A FIELD STUDY CONCERNING THE EFFECT OF ZINC UPON TRYPTOPHAN AND PROTEIN IN TWO VARIETIES OF NAVY BEANS, *PHASEOLUS VULGARIS* (L.)

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Samuel J. Woods

1968

ABSTRACT

A FIELD STUDY CONCERNING THE EFFECT OF ZINC UPON TRYPTOPHAN AND PROTEIN IN TWO VARIETIES OF NAVY BEANS, PHASEOLUS VULGARIS (L.)

by Samuel J. Woods

Two varieties of navy beans were exposed to zinc stress for different lengths of time in the field. Zinc was applied to the soil in a water solution of zinc sulfate ($ZnSO_4$).

In Sanilac, zinc deficiency symptoms appeared in untreated plants two weeks after planting. No visible zinc deficiency symptoms appeared in Saginaw during the course of the experiment.

The total quantity of protein produced in whole-plant tissue was greater for Saginaw when zinc was not applied. Zinc application to Sanilac resulted in an increased amount of foliage protein.

Protein percentage decreased in both varieties with advanced maturity. However, longer periods of zinc stress were associated with higher whole-plant protein percentages in Sanilac, whereas the period of zinc stress had little effect upon the protein percentage of Saginaw.

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The tryptophan percentage of Kjeldahl protein was measured in whole-plant tissue and in the seed. No significant differences in tryptophan percentage due to zinc treatment were obtained in the whole-plant tissue of either variety. The seed proved to be a stronger indicator of tryptophan variability. As zinc was applied at successively later dates during the season, the tryptophan composition of the seed in both varieties increased.

No statistically meaningful relationships were obtained between zinc concentration and tryptophan percentage in the foliage or seed of either variety. A strong positive correlation existed between zinc concentration and protein percentage in the seed of Saginaw.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop Science

1968

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. M. W. Adams, not only for his help in preparing this manuscript, but also for his sincere interest in the author's intellectual development. Drs. Selma Bandemer and E. J. Benne were very instrumental in providing the technical assistance needed for performing the chemical analyses.

Special gratitude is offered to the author's wife for her unselfish contributions to this work.

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INTRODUCTION

In Michigan's Thumb area, the use of large quantities of phosphorus has seriously reduced the zinc supplying capability of the soil. Of all the different crops grown in the area, the navy bean has proved most susceptible to zinc deficiency.

Differential response to zinc-deficient conditions has been observed among navy bean varieties. The most dramatic contrast exists between the variety Saginaw, which appears to grow and develop normally under zinc-deficient conditions, and Sanilac, a very susceptible variety, responding to zinc-deficiency by displaying short internodes, small, deformed leaves, interveinal necrotic lesions, few flowers and delayed maturity.

Major differences between the two varieties in response to low levels of zinc appeared to be in the growth processes. Therefore, it was postulated that varietal differences existed with respect to certain physiological manifestations of growth.

For several years plant physiologists have known that zinc plays a role in auxin synthesis. Strong evidence now exists that zinc is required for the synthesis of the amino acid, tryptophan, which serves as a natural precursor for auxin. Zinc also has been shown to affect protein synthesis by being essential for the production of RNA.

This study was conducted with four main objectives in mind:

(1) To compare the total protein contents of Saginaw and Sanilac after subjection to zinc-deficient conditions for different periods of time in a field situation.

(2) To compare the two varieties with respect to protein quality by analyzing for tryptophan content.

(3) To provide evidence for a relationship between zinc concentration, protein percentage, and tryptophan content in whole-plant tissue.

(4) To analyze the seed of both varieties to provide evidence for a possible zinc, protein, tryptophan relationship.

REVIEW OF LITERATURE

Zinc in relation to tryptophan synthesis

Information concerning the relationship of zinc to modern plant nutrition was practically nonexistent before 1940. One of the first to explore the physiologic role of zinc in modern plant nutrition was Skoog (13). Working with tomato, *Lycopersicum esculentum*, and sunflower, *Helianthus annuus*, Skoog discovered that plants deprived of zinc in a Hoagland nutrient solution showed depressed auxin content. Both species were placed in a zinc-deficient nutrient solution until the older leaves became necrotic. At this point zinc at a concentration of .01 m/l was added to the nutrient solution. After only a twenty-four hour period the auxin content of both species began to increase. The disappearance of auxin in zinc deficient plants was accompanied by an increase in peroxidases. Skoog concluded that zinc had an indirect effect by preventing auxin oxidation.

Tsui (16) explored the relationship between zinc and auxin more thoroughly. Working with tomato (variety, John Baer), he found that in addition to the auxin content gradually decreasing on exposure to a zinc-deficient medium, the tryptophan level fluctuated as well. The tryptophan level of zinc-deficient plants was found to be lower when compared to the level found in normal plants. When zinc was supplemented in the deficient nutrient medium, the tryptophan level increased.

In a subsequent experiment Tsui (16) subjected tomato leaf disks to tryptophan infiltration. An increase in auxin measurement after tryptophan infiltration indicated that there must have been an enzyme present in the leaf tissue capable of transforming the added tryptophan to a growth substance. The capacity for tryptophan conversion was essentially the same in both zinc-deficient and sufficient tissue. Tsui concluded that zinc is not required principally for the synthesis of auxin. This left the possibility that zinc affected tryptophan biosynthesis, which could serve as a precursor for auxin.

Extended work on the relationship between zinc and tryptophan was to no avail until a pathway for tryptophan biosynthesis in higher plants had been established. Available enzyme separation techniques were inadequate to define a pathway in plants. Therefore, emphasis was placed on establishing a pathway in microorganisms with later application to higher plants. Yanofsky (14), with the use of Escherichia coli mutants for tryptophan synthesis, discovered a functional pathway in bacteria. It was discovered that an enzyme (tryptophan synthetase) was responsible for the condensation of indole and serine during the last stage of tryptophan synthesis.

Basic information gained from Yanofsky's microbial work made it possible to continue the search for a similar mechanism in higher plants. Greenberg and Galston (3) found the first evidence for the indole-serine condensation in Alaska Pea extracts, Pisum sativum. By the use of Ehrlich's reagent, indole was measured before and after subjection to the extract. Results showed that indole disappearance was greater with the addition of D-L-serine. Black Valentine bean seedlings were also investigated but no clear evidence for the indole-serine reaction could be found. However, Mudd and Zalik (10) demonstrated the dependency of indole disappearance on serine concentration using tissue slices from various parts of the tomato plant.

Crown gall tissues of Boston Ivy, Parthenocissus tricuspidatus, were cultured by Klein (8) on a zinc-deficient nutrient medium. Two types of cultures were used: cultures receiving no exogenous auxin and those receiving exogenous auxin. The cultures receiving auxin grew normally while growth of the cultures receiving no auxin was decreased by 50 percent. Results showed that exogenous tryptophan permitted normal growth also.

The effect of zinc on the biosynthesis of tryptophan has been studied in barley (9). Plants were cultured in several different concentrations of zinc. Maximum growth, as measured by plant height, occurred at a zinc concentration of 8×10^{-4} M. Maximum tryptophan concentration in the plant occurred with optimum zinc concentration in the nutrient medium. Tryptophan content fell below that of the control when an 8×10^{-2} M concentration of zinc was used in the nutrient medium.

Zinc in relation to protein synthesis

Growth is manifested by cell division and (or) enlargement. Steward (15) reported that some form of protein synthesis always occurs with the growth process.

