Zinc Nutrition and Starch Metabolism in *Phaseolus vulgaris* L. 1

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**ABSTRACT**

The effect of Zn nutrition on leaf starch metabolism was studied in two navy bean (*Phaseolus vulgaris* L.) varieties, Sanilac and Saginaw. Sanilac is much more susceptible to Zn deficiency than is Saginaw. The variables examined in these two strains were starch content, the activity of soluble starch synthetase (ADP-glucose:starch α-1,4-glucosyltransferase, EC 2.4.1.1b), and the size and number of starch grains. All of these variables decreased during Zn deficiency. The reductions were much greater in Sanilac than in Saginaw. Thus, positive correlations exist between the relative changes in these variables in Sanilac and Saginaw under low Zn and their genotypic difference in growth response to low Zn. These results are taken to purport that the above observations most likely represent characteristic responses to Zn deficiency. We therefore suggest that, as a possible role in plant metabolism, Zn is involved in starch formation.

Zinc is well known as an essential mineral nutrient for normal growth of plants. However, our knowledge of the role of Zn in plant metabolism is scanty and fragmentary (3, 14, 21). There is evidence that Zn may play a role in starch metabolism in plant cells (15, 20, 22). Starch grains were absent or decreased in size and number in Zn-deficient tomato (15), bean (20, 22), and spinach leaves (22).

Whether the reduced content of starch is specific for Zn deficiency has not been examined. In an attempt to resolve this question, we took advantage of the availability of two navy bean (*Phaseolus vulgaris* L.) varieties, Sanilac and Saginaw, whose growth response to Zn is different. Sanilac is sensitive to Zn deficiency, whereas Saginaw can tolerate it (4, 13). Our measurements of the effects of Zn on starch content, the activity of soluble starch synthetase (ADP-glucose:starch α-1,4-glucosyltransferase, EC 2.4.1.1b), and the size and number of starch grains were then related to the genotypic difference of these two varieties in their growth response to Zn. We found that the relative reduction in these measurements in Sanilac and Saginaw paralleled the varietal difference in their growth response to Zn. The observations reported herein most likely represent characteristic responses to Zn deficiency.

**MATERIALS AND METHODS**

**Plant Culture.** Seeds of Sanilac and Saginaw varieties of navy beans (*Phaseolus vulgaris* L.) were germinated in vermiculite in the dark at 24°C for 4 days, followed by their transfer to a growth chamber under a photoperiod of 16 hr light at about 2000 ft-c and 8 hr dark. Sixteen high-output fluorescent lamps (F7T2 12 CW/HO) and 10 25-w incandescent light bulbs were the sources of light. Temperature of the growth chamber during the light and dark was 28 and 20°C, respectively. Relative humidity varied from approximately 85% during the light to about 65% during the dark. On the 6th day, the seedlings were removed from vermiculite, the roots were thoroughly washed in distilled H2O, and the plants were transferred to beakers containing distilled H2O. On the 7th day, the seedlings were transferred to aerated nutrient solutions in Plexiglass containers (8 plants/about 3 liters). The basic nutrient solution contained 5.9 mM Ca(NO3)2, 0.58 mM K2HPO4, 0.58 mM NH4H2PO4, 5.2 mM KNO3, 1.8 mM MgSO4, 0.58 mM KCl, 46 µM H3BO3, 0.1 µM Na2MoO4, 0.32 µM CuSO4, 9.1 µM MnSO4, and 8.9 µM FeEDTA (an equimolar complex of FeSO4 and Na2EDTA). The corresponding concentrations of micronutrients in µg/ml are 0.5, B; 0.01, Mo; 0.02, Cu; 0.5, Mn; 0.5, Fe. Zinc sulfate was added to give a concentration of 0, 0.002, 0.02, or 0.2 µg Zn/ml. A Zn concentration of 0.2 µg/ml represents the optimum concentration required for growth. The solution, which was renewed weekly, was adjusted to pH 6.5 using 0.75 N NaOH. All chemicals used were reagent grade. Deionized distilled H2O was redistilled in an all-glass apparatus before use. The incipient Zn deficiency symptoms developed within a week to 10 days under Zn-deficient conditions (0 to 0.002 µg Zn/ml), with marked deficiency symptoms developing in 2 to 3 weeks.

