

THE EFFECT OF VARIATIONS IN LIGHT INTENSITY AND IRON-MANGANESE RATIO OF THE CULTURE SOLUTION ON GROWTH OF SOYBEANS

> Thesis for the Degree of M. S. MICHIGAN STATE COLLEGE Caroline Baumgras Gray 1950

#### This is to certify that the

thesis entitled Effect of Variations in Light Intensity and the From manganese Ratio of the Culture Solution on From the of Soydeans presented by

Caroline B. Gray

has been accepted towards fulfillment of the requirements for

M. S. degree in Plant Physiology

Tembauer

Major professor

Date aug 2/ 1950

**O**-169

#### THE EFFECT OF VARIATIONS IN LIGHT INTENSITY AND IRON-MANGANESE RATIO OF THE CULTURE SOLUTION ON GROWTH OF SOYBEANS

By

## CAROLINE BAUMGRAS GRAY

#### A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

.

.

.

: . · ·

#### ACKNOWLEDGEMENTS

The author is grateful to Dr. G. P. Steinbauer, under whose supervision this investigation was carried out, for his helpful advice and criticisms throughout the entire study. To Dr. E. F. Woodcock, for his technical assistance with the histological portion of this problem, go many thanks. Acknowledgement is made to Mr. C. L. Gilly, Dr. W. B. Drew and Dr. G. B. Wilson for reading the manuscript. I also thank Mr. C. W. Reimer for his help in making the statistical computations.

## TABLE OF CONTENTS

•

																						Page
I.	INT	ROD	UCT	ION	•	•	٠	••	•	•	•	•	•	•	•	•	•	•	٠	•	٠	l
II.	HIS	TOR	ICA	L R	EV.	IE.	10	FL	ITI	IR.	)TI	JRI	2	•	•	•	•	•	•	•	•	2
	Α.	Ir	ona	and	Ma	ang	gan	ese	Re	ele	atj	Loi	ısł	nij	<b>s</b>	•	•	•	•	•	•	2
	Β.	E <b>f</b> :	fec	ts	of	Li	igh	t I	nte	ens	sit	cie	es	•	•	•	•	•	•	•	•	5
	с.	Hi	sto	log	ica	al	Fi	ndi	ngs	3	•	•	•	•	•	•	•	•	•	•	•	6
III.	MATI	ERI	ALS	AN	DI	Π	THO	DS	•	•	•	•	•	•	•	•	•	•	•	•	•	7
	Å.	Gei	nera	al	Cu.	ltu	ı.e	•	•	•	•	•	•	•	•	•	•	•	•	•	•	7
	Β.	Cu	ltu	re	Sol	Lut	io	ns	•	•	•	•	•	•	•	•	•	•	•	•	•	9
	с.	Hai	rve	st	•	•	•	•••	•	•	•	•	•	•	•	•	•	•	•	•	•	12
IV.	RESI	ULT:	3	•••	•	•	•	•••	•	•	•	•	•	•	•	•	•	•	•	•	•	15
	A.	Gro	oss	Ob	sei	c v e	ati	ons	•	•	•	•	•	•	•	•	•	•	•	•	•	16
	в.	His	sto	log	ice	al	Rea	sul	ts	•	•	•	•	•	•	•	•	•	•	•	•	19
	с.	Pla	ant	Me	ຄຣເ	ıre	ene	nts	•	•	•	•	•	•	•	•	•	•	•	•	•	22
	D.	Dry	y We	eig	ht	De	ita	•	•	•	•	•	•	•	•	•	•	•	•	•	•	24
۷.	DIS	CUSS	SIOI	ν.	•	•	•	•••	•	•	•	•	•	•	•	•	•	•	•	•	•	26
	Α.	Gro	ss	ОЪ	sei	rve	ati	ons	•	•	•	•	•	•	•	•	•	•	•	•	•	26
	в.	His	sto	log	ice	al	Rea	sul	ts	as	3 ]	['he	эy	Cc	rı	el	at	e	wj	ltł	ı	
		Gro	oss	0b	sei	٢Ve	ati	ons	•	•	•	•	•	•	•	•	•	•	•	•	•	28
	с.	Pla	ant	Me	ası	ire	eme	nts	•	•	•	•	•	•	•	•	•	•	•	•	•	30
	D.	Dry	7 We	eig	ht	Da	ita	an	1 (	lon	ipu	ιtε	ti	or	ıs	•	•	•	•	•	•	31
VI.	CONC	CLUS	SIOI	NS	•	•	•	•••	•	•	•	•	•	•	•	•	•	•	•	•	•	32
VII.	SUM	MARY	Z		•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	33
VIII.	BIBI	LIOC	FRAI	PHY	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	43

#### INTRODUCTION

Trace elements are chemical elements which, although essential to the growth of plants, are required only in minute quantities (20). During the last twenty to thirty years it has been learned that these elements perform definite physiological functions and that they must be available to the plant within a certain ratio to perform these functions at optimum efficiency. Iron and manganese are two such elements which, if totally absent or if present in an improper proportion, produce severe pathological conditions and cause considerable reduction in yield of an economic crop (4).

In 1941 Shive (18) attacked the iron and manganese problem with respect to soybeans in an effort to determine the optimum ratio of the two elements for this crop. From results obtained by him the ratio was found to lie between 1.5 and 2.5 in a water culture solution under the conditions of his experiment.

The primary purpose of this investigation has been to observe the differences in response of soybean plants to several different proportions of iron and manganese as affected by variations in light intensity.

#### HISTORICAL REVIEW OF LITERATURE

#### Iron Manganese Relationships

As early as 1897 manganese was recognized as a trace element with a definite physiological function by the French scientist, Bertrand (20). He found manganese to be present in the oxidizing enzymes of plants and subsequently discovered that small amounts of manganese salts stimulate the oxygen carrying power of these catalytic agents.