A major prerequisite for protein synthesis is the presence of ribonucleic acid (RNA). Working with cultures of *Rhizopus nigricans*, Wegener and Romano (17) discovered that the addition of zinc immediately stimulated RNA synthesis. After a brief period, protein synthesis increased at the same rate as RNA synthesis. The pattern observed for growth was similar to that found for protein synthesis. Key and Barnett (7) suggested that the RNA synthesis required for growth is restricted to a certain type called messenger RNA. The use of specific metabolic inhibitors revealed a probable relationship between auxin-induced growth and protein synthesis. Actinomycin D, a strong inhibitor of RNA and protein synthesis, was applied to mature soybean (Glycine max) hypocotyls. When auxin and inhibitor were added simultaneously to the plant tissue, inhibition of auxin-induced growth paralleled the inhibition of RNA and protein synthesis.

Both messenger and soluble ribonucleic acids are needed for protein synthesis in the living cell. Hall and Cocking (4) discovered that an enzyme, RNA-ase, was effective in degrading both m RNA and s RNA, which in turn impaired the incorporation of amino acids into protein. Further investigation resulted in the discovery that both copper sulphate and zinc acetate were effective inhibitors of crystalline RNA-ase.

Kessler (6) established RNA-ase as a guide for the determination of zinc deficiency in citrus. By assuming that zinc was either directly or indirectly responsible for protein synthesis, Kessler assayed zinc-deficient leaves for zinc and RNA-ase activity. One half of the leaf was used for zinc determination and the other half for measuring the percentage of substrate (RNA) hydrolysis per hour. In healthy leaves, regardless of variety, species, or location, RNA-ase activity was below 40 percent of total substrate hydrolysis per hour. In zinc-deficient leaves the percentage of total substrate hydrolysis was higher than 50. High RNA-ase activity was associated with 16 ppm zinc in orange trees. Only 10 ppm in Delicious apples produced the same effect. Kessler concluded that optimum levels of zinc in the leaf keeps RNA-ase activity at a minimum, thus promoting RNA and protein synthesis.

Nason (11) investigated total protein levels in Neurospora as affected by zinc. A dramatic decrease in total protein was experienced in zinc-deficient mycelia as compared to mycelia cultured on zinc-deficient media.

MATERIALS AND METHODS

Field management

This investigation was conducted on the Clifford Schiann farm, five miles east of Saginaw, Michigan. The experimental site consisted of a calcareous Wisner clay loam soil. To insure the lack of available zinc before treatment, superphosphate, at the rate of 91 kilograms/hectare was broadcast and worked in. One hundred and seventy kilograms/hectare of 5-20-20 fertilizer was applied in a band one inch to the side and two inches below the seed. Plots were planted with a one row International Harvester planter modified for experimental use. Seeds were spaced approximately five centimeters apart in the row with 1.8 meters between rows. Weed control consisted of a pre-plant application of Eptam at the rate of 3.10 kilograms of active ingredient per hectare. During the growing season each plot was cultivated twice with a standard two row cultivator.

The experimental design consisted of a split-plot arrangement with varieties as main plots. Each main plot was divided into 8 individual one-row subplots representing eight successive weeks of zinc application. Zinc application dates were replicated five times.

Zinc sulfate ($ZnSO_4$) dissolved in well water was applied by bucket to individual plots at an approximate rate of 12.7 kilograms/hectare. For this soil 6.75 kilograms/hectare is barely adequate for growth of Sanilac. Trenches were made on both sides of the row to prevent run-off.

Procedure for sampling of plant material

Four zinc application dates, in addition to an untreated check, were sampled at three separate times during the growing season. The first sampling was July 13 with two subsequent samplings at two week intervals.

Samples consisted of three plants taken in succession within the one-row plot. To minimize contamination, each plant was carefully put through a series of washings. Plants were first submerged in tap water, followed by rinsing in a dilute HCl solution to render soluble all free zinc clinging to roots and foliage. Finally, each sample was rinsed in deionized water and placed in clean paper bags. The bags were then placed in a forced air dryer set at 80 C. for a 36 hour period. Upon drying, each whole plant (including roots) was ground in a Wiley mill using a 60 mesh screen. After grinding, each sample was revolved in a large piece of wrapping paper to insure proper mixing. Samples were then placed in a sealed glass wash bottle to await chemical analysis. During the grinding procedure, the samples undoubtedly took up moisture. Therefore, before chemical analysis, samples were redried at 90 C. for six hours and placed in desiccators until weighing.

Tryptophan analysis

The method used for tryptophan analysis was a modification from Roth (12) by Dr. Selma Bandemer of the M. S. U. Biochemistry Department. One-half gram of plant material was weighed out, using a triple beam analytical balance, and placed in a 125 ml. Erlenmeyer flask for digestion. The acid solution used to digest the plant material consisted of 98 ml. concentrated sulfuric acid (H_2SO_4), 63 ml. water, and 84 ml. of concentrated nitric acid (HNO_3). Forty ml. of the acid solution was poured into each flask. The flasks were then placed on an 85 C. steam bath for 18 hours. During the first two hours of digestion a heavy brown vapor of nitrous oxide evolved. After several hours all that remained of the plant material was a gelatinous precipitate of silica. After digestion, contents of each 125 ml. flask were washed into a 50 ml. volumetric flask and brought up to volume using deionized water. Upon cooling, the solution was filtered through Whatman no. 50 hardened filter paper into a 50 ml. glass stoppered Erlenmeyer flask. The filtered acid solution was then read at 440 millimicrons in a Bauch and Lomb spectrophotometer.

Zinc determination

Zinc determination was accomplished with a Perkin-Elmer 303 atomic absorption spectrophotometer. In order to obtain zinc in large enough quantities for analysis, a 0.3-1.0 gram sample was placed in a crucible for dry-ashing at 500 C. for 18 hours. The ash was then dissolved in 5 ml. of 2 N HCl and filtered through Whatman no. 2 filter paper into a 125 ml. Erlenmeyer flask. Contents of the flask were brought up to a 50 ml. volume by the use of an automatic pipet. Samples were then read at a wavelength of 214 millimicrons.

Protein determination

The official Kjeldahl procedure, as outlined in the AOAC manual (1), was used to determine percent amino nitrogen. The nitrogen percentage was then multiplied by a factor of 6.25 to obtain total protein.

Table 1.--A guide to codes representing the various zinc application dates.