When the bean plants were field-grown, they were grown as described previously (8) on the Zn-deficient soil near Saginaw, Mich., where both Saginaw and Sanilac varieties repeatedly exhibited differential growth response to low Zn (13). A description and analysis of this soil, with a pH of about 7.9 has been reported (13). The available levels of P, K, Ca.
and Mg were 42, 343, 9568, and 624 kg/ha, respectively, when determined after extraction of P by 0.03 N HNO₃ and 0.025 N HCl and of Ca, K, and Mg by 1.0 N CH₃COONH₄, at pH 7.0. Prior to planting, two levels of Zn were established in adjacent plots by broad applications of ZnSO₄·7H₂O at rates of 1 and 10 kg/ha, followed by disking into the soil. At planting time both areas received a band application of a 5-20-20 (non-Zn) fertilizer containing 2% Mn, at the rate of 208 kg/ha.

### Determination of Leaf Zn Content
Leaves were removed from the 4-week-old plants, dried and weighed, and then digested with HNO₃ and HClO₄ in a platinum crucible. A few drops of HF were added to remove silica and drops of HF evaporated to dryness. The residues redissolved in approximately 5 ml of 0.1 N HCl were brought to a volume of 25 ml. Zinc was determined by atomic absorption spectrometry. Two sets of samples were analyzed. The accuracy of the individual measurements was ± 5%. Since, within the experimental error, the Zn content of the leaves from the upper half of the plants was apparently the same as that of the leaves from the lower half, the average values of upper and lower leaves are given. As an exception, the Zn content of the upper leaves is presented for the normal Saniacl plants grown at 0.2 μg Zn/ml, because the lower leaves contained 1.8 times as much Zn as the upper leaves.

### Starch Determination
Leaves were collected from the upper half of 2.5- to 3.5-week-old plants grown in nutrient solution. Eight leaf discs of 9 mm were punched from the interveinal region with a cork borer. A sample of eight discs from a single plant weighed approximately 0.1 g. Four to five samples were used for each Zn treatment. Starch was determined by the procedure of Carter et al. (2). The sample was extracted three times with boiling 80% ethanol, dried, and ground in the 0.1 M acetate buffer (pH 4.8). Starch was hydrolyzed to glucose by incubation with 2 mg/ml of amyloglucosidase (grade II from Rhizopus, Sigma Chemical Co.). Glucose was then determined by the glucose oxidase method (Enzyme Manual by Worthington Biochemical Corp.). Each determination was run in duplicate.

### Enzyme Extraction
Leaves were sampled from the upper half of plants grown in nutrient solutions. The leaf samples from at least four different plants were pooled for enzyme extraction. Leaf samples were ground with 5 volumes (v/w) of buffer in a prechilled mortar until the final slurry was freely pipettable with a 1-ml serological pipet (approximately 3.5 min). The buffer consisted of 5 mM mercaptoethanol in 50 mM tris-HCl (pH 7.5). Insoluble PVP (polyacrylamide) was added in the amount of 60% of leaf weight during homogenization. The homogenate was centrifuged at 1,000g for 5 min. The supernatant fluid was recentrifuged at 40,000g for 30 min. The final supernatant liquid was used as an enzyme source.

When field-grown plants were used, all trifoliate leaves were excised from 3- to 4-week-old plants. Leaf tissues were cut into small pieces and dropped into liquid nitrogen. From the freshly frozen material acetone powder was first prepared by grinding with cold acetone (−70°C) in a Waring Blendor for 5 min. Homogenates were poured into an Erlenmeyer flask and kept in a freezer (−25°C) for 24 hr. The supernatant liquid was removed by suction filtration, and the residue washed three times with acetone (−30°C), and three times with acetone-ether (1:1) at −70°C. The enzyme was extracted by suspending 300 mg of the acetone powder in 3 ml of the buffer (above) in the presence of 180 mg of insoluble PVP for 1 hr at 4°C, with stirring every 15 min. The suspension was filtered through a glass fiber filter, followed by the centrifugation procedure described above. The final supernatant fluid was used as an enzyme source.

