EFFECT OF THE FORM OF THE AVAILABLE NITROGEN ON THE CALCIUM DEFICIENCY SYMPTOMS IN THE BEAN PLANT

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(WITH THREE FIGURES)

Introduction

Calcium has been found to be one of the most important mineral elements needed for normal plant growth. In its absence plants exhibit very severe deficiency symptoms usually very early and if calcium is withheld further, death always results. NIGHTINGALE et. al. (7) made a detailed study of the responses of the tomato plant to calcium deficiency. The reason that calcium deficiency has a more severe effect on a plant than the deficiency of almost any other single element is probably in part because calcium has been found to have many functions in growth and development. These various rôles of calcium will not be discussed here, with the exception of one; the effect of calcium on nitrogen metabolism, as it is directly concerned with this report.

After ECKERSON (2) devised a method for measuring reductase activity, the plant's capacity to reduce nitrates to nitrites, which is the first phase of protein synthesis, she later studied the conditions which affect nitrate reduction and found calcium among other things to be essential (3). Other workers have also found that calcium malnutrition impairs normal nitrogen metabolism. BURRELL (1) found soybeans grown without calcium to accumulate nitrates in the leaves, and to have a much smaller amount of insoluble and amino acid nitrogen than plants grown with calcium; he believes this condition is brought about by reduced nitrate reduction. Ginsburg and Shive (5) on the other hand, also working with the soybean believe that calcium has no effect on the protein content. HIBBARD and GRISBLY (6) found calcium-deficient peas to contain smaller amounts of protein than the control plants but believe this is caused by the general disturbance of the plants rather than by the lack of any particular element. Presenting calcium and nitrogen analyses of a large number of plants PARKER and TRUOG (8) found a very close correlation between the calcium and nitrogen content.

If minus calcium plants lose their capacity to reduce nitrates and synthesize proteins, they are essentially minus nitrogen as well as minus calcium. The typical calcium deficiency symptoms then must be caused by both of these factors.

The question as to just what symptoms would develop with calcium deficiency if nitrate reduction were made unnecessary, prompted these
experiments in which a reduced form of nitrogen was supplied to the plants, and compared to those receiving oxidized nitrogen.

**Materials and methods**

The plant used in this study was the Dwarf Red Kidney bean, *Phaseolus vulgaris*. The seeds were planted in flats of unused quartz sand receiving only distilled water and allowed to germinate and grow in the greenhouse. After about 7 days plants of uniform size were transplanted to 1.5-liter glazed earthenware pots filled with unused quartz sand. The holes in the center of the pots were covered with small amounts of glass wool to insure good drainage, and to keep the sand from washing through. Two plants were planted in each pot. One day after transplanting (8 to 9 days after planting the seeds) the plants received the first application of nutrients, and every second day thereafter. Merck's reagent quality chemicals were used for all solutions. The reduced forms of nitrogen used in the first preliminary experiments were urea, and ammonia in the form of NH₄Cl. Ammonia proved to be very toxic to the bean plant, causing severe burning of the leaves and eventual death. This symptom was clearly evident within a week after the first application. Concentrations of NH₄Cl from 0.015 to 0.0045 M were tested, and the nutrient solutions were run at pH 4.7 and 6.0. Since all of these solutions containing ammonia proved to be very toxic, the remainder of the experiments were devoted to the use of urea as compared to NO₃ nitrogen.

Only a few references can be found in the literature concerning the use of urea in growing plants. Pirschle (9) grew several different plants in water culture supplying urea to some and expresses the opinion that urea can be changed to ammonia inside the plant independently of bacterial action. Yamaguchi (10) grew corn seedlings in sterile culture with urea as a source of nitrogen. He reports that urea was absorbed and was a readily available source of nitrogen for plant growth.

The following groups of plants were grown in each experiment: (1) +Ca, +NO₃; (2) −Ca, +NO₃; (3) +Ca, +CO(NH₄)₂; and (4) −Ca, +CO(NH₂)₂. Twenty-eight to 30 plants were grown under each nutrient in each experiment. The minus-calcium plants received no calcium whatsoever at any time during their growth.