Code		Application date
T_0	. . . .	June 15
T_1	. . . .	June 22
T_2	. . . .	June 29
T_3	. . . .	July 6
T_4	. . . .	July 13
T_5	. . . .	July 20
T_6	. . . .	July 27

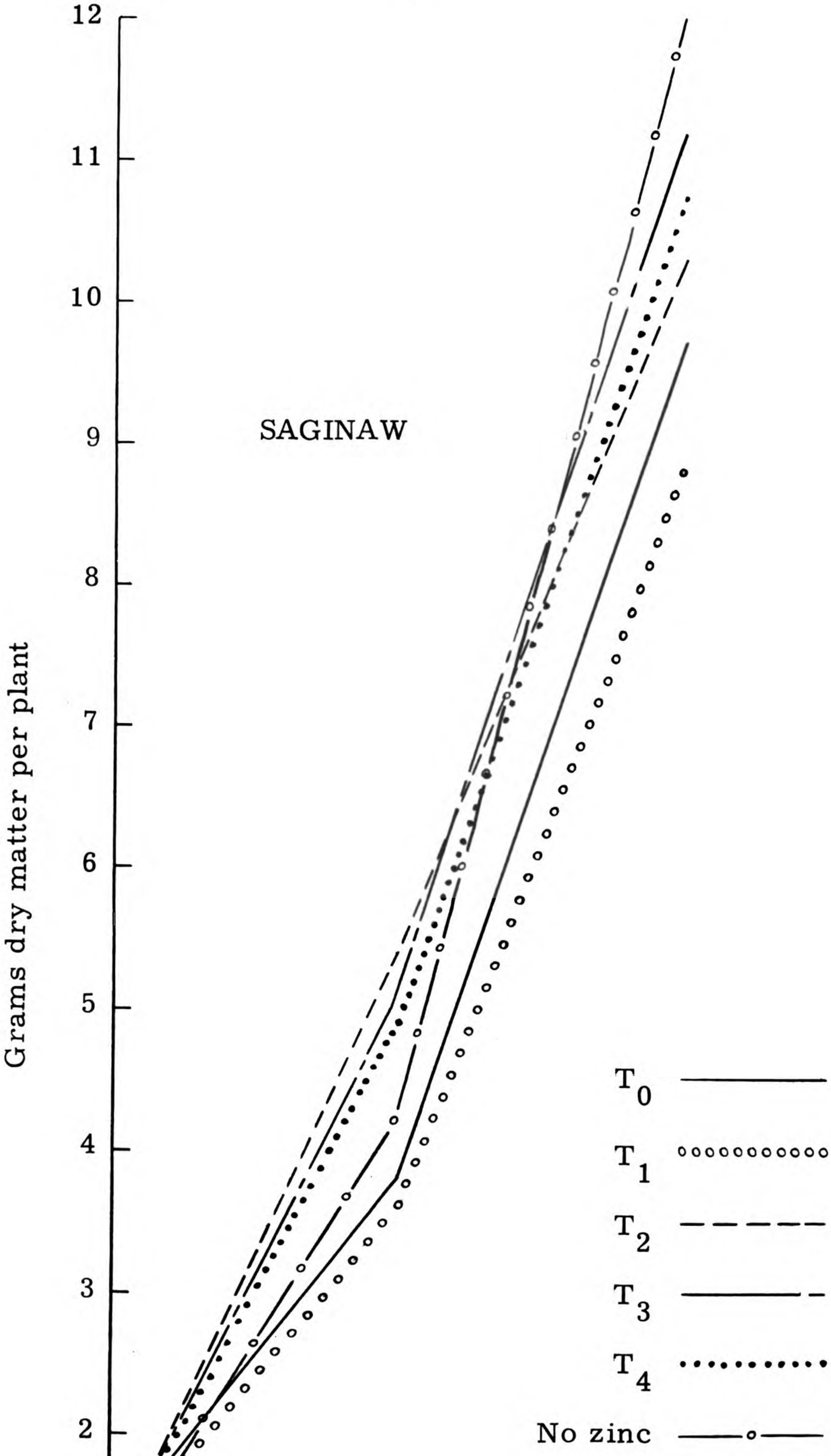
RESULTS

Varietal growth and development

Varietal dry matter accumulation patterns for three sampling dates are presented in Figure 1. Saginaw displayed a significantly higher dry matter accumulation for two of the three sampling dates than Sanilac. Means representing dry matter per plant were lower for Saginaw when zinc was added than when no zinc was applied. However, the differences were not statistically significant, suggesting that zinc application had little effect upon the growth of Saginaw.

Plots of Sanilac treated at T_1 (one week after planting) had a more rapid rate of dry matter accumulation in July as compared to the other application dates. However, the same treatment date showed a large decrease in the rate of dry matter accumulation during the July 27 to August 10 interval. All other treatments, including the check, displayed their most rapid rate of dry matter accumulation during the July 27 to August 10 period. The effect of the early post-emergence applications of zinc on Sanilac is illustrated in Figure 3. Application of zinc to Sanilac at planting time resulted

Figure 1. -- Varietal dry matter production patterns for five zinc application dates.


SAGINAW
Grams dry matter per plant
12
11
10
9
8
7
6
5
4
3
2
1
Jly 13
Jly 27
Aug 10
T_0
T_1
T_2
T_3
T_4
No zinc

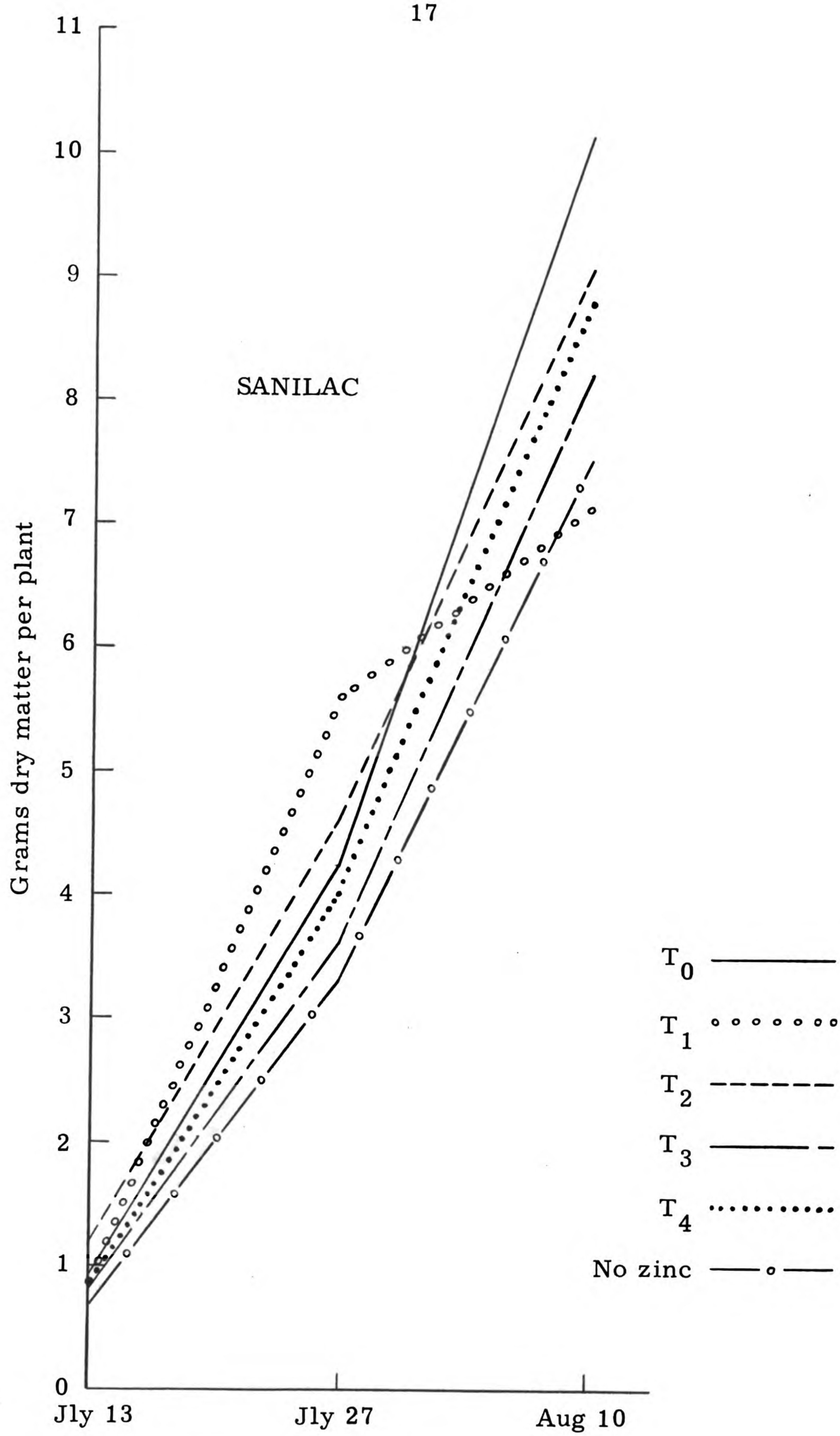


Figure 1. -- Continued.