### Enzyme Assay
The filter paper method of Thomas et al. (19) originally developed for glycogen synthetase was adopted for the assay of starch synthetase. The incubation mixture, modified after Ghosh and Preiss (7), consisted of 1 mM ADP 1C glucose (1.1 × 10⁶ dpm/μmole), 50 mM N-tris(hydroxymethyl)methyl glycine, i.e., Tricine (pH 8.5), 24 mM KCl, 10 mM dithiothreitol, 5 mM EDTA, amylopectin (1.25 mg/ml), and enzyme in a final volume of 90 μl. After a 10-min incubation period at 30°C, the reaction was terminated by spotting a 75-μl aliquot onto a small square (2 × 2 cm) of filter paper (Whatman 31 ET) and dropping the paper into ice-cold 66% (v/v) ethanol. Up to 30 papers could be added to 300 ml of the aqueous ethanol. The incorporation of 1C-glucose from ADP 1C-glucose onto primer was then determined. Approximately 5 min after the addition of the last paper, the 66% ethanol was removed and fresh 66% ethanol (room temperature) was added and the papers were washed by stirring on a magnetic stirrer (about 60 rpm). The wash beaker was equipped with a stainless steel screen to protect the papers from the stirring bar. After washing for 30 min, the 66% ethanol was changed and papers washed twice for 20 min with 66% ethanol. Finally, the filter papers were washed with acetone for 5 min to remove ethanol and water. The filter papers were then dried, placed in scintillation vials containing 15 ml of 0.5% (w/v) 2,5-diphenyloxazole in toluene, and counted in a liquid scintillation spectrometer. The counting rate was corrected for background radioactivity estimated from filter papers containing incubation mixture without enzyme which was carried through the same washing procedure. In all of our experiments, quenching (measured by isotope channels-ratio method) was constant. Since all of the radioactive material remained on the filter paper, the paper was removed and both the vial and scintillation fluid were reused. When the volume of the scintillation fluid was reduced to about 7 ml due to repeated removal of filter papers, the scintillation fluid was replaced with 15 ml of fresh solution. The activity of starch synthetase thus determined was linear with respect to time (at least 15 min) and protein concentration (up to 16 μg/90 μl).

ADP (U-14C)-glucose was obtained from Calatonic (Los Angeles, Calif.). Unlabeled ADP glucose, amylopectin (soluble starch, grade II), and Tricine were obtained from Sigma Chemical Co.

### Protein Determination
Protein was determined according to the method of Lowry et al. (9) with crystalline BSA as a reference.

### Electron Microscopy
Leaf samples were taken from the upper half of 3- to 4-week-old plants. The leaf discs of 1.5 mm were punched from the interveinal region of each leaf with a Pasteur pipet. The fixation and dehydration of the leaf tissue were carried out at 4°C in the dark to reduce the loss of Chl. The discs were immediately transferred to the ice cold fixing medium, consisting of 5% glutaraldehyde, 0.4 M sucrose, 0.01 M phosphate buffer (pH 7.5), and 0.02 M MgCl₂. Samples were fixed for 2 to 4 hr, then rinsed twice with 5 mM phosphate buffer (pH 7.5) and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.5) for an additional 8 to 10 hr. Then the samples were washed twice with 50% ethanol and dehydrated in a graded ethanol series (50, 70, 80, 90, 95, and 100%) for 2 hr each. Beyond the 95% ethanol step the samples were processed at room temperature. After dehydration, the samples were processed in two changes of propylene oxide for 1 hr each, and then transferred to 1:1 mixture of Araldite resin and propylene oxide. At a 3-hr interval the ratio of resin to propylene oxide was raised to 2:1 then to 3:1. After 3 hr, the samples were transferred to fresh resin for 8 to 10 hr. The
samples were then embedded in large Beem pyramid-tip capsules using fresh resin and polymerized in three steps (35, 45, and 62 C) for 12 hr each. Thin sections were cut on a LKB ultramicrotome and mounted on 200 or 400 mesh copper grids. The sections were stained with uranyl acetate and lead citrate and then coated with a thin carbon film. Micrographs were taken in a Siemens 1A electron microscope at 60 kv. Primary magnifications ranged from 500 to 18,000.

