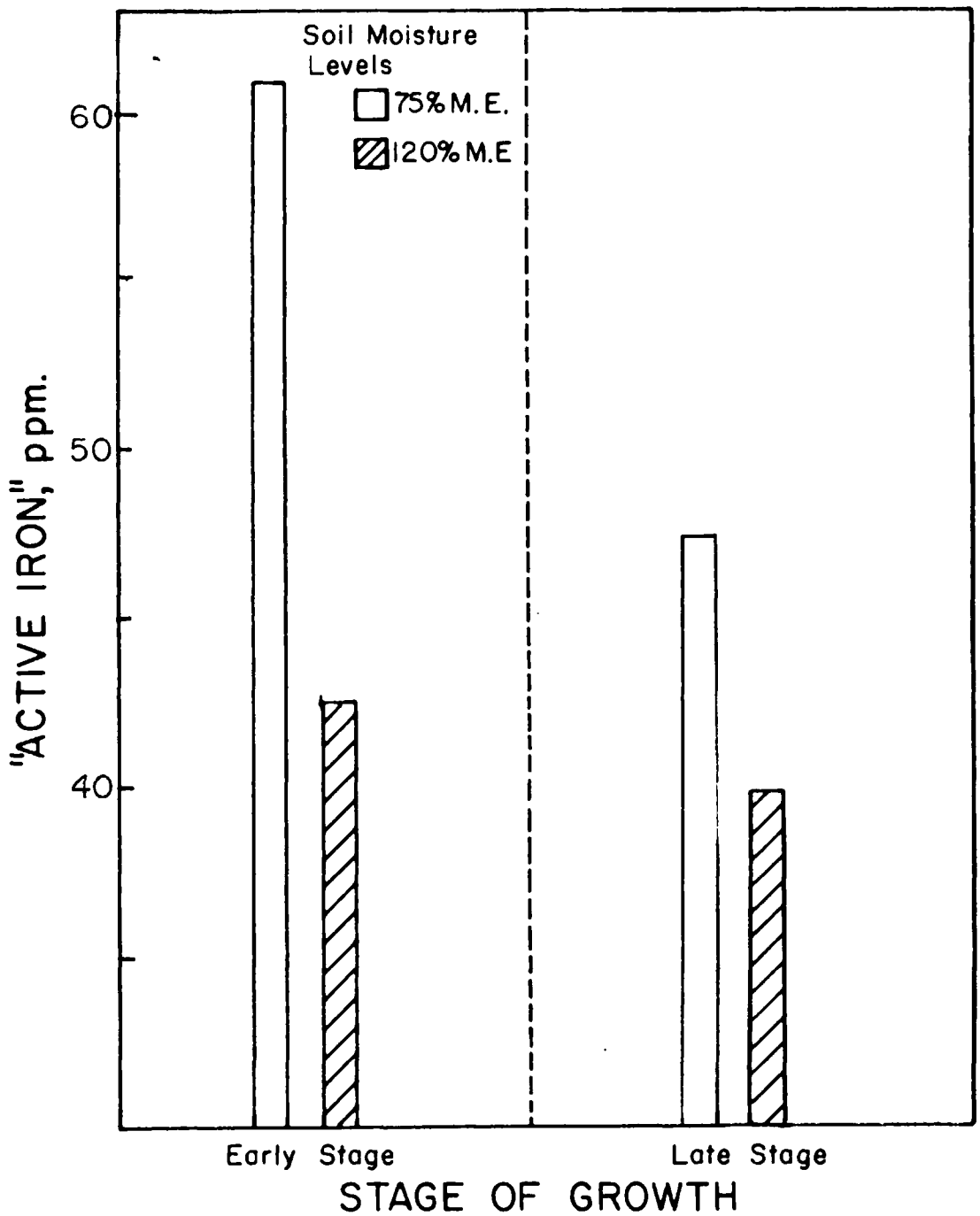

Fig. 8—Average concentration of "active iron" in soybean leaf tissues at two stages of growth as affected by soil moisture level.

leaves. The content of active iron in the chlorotic leaves did not change appreciably with increasing plant age.

Also, the percent of the total iron which was active was significantly less than that in nonchlorotic leaves only at the early stage of growth as shown in table 10 and figure 9. The percent values for this iron fraction did not change much in chlorotic leaves with respect to the stage of growth.

Water-soluble nitrogen compounds of the leaf and stem tissues

Soluble nitrogen compounds which are associated with physiological changes in plant cells was determined in chlorotic and nonchlorotic plant tissues.

The percent total nitrogen containing substances (total N), protein nitrogen (protein N) and nonprotein nitrogen (nonprotein N) in the water extract of the stem tissues are shown in table 11 and figure 10. There was no significant difference in the percentages of the nitrogen forms extracted with water in chlorotic and nonchlorotic stem tissues. The percent water-soluble nitrogen compounds did not change appreciably in the stem with respect to the age of plants.

The percentages of water-soluble nitrogen compounds in the leaf tissues were much higher than those in the stem tissues. The percent total N, protein N and nonprotein N in the leaf tissues are presented in table 12 and graphically shown in figure 11. The nitrogen

Table 10. "Active Iron" in Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Stage of growth	Soil moisture level	Percentage active iron of the total			
		Replicates			Mean
		1	2	3	
	% of M.E.	percent			
Early stage	75	40.1	45.6	49.8	45.2
	120	30.7	36.4	42.1	36.4
Late stage	75	47.0	41.9	38.1	42.3
	120	26.1	48.5	28.6	34.4