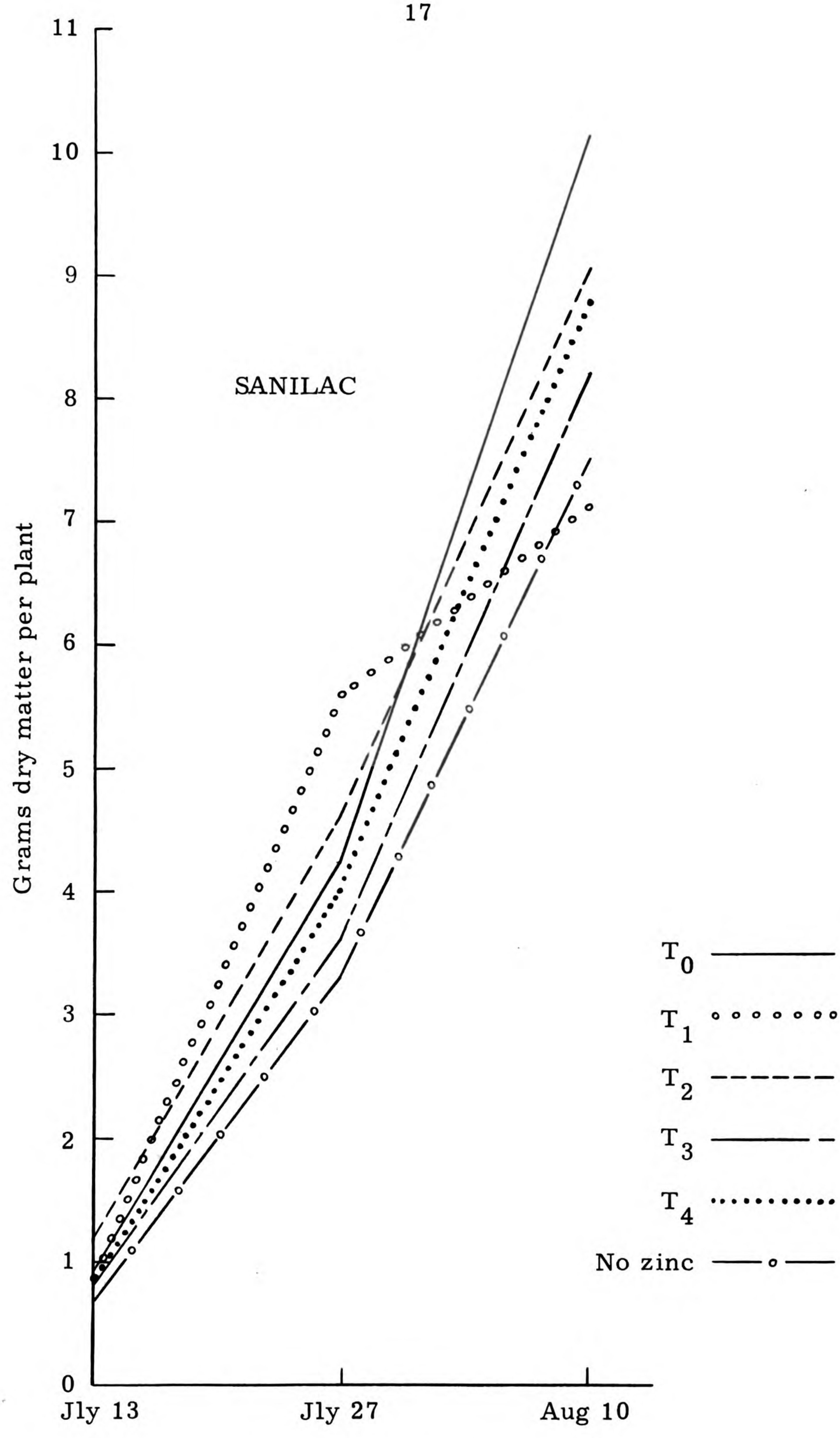


Figure 1. -- Continued.

Figure 2. -- Zinc deficiency in Sanilac three weeks after planting.

Figure 3. -- Sanilac treated one week after planting (right) and untreated (left) six weeks after planting.

in a 30 percent increase in dry matter over the check on August 10. Treatment at T_2 resulted in 20 percent more dry matter per plant on August 10 than those untreated.

Whole-plant analysis for protein and tryptophan

Tables 2 and 3 present treatment means for protein percentage and tryptophan composition of protein, respectively. A

Table 2. -- Varietal protein percentage based on whole-plant samples for three harvesting periods representing five different zinc application dates.

Zinc application date	Variety					
	Saginaw			Sanilac		
	Jly 13[a]	Jly 27[b]	Aug 10	Jly 13	Jly 27	Aug 10
T_0	24.73	22.34	18.86	24.46	22.28	18.96
T_1	23.75	22.34	18.52	22.19	20.98	17.25
T_2	23.31	22.40	17.54	23.25	21.71	17.05
T_3	24.87	22.93	17.81	24.44	22.37	17.98
T_4	-----	21.75	17.44	-----	23.23	17.72
No zinc	24.69	23.19	19.12	25.10	23.80	20.28

[a]July 13 data represents the mean of three replications.

[b]July 27 and August 10 data represents mean of five replications.

Table 3.--Varietal tryptophan composition expressed as a percentage of the protein found in whole-plant samples.

Zinc application date	Variety					
	Saginaw			Sanilac		
	Jly 13[a]	Jly 27[b]	Aug 10	Jly 13	Jly 27	Aug 10
T_0	2.79	2.65	3.14	2.54	2.58	3.04
T_1	3.22	2.77	3.07	2.52	2.72	2.91
T_2	2.84	2.89	2.95	2.63	2.72	3.40
T_3	2.74	2.98	2.93	2.52	2.78	3.08
T_4	----	2.74	3.02	----	2.73	3.09
No zinc	2.69	2.75	3.53	2.49	2.82	2.95

[a]July 13 data represents the mean of three replications.

[b]July 27 and August 10 data represents the mean of five replications.

separate analysis of variance for the data appearing in Tables 2 and 3 was performed for each sampling date. A summary of these analyses is found in Table 4.

Protein percentage was greatly affected by zinc application date for each sampling period. A significant varietal difference occurred in total protein percentage for the July 13 sampling date. Interactions between zinc application date and variety were not significant for either tryptophan or total protein percentage.

Table 4. -- Summary of the analysis of variance performed at three sampling periods for tryptophan percentage of protein and protein percentage in whole-plant tissue.

Source of variation	Tryptophan			Total protein percentage		
	Jly 13	Jly 27	Aug 10	Jly 13	Jly 27	Aug 10
Variety	*			*		
Zinc application date				**	**	**
Variety X zinc application						

*--Significant at .05 level.