Various concentrations of the salts making up the nutrient solutions were tried and one of the more dilute ones was finally chosen. An attempt was made to keep the MgSO₄ and KH₂PO₄ rather low so that they would not become excessively toxic in the absence of calcium and over-emphasize the true calcium deficiency symptoms and yet not have them too low to be limiting for best growth. Gauch (4) found magnesium to be very toxic in the absence of calcium, and by lowering it considerably the −Ca plants were
able to live longer, take up more NO₃ and other minerals and synthesize more materials including nitrogen compounds.

The composition of the nutrient solutions used is given in table I.

### TABLE I
**Composition of nutrient solutions***

<table>
<thead>
<tr>
<th>Solution</th>
<th>Reaction of Solution</th>
<th>Molar concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Ca(NO₃)₂</td>
</tr>
<tr>
<td>+ Ca</td>
<td>4.7</td>
<td>0.0045</td>
</tr>
<tr>
<td>+ NO₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Ca</td>
<td>4.7</td>
<td>0.0045</td>
</tr>
<tr>
<td>+ CO(NH₂)₂</td>
<td>4.9</td>
<td>0.0045</td>
</tr>
<tr>
<td>- Ca</td>
<td>5.1</td>
<td>0.0045</td>
</tr>
<tr>
<td>+ CO(NH₂)₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All solutions also contain the following micro-nutrients: 0.5 p.p.m. B as H₂BO₂; 0.5 p.p.m. Fe as ferrie citrate; 0.5 p.p.m. Mn as MnCl₂; 0.5 p.p.m. Zn as ZnCl₂; and 0.125 p.p.m. Cu as CuCl₂.

**Experimentation**

The experimental data here presented consists of a report of but two experiments, as the preliminary experiments conducted gave essentially the same results.

Experiment I was started in June, 1939, and the plants were harvested 30 days after planting, about the time flower buds began to appear. Experiment II was set up in September, 1939, and the plants were allowed to grow to maturity, 52 days after planting. Wet and dry weights were taken of all plants, and photographs were taken of the plants in experiment II at the time of maturity and at the age of 30 days. Notes were kept on the appearance of the plants in the course of the experiment. No chemical analyses were made at this time.

**Experiment I.—** About 15 days after time of planting, the -Ca, + NO₃ plants showed definite symptoms. The plants were 12 to 15 cm. tall and had less leaves than the +Ca plants. The old leaves were dark and the young leaves very chlorotic at this time. The +Ca, + NO₃ plants were 15 to 20 cm. tall, had more and larger leaves than the former group, and were grass green in color. The +Ca, + CO(NH₂)₂ plants were 12 to 15 cm. tall, and were darker green in color than the +Ca, + NO₃ group. They had also fewer new leaves than this latter group; some of the older leaves were slightly yellow, and in some cases very slightly burned at the margins only. The -Ca, + CO(NH₂)₂ plants at this time were 12 to 15 cm.
tall, had fewer leaves than the + Ca, + NO₃ group, and the new leaves were a little smaller. The plants on the whole had a very good appearance and were exceptionally dark green. They had the darkest green color of the entire group. The new leaves also were very dark green (the new leaves are usually lighter than the old leaves). There was no burning of leaves nor injury of any kind at this time. The contrast of this group with the −Ca, + NO₃ group was very striking.