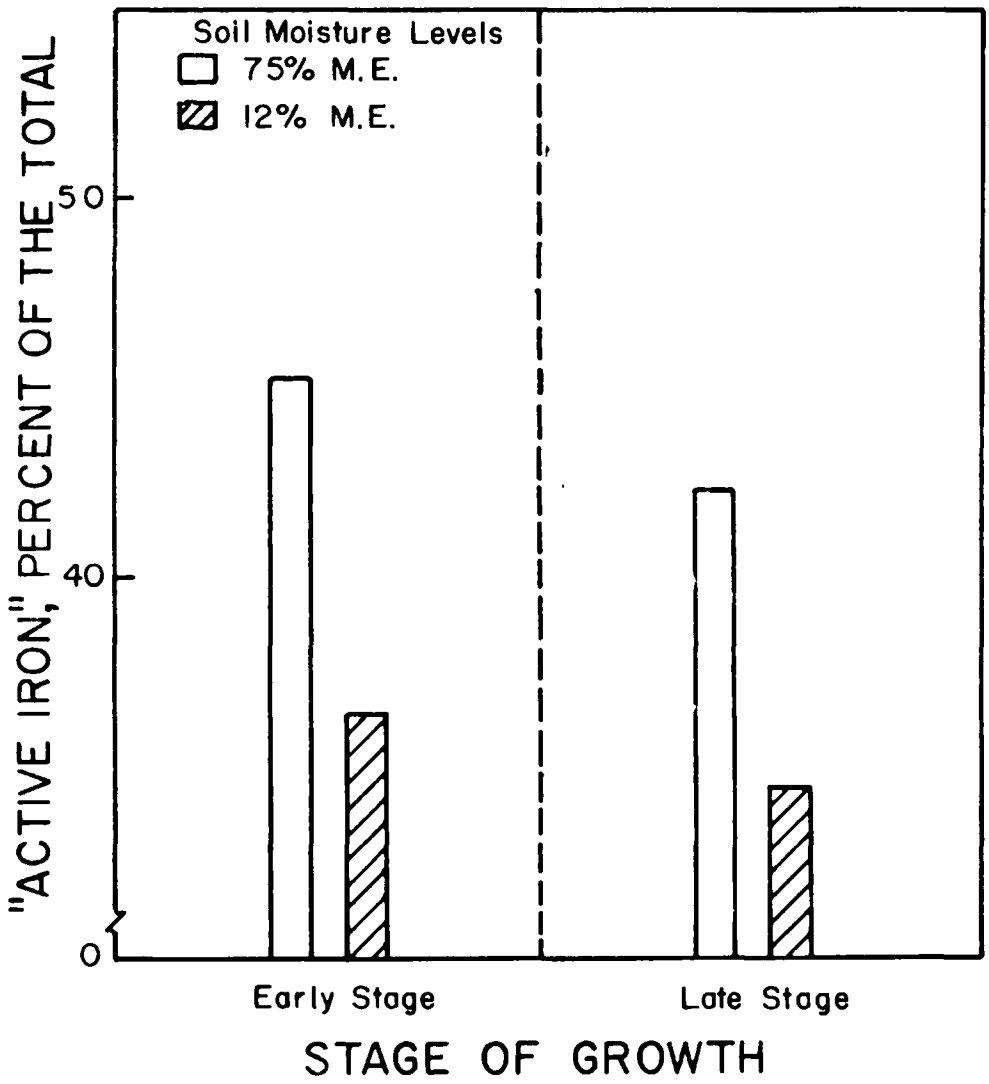

Fig. 9—Average percentage of "active iron" in soybean leaf tissues at two stages of growth as affected by soil moisture levels.

Table 11. Water-soluble Nitrogen Compounds in Soybean Stem Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of nitrogen	Soil moisture level	Nitrogen compounds							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	percent							
Total N containing substances	75	2.63	2.43	2.52	2.54	2.26	2.49	2.40	2.38
	120	2.61	2.44	2.45	2.50	2.42	2.41	2.34	2.39
Protein N	75	0.37	0.41	0.37	0.38	0.21	0.24	0.24	0.23
	120	0.28	0.30	0.31	0.30	0.30	0.29	0.29	0.29
Nonprotein N	75	2.26	2.02	2.14	2.18	2.05	2.25	2.16	2.15
	120	2.33	2.14	2.14	2.20	2.12	2.12	2.05	2.10

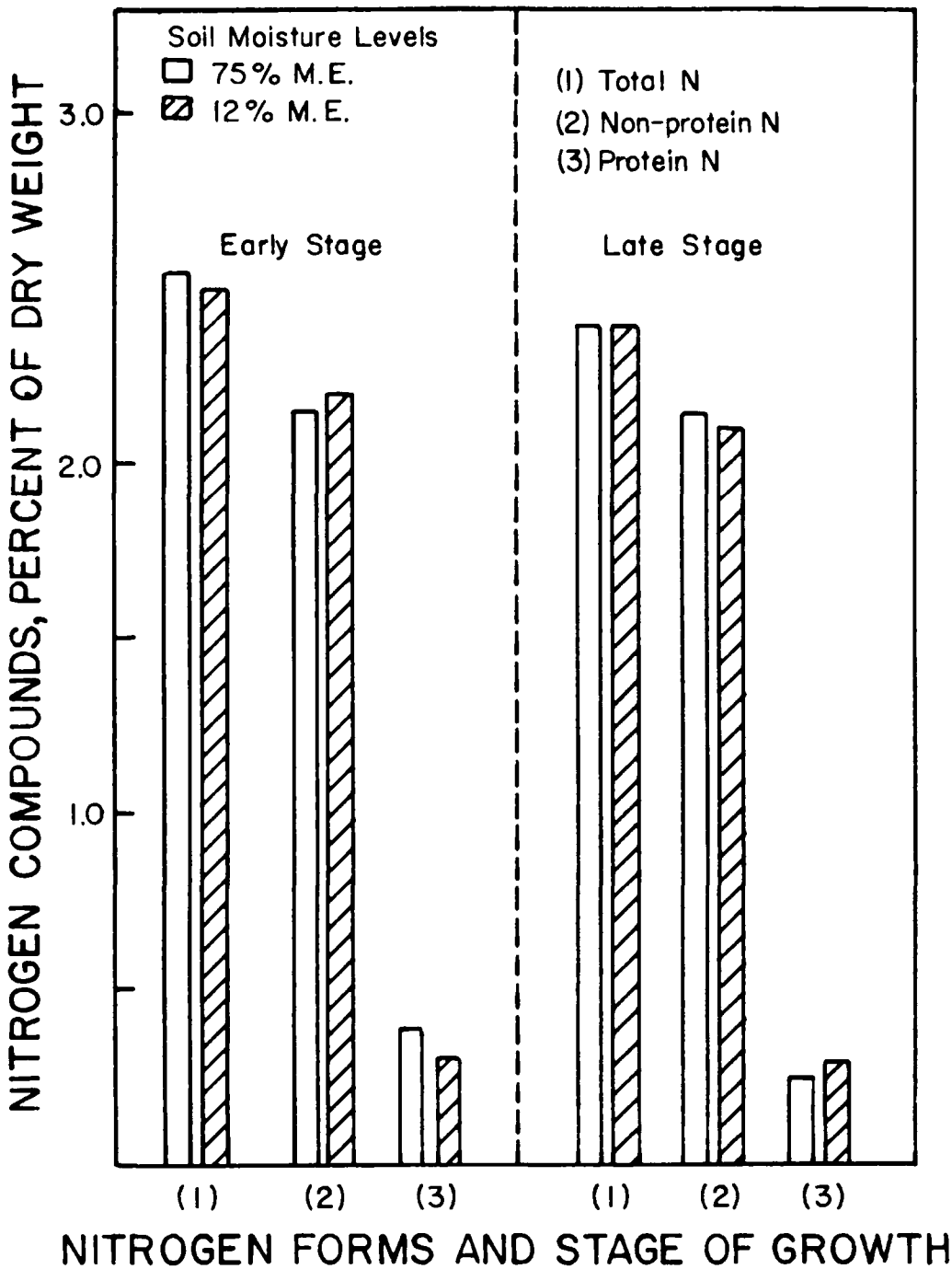

Fig. 10—Average percentage of water-soluble nitrogen compounds in soybean stem tissues at two stages of growth as affected by soil moisture level.

Table 12. Water-soluble Nitrogen Compounds in Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of nitrogen	Soil moisture level	Nitrogen compounds									
		Early stage					Early stage				
		Replicates				Mean	Replicates				Mean
		1	2	3	4*		1	2	3	4*	
	% of M.E.	percent									
Total N containing substances	75	16.7	17.7	17.7	18.7	17.7	14.5	12.2	13.9	14.5	13.7
	120	18.7	18.9	19.8	18.6	19.0	13.3	14.0	10.1	12.2	12.4
Protein N	75	10.61	11.2	12.0	10.9	11.1	7.7	5.2	7.3	7.0	6.8
	120	12.0	12.1	11.9	12.5	12.1	6.5	7.6	5.3	6.6	6.5
Nonprotein N	75	6.1	6.5	5.5	6.8	6.2	6.8	7.0	6.7	7.5	7.0
	120	6.7	6.8	6.8	6.6	7.1	6.8	6.4	5.4	5.5	6.0

The data are expressed on oven-dry weight basis.