Kelley (8), working with pineapples, discovered that although a low concentration of manganese salts caused stimulation of oxidizing enzymes, higher concentrations caused a marked toxicity. An examination of leaves showing toxicity symptoms revealed marked plasmolysis, especially in the palisade cells. The starch content decreased, the plastids began to disappear and the palisade cells became filled with calcium oxalate crystals. Kelley explained his results by refuting the Bertrand theory of relationship between the activity of the oxidizing enzymes and the percentage of manganese in the soil. He did not believe that chlorosis could be completely accounted for on the basis of excessive autooxidation but that excessive manganese causes a disturbance of the mineral balance. He believed that one important effect of manganese is to modify the osmotic absorption of lime and magnesium and that the toxic results are primarily caused by these effects.

In 1917 Johnson (6, 7) found that chlorosis of pineapples

on the highly manganiferous soils of Oahu in the Hawaiian Islands was due to a depression in the assimilation of iron.

Hopkins in 1930 (5) found that manganese would not replace iron in the nutrition of <u>Chlorella</u> and that a number of other elements would not replace manganese. To explain the function of manganese he offered the hypothesis that this element tends to control the ratio of ferrous to ferric iron. He maintained that manganese brings about the reoxidation of iron after its reduction in the plant to the ferrous state. From this viewpoint it may be surmised that the ratio of manganese to iron in the plant is of more importance than the absolute concentration of manganese.

In 1941 the relationship was studied by Shive (18). He based his study on two important points: first, active iron exists in the ferrous state and, second, the oxidizing potential of manganese is higher than that of iron. Iron absorbed into the plant in the ferric state is immediately reduced by "powerful reducing systems" unless manganese is present as a counter reactant. At the present time, this appears to be a generally accepted idea but the sequence of events involved in the reaction is still unknown.

The oxidation of ferrous to ferric iron and the precipitation as ferric organic compounds is determined by the relative quantity of manganic ions in the system. Good growth was achieved with a wide range of iron concentrations in the nutrient substrate but only when the iron was accompanied by

a corresponding range in the concentrations of manganese. A similar relationship exists within the tissues of the plant between active iron and active manganese.

A year later Somers and Shive (19) ran a series of experiments on soybean to determine the ratio of iron to manganese associated with good growth and development of the plants. Eighteen various combinations of iron and manganese were used in their culture solutions. The iron varied from 0.005 ppm to 3.00 ppm and the manganese from 0.0 to 5.00 ppm. Approximate determinations of both soluble and insoluble iron and manganese in the tissues as well as dry weights were taken. Plants growing in solutions containing 0.002, 0.250 and 2.00 ppm manganese respectively were all normal and possessed about the same dry weight providing in each case the ratio of iron to manganese was within the range 1.5 to 2.5. Pathological symptoms appeared whenever the ratio was outside of this range.

If the ratio were above 2.5 the condition might be considered either manganese deficiency or iron excess. Under such a condition the upper leaves showed a fading of the green color between the veins followed by the development of small brown necrotic areas. Roots showed no visible symptoms other than being smaller than those of normal plants.

If the ratio were below 1.5 the condition was considered one of iron deficiency or manganese excess and the symptoms

of toxicity were a slight discoloration of the roots accompanied by yellowing and slight curling of the upper leaves. Later the roots progressively darkened in color, and the yellowing of the upper leaves continued. The new leaves appeared almost white. Large necrotic spots soon appeared on most of the chlorotic leaves; this was followed by the death of the apical meristem of the shoot. Root growth ceased soon after the first symptoms appeared on the leaves.

#### Effects of Light Intensities

J. M. Arthur (1) carried out experiments on plant growth under shading cloth. Plants studied were salvia, sunflower, buckwheat, dahlia and tobacco; these were grown, during late May, June and July, under light conditions ranging from 100% to 35% of total solar radiation. All plants grown in June and July except salvia produced higher dry weight when shaded than when grown in open sunlight. Full light intensity, he concluded, is apparently too high for maximum dry weight during June and July for a number of plants but is optimum for others.

McCool (11) studied the effect of light intensity upon the injurious effect of manganese on soybean and buckwheat in field plots and on soybean, snapbean, and tobacco in the greenhouse. He found that visible injury to plants grown in manganese treated soil decreased as the light intensity became less. The manganese content of leaves of soybean grown in

the greenhouse decreased as the degree of shading increased. The decrease was most marked on the lowermost leaf on each plant and less marked on the second and third leaves. The percent of manganese in the stems of plants grown in culture solutions containing 400 ppm of manganese varied but little. At 600 ppm the content of manganese in the stems increased somewhat with shading. The manganese content in the roots was high in snapbeans and tobacco; shading did not decrease the content in these plants as in tobacco.

## Histological Findings

In 1941 Ethel Etlinge (3) performed some experiments to determine the effect of manganese deficiency upon the histology of tomato. She found that in manganese deficient leaves the plastids, especially those in the palisade cells, were the first part of the leaf to show injury. Injury to plastids began by a gradual loss of starch grains followed by a development of vacuoles. At first, isolated palisade cells were the only necrotic cells. Necrosis then developed in groups of palisade cells, and finally the injury spread to the spongy parenchyma and the epidermis.

Manganese deficient stems were smaller in diameter and were found to contain less xylem. Often the xylem cells present were plugged with coagulated materials.

Struckmeyer and Berger in 1950 (21), following the procedures used by Etlinge (3), studied the effect of manganese

toxicity on the histological structure of potato stems and leaves. Plants were grown in a solution containing 200.5 ppm of manganese. The internal sign of toxicity in the stem was a collapse of cells of the epidermal and cortical region corresponding to the brown necrotic streaks on the surface of the stem. Parenchyma cells seemed to be affected first from the cortex through the ray to the pith. There was a progressive degeneration and finally a collapse of these cells. As the ray parenchyma cells collapsed, some of the adjacent vascular tissue also became affected. Cells of the internal phloem proliferated to form a mass of disorganized tissue and within this region some cells enlarged while others were being crushed and obliterated.