RESULTS

Effect of Zn Nutrition on Leaf Starch Content. The starch content decreased under Zn-deficient conditions in both varieties (Table I). The reduction was much greater in Sanilac (94%) than in Saginaw (37%), reflecting the genotypic difference in their growth response to Zn-deficiency. When the plants were allowed to recover from Zn-deficiency in the complete nutrient solution for 10 days, the starch content increased to 75 and 88% of the normal level in Sanilac and Saginaw, respectively.

The leaf Zn content under low Zn conditions decreased by 80% and 43% in Sanilac and Saginaw, respectively (Table I). Thus, the relative reduction in both starch and Zn content under low Zn conditions follows a similar trend, and reflects the varietal difference of Sanilac and Saginaw in their growth response to Zn. When the plants were grown at an intermediate Zn level (0.02 μg Zn/ml), the leaf Zn content was 20 μg/g dry weight in both Saginaw and Sanilac. At this Zn treatment, the starch content was not determined. However, it appeared to be approximately the same in both varieties as judged from the electron microscopic study (Table IV).

Effect of Zinc Nutrition on Soluble Starch Synthetase Activity. Starch synthetase activity decreased under the stress of Zn deficiency and it was significantly more pronounced in the Sanilac variety than in Saginaw (Table II). The enzyme activity under Zn deficiency was decreased to 63% and 36% of the normal level in Saginaw and Sanilac, respectively. Such a differential response of starch synthetase to Zn deficiency in these two varieties parallels the genotypic difference in their growth response to low Zn.

The results from the field-grown plant material were quite compatible with those of the growth chamber experiments (Table III). Under low Zn condition, starch synthetase activity was slightly decreased in Saginaw and was markedly decreased in Sanilac. The varietal difference in response to low Zn seems to be more pronounced under the field conditions than in growth chamber.

Electron Microscopic Study of Effect of Zn Nutrition on Starch Metabolism. Electron microscopic examination of the relationship between Zn nutrition and the size and number of starch grains is summarized in Table IV, and typical micrographs are presented (Figs. 1–4). This portion of the study was not intended to analyze quantitative differences but to show a general trend in the effect of Zn nutrition on starch metabolism in Sanilac and Saginaw varieties. In Sanilac both the size and number of starch grains decreased under low Zn conditions (Fig. 1 versus 2), whereas these two variables changed little in Saginaw (Fig. 3 versus 4). Such a varietal difference in starch metabolism in response to Zn is suggestive

<table>
<thead>
<tr>
<th>Variety</th>
<th>Zn Status</th>
<th>Starch Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn Content μg/g</td>
<td>Starch Fresh wt of</td>
</tr>
<tr>
<td></td>
<td>dry wt</td>
<td></td>
</tr>
<tr>
<td>Saginaw</td>
<td>Normal</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Recovered from</td>
<td></td>
</tr>
<tr>
<td></td>
<td>deficiency</td>
<td></td>
</tr>
<tr>
<td>Sanilac</td>
<td>Normal</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Recovered from</td>
<td>158 ± 13 (5)</td>
</tr>
</tbody>
</table>

Table II. Effect of Zn Nutrition on Starch Synthetase Activity in Two Navy Bean Varieties Grown in Growth Chamber

The plants were grown in the complete nutrient solution for 3 weeks, except that Zn concentration was 0.002 and 0.2 μg/ml for the deficient and normal level, respectively. The starch synthetase activity was assayed by the filter paper method described in the text. Each figure is an average with the number of experiments in parenthesis and variation given as the standard error of a mean.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Zn Status</th>
<th>Starch Synthetase Activity</th>
<th>μmol glucose/g fresh wt/10 min</th>
<th>% of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saginaw</td>
<td>Normal</td>
<td>2.1 ± 0.5 (4)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>1.6 ± 0.4 (4)</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Sanilac</td>
<td>Normal</td>
<td>2.2 ± 0.3 (4)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>0.8 ± 0.3 (4)</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Effect of Zn Nutrition on Starch Synthetase Activity in Two Navy Bean Varieties Grown in Field