* Replicate No. 4 is a combination of the remaining plant material of replicates 1, 2, and 3.

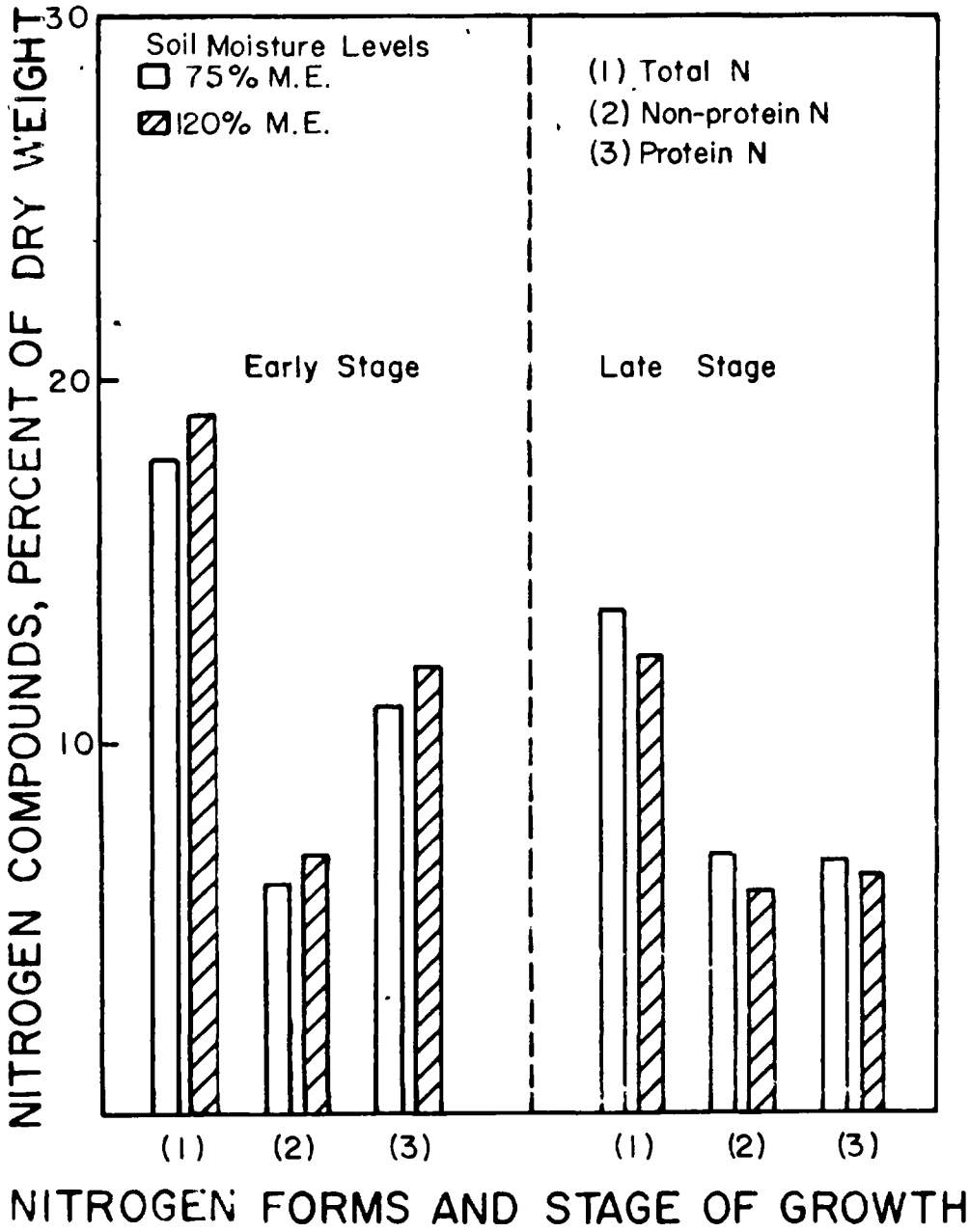

Fig. 11—Average percentage of water-soluble nitrogen compounds in soybean leaf tissues at two stages of growth as affected by soil moisture level.

fractions for chlorotic and nonchlorotic leaf tissues did not differ. In general, total N and protein N decreased significantly at the late stage of growth regardless of plant appearance; however, the nonprotein N remained the same at the two stages of growth.

Salt-soluble nitrogen compounds of leaf and stem tissues

The salt-soluble nitrogen fractions of stem tissues are shown in table 13 and figure 12.

The salt solution extracted more nitrogen compounds from the stem tissues than did water. However, no significant difference occurred with respect to the nitrogen content of the salt-soluble fraction in the stems of chlorotic and nonchlorotic plants. The data show a significant decrease in salt-soluble nitrogen compounds from the early to the late stage of growth.

The salt solution did not extract more nitrogen compounds from leaf tissues as it did with stem tissues. No significant difference was detected with respect to salt-soluble nitrogen fractions in chlorotic and nonchlorotic leaf tissues. In general, there was a significant decrease in the total salt-soluble nitrogen compounds as well as the protein fraction with the increase in plant age. However, the nonprotein nitrogen fraction remained the same. The data are shown in table 14 and graphically presented in figure 13.

Table 13. Salt-soluble Nitrogen Compounds in Soybean Stem Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of nitrogen	Soil moisture level	Nitrogen compounds							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	percent							
Total N containing substance	75	3.17	3.29	3.03	3.17	2.45	2.58	2.59	2.54
	120	3.28	3.13	3.03	3.14	2.54	2.65	2.43	2.54
Protein N	75	0.77	0.86	0.80	0.81	0.55	0.50	0.50	0.52
	120	0.75	0.86	0.77	0.79	0.52	0.56	0.43	0.50
Nonprotein N	75	2.40	2.43	2.23	2.36	1.90	2.08	2.09	2.02
	120	2.53	2.27	2.26	2.35	2.02	2.09	2.00	2.04

The data are expressed on oven-dry weight basis.