In early stages healthy cortical cells produced wound cambium which separated injured outer and normal inner tissue. However, since these cells did not become suberized, the symptoms were not prevented from spreading.

As for the leaves, the authors merely mention that dark brown areas of collapsed cells appeared between the veins.

#### MATERIALS AND METHODS

#### General Culture

Soybeans were chosen as the crop plant for this work because they have been shown to require a relatively high amount of manganese for optimum growth (19). Seeds, from a lot being

sold to farmers for field sowing, were obtained from the R. J. Carl Elevator, Lansing, Michigan.

Plants were grown in two lots on an open bench in an unshaded greenhouse on the campus of Michigan State College. Each lot was grown for a period of five weeks. Temperatures, ventilation and humidity were those used for general cool house greenhouse crops (9). General weather conditions varied to some extent. The first lot, placed in containers on March 22, was grown under cloudy, rainy, rather cool conditions while the second lot, placed in containers on April 30, received much more sunshine and the temperatures were generally higher.

34.31

Seeds were germinated by the wet towel method using a solution containing 0.1 gram  $LH_2FO_4$ , 4 grams  $Ca(EO_3)_2$  and 1 gram  $M_6SO_4$  per liter (14). As soon as the seeds had sprouted, the seed coats were removed as they have been found by LeHargue (12) to contain a large accumulation of manganese. Seedlings were allowed to grow until the hypocotyls reached a length of between 5 and 6 cm. before transfer to the culture solution.

Two seedlings were placed in each of sixty culture containers. These containers were prepared by painting 1 quart glass jars with aluminum paint to provide a light shield. Such a shield tends to limit algal growth. After painting, the jars were thoroughly cleaned with a 5% solution of  $H_2SO_4$ and washed in hot soap suds. Cardboard inserts were removed from the bakelite caps and 2 holes 3/8 inches in diameter were bored on opposite sides of the caps. Plants were placed through these holes, roots first, after the hypocotyls reached

a length of 5 to 6 cm. and were held in place by cotton wads.

The sixty containers, used for each lot, were divided into four groups of fifteen. Each group was filled with either solution A, B, C or D as described below. Five containers from each group were placed on the open greenhouse bench. Another five were placed in light cages under medium shade and the remaining five containers from each group were placed in light cages under heavy shade.

Light cages were built of light weight 1" x 1" lumber. The frames were 20 inches square and 30 inches high. The cage for heavy shade was covered with six layers of white cheese cloth with overlapping only at the edge of the frame. A loose flap was left on the front edge for ease in removing culture jars. The second frame for medium shade was built in the same manner and covered with two layers of white cheese cloth.

Light meter readings were taken on May 23 at 9:15 a.m. at various places over the growing areas. Averages for the three conditions were as follows:

full sunlight,	2650	foot	candles
medium shade,	1350	foot	candles
heavy shade,	1000	foot	candles

Container numbers, culture solutions and light conditions are summarized in Table 1.

# Culture Solutions

Concentrations of iron and manganese used in this study

## TABLE I

# Outline of culture solutions, container numbers and light conditions followed in this study

Solution	Manganese Content	Containers	Light Condition
A B C D	0.002 ppm 0.010 ppm 2.00 ppm 20.00 ppm	04 59 1014 1519	Full light """ """
A B C D	0.002 ppm 0.010 ppm 2.00 ppm 20.00 ppm	2024 2529 3034 3539	Medium shade n n n n n n
A B C D	0.002 ppm 0.010 ppm 2.00 ppm 20.00 ppm	4044 4549 5054 5559	Heavy shade """ """

.

were adapted from Shive's (18) work on iron and manganese ratios. In that paper 2.00 ppm manganese was assumed to be optimum when the iron concentration was 3.00 ppm and the other concentrations of manganese were derived on the basis of this as a mid point (10).

The culture solution for this work contained the following salts:

> 0.002M  $\text{KH}_2\text{PO}_4$ 0.001M  $\text{NH}_4\text{NO}_3$ 0.002M  $\text{Ca}(\text{NO}_3)_2$ 0.001M  $\text{MgSO}_4$

All solutions contained, in addition, 3.00 ppm of iron supplied as  $FeSO_4$ . Manganese was added in the form of  $MnSO_4$  as follows:

Solutions A	,	0.002	ppm
Solutions E	3,	0.010	ppm
Solutions C	;,	2.000	ppm
Solutions D	), 2	20.000	ppm

All salts in the culture solutions with the exception of the iron and manganese were used in the same concentration as those suggested by Trelease and Trelease (22, 23) to maintain a pH of 5.1.

The optimum pH for maintenance of maximum iron and manganese availability is 5.1 according to Shear, et al (16). Since there was no means available in the present experiment for producing a continuous flow of solution to maintain a constant pH, another method had to be found.

The work of Trelease and Trelease (23) demonstrated that when a NO/NH<sub>4</sub> ratio of suitable value was used a physiologically balanced solution was obtained and the hydrogen ion concentration remained constant over an eight day period. By balancing the partial concentration of nitrate (the absorption of which removes H ions from the solution) against the ammonia (the absorption of which removes OH ions from the solution), the factors which tend to increase acidity are exactly opposed to those which tend to decrease acidity. For a pH of 5.1 this ratio was 90 NO<sub>3</sub>/10 NH<sub>4</sub> for the first 48 days of growth. Had the plants been grown beyond this period the ratio would have had to have been changed since the ratio to maintain stable pH becomes lower and lower with the age of the plants.