**--Significant at .01 level.

Figure 4 illustrates the whole-plant protein percentage relationship between Saginaw and Sanilac for three sampling dates. Both varieties tended to decrease in protein percentage with maturity. When zinc was not applied to either variety, Sanilac possessed a higher protein percentage. Zinc application lowered the protein percentage of both varieties. The decrease in protein as a result of zinc application was more pronounced in the Sanilac variety. With the exception of the preemergence treatment, a decrease in protein of Sanilac occurred with earlier dates of zinc application. Although

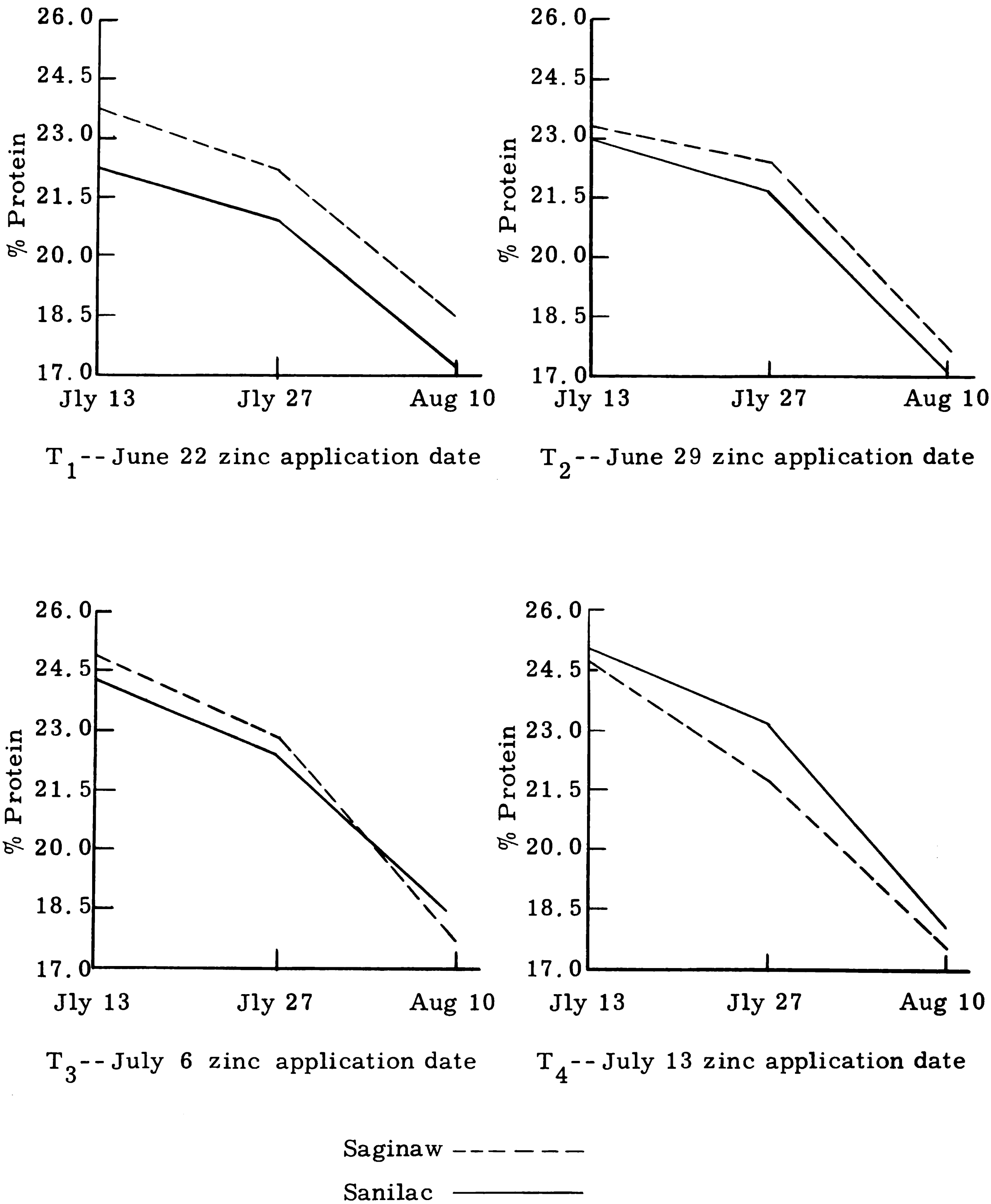


Figure 4.--Varietal protein percentage relationships for five different zinc treatments at three sampling dates.

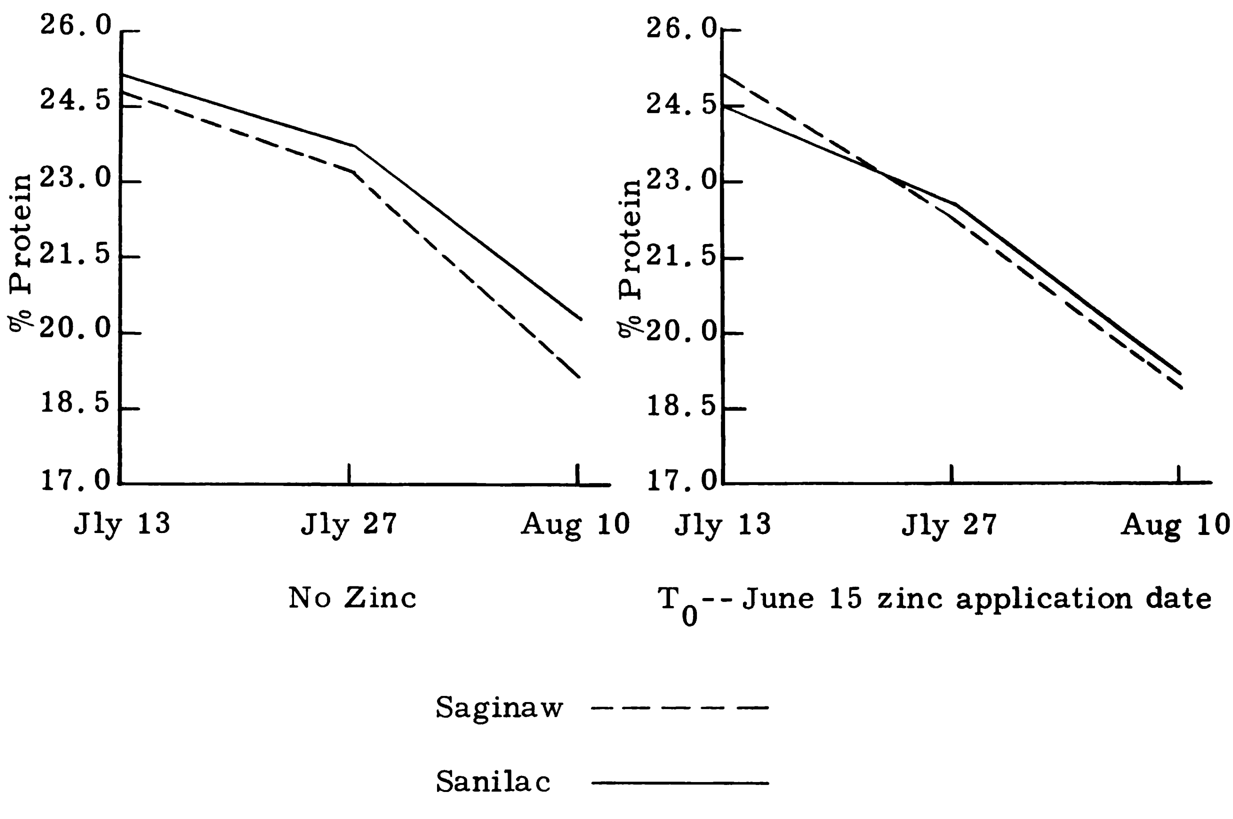


Figure 4. -- Continued.

somewhat variable, the pattern of response for the protein percentage of Saginaw remained relatively constant among treatments. Preemergence application of zinc had the effect of reducing the magnitude of difference in protein percentage between varieties.