Fifteen days later (30 days after planting) this series of plants was harvested. At this time flower buds had formed on all plants except the −Ca, + NO₃ group. The + Ca, + NO₃ plants were very vigorous, green and had a good development of roots which were yellowish brown in color. The −Ca, + NO₃ plants showed considerable injury. They were very chlorotic and the tips were completely dead. The roots were poorly developed and had a dark brown color. The epidermis was loose and had been lost from most of the roots. The + Ca, + CO(NH₂)₂ plants were smaller than the + Ca, + NO₃ plants and not as vigorous. The leaves were paler green in color and some of the lower leaves were chlorotic. The roots were fairly well developed and very light in color, in fact the lightest of all four groups. The −Ca, + CO(NH₂)₂ plants were still dark green in color, although the lower leaves were somewhat burned and their tips beginning to die. The upper parts of the plants were in very good condition with no injury to the growing tips. The roots were fairly well developed and had a yellowish brown color and no loose epidermis. They were lighter in color than those of the + Ca, + NO₃ group and slightly darker than the + Ca, + CO(NH₂)₂ group. The appearance of both the top portion and the roots of the −Ca, +CO(NH₂)₂ group was decidedly not characteristic of the usual −Ca symptoms.

Data on the plants of experiment I are presented in table II.

Experiment II.—The plants in this experiment were grown under the same conditions as those in experiment I, but they were grown to maturity. The following description applies to plants at 19 days of age. The + Ca, + NO₃ plants were 25 to 27 cm. tall, with large green leaves. The new leaves were lighter than the old leaves. The + Ca, + CO(NH₂)₂ plants were 23 to 26 cm. tall and were about like those in the former group except that the new leaves were not lighter than the old leaves. The −Ca, + NO₃ plants were 21 to 27 cm. tall, and the upper leaves were rather pale (paler than those of the + Ca, + NO₃ group) and some had grayish spots. The plants in general were almost as tall as the + Ca, + NO₃ group but were not as vigorous and had not attained as much growth. The −Ca, + CO(NH₂)₂ group was 24 to 27 cm. tall and very good in appearance. The plants had a dark green color; none of the leaves, including the new leaves, were light. The leaves were slightly smaller than those of the + Ca, + NO₃ group, but otherwise growth was about equal in both groups.
### TABLE II
DATA OF EXPERIMENT I, PLANTS AT 30 DAYS
WEIGHTS ON 30-PLANT BASIS

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Average Height</th>
<th>Wet Top Weight</th>
<th>Wet Root Weight</th>
<th>Dry Top Weight</th>
<th>Dry Root Weight</th>
<th>Percentage Dry Weight Entire Plant</th>
<th>Percentage Dry Weight Tops</th>
<th>Percentage Dry Weight Roots</th>
<th>Wet Top-Root Ratio</th>
<th>Dry Top-Root Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Ca</td>
<td>cm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ NO₃</td>
<td>55</td>
<td>596.50</td>
<td>195.21</td>
<td>72.15</td>
<td>19.85</td>
<td>11.62</td>
<td>12.09</td>
<td>10.16</td>
<td>3.05</td>
<td>3.63</td>
</tr>
<tr>
<td>+ Ca</td>
<td>15</td>
<td>100.20</td>
<td>42.30</td>
<td>17.20</td>
<td>3.75</td>
<td>14.70</td>
<td>17.16</td>
<td>8.86</td>
<td>2.36</td>
<td>4.58</td>
</tr>
<tr>
<td>+ CO(NH₂)₂</td>
<td>40</td>
<td>344.06</td>
<td>97.96</td>
<td>36.51</td>
<td>8.30</td>
<td>10.13</td>
<td>10.61</td>
<td>8.47</td>
<td>3.51</td>
<td>4.40</td>
</tr>
<tr>
<td>+ Ca</td>
<td>20</td>
<td>170.62</td>
<td>76.59</td>
<td>26.72</td>
<td>6.53</td>
<td>13.45</td>
<td>15.66</td>
<td>8.52</td>
<td>2.22</td>
<td>4.16</td>
</tr>
<tr>
<td>- Ca</td>
<td>20</td>
<td>170.62</td>
<td>76.59</td>
<td>26.72</td>
<td>6.53</td>
<td>13.45</td>
<td>15.66</td>
<td>8.52</td>
<td>2.22</td>
<td>4.16</td>
</tr>
</tbody>
</table>
Thirteen days later (31 days after planting) photographs were taken of the plants in this experiment which are shown in figure 1.