Solutions in all cases were changed at the end of seven days, the new solutions being freshly made at the time of change. Solutions were made in 15 liter lots. Salts were dissolved individually in cold double distilled water and the solution brought to volume. Double distilled water was also used as a wash for all glassware involved.

#### Harvest

#### Gross observations:

Condition of the plants was carefully noted weekly at the time of general solution changes. Cultures were checked for the first appearance and the development of pathological

symptoms such as development of chlorotic areas, appearance of brown necrotic spots, general stunting of growth, lack of vitality of the growing points and the browning of the roots. A complete record of the over all condition of the test plants was taken at the time of final harvest for both lots one and two.

#### Histological sampling:

The first plants were removed for histological sectioning on April 5, 1950. Samples were taken from the primary root, from the secondary root at a point where secondary thickening had occurred, from the stem, from the cotyledons and from the leaf through a vein. Sections were placed immediately into chrom acetic acid killing solution (24). Sections were then aspirated a suitable length of time to remove all air and were left over night in the chrom acetic acid to allow the killing process to go to completion. A 24 hour washing period in a continuous flow of cool water bath followed. Dehydration was accomplished by passing the sections at 4 to 6 hour intervals through a series of alcohols starting at 35% and ending at 100%. Xylol was then introduced in a like manner until the sections were in 100% xylol. Imbedding in household paraffin (Parowax) followed. Sections were kept in the paraffin oven in a 100% paraffin solution for 4 days.

Paraffin blocks were made by the use of paper boats  $1\frac{1}{2} \ge 25/8$  inches in size and were then mounted on wooden

microtome blocks.' Sections, cut on a rotary microtome, were of the following thicknesses: leaves and cotyledons, 12 microns; roots, 18 microns; stems, 22 microns.

Sections were mounted on standard glass microscope slides using an egg albumen, glycerine fixative and a 2% formalin solution. All sections were stained with a 1% aqueous solution of methyl green and a 70% alcoholic solution of bismark brown. Methyl green is a specific stain for lignified tissues and bismark brown for parenchyma tissues.

The second set of histological samples was taken at the time of general harvest on April 26, 1950. The same procedure was followed with one exception. No samples of cotyledons were taken since by this time the cotyledons on most specimens had abscissed. A third series of samples were taken in the same way at the time of harvest of lot two on June 4. Samples were carried to the 70% alcoholic dehydration stage pending necessity for further processing.

#### Plant measurements:

At the time of final harvest, on April 26 for lot one and June 4 for lot two, plants were cut with a sharp razor blade just below the cotyledons in order to remove them from the culture jars without injury. Separate measurements were taken, in centimeters, of the portion of the plant above the cotyledons and the portion of the plant below the cotyledons for each plant involved. In the latter case, the measurement was to the tip of the longest root. Measurements of the two plants in each jar were added together, again keeping the upper and the lower portions separate. Measurements throughout the rest of the paper will therefore be the total of the two plants in each jar.

#### Dry weight computations:

New 200 cc beakers were washed in hot soap suds and dried in a  $90^{\circ}$ C. oven until a constant weight was obtained. After measurement of the test plants they were carefully patted with absorbent toweling to remove any adhering culture solution. The plants were then cut up as necessary and those from each culture jar were placed in individual beakers. There were four beakers for each solution used in the experiment. These beakers were then removed to a drying oven maintained at  $90^{\circ}$ C. for a period of one week. Dry weights were taken until they were constant through the second decimal place.

#### RESULTS

Results of this experiment were tabulated in four differend forms: gross observations, histological results, plant measurements as computed for the portions above and below the cotyledons, as well as the total length and dry weight data. Results will be treated in this order in this section of the paper.

#### Gross Observations

Jars A0 through A4 contained plants grown in solution A (containing 0.002 ppm of manganese) and exposed to full sunlight. On April 26 plants in this group appeared stocky and firm. The hypocotyl in all cases was split on either side of the jar opening. The roots were sturdy with much secondary and tertiary branching in the last week of growth, however, the roots appeared to have a tendency to brown. The first, second and third leaves developed a severe chlorosis between the veins. The veins remained a rich, dark green. The fourth leaves appearing at the growing point were very yellow with a tendency to dry. Growth was beginning to be less vigorous with much shortened internodes.

Plants in jars B5 through B9 were grown in solution B (containing 0.010 ppm manganese) and exposed to full sunlight. These plants appeared sturdy and strong until the unfolding of the second leaf. These leaves showed a definite yellow chlorosis. Minute but profuse brown necrotic areas appeared first on the second and later on the first leaves also. Growing points were stunted but not dying as in jars A0--A4. Leaves generally were small and chlorotic. Roots were creamy in color, profuse and much branched. As in A0--A4, the hypocotyls were split at the jar opening.

Growth was generally sturdy in jars Cl0 to Cl4. These plants were grown in solution C (containing 2.00 ppm manganese) and received full sunlight. All leaves appeared green

.

with little or no chlorosis. There was some evidence, however, of the development of slight brown necrotic areas. Growing points were sturdy and new leaves were normal. Root growth was not quite as heavy as in the two preceding groups with considerable secondary but no tertiary branching. Roots were creamy in color and again the hypocotyls were split.

Plants in jars D15 through D19 were grown in solution D (containing 20.0 ppm manganese) and were exposed to full sunlight. The plants were extremely stunted and this stunting appeared with the first growth made by the plants after being placed in the solution. As soon as the first leaves unfolded, growth appeared to have stopped; there was no further enlargement of the leaves which were extremely wrinkled and profusely covered with brown necrotic areas. Roots were sparse with no secondary branching. They were very brown and slimy. This reaction was an immediate one. Hypocotyls in this case remained intact.

Jars A20 through A24 contained plants in solution A and exposed to medium shade. Plants were apparently firm and vigorous. The first leaves were uniformly green. The second leaves, however, showed a strong yellowing between the veins. The growing points remained alive but the new leaves were more severely chlorotic than the second. Cream colored roots were fairly heavy with much secondary but little tertiary branching. Hypocotyls were split.