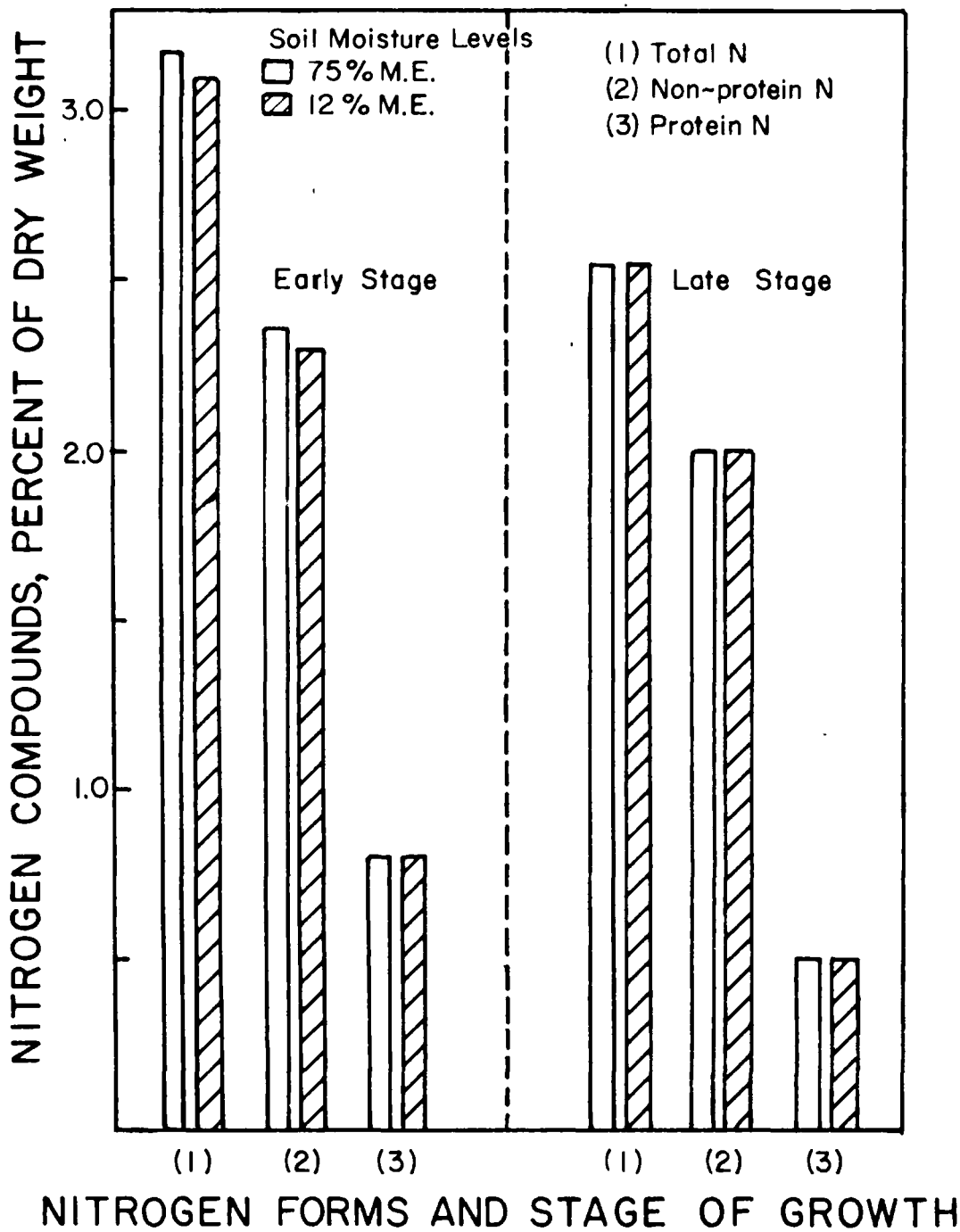

Fig. 12—Average percentage of salt-soluble nitrogen compound in soybean stem tissues at two stages of growth as affected by soil moisture levels.

Table 14. Salt-soluble Nitrogen Compounds in Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of nitrogen	Soil moisture level	Nitrogen compounds									
		Early stage					Late stage				
		Replicates				Mean	Replicates				Mean
		1	2	3	4*		1	2	3	4*	
	% of M.E.	percent									
Total N containing substances	75	18.9	18.9	18.0	18.7	18.7	14.0	13.9	13.9	14.5	14.1
	120	18.7	20.9	20.3	20.8	20.2	13.3	14.3	13.0	13.9	13.6
Protein N	75	10.9	11.4	12.1	11.8	11.5	7.6	10.7	7.5	7.2	8.3
	120	13.0	13.2	12.9	13.5	13.1	7.8	8.6	7.8	7.5	7.9
Nonprotein N	75	8.0	7.5	5.9	7.1	7.1	6.4	3.2	6.4	7.3	5.8
	120	5.7	7.7	7.3	7.4	7.1	5.5	5.7	5.2	6.4	5.7

The data are expressed on oven-dry weight basis.

* Replicate No. 4 is a combination of the remaining plant material of replicates 1, 2, and 3.

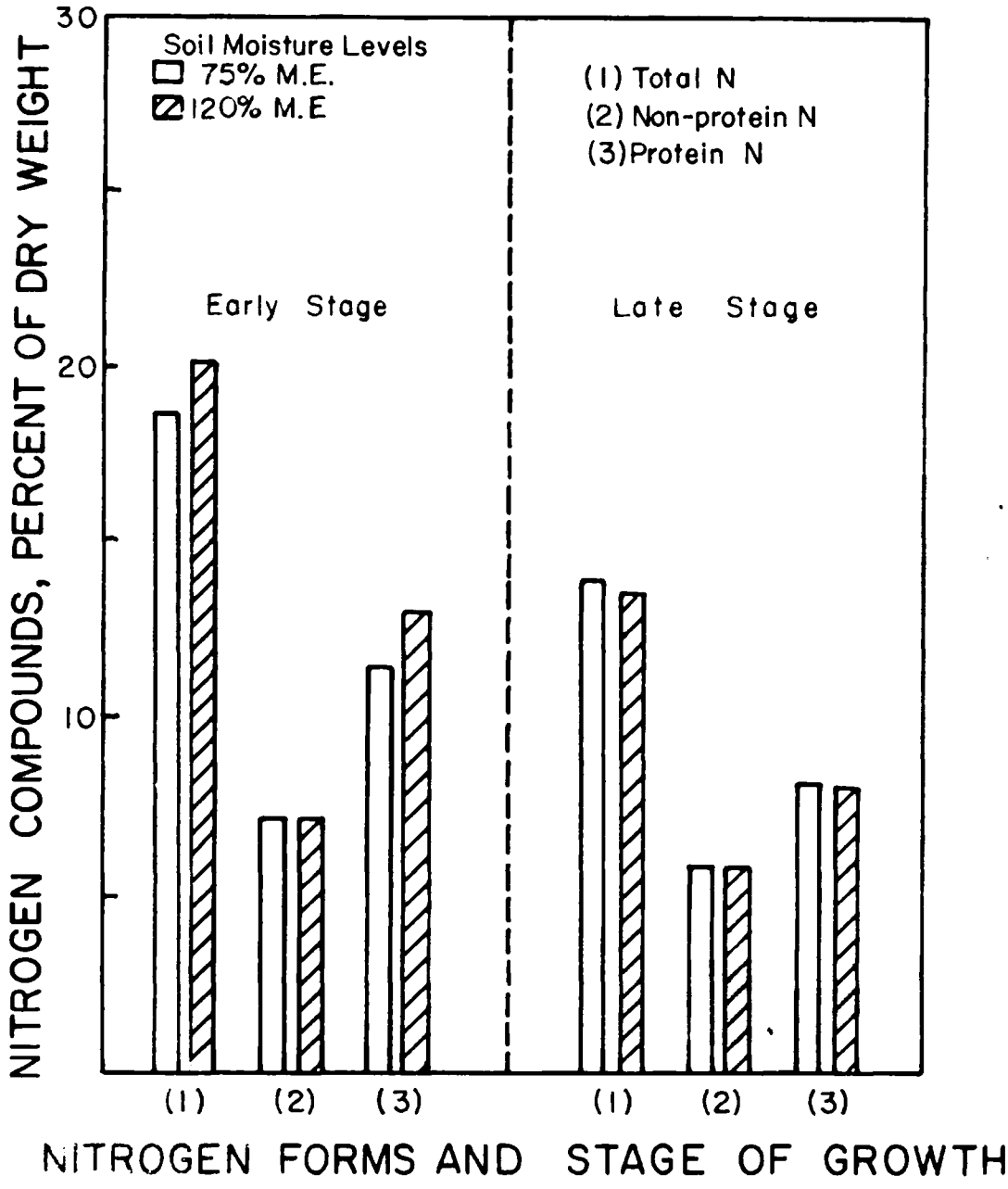

Fig. 13—Average percent of salt-soluble nitrogen compounds in soybean leaf tissues at two stages of growth as affected by soil moisture level.