The low and adequate Zn plants were grown in the Zn-deficient soil supplemented with 1 and 10 kg of ZnSO₄·7H₂O per hectare, respectively. The enzyme was extracted from acetone powders prepared from the leaves of plants grown under different Zn status. The starch synthetase activity was assayed by the filter paper method described in the text. Each figure is an average of two separate determinations made on different extracts with variation given as the standard error of a mean.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Zn Status in Soil</th>
<th>Starch Synthetase Activity</th>
<th>μmol glucose/10 min</th>
<th>% of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saginaw</td>
<td>Adequate</td>
<td></td>
<td>2.3 ± 0.3 (2)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td></td>
<td>2.0 ± 0.4 (2)</td>
<td>87</td>
</tr>
<tr>
<td>Sanilac</td>
<td>Adequate</td>
<td></td>
<td>3.5 ± 0.2 (2)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td></td>
<td>0.7 ± 0.5 (2)</td>
<td>20</td>
</tr>
</tbody>
</table>
of a close relationship between Zn nutrition and starch formation.

A corollary finding from the micrographs was the increase in the size and number of osmiophilic globuli (lipoïd globuli or plastoglobuli) under low Zn conditions (Fig. 1) particularly in Sanilac variety. A general degeneration of chloroplast structure is also easily noticeable. This was particularly true for Sanilac when grown under low Zn conditions (Fig. 1). In these chloroplasts the number of grana was reduced and its structure was indistinct and irregular.

**DISCUSSION**

Sanilac and Saginaw beans had a differential growth response to low Zn similar to that previously reported (4, 13). The varietal difference was evident from more pronounced Zn-deficiency symptoms and greater retardation in growth in Sanilac than in Saginaw under low Zn conditions in both the growth chamber and field. The leaf Zn content of Sanilac and Saginaw under low Zn conditions, 10 and 18 µg/g dry weight, respectively (Table I), is also compatible with the value (15 µg/g dry weight or less) reported for other Zn-deficient tissues (23). These two navy bean varieties were then employed in the present study for a further understanding of the role of Zn in plant metabolism by relating the effect of Zn nutrition on starch metabolism to the genotypic characteristics of these beans.

Starch content was studied in the two strains under normal and low Zn conditions (Table I). The observed reduction in starch content under Zn-deficient conditions is consistent with reports on other plant species (15, 20, 22). Moreover, a correlation between the relative reduction in starch content in Sanilac and Saginaw under low Zn, and their genotypic difference in sensitivity to low Zn, suggests that a marked decrease in starch content may be associated with Zn deficiency.

The decrease in starch content could reflect either primary or secondary effect of Zn deficit. The starch content of leaves was directly proportional to the amount of CO₂ absorbed (17). Thus, the reduction in ribulose di-P carboxylase activity during Zn deficiency which we reported previously (8) could result in a decrease in starch content. For a further analysis, we examined the effect of Zn nutrition on starch synthetase activity.

The reduction of starch synthetase activity (Tables II and III) as determined by the filter paper method was compatible with the reduction of starch content (Table I). Again, a good correlation between the effect of Zn deficit on starch synthetase in Saginaw and Sanilac and their differential growth response to low Zn is suggestive of an intimate relationship between Zn nutrition and starch formation.

The apparent amplification of the varietal differences between Saginaw and Sanilac in their response to low Zn under the field conditions may have resulted from the higher light intensity and temperatures in the field than in the growth chamber. Under these conditions, Zn deficiency symptoms are known to be more conspicuous (23).

The mechanism by which Zn deficit decreases starch synthetase activity is not known. The reduction could result from a decreased synthesis of the enzyme under low Zn conditions or from a reduced activity under these conditions. We have some evidence for decreased protein synthesis. The reduction in protein synthesis during Zn deficiency was more drastic in Sanilac than in Saginaw. The incorporation of ¹⁴C-leucine into protein (70% ethanol-insoluble fraction) under low Zn (0.002 µg Zn/ml) was decreased to 57% and 78% of the normal level in the leaf discs of Sanilac and Saginaw, respectively. (The details will be reported elsewhere). If Zn deficiency reduces the biosynthesis of starch synthetase, a slight decrease in the leaf Zn content below a minimum required may result in a drastic reduction in starch formation. As to the possible effect of Zn on the activity of starch synthetase, Cardini and Frydman (1) noted that 7 mM ZnSO₄ completely inhibited the activity of the soluble starch synthetase purified (50-fold) from sweet corn kernels. A similar study needs to be done at the physiological or nutritional level of Zn, i.e., in µM concentrations.