Jars B25 through B29 contained plants with vigorous growth. These were growing in solution B and exposed to medium

shade. The second leaves developed a yellow color uniformly throughout. To some extent, fine brown pin point lesions were found on the second and unfolding leaves. Growing points were developing more nearly normal than in jars A20--A24 except the young leaves which were opening chlorotically. Roots were fairly full and creamy color. Hypocotyls were split.

Normal sturdy plants of good vigor were found in jars C30 through C34. C solution and medium shade were the conditions under which they were grown. Roots were moderately abundant with short tertiary branching. They were creamy in color. The hypocotyls split.

Plants in jars D35 through D39 were completely stunted. There was no development beyond the unfolding of the first leaf. Leaves as well as the hypocotyls were covered with brown pin point lesions. Roots were very brown with only sparse secondary branching. The hypocotyls remained intact.

Plants grown in solution A and exposed to heavy shade were in jars A40--A45. Growth was rank and the stems were spindly throughout. There was a slight tendency toward chlorosis between the veins of the third leaves. Roots were white and very sparse with some secondary but no tertiary branching. Hypocotyls remained intact.

Plants from B45 through B49 were also delicate stemmed but with rank growth. The plants were grown in solution B and exposed to heavy shade. A general yellowing of the upper and third leaves was becoming apparent with definite chlorotic spots between the veins developing. Creamy roots were not numerous but exhibited secondary branching and the beginnings of tertiary branching. Hypocotyls were not split.

Jars C50--C54 contained plants grown in solution C in heavy shade. Although growth was rank, the stems were delicate and spindly. Plants were normal throughout as to color. Roots appeared to be the same as in plants A40 through A49.

Plants in jars D55 through D59, although grown in heavy shade, had the same toxicity symptoms as the other plants grown in solution D.

The experiment was repeated exactly with lot two which was harvested on June 4, 1950. Plants grown in this lot were stockier and of far better vigor than those of lot one. It is believed that this result was due directly to a difference in general weather conditions which were much more favorable during the later growing period. General pathological symptoms were very similar in both lots and developed at about the same rate under both sets of conditions.

#### Histological Results

Stained sections of the soybean test plants showed the greatest effects from the manganese and light variations to exist in the leaves. Plants grown in solutions A and B produced leaves which were very similar in appearance when stained with methyl green and bismark brown. Leaves generally were indented at the veins producing a crinkled appearance which was much more noticeable microscopically than macroscopically. Chlorosis appears to start at the epidermis, usually the upper epidermis above the palisade parenchyma, and progresses inwardly. Shrinkage and collapse was followed by final disintegration of the epidermal cells. Cells in the collapsed condition took a very heavy methyl green stain.

Palisade cells were next affected. Walls of this layer thickened and crumbled. As soon as the palisade cells showed any reaction the chloroplasts began to disappear. By the time the walls began to crumble almost all chloroplasts had disintegrated. Spongy parenchyma did not show the effect until the lower epidermis began to disintegrate. Veins appeared to be unaffected and parenchyma tissue immediately surrounding them remained intact.

Plants grown in solution C produced leaves which in cross section revealed cells in their normal condition. A palisade layer and a spongy parenchyma layer made up the mesophyll of the blade. The palisade tissue was formed by a single layer of elongate parenchyma cells at right angles to the epidermis and separated by only a few intercellular spaces. Coming into contact with the palisade layer was the spongy parenchyma whose cells were large and numerous in this area. This spongy tissue contained about four layers of cells which did not have as many chloroplasts as the palisade cells. The upper epidermis was about  $l_2^1$  times as thick as the lower epidermis. No chloroplasts were found in these cells. The midrib projected prominently on the lower surface of the blade but less so on the upper surface. The vascular tissue, containing xylem and phloem, was semicircular in form with the open part toward the upper epidermis (2).

Group D produced leaves with large areas of collapsed cells which had stained heavily with methyl green. Disintegration usually started at the upper epidermis and passed progressively through the palisade parenchyma and spongy parenchyma to the lower epidermis.

No abnormalities were observed in the stems and roots of plants grown in solutions A, B and C. Cross sections of the roots showed vascular tissue in a tetrarch arrangement forming a central core. It was limited on the outside by an endodermis and a cortex. The pericycle, opposite the phloem, was one cell thick but several cells thick opposite the xylem. The cortex contained about 15 rows of parenchyma cells among which were numerous intercellular spaces. The epidermis in all cases was broken away in the preparation of the slides.

There were generally 12 collateral bundles in the stems. The pith area was small. The pericycle was lignified where it came in contact with the phloem. The cortex consisted of several rows of large parenchyma cells.

Stems of plants grown in solution D showed small areas of collapsed tissues in the cortex which corresponded to the necrotic areas on the outer surface. Roots also showed the results of excess manganese. Disintegration started with cortical parenchyma and progressed inward with the continuation of the treatment. Secondary roots corresponded in their

appearance to the primary roots of the respective plants.

Cotyledons in no case showed any variation from the normal condition.

#### Flant Measurements

Table II shows the compiled averages of lengths of portions above and below the cotyledons and total lengths for plants grown in the four solutions under the three light conditions. All measurements were made in centimeters.

From this table it will be noted first that, in all lots and under all light conditions, plants grown in solution C were superior in length of the upper portion of that plant except under heavy shade where plants grown in solution B were superior both in lots one and two. In plants grown in solutions A, B and C, lengths of the part above the cotyledons in lot one exceeded the length in lot two. In plants grown in solution D, lot one exceeded lot two in length of the upper portions except under heavy shade in which case the condition was reversed.