The percent soluble iron on soluble protein nitrogen basis

Bennet (1) has suggested the use of protein nitrogen as a basis for calculating iron in plant tissues instead of the use of oven-dry weight where the weights were found to differ in chlorotic and nonchlorotic tissues.

The percent water and salt-soluble iron on a protein nitrogen basis of the extracts were calculated. Table 15 and figure 14 show this relationship in extracts from stem tissues. The data show a significant decrease in the percent of water-soluble iron of chlorotic stem tissues as compared to nonchlorotic tissue at the second stage of growth. The iron percent on protein basis decreased significantly only in chlorotic stem tissue with increasing plant age. The percent iron of the salt extract increased significantly only in nonchlorotic leaves at the late stage of growth. No significant difference was detected between chlorotic and nonchlorotic stem tissues with respect to the percent salt-soluble iron on protein nitrogen basis.

The percent iron on protein nitrogen basis of the water and salt leaf tissue extracts are shown in table 16 and figure 15. The data show a consistent relationship between water-soluble iron of the leaf tissues and the degree of green color development when iron was expressed as percent of protein nitrogen. The percent iron was significantly less in

Table 15. Percent Water- and Salt-soluble Iron on Soluble Protein Nitrogen Basis for Soybean Stem Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Iron/protein N X 10^{-2}							
		Early stage				Late stage			
		Replicates			Mean	Replicates			Mean
		1	2	3		1	2	3	
	% of M.E.	percent							
Water soluble	75	78	46	41	55	58	46	52	52
	120	42	62	72	59	32	22	38	31
Salt soluble	75	23	29	22	25	40	45	49	45
	120	26	23	23	24	45	44	25	38

Method of calculating iron in plant tissues.

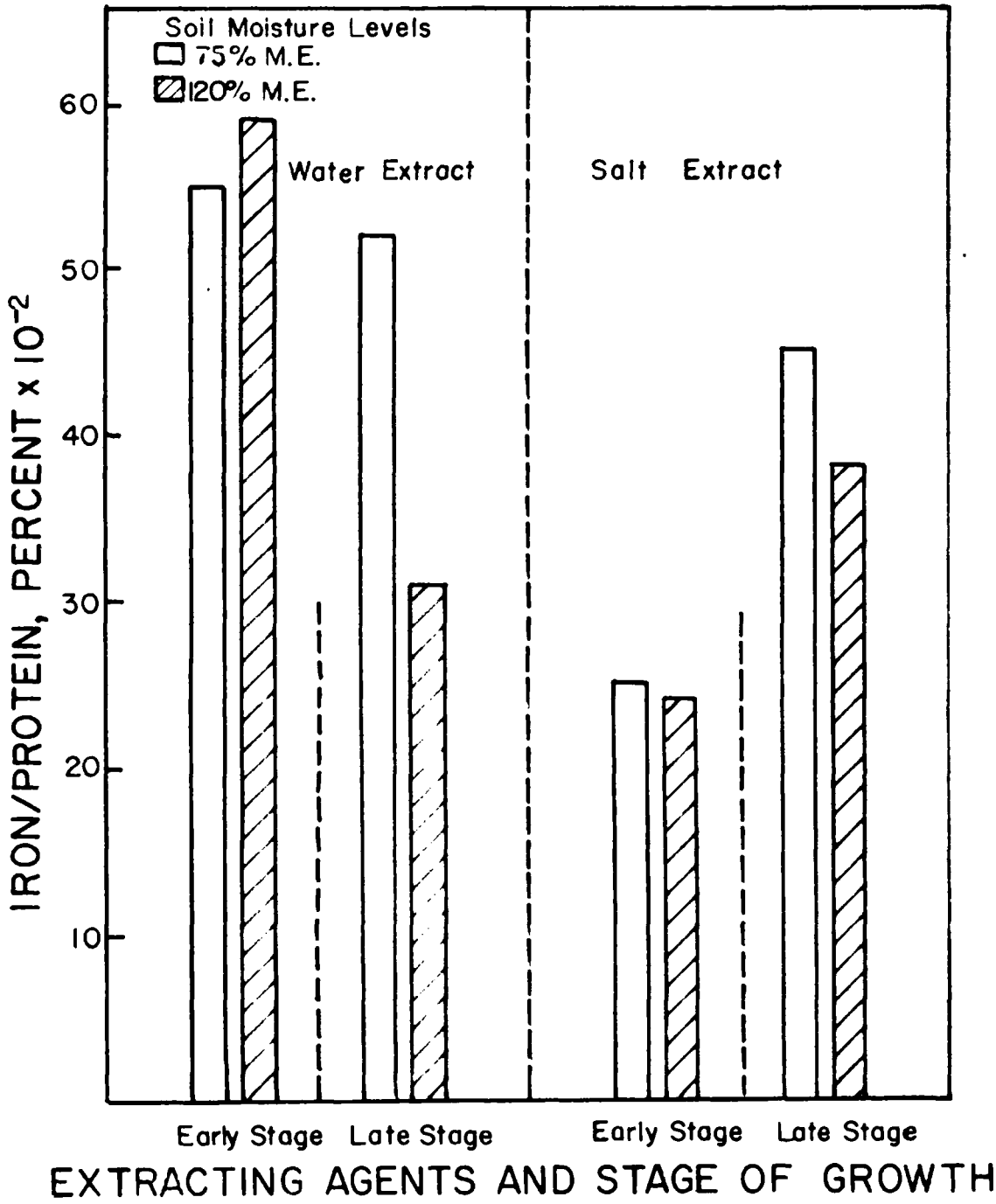

Fig. 14—Average percent of water and salt soluble iron of soluble protein nitrogen in stem tissues at two stages of growth as affected by soil moisture level.

Table 16. Percent Water- and Salt-soluble Iron on Soluble Protein Nitrogen Basis for Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Iron/protein N X 10^{-2}					
		Early stage			Late stage		
		Replicates		Mean	Replicates		Mean
		1	2		1	2	
	% of M.E.						
Water soluble	75	2.1	2.2	2.1	3.8	2.3	3.1
	120	3.1	1.1	1.2	2.1	2.1	2.1
Salt soluble	75	2.2	2.4	2.3	2.1	2.8	2.5
	120	1.6	1.6	1.6	2.1	2.4	2.4

Method of calculating iron in plant tissues.