On July 13 Saginaw possessed a higher tryptophan content than Sanilac for all zinc application dates (Table 4). The varietal difference in tryptophan percentage disappeared as the growing season progressed. An increase in tryptophan content occurred in both varieties as a result of plant growth and development. This increase was observed for each application date, and was more pronounced in Sanilac. Differences in tryptophan percentage resulting from zinc application dates were not significant for either variety.

Whole-plant zinc analysis

Zinc content based upon whole-plant analysis proved to be extremely variable (Table 5). Saginaw possessed a significantly higher zinc content for the first two sampling dates, but not on August 10.

Correlation coefficients were small, positive, and insignificant for both varieties with regard to zinc concentration and tryptophan content (Table 6). This suggests only a small degree of relationship between the two variables. The correlation between

Table 5.--Zinc concentration in micrograms per gram of dry matter for Saginaw and Sanilac, based upon whole-plant analysis.

Zinc application date	Variety					
	Saginaw			Sanilac		
	Jly 13	Jly 27	Aug 10	Jly 13	Jly 27	Aug 10
T_0	69.00[a]	87.80	70.80	58.42	69.60	87.60
T_1	106.33	72.10	76.90	87.41	65.50	54.80
T_2	104.00	72.70	93.70	63.16	63.20	68.90
T_3	99.23	81.60	70.90	72.83	65.87	61.00
T_4	------	110.00	80.80	-----	85.00	79.70
No zinc	57.25	55.40	105.60	63.80	75.60	75.30

[a]Data represents mean of five replications.

Table 6.--Correlation coefficients relating zinc, tryptophan, and protein in whole-plant tissue.

Correlation	Variety	
	Sanilac	Saginaw
Zinc and tryptophan	.127	.385
Zinc and protein	-.046	-.129
Protein and tryptophan	-.877**	-.537**

**--Significant at the .01 percent level.

zinc and tryptophan was greater for Saginaw than Sanilac. Only a small negative relationship existed between zinc concentration and protein percentage in both varieties.

A very strong negative correlation existed between protein percentage and tryptophan content. Although both varieties decreased in protein percentage and increased in tryptophan content over the time-course of the experiment, the tendency proved greater for Sanilac (Tables 2 and 3).

<u>Zinc application vs. protein content of Sanilac</u>

A larger quantity of protein occurred in plants treated with zinc at T_0 (Figure 5). When zinc was applied at T_1 the rate of protein accumulation during July was high when compared to the other treatments, but decreased markedly during early August. Only a small variation in protein content was observed when zinc was applied subsequent to T_1.

<u>Zinc application vs. protein content of Saginaw</u>

Protein content remained low at all three sampling dates when zinc was applied at T_0 and T_1. A moderate increase occurred with the later treatments. Plants remaining untreated contained a

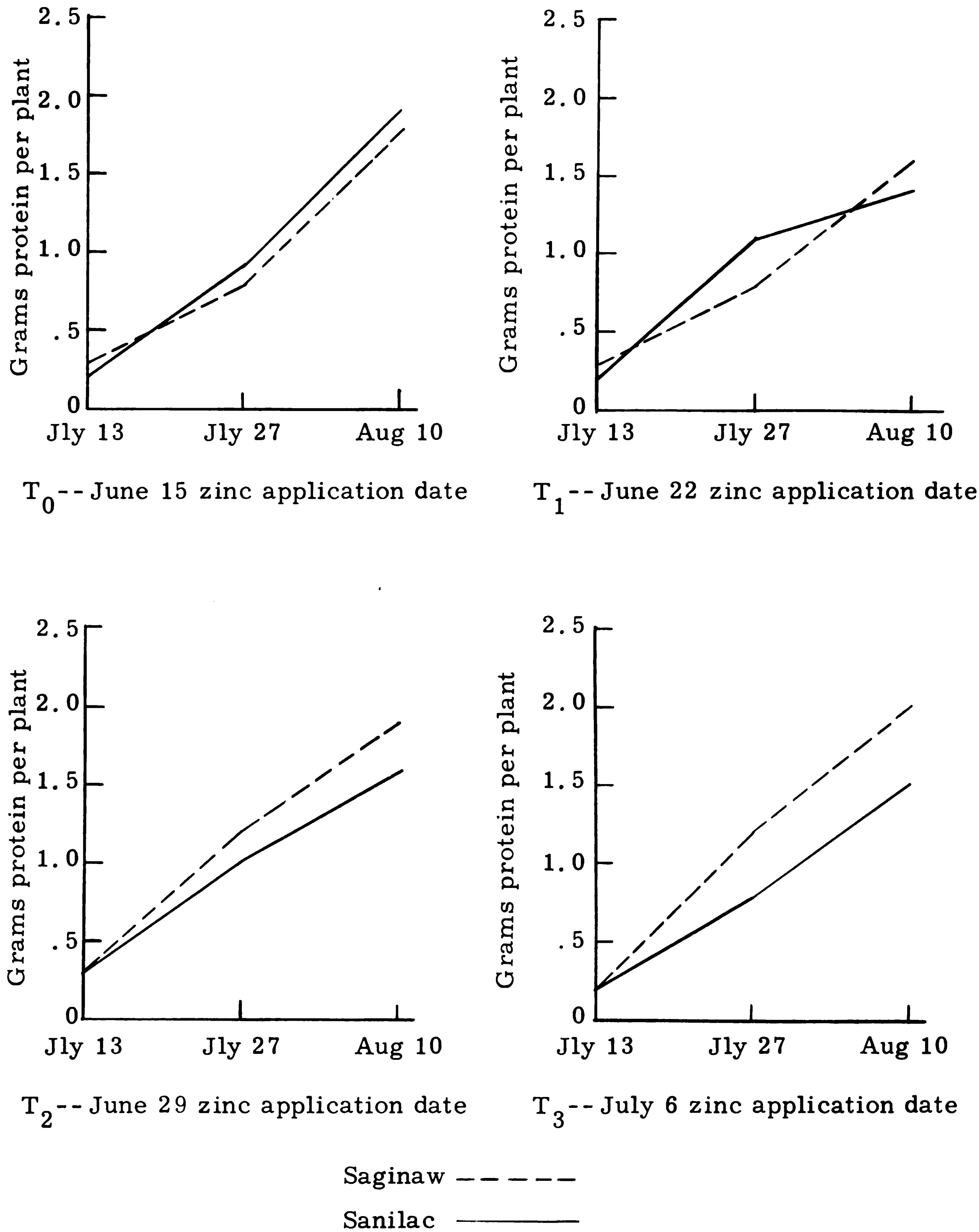
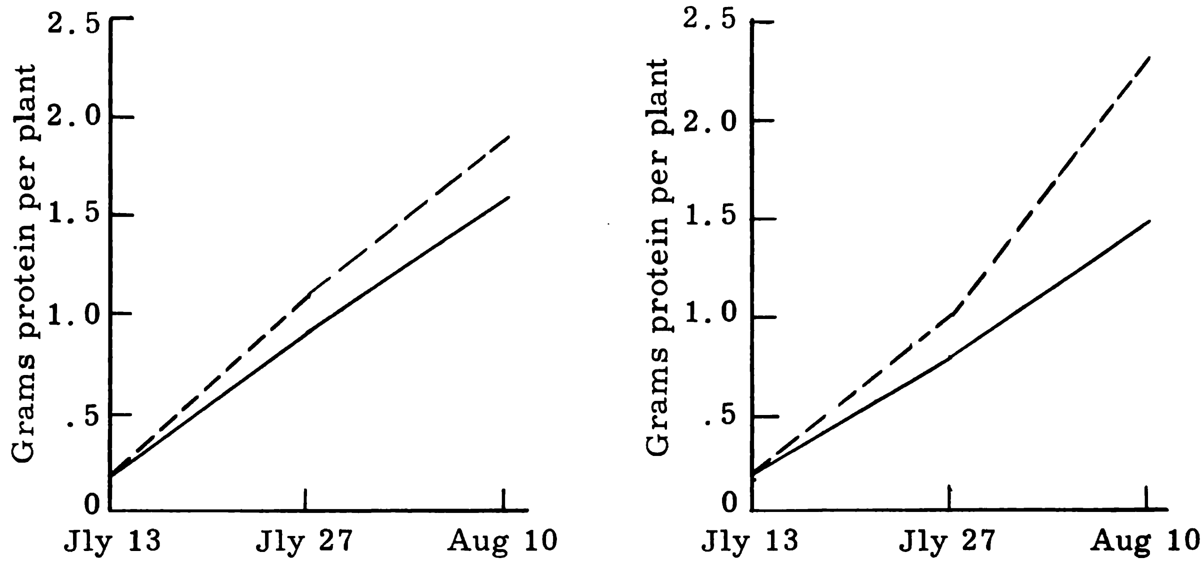