Lengths of the portions below the cotyledons were little affected within their lot even including the plants grown in solution D (See Figure 2). Neither light intensity nor concentration of manganese seemed to affect the actual length. To a certain extent, length of the lower portions of lot one did exceed those of lot two.

The effect of variation in manganese concentration and

Lig	ht	Manganese		Leng	ths	
Condi	tion	Content-ppm	Lot	Above cot.	Below cot.	Total
Full 1	ight	0.002	1	33.9	59•5	93 <b>.4</b>
"	"		2	19.0	52•4	71 <b>.4</b>
11	n	0.010	1	32.4	60.1	92 <b>.5</b>
11	n		2	16.3	48.7	65 <b>.</b> 0
11 17	11 17	2.00	12	37.3 19.4	56.3 53.1	93.6 72.5
77	17	20.00	1	5 <b>.1</b>	57.9	63.0
77	17		2	7 <b>.</b> 2	40.4	47.6
Medium	n shade	0.002	1	43.3	64 <b>.4</b>	107.7
"	"		2	26.9	51 <b>.</b> 5	78.4
17	11	0.010	1	47.9	60.0	107.9
17	11		2	26.8	52.5	79.3
11	11	2.00	1 ·	47.9	62 <b>.2</b>	101.1
17	11		2	27.4	48.4	75.8
17	17	20.00	1	4.9	51.5	56.4
17	17	"	2	9.1	31.2	40.3
Heavy	shade	0.002	1	61.3	56.2	117.5
"	"		2	35.8	44.6	80.4
17	11	0.010	1	70.2	52.4	122.6
17	11		2	41.8	44.2	86.0
17	17	2.00	1	63.2	62.2	125.4
17	17		2	39.8	51.1	90.9
17	17	20.00	1	5.1	40.9	46.0
17	17	"	2	6.9	44.6	51.5

Variations in length of soybean plants when grown under varying conditions of light intensity and manganese content. All measurements are in centimeters. light intensity upon total length of the soybean plants has been shown in graphic form in figures 3 and 4. Since these graphs represent the averages of the sums of the upper and lower portions of the plants, and since the roots vary but little, the general trend of growth follows closely that of the upper portions (See Figure 1) of the plant.

#### Dry Weight Data

Average dry weights were computed for the groups of plants grown in solutions A, B, C and D of lots one and two under the three light intensities. The data are presented in Table III.

As in the case of the plant lengths it may also be observed here that plants in lot two in all cases exceeded the weight of plants in their respective groups of lot two. Greatest weights were recorded for plants grown in solution C at all three light intensities. Greatest weights were also recorded in both lots one and two in full sunlight with one exception: in lot two, plants grown in solution D, the greatest weight was recorded in medium shade.

Manganese appears to have an unusual effect on both lots of soybean plants when data on light conditions and weight are correlated. Plants grown in solution C consistently had higher weights than those grown in solutions A, B and D and plants grown in solution D consistently had lighter weights than all the rest. However, on graphs (Figures 5 and 6), prepared on the basis of light and on the basis of manganese

# Table III

# Dry weights of two lots of soybean plants. Each figure represents the average weight of eight plants.

Manganese			Light conditions				
Content	Lot	Full light	Medium shade	Heavy shade			
nAn							
0.002 ppm	1	1.67*	1.42*	1.13*			
	2	1.18	1.04	.80			
<b>"</b> B"			-				
0.010 ppm	1	1.44	1.19	•90 <b>7</b>			
	2	1.03	•93	•70			
<b>#C</b> #		-		-			
2.00 ppm	1	1.81	1.61	1.22			
	2	1.35	1.23	.93			
иDи							
20.00 ppm	1	.332	.303	.272			
	2	•34	.52	.25			
			-				
**L.S.D. <	1%	<b>±</b> .19	± .32	<b>±</b> .48			
**L.S.D. <	5%	<b>±</b> .14	<b>±</b> .24	<b>±</b> .36			

\*

V V

.

Base numbers. Pertain to lot one only. \*\*

content, it may be observed that group B falls far below group A in dry weight even though the culture solution contained more manganese than A and less manganese than C.

#### DISCUSSION

#### Gross Observations

From general observations it is apparent that solution C was consistently the better solution under all three light conditions used in this experiment since in most cases, no chlorotic effects at all were evident. This was to be expected for the results are directly in line with those of Somers and Shive (19). In 1942 they substantiated the earlier results of Shive (18) who found that iron and manganese must be present in a certain ratio for optimum growth. That ratio was found to lie between 1.5 and 2.5. Iron was used in solution C at the rate of 3.00 ppm and manganese at the rate of 2.00 ppm, the ratio being 1.8. Solution B had a ratio of 300, solution A had a ratio of 1500 and solution D a ratio of 0.15. Above 1.5 manganese was deficient and a chlorosis resulted from an excess of iron. Under these conditions, the manganese counter reactant for the natural reducing systems in the plant was absent and the ferric iron was easily reduced to the active ferrous state. In solution D, where the ratio was less than 1.5, just the reverse was true.

The severe chlorosis between the veins of the leaves,

with occasional development of small brown necrotic areas and eventual inactivation of the growing points are results consistent with those of Somers and Shive for manganese deficiency (18). However, in solutions with a manganese toxicity they found plants to develop a general chlorosis throughout the leaves which became so severe that the new unfolding leaves were almost white. Death of the growing points followed. The first visual sign of toxicity in their work was the immediate browning of the roots.

In the manganese toxic solution (D) the severe browning of the roots was the only symptom consistent with those of Somers and Shive. The leaves in this case were not chlorotic but instead were densely covered with minute necrotic areas and were extremely crinkly and curled. The entire plant was severely stunted. A partial explanation may lie in the fact that their toxic solution had a Fe/Mn ratio of 0.6 while this one had a ratio of 0.15. The concentration of manganese in the latter was considerably higher and may account to some extent for the difference in the results.