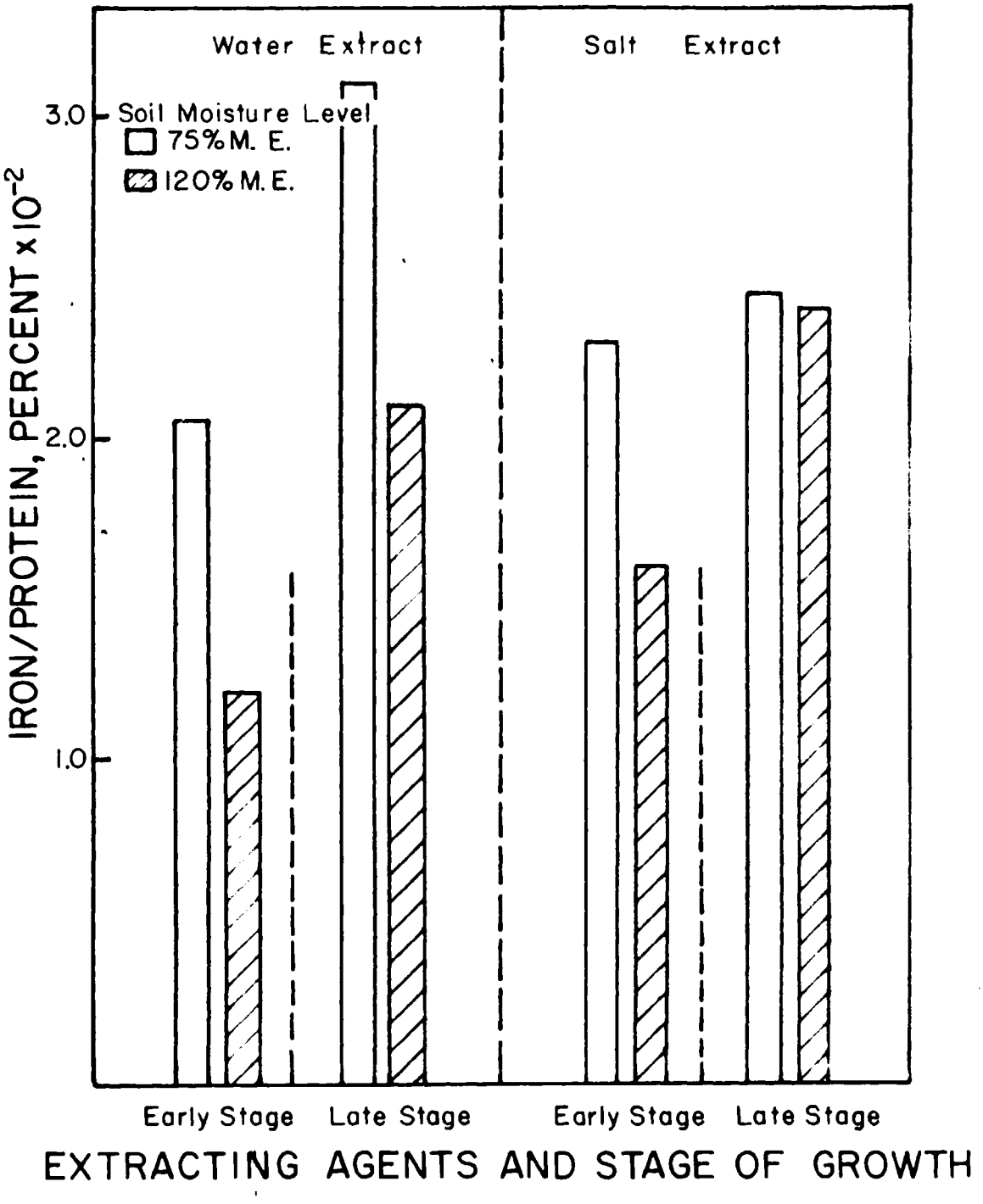

Fig. 15—Average percent of water and salt soluble iron of soluble protein nitrogen in leaf tissues at two stages of growth as affected by moisture level.

chlorotic leaves than nonchlorotic ones for water-soluble iron. The percent iron on protein basis of the salt extract was low in the chlorotic leaves only at the early stage of growth.

Trichloroacetic acid-soluble and insoluble iron in water and salt extracts from leaf tissues

Iron was determined in the trichloroacetic acid (TCA) fractions of water and salt extracts in an effort to determine the fraction that caused the difference in chlorotic and nonchlorotic total soluble iron. The total water-soluble iron, TCA-soluble iron and TCA-insoluble iron values are presented in table 17 and figure 16. The data show that the concent of TCA-insoluble iron compounds was the same in chlorotic and nonchlorotic leaves, while the calculated TCA-soluble iron fraction was high in nonchlorotic leaves. The salt-soluble extract of the leaf tissues did not show any appreciable difference in the TCA-iron fractions in chlorotic and nonchlorotic leaves, table 18 and figure 17.

Negligible amounts of iron were found in the TCA-insoluble fraction of the water and salt extracts from stem tissues. Therefore, most of the water and salt-soluble iron in the stem tissues was in a form soluble in TCA.

Table 17. Water-soluble, Trichloroacetic acid-insoluble, and Soluble Iron in Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Iron concentration					
		Early stage			Late stage		
		Replicates		Mean	Replicates		Mean
		1	2		1	2	
	% of M. E.	ppm.					
Total water soluble Fe	75	22.6	23.6	23.1	23.8	17.0	20.4
	120	16.2	13.1	14.7	16.1	13.7	14.9
TCA insoluble Fe	75	3.0	3.0	3.0	5.4	2.9	4.2
	120	5.8	2.1	4.0	5.3	2.1	3.7
TCA soluble Fe	75	---	---	20.1	---	---	16.2
	120	---	---	10.7	---	---	11.2

The data are expressed on oven-dry weight basis.