Figure 5. -- Varietal protein quantity relationships for several different zinc treatments at three sampling dates.



T_4-- July 13 zinc application date

No Zinc

Saginaw - - - - -

Sanilac ———

Figure 5. -- Continued.

larger quantity of protein on August 10 than any particular zinc treatment.

Seed tryptophan analysis

The percentage of tryptophan found in total seed protein for four zinc application dates is presented in Table 7. A functional

Table 7.--Varietal mean tryptophan percentages for four zinc application dates.

Zinc application date	Variety		Treatment mean
	Saginaw	Sanilac	
June 15	1.94	1.82	1.88
June 29	1.97	2.25	2.11
July 13	2.21	2.26	2.24
July 27	2.36	2.48	2.42
Varietal mean	2.12	2.21	

analysis of variance summarizing the data in Table 7 appears in Table 8.

Significant differences between varieties regarding tryptophan percentage could not be discerned. The variety-treatment interaction was not significant, which would indicate that both varieties displayed the same pattern throughout the analysis.

Table 8. -- Functional analysis of variance for tryptophan composition of seed protein.

Source	S. S.	D. F.	M. S.	F.
Variety	0.0368	1	0.0368	0.6095
Replication	0.1982	2	0.0991	1.6404
Error A	0.1208	2	0.0604	
Zinc application date	0.9393	3	0.3101	14.6004***
(A)		1	.5370	26.85 **
(B)		1	.3450	17.25 **
(C)		1	.0460	2.30
Variety × zinc	.1178	3		1.85
Error B		12		
Total	1.6588	23		

(A)--July 27 vs. the average of June 15, June 29, and July 13.
(B)--June 15 vs. the average of June 29 and July 13.
(C)--June 29 vs. July 13.

**--Significant at .01 level.

***--Less than .01% level of significance.

A very significant difference in tryptophan composition due to zinc application was detected in the seed of both varieties. Therefore, a set of orthogonal comparisons was used to render the differences meaningful. Seed from the July 27 application date was significantly higher in tryptophan than the average of all other dates. When zinc was applied immediately after planting, the tryptophan percentage proved to be significantly lower than the average of the June 29 and July 13 dates. No significant difference in seed tryptophan percentage could be found between the June 29 and July 13 application dates.

Zinc content was less variable between treatments and varieties when based upon seed analysis (Table 9). The difference

Table 9. -- Seed zinc content of Saginaw and Sanilac as affected by four different dates of zinc application.

Zinc application date	Variety	
	Saginaw	Sanilac
T_0	37.23	39.66
T_2	34.91	53.83
T_4	45.16	40.50
T_6	39.25	40.50

between varietal means was not statistically significant. No difference between treatment means were observed.

Seed protein analysis

Seed samples from plants selected from zinc application dates were analyzed for protein percentage. Results from the protein analysis are summarized in Figure 6.