That deficiency symptoms were delayed and seemed to be of less severity under medium shade and even to a greater extent under full shade seems to be established. The work of McCool (11) states that the manganese content of the leaves of tobacco decreased with a decrease of light intensity. Toxicity symptoms also decreased with a lower light intensity. The final yield of the tobacco plants grown on soil to which excess manganese sulfate was added increased by shading the

plants. Apparently, there is a definite relationship between the assimilation of manganese and the light intensity.

Stems varied little from normal except in plants grown in solution D under all light intensities when they did show small necrotic areas. Since they have not been mentioned in the literature it is probable that the reaction of the stems is of little value as an indicator.

Roots apparently are normally creamy in color as this was the case not only in solution C but also in solutions A and B. On the other hand, severe stunting of the shoot and extensive browning of the roots are characteristic of manganese toxicity.

The split hypocotyls appeared consistently in plants which were growing most vigorously. This may be due to the constricted area and the rough edges through which they grew. It is not felt that the splitting was due to the manganese concentrations or light intensity.

### Histological Results

Chlorosis between the veins of the leaves and gradual bleaching of the growing point appears to be due to the inactivation and disintegration of the chloroplasts. This type of reaction is characteristic of a manganese deficient condition (3). The chlorosis between the veins is easily shown histologically for the palisade cells between the veins are the first to be affected. As chlorosis becomes more apparent externally, the spongy parenchyma cells become affected as

did the palisade layer. Iron which is reputedly a catalyst for the synthesis of chlorophyll (14) apparently becomes toxic. This is brought about by its excess quantity due to the deficiency of the manganese. Too much iron, it appears, is capable of causing the breakdown of chlorophyll.

Struckmeyer and Berger (21), as well as Etlinge (3), in discussing the histological effects of manganese toxicity and deficiency on plants grown for about 60 days both considered in detail the reaction on the stems. Stems of plants grown and harvested at the end of 35 days in the present experiment were little affected. The length of the growing period may be a factor in this case. Symptoms in stems apparently show up considerably after those in the leaves.

In every case where stained histological sections were made through collapsed tissue the methyl green stain was deep and intense. It is not believed that this is due to the production of lignin but rather to the difficulty of properly washing the stain out of cells in this condition.

Cross sections through roots of plants grown in manganese toxic solutions did not show the internal structure of the brown slimy surface because in all cases this was torn away in processing. However, it has been said (18) that the browning of the roots is associated with the darkening of the nuclei and cytoplasm of the cortical cells, along with the occurrence of numerous unidentifiable brown masses in the cells. This might very well be the case here since cells were torn through the cortical region and those cells which remained were in a normal condition.

#### Flant Measurements

From the data compiled on the length of the plant as affected by mangenese and light variation it would appear that solution C with medium shade was optimum under the conditions of this experiment.

From the data compiled on the dry weight basis from the same plants it would appear that group C under conditions of full sunlight were optimum.

Since the advantage in the first instance appeared as excess length rather than weight it may be assumed that the latter is the more correct basis of comparison. According to Meyer and Anderson (13) the attenuated growth of stems of etiolated plants is due chiefly to an increase in the length of their component cells as compared with cells of similar tissues developed in the light. They suggest that substances similar to hormones are synthesized in plants under the influence of light and that it is the absence of these substances which permits the development of the etiolation characteristics. This may help to explain the apparent lack of effect of light intensity upon the roots. Any light at all according to Shirley (16) allows for the manufacture of a certain amount of the hormone like substance. As soon as any at all is manufactured the roots proceed to grow in a normal manner.

These results are in direct accordance with those of Shirly (16, 17) who found that, in general, absolute dry weight increased with increase in light intensity up to full sunlight, providing other factors were not limiting. Maximum height of the plants and maximum leaf area were attained, however, at light intensities considerably below that of full summer sunlight.

#### Dry Weight Data and Computations

It is felt by the writer that dry weight generally is a more valid standard for judging the condition of the plants in this experiment than measurements of length. The single factor, light, is responsible for too great a percentage of the differences in lengths. With reference to the data presented in Table III it will be noted that factors for determining significant differences at the 1% level and 5% levels have been computed. From this standpoint it is seen that the excellence of solution C on the basis of increase in dry weight is significant only under full sunlight. The pronounced loss of weight in solution D is significant under all three light conditions.

From these data it may be surmised that light retards either the absorption of excess iron or the reduction of iron to the ferrous state. Light, on the basis of this assumption, has little effect on manganese absorption.

The fact that solution B gave better results in length of the portion above the cotyledon than solution C in heavy shade (Figure 1) may be considered an experimental error, but the fact that solution B gave poorer results in dry weight than solution A at all light intensities remains unexplained. The condition is too consistent to be considered an error. Theoretically, point B should lie on a line between A and C on the graph in figure 6, as the manganese concentration of that solution is greater than A and less than C.

#### CONCLUSIONS

From the foregoing discussion it may be concluded that iron and manganese in certain definite proportions are necessary to the life of a plant. These proportions may vary under different environmental conditions. Light appears to affect the optimum Fe/Mn ratio. The onset of deficiency symptoms are retarded with a decrease in light intensity.

Dry weights seem to be the most valid of the measurements of differences used in this work.

Symptoms of manganese deficiency are distinct from those of manganese toxicity. Manganese deficient plants show severe chlorosis between the veins of the leaves with an eventual death of the growing point. Manganese toxic plants are severely stunted in growth and produce roots which are brown and slimy in appearance. The leaves in this case are stunted and profusely covered with minute pin point lesions.

Chlorosis corresponds to the disintegration and final destruction of the chloroplasts. Symptoms appear first in the palisade parenchyma and gradually spread throughout the spongy parenchyma except for the cells immediately surrounding the veins. These cells, together with the veins, appear healthy in every respect.