Following is a description of the plants at this date. The +Ca, +NO₃ plants were 40 to 60 cm. tall, had large green leaves, and many flower buds. The +Ca, +CO(NH₂)₂ plants were 40 to 50 cm. tall, and the leaves were smaller than those of the preceding group. The leaves were also decidedly mottled, somewhat resembling either iron or magnesium deficiency. Many flower buds were present. The −Ca, +NO₃ plants were 25 to 30 cm. tall and very poorly developed. The upper leaves were yellow, with gray and brown necrotic spots. All the leaves were small and a few poorly developed flower buds were present. The −Ca, +CO(NH₂)₂ plants were 30 to 45 cm. tall and their general appearance was rather good. Their color was still fairly dark green and many flower buds were present. Some leaves, however, at this time had become mottled with very small yellowish, grayish, to brownish spots. The veins, particularly, of some leaves were becoming discolored. The stems of some plants also showed small brown spots. Many of the younger leaves showed their first injury and discoloration at the veins and pulvinus region. This response was also observed by Gauch (4). Most of the flower buds were rather well developed.

Four days later (35 days after planting) the −Ca symptoms of the −Ca, +CO(NH₂)₂ plants, although much delayed as compared to that of the −Ca, +NO₃ plants, were becoming very evident and rather severe. The growing tips of a few plants were dead or dying and several of the leaves had become pale and developed grayish or brownish spots. The plants, however, were still rather green with many uninjured flowers and in decidedly better condition than the −Ca, +NO₃ plants.

Nine days later (44 days after planting) the following observations were made: The +Ca, +NO₃ plants had all set fruit, and were in good condition. The +Ca, +CO(NH₂)₂ plants had by this time developed very mottled and chlorotic leaves but the plants otherwise were fairly sturdy in appearance and were bearing fruit in about the same abundance as the plants in the +Ca, +NO₃ group. The upper portions of the −Ca, +NO₃ plants were all dead at this time and all their leaves were rather chlorotic. Some of the plants had flowered but none produced fruit. The −Ca, +CO(NH₂)₂ plants were in a much less severe condition. The very uppermost portion of the tips of slightly over half of the plants in this group were dead and the upper leaves were yellowed, some having grayish brown spots. The other leaves still had a rather dark green color, even though some of these same leaves had small spots. All the plants of this group had flowered, and several were bearing fruit.

Eight days later (52 days after planting), these plants were harvested. Photographs were again taken at this time and are shown in figure 2.
Fig. 1. Experiment II. Plants at 31 days.
Fig. 2. Experiment II. Plants at maturity (52 days).
A brief description of the plants at this date follows: The + Ca, + NO₃ plants were about 60 cm. tall, had large leaves and bore many beans on each plant. The roots were well developed, and light brown in color. The leaves of the + Ca, + CO(NH₂)₂ plants were very chlorotic and smaller than those in the preceding group, and most of the lower leaves were dead. All of the plants produced fruit. The roots were well developed, and yellowish brown in color. They were just slightly lighter in color than those of the + Ca, + NO₃ plants. The − Ca, + NO₃ plants were very badly injured. The tops of all plants were dead and all the leaves, even the lowermost, were partially injured. None of the plants produced fruit. The roots were very poorly developed, and dark brown in color. The epidermis of the roots was loose, giving the roots a slimy texture. The base of the stem (lower hypocotyl) was also dark brown and necrotic. The − Ca, + CO(NH₂)₂ plants were much larger and in not nearly as severe condition as those deficient in calcium but receiving nitrate. The leaves had spots showing injury, but they still retained their dark, almost blue-green color. The tips of almost half the plants were still living. Fruit was produced by 25 per cent. of the plants in this group (8 out of 32 plants). The fruit was small in most cases (largest bean pod produced was 7 cm. long) and few per plant, but not entirely absent as in the case of the − Ca, + NO₃ plants. The roots were fairly well developed. There was considerably better growth than that produced by the − Ca, + NO₃ plants, but less than that produced by either of the + Ca groups. They were darker in color than those of the + Ca plants but considerably lighter than those of the − Ca, + NO₃ plants. The epidermis seemed in good condition and no slimy texture was evident. There were no necrotic areas at the base of the stem.