Potassium has also been known to be involved in starch metabolism. The decrease in starch content during K deficiency (5) has been explained by the fact that K is required for maximum activity of starch synthetase (10, 11). Potassium content of the plant materials used in the present work was not determined. However, the plants received adequate K during their growth, and K (25 mM) was included in the enzyme assay mixture.

Recently, Tanaka and Akazawa (18) and Ozbun et al. (12) showed that starch synthetase exists in multiple forms in rice seeds, and leaf extracts of rice, maize, and spinach. It would be interesting to determine if bean plants contained isoenzymes of synthetase and if all of them would be affected in the same way by Zn deficiency.

Similarly, whether Zn deficiency affects primarily the soluble or starch granule-bound form, or both, of starch synthetase was not examined. Frydman and Cardini (6) pointed out that the two forms may be modifications of the same enzyme and that, particularly in leaves, the two forms had the same substrate specificity for ADP glucose. The soluble form of starch synthetase represented at least 80% of the total starch synthetase activity in the leaves of rice, tobacco, tomato, kidney beans, sorghum, barley, and sugar beet (16).

Electron microscopic observations provide further evidence of a close relationship between Zn and starch formation. The decrease in the size and number of starch grains is consistent with the reports by others (15, 20, 22). Furthermore, it is again our unique plant materials that provide a further documentation for such a relationship. The decrease in the size and number of starch grains under low Zn was much more drastic in the low-Zn sensitive variety, Sanilac, than in the tolerant Saginaw variety.

Based upon the electron micrographs, it is difficult to

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**Table IV. Summary of Electron Microscopic Study of Effect of Zn Nutrition on Starch Formation in Two Navy Bean Varieties**

The summary is based upon the observations of several micrographs for each Zn treatment. The plants were grown in the complete nutrient solution with varying Zn concentrations for 3 to 4 weeks.

<table>
<thead>
<tr>
<th>Zn Added to Nutrient Solution</th>
<th>Leaf Starch Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/ml</td>
<td>size</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>None</td>
<td>Large</td>
</tr>
<tr>
<td>0.002</td>
<td>Large</td>
</tr>
<tr>
<td>0.02</td>
<td>Large</td>
</tr>
<tr>
<td>0.2</td>
<td>Very large</td>
</tr>
</tbody>
</table>

1 Arbitrary and relative to the size of starch grains in the chloroplasts of the Sanilac plants grown at optimum Zn level (0.2 µg/ml) (after adjustment for magnification).
Fig. 1. A typical chloroplast section from the Sanilac plant grown without Zn (× 16,500). The following abbreviations are used for figures: Ag, abnormal granum; G, granum; CM, chloroplasts membrane; C, paracrystalline body; PG, plastoglobulus; S, starch grain; ST, stroma; T, large thylakoid; R, chloroplast ribosome; CR, cytoplasmic ribosome; F, phytoferritin; ER, endoplasmic reticulum; Mi, mitochondrion; W, cell wall.

Fig. 2. A typical chloroplast section from the Sanilac plant grown at 0.2 μg Zn/ml. × 23,500.
Fig. 3. Sections of two chloroplasts from the Saginaw plants grown without Zn. X 42,000.

Fig. 4. A typical chloroplast section from the Saginaw plant grown at 0.2 g Zn/ml. X 40,000.
ascertain the comparative effect of Zn on the structure and starch formation of the chloroplast. Wherever starch formation was absent, there occurred a general degeneration of chloroplast structure (Fig. 1). Perhaps, time course studies could provide a clarification.

The present study provides some evidence for a close relationship between Zn nutrition and starch formation. Whether Zn effect on starch formation is primary or secondary is yet to be clarified. Moreover, much work remains to elucidate the role of Zn in plant metabolism.

LITERATURE CITED