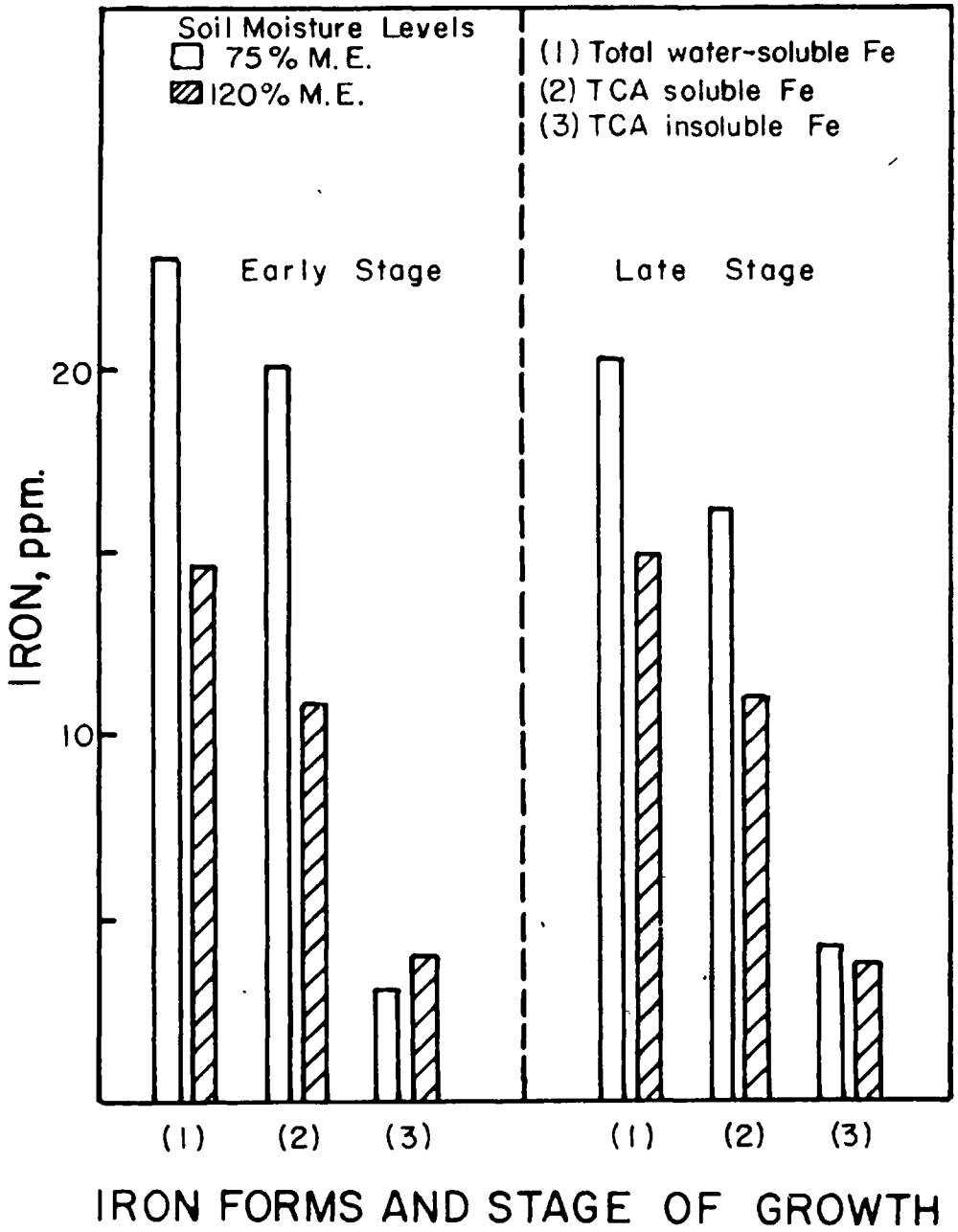

Fig. 16–Average concentration of water-soluble, trichloroacetic acid-insoluble and soluble iron in soybean leaf tissues at two stages of growth as affected by soil moisture level.

Table 18. Salt-soluble, Trichloroacetic acid-insoluble, and Soluble Iron in Soybean Leaf Tissues at Two Stages of Growth as Affected by Soil Moisture Level.

Forms of iron	Soil moisture level	Iron concentration					
		Early stage			Late stage		
		Replicates		Mean	Replicates		Mean
		1	2		1	2	
	% of M.E.	ppm.					
Total salt soluble Fe	75	26.9	23.6	25.2	22.9	21.4	22.1
	120	20.6	20.5	20.6	20.6	19.0	19.8
TCA insoluble Fe	75	9.9	4.0	7.0	2.9	2.9	2.9
	120	2.9	2.9	2.9	2.0	2.0	2.0
TCA soluble Fe	75	---	---	18.2	---	---	19.2
	120	---	---	17.7	---	---	17.8

The data are expressed on oven-dry weight basis.

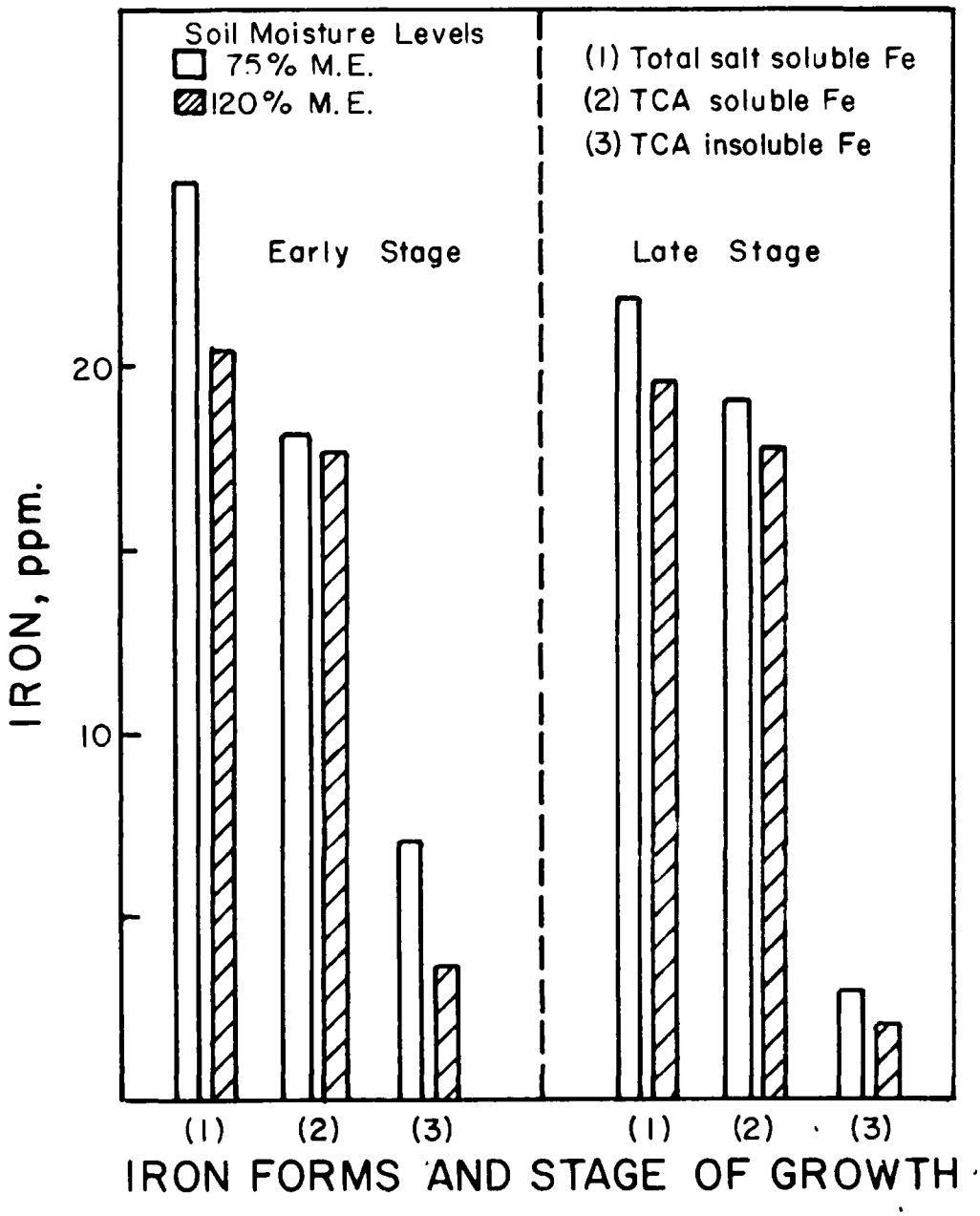

Fig.17–Average concentration of salt-soluble, trichloroacetic acid-insoluble and soluble iron in soybean leaf tissue at two stages of growth as affected by soil moisture level.