Table III contains the data of experiment II.

Discussion

From the results obtained in these experiments two things are evident:

1. Under normal conditions including the presence of calcium, urea is not as good a source of nitrogen as is the nitrate form for growth of the bean plant. 

2. In the absence of calcium much better growth is made by the bean plant with urea than with nitrates.

It was not determined what factors were responsible in producing poorer growth with urea in the presence of calcium than with nitrate. The reactions of the two solutions did not differ greatly, as is recorded in table I. The form of the available nitrogen, however, has a very pronounced effect on the calcium deficiency symptoms. With urea, the calcium deficiency symptoms are much delayed and when they become evident they are very much less severe. The weights of the − Ca, + CO(NH₂)₂ plants were about 60 per cent. greater than those of the − Ca, + NO₃ plants. The former plants pro-
TABLE III
DATA OF EXPERIMENT II, PLANTS AT MATURITY (52 DAYS)
WEIGHTS ON 30-PLANT BASIS

<table>
<thead>
<tr>
<th>NUTRIENT</th>
<th>AVERAGE HEIGHT</th>
<th>WET* TOP WEIGHT</th>
<th>WET ROOT WEIGHT</th>
<th>WET FRUIT WEIGHT</th>
<th>DRY* TOP WEIGHT</th>
<th>DRY ROOT WEIGHT</th>
<th>DRY FRUIT WEIGHT</th>
<th>PERCENT-AGE DRY WEIGHT ENTIRE PLANT</th>
<th>PERCENT-AGE DRY WEIGHT TOPS</th>
<th>PERCENT-AGE DRY WEIGHT ROOTS</th>
<th>PERCENT-AGE DRY WEIGHT FRUIT</th>
<th>WET† TOP-ROOT RATIO</th>
<th>DRY† TOP-ROOT RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Ca</td>
<td>cm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>+ NO₃</td>
<td>60</td>
<td>463.50</td>
<td>174.06</td>
<td>307.70</td>
<td>83.05</td>
<td>18.65</td>
<td>22.86</td>
<td>13.18</td>
<td>17.92</td>
<td>10.71</td>
<td>7.56</td>
<td>4.43</td>
<td>5.68</td>
</tr>
<tr>
<td>− Ca</td>
<td>29</td>
<td>137.93</td>
<td>48.16</td>
<td>None</td>
<td>24.38</td>
<td>5.11</td>
<td>5.51</td>
<td>15.84</td>
<td>17.68</td>
<td>10.61</td>
<td>−</td>
<td>2.86</td>
<td>4.77</td>
</tr>
<tr>
<td>+ Ca</td>
<td>53</td>
<td>237.23</td>
<td>98.03</td>
<td>62.39</td>
<td>44.16</td>
<td>8.67</td>
<td>4.71</td>
<td>14.47</td>
<td>18.61</td>
<td>8.84</td>
<td>7.55</td>
<td>3.06</td>
<td>4.91</td>
</tr>
<tr>
<td>− Ca</td>
<td>43</td>
<td>183.05</td>
<td>76.45</td>
<td>4.85</td>
<td>39.84</td>
<td>8.91</td>
<td>0.51</td>
<td>18.63</td>
<td>21.76</td>
<td>11.65</td>
<td>10.52</td>
<td>2.46</td>
<td>4.53</td>
</tr>
</tbody>
</table>

* Fruit not included.
† Fruit included.
duced fruit while the latter did not. Flowering and fruiting is very striking for a minus-calcium plant. Equally striking was the good condition of the roots in the calcium deficient plants receiving urea.

These experiments then may be considered as additional evidence that