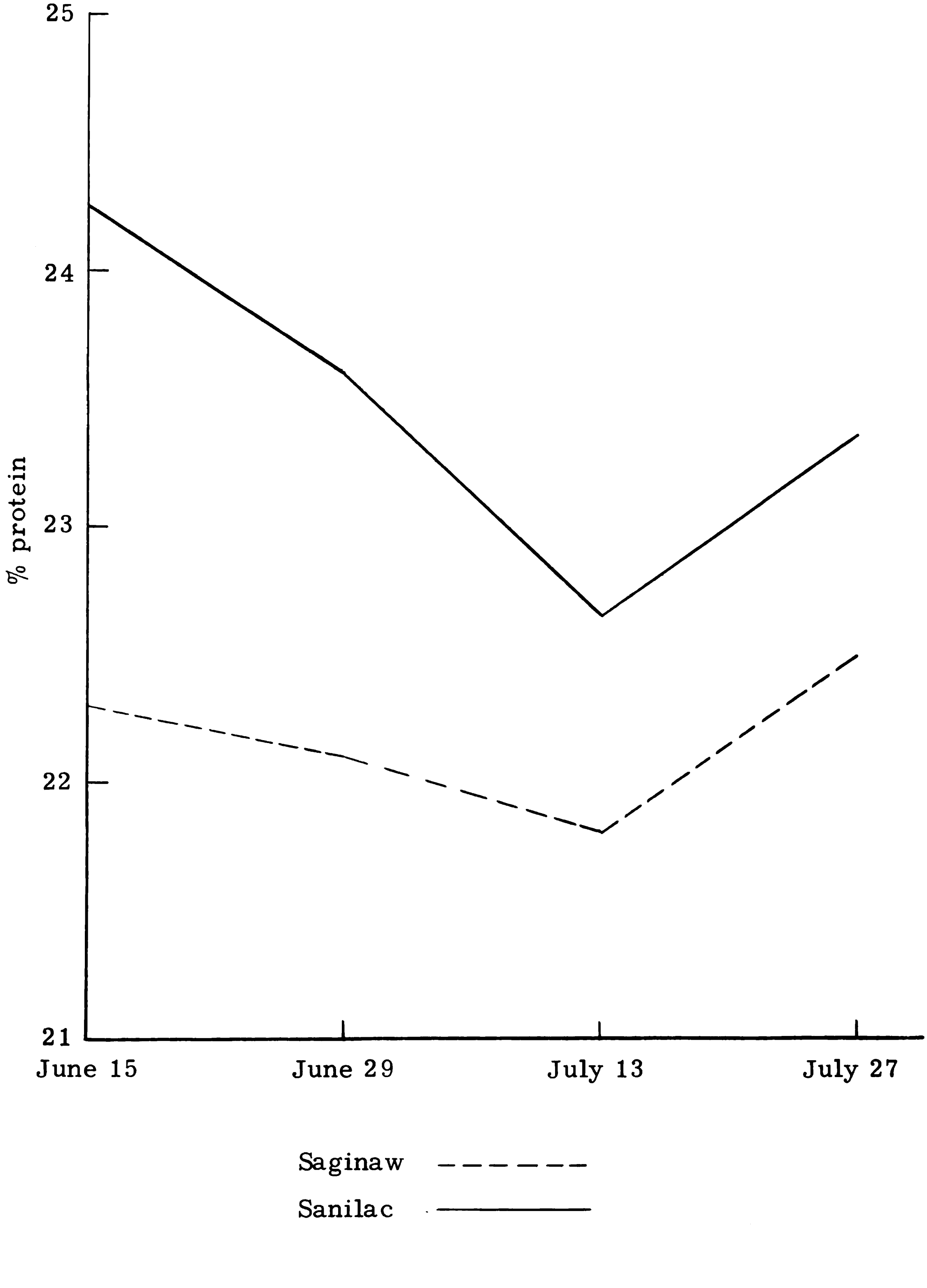


Figure 6. -- Seed protein percentages for Saginaw and Sanilac when treated with zinc on four different application dates.

The Sanilac variety was higher in seed protein percentage for all treatment dates selected. Both varieties followed the same general pattern over all treatment dates. Seed protein percentages decreased with the delay of treatment date until July 13, and increased markedly for the July 27 treatment date.

Table 10.--Correlations between zinc concentration, tryptophan content, and protein percentage in the seed of Saginaw and Sanilac.

Correlation	Variety	
	Saginaw	Sanilac
Zinc and tryptophan	+.180	-.116
Zinc and protein	+.661*	-.048
Protein and tryptophan	+.422	-.335

*--Significant at .05 percent level.

With one exception, no meaningful relationships were found between zinc content, tryptophan percentage, and protein percentage in the seed of either variety. A rather substantial and significant correlation was recorded between zinc content and protein percentage for Saginaw. It is interesting to note that all coefficients were positive for Saginaw and negative for Sanilac.

DISCUSSION

Plant growth and development

Sanilac demonstrated a need for supplemental zinc soon after emergence. Plants showing deficiency sumptoms took approximately two weeks to recover after zinc was applied. Conversely, Saginaw lacked deficiency symptoms in the early stages of growth and showed little response to added zinc. This would suggest that the requirement for zinc in the early developmental stages is more critical for Sanilac than for Saginaw.

Soils with a large clay content tend to lose their structure when wetted. Lack of macro-porosity upon redrying reduces aeration of plant roots, resulting in poor nutrient uptake. It is believed that reduced growth of Saginaw experienced when zinc in solution was applied early was caused by altered soil structure. Development of a more extensive root system before zinc application enabled the plants to deal more effectively with the aeration problem.

Several possibilities exist to explain why Sanilac reacted favorably to early zinc treatment: (1) A difference in tolerance to low levels of soil aeration exists between varieties, especially during the early growth period. (2) The need for zinc was the most limiting factor for successful plant growth of Sanilac. (3) A combination of statements (1) and (2).

Protein analysis

Early postemergence zinc treatment permitted normal plant development of Sanilac (Figure 3). Plants receiving no supplemental zinc flowered two weeks later than those treated at T_1 and T_2.

The effect of zinc application upon plant maturity of Sanilac was also reflected in foliage protein percentage. With the exception of T_0, increased protein percentage occurred with extended zinc stress. One of the most pronounced symptoms of zinc deficiency is a tendency for the plants to remain at a juvenile stage of growth. The protein percentage of young whole-plant tissue tends to be higher than in older tissue, which possesses a greater amount of carbohydrate in the form of secondarily thickened cell walls. A clear effect of zinc application upon the protein percentage of Saginaw was not discerned.

Several workers have demonstrated that protein synthesis continues throughout the main course of cell division and enlargement (15). This would explain why patterns observed for the total quantity of protein per plant strongly reflected the dry matter response.

Tryptophan analysis

Both varieties increased in seed tryptophan content with successively later dates of zinc application. Tentatively, this can be explained by principles of plant development. Early in the season, growth is taking place at a rapid rate. Tryptophan is being utilized in large quantities in regions of meristematic activity. As the growth rate decreases with increased plant maturity the developing reproductive structures become the major recipients of plant metabolites, such as tryptophan. Any increase in tryptophan synthesis would be detected in the developing seed.

Results obtained from the seed tryptophan analysis suggest the possibility of altering the tryptophan content of bean seeds by manipulating the available zinc supply in a field situation.

Results obtained from the foliage tryptophan analysis proved to be inconclusive. A natural tendency for protein to increase in tryptophan composition with plant development may have masked any effect that zinc had upon tryptophan content. Analysis of a particular organ taken from a specific position on the plant may have been more effective.

Relationships between zinc, tryptophan and protein

Negative correlations observed between protein percentage and tryptophan content were expected, based on the data of Tables 2 and 3. The protein percentage decreased with advanced maturity, while the quality of this protein was changed by increases in tryptophan composition. Steward and his co-workers found that plant proteins are dynamic with respect to composition.

A positive correlation was observed between zinc content and protein percentage in the seed of Saginaw, whereas practically no relationship between the two variables was recorded for Sanilac. These results, while statistically significant, are difficult to interpret biologically. If zinc were closely related to protein synthesis for different plant species, as supported by the literature, it would seem logical to expect the same relationship to exist between varieties of the same genus and species.

SUMMARY AND CONCLUSIONS

Several plant responses were evaluated in two varieties of navy beans (Saginaw and Sanilac) under differential conditions of zinc stress in the field. Whole-plant and seed analyses for percentage and total protein, tryptophan composition, and zinc content were performed at three different sampling dates during the growing season.

(1) Saginaw and Sanilac responded differently in a field situation of low zinc availability. Sanilac required greater amounts of zinc in the early stages of growth than could be supplied naturally by the soil, whereas Saginaw developed normally during this time.

(2) The tryptophan content of both varieties, based upon whole-plant analysis, increased with advancing plant maturity. This phenomenon may have obscured any strong zinc-tryptophan relationship.

(3) The seed of both varieties proved to be a more precise indicator of tryptophan variability attributed to zinc treatment.

(a) An increase in seed tryptophan content was observed in both varieties when zinc was applied at successively later dates.

(b) Water applied with the zinc may have interacted to produce at least some of the effect attributed to treatment.

(4) Relationship between zinc, tryptophan, and protein.

(a) No meaningful relationships between zinc and tryptophan were observed in the seed or in whole-plant tissues of either variety.

(b) Inconsistent relationships with respect to variety were recorded between zinc content and protein percentage in the seed. Saginaw possessed a strong positive relationship, while no relationship was observed in Sanilac.

(c) Negative relationships between tryptophan and protein percentage were found in whole-plant tissue of both varieties. This was a consequence of plant maturity.

(5) In Sanilac, higher whole-plant protein percentages were associated with longer periods of zinc stress, with the exception of treatment at planting time, while the protein percentage of Saginaw remained stable among zinc treatments.

(6) Total quantity of protein per plant was increased when zinc was applied to Sanilac. A decreased amount of protein per plant occurred with zinc application to Saginaw.

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