DISCUSSION

Soil iron

The results of the extraction of soil iron by EDTA and EDDHA agree with the studies of Wallace (50) for a calcareous soil. It has been reported that EDTA extracts less iron than EDDHA due to the great affinity of the former for calcium (50) or as a result of more hydrolysis of FeEDTA in the presence of calcium ions (28). Metwalley and Pollard (30) found an increase in calcium content of the soil solution with increasing water content of the soil. Thus, the significant decrease reported here for soil iron extracted with EDTA from soil at the high moisture level could be attributed to a relative increase in the free calcium ions.

The decrease in the amount of iron extracted with ammonium acetate, pH 3, as compared with water, pH 6, could be attributed to iron forms that were insoluble at the lower pH. Soluble organic forms of iron are usually soluble at high pH values. Water plus anion exchange resin also extracted less iron than water. This difference could be due to organic iron form or forms which possess a net negative charge and were held on the anion exchange resin. Thus, decomposing organic matter in calcareous soils under different moisture levels may result in

different amounts of soluble organic matter being produced which differ in their chelate stability for iron. The difference in stability of these organic iron forms may influence iron availability for plants.

Plant iron

The development of lime-induced chlorosis of soybean plants grown under 120 percent moisture equivalent level is in agreement with the interaction observed to occur under high soil moisture levels (9, 10, 40).

The data have shown that the iron concentrations of leaf and stem tissues were the same in chlorotic and nonchlorotic plants. Also, the ratio of total iron in the leaves to total iron in the stem of chlorotic plants did not differ from nonchlorotic plants. This indicates that lime-induced chlorosis is neither a matter of total iron in the tissues nor to impaired translocation of iron from stem to leaves. However, this may be in agreement with the hypothesis that iron is not equally active within chlorotic and nonchlorotic plants (6, 8, 36). The percent of the total iron which was water-soluble was significantly less in chlorotic stem tissues than nonchlorotic stem tissues at the late stage of growth. Water-soluble iron in leaf tissues was significantly less (on both concentration and percent of the total iron basis) only at the early stage of growth. In general, the values of water-soluble iron were less in chlorotic tissues than nonchlorotic when the iron was expressed on a

protein nitrogen basis. Maier and Catteni (26) postulated that the decrease in water-soluble iron in chlorotic tissues could be attributed to a difference in the chemical forms of iron or to unequal distribution of iron in plant cells. Perur (37) found that iron accumulated in the cell wall fraction of soybean and corn leaves as compared to the chloroplast or cytoplasm fraction.

The salt-soluble iron values did not show any significant difference between chlorotic and nonchlorotic tissues even when the values were expressed on a protein nitrogen basis. The lack of difference between chlorotic and nonchlorotic tissues in the amount of salt-soluble iron as it was found in water extract may be due to the precipitation or insolubility of certain iron compounds in the salt solution. However, the salt solution may dissolve iron compounds which are not soluble in water.

The data presented here for 1.0 N hydrochloric acid-soluble iron in leaf tissues showed that the active iron fraction of chlorotic leaves was less than that of nonchlorotic leaves. This indicates that the iron fraction soluble in 1.0 N hydrochloric acid was correlated with lime-induced chlorosis developed under high moisture level. Since the data have shown that the amount of active iron exceeds the amount of iron extracted with water, which represents the cytoplasmic protein (26, 38), it is clear that part of this active iron is present in another fraction of the cell. This portion of the cellular iron may be in the

chloroplast fraction. Perur (37) in his fractionation studies of corn and soybean leaves found that the difference in iron content between chlorotic and nonchlorotic leaves was observed only in the chloroplast fraction. Bennett (1) also suggested that active iron is associated with active protein in the chloroplast fraction which is active in chlorophyll formation.

The decrease in active iron level in nonchlorotic leaves at the late stage of growth is in agreement with that found by Bennett (1). Since Reuther and Crawford (40) showed that plants grown under normal moisture level have about 40 percent more chlorophyll than the high moisture treated plants. Thus, the development of lime-induced chlorosis in soybean plants may indirectly be related to the effect of high moisture on the amount of iron that is active in chlorophyll formation. Several investigators (2, 8, 31) have reported that iron can be inactivated internally in plant tissue by increasing phosphorus and calcium content in the tissue. Phosphorus and calcium uptake from the soil by different plants was reported (4, 17, 30) to be increased by high moisture level. Thus, an increased uptake of these ions by soybean plants grown under a high moisture level may possibly result in a physiological disturbance which could lead to the accumulation of "inactive forms of iron" within plant tissue. The inactive fraction which could be organic or inorganic may accumulate in the cell wall, and consequently

the iron content of the protoplasm of chlorotic plant cells would be low.

With respect to soluble nitrogen compounds, the percentage water-soluble nitrogen fractions were much the same in chlorotic and nonchlorotic plant tissues. This is in agreement with the studies of Perur et al. on soybean and corn plants (38). The salt solution extracted more nitrogenous compounds than did water from stem tissues; however, all the nitrogen fractions were the same in chlorotic and nonchlorotic tissues. Also, there was no difference between chlorotic and nonchlorotic leaves with respect to their nitrogen content of the salt-soluble fraction.

The data for the TCA iron fraction of water extracts showed that the concentration of TCA-insoluble iron compounds was the same in chlorotic and nonchlorotic leaves while there was a difference in the TCA-soluble fraction. The concentration of TCA-soluble iron fraction was less in chlorotic than nonchlorotic leaf tissues. Increasing the concentration of the TCA-soluble iron in the water extract of the leaf tissues may be a characteristic of nonchlorotic leaves. The TCA-soluble iron fraction in the salt extract of leaf tissues was about the same for chlorotic and nonchlorotic leaves.

SUMMARY

Experiments were conducted to determine whether soil moisture level affected the chemical forms of iron in plant and soil. Soybean plants, variety Lee, were grown on Adelanto loam soil under greenhouse conditions. Two different moisture levels, namely 75 percent and 120 percent of the moisture equivalent, were maintained in soil samples placed in individual containers. The same moisture treatments were used for separate soil samples on which the plants were grown.

Soybean plants grown under the high moisture level were chlorotic while those under the low moisture level were normally green in appearance. Plant samples were taken at two stages of growth.

Extractable soil iron was determined in the moisture treated soils by using different extracting agents. There was a significant decrease in soil iron extracted with 10^{-4} M EDTA from soil at the high moisture level which could be attributed to a relative increase in the free calcium ions.

Chlorotic and nonchlorotic leaf and stem tissues were analyzed for total iron, water- and salt-soluble iron, and 1.0 N hydrochloric acid-soluble leaf iron ("active" iron). In the water- and salt-soluble

extracts from leaf and stem tissues, the nitrogen compounds as well as the trichloroacetic acid-soluble and -insoluble iron fractions were determined.

The chemical analysis showed the presence of equal amounts of total iron and less amounts of water-soluble and "active" iron in chlorotic tissues as compared to nonchlorotic tissues. The difference found between chlorotic and nonchlorotic plants in the amount of iron in the water extract of the leaf tissues was in the trichloroacetic acid-soluble fraction. The content of water and salt-soluble nitrogen compounds were approximately the same in chlorotic and nonchlorotic plants. This indicated a possible difference between chlorotic and nonchlorotic plants in the chemical forms and/or distribution of iron within plant.

The development of lime-induced chlorosis by the use of high moisture levels could be attributed to an indirect effect on the chemical forms of iron by increasing the plant uptake of interfering ions.

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