#### SULMARY

- Soybeans were grown in standard water cultures containing 3.00 ppm Fe with variation in the Mn content as .002, .010, 2.00 and 20.00 ppm and exposed to comparative light intensities of 2650 foct candles, 1350 foot candles and 1000 foot candles.
- At the time of harvest data were recorded as: gross observations, histological results, plant measurements in centimeters and dry weights.
- 3. Plants grown in solutions containing .002 and .010 ppm of manganese and exposed to full sunlight (2650 F. C.) produced typical manganese deficiency symptoms. Plants grown in the same solutions under 1350 F. C. produced slight deficiency symptoms and under 1000 F. C. showed a tendency towards a deficient condition.
- 4. Flants grown in 2.00 ppm of manganese were normal under all light conditions.
- 5. At 20.0 ppm of manganese plants under all light intensities produced severe toxicity symptoms.
- 6. Histological sections through leaves showing manganese deficiencies indicate that chlorosis is accompanied by a breakdown of the chloroplasts of the palisade cells followed by a similar disintegration in the spongy parenchyma.
- 7. Histological sections through leaves with manganese toxicity symptoms showed a progressive disintegration of the parenchyma tissue beginning on the upper epidermis and passing progressively through the palisade layer at the

spongy parenchyma and the lower epidermis. This tissue breakdown corresponded directly to the brown necrotic areas on the surface of the leaves.

## Description of Figures

- Figure 1. Average length of the portion above the cotyledons of soybean plants grown in solutions A, B, C and D--lot 1.
- Figure 2. Average length of the portion below the cotyledons of soybean plants grown in solutions A, B, C and D--lot 1.



7 F

# Description of Figures

.

- Figure 3. Total length of soybean plants grown in solutions A, B, C and D of lot 1.
- Figure 4. Total length of soybean plants grown in full sunlight, medium shade and heavy shade of lot 1.



Description of Figure

Figure 5. Average dry weights of soybean plants grown in solutions A, B, C and D of lot 1.





Description of Figure

Figure 6. Average dry weights of plants grown under full sunlight, medium shade and heavy shade of lot 1.

#### BIBLIOGRAPHY

- Arthur, J. M. and Stewart, W. D. Plant growth under shading cloth. Amer. Jour. Bot. 18: 897. 1931.
- Doutt, Margaret T. Anatomy of <u>Phaseolus vulgaris</u>, var. Black Valentine. Michigan State College Agric. Exp. Sta. Bull. 128, 31 p. 1932.
- Etlinge, Ethel. Effect of manganese deficiency upon the histology of <u>Lycopersicum esculentum</u>. Plant Physiol. 16: 189-195. 1941.
- 4. Hambidge, Gove, et al. Hunger signs in crops, A symposium.
  327 p. Judd and Detweiler, Washing, D. C. 1941.
- 5. Hopkins, E. F. Manganese, an essential element for a green alga. Amer. Jour. Bot. 17: 1047. 1930.
- 6. Johnson, M. O. Manganese as a cause of the depression of the assimilation of iron by pineapple plants. Ind. Eng. Chem. 9: 47-49. 1917.
- 7. -----. Manganese chlorosis of pineapples; its cause and control. Hawaii Agric. Exp. Sta. Bull. 52. 1924.
- Kelley, W. P. Function of manganese in plants. Bot. Gaz. 57: 213-227. 1914.
- 9. Laurie, Alex and Kiplinger, D. C. Commercial flower forcing. The Blakiston Co., Philadelphia, Pa. 1944.
- 10. Marsh, R. P. and Shive, J. W. Adjustment of iron supply to requirements of soybean in solution culture. Bot. Gaz. 80: 390-409. 1925.

- 11. McCool, M. M. Effect of light intensity upon manganese content of plants. Boyce Thompson Inst. Contrib. 7: 427-437. 1935.
- 12. McHargue, J. S. The occurrence and significance of manganese in the seed coats of various seeds. Amer. Chem. Soc. Jour. 36: 2532-2536. 1914.
- Meyer, B. S. and Anderson, D. B. Plant physiology. 696 p.
   D. Van Nostrand Co., Inc., New York. 1939.
- 14. Miller, Edwin C. Flant physiology. McGraw-Hill BookCo., New York. 1938.
- 15. Shear, G. M. <u>et al</u>. How soil reaction affects the supply of plant foods. Virginia Polytech. Bull. 136. 18 p. 1947.
- 16. Shirley, H. L. The influence of light intensity and light quality upon the growth of plants. Amer. Jour. Bot. 16: 354-390. 1929.
- 17. ------ The effects of light intensities upon plants. <u>In</u> Biological effects of radiation. 2: 729-762. McGraw-Hill Book Co., New York. 1936.
- 18. Shive, J. W. Significant roles of trace elements in the nutrition of plants. Plant Physiol. 16: 435-445. 1941.
- 19. Somers, I. I. and Shive, J. W. The iron manganese relation in plant metabolism. Plant Physiol. 17: 582-602. 1942.
- 20. Stiles, Walter. Trace elements in plants and animals. 189 p. Cambridge University Fress, Cambridge, England. 1946.

- 21. Struckmeyer, Esther and Berger, K. C. Histological structure of potato stems and leaves as influenced by manganese toxicity. Plant Physiol. 25: 114-119. 1950.
- 22. Trelease, Sam F. and Trelease, Helen M. Physiologically balanced culture solutions and stable hydrogen-ion concentration. Science 78: 438-439. 1933.
- 23. ----- and ----- Changes in hydrogen-ion concentration of the culture solutions containing nitrate and ammonia nitrogen. Amer. Jour. Bot. 22: 520-542. 1935.
- 24. Woodcock, E. F. Laboratory manual for plant histology. Michigan State College, East Lansing, Michigan. Mimeographed. 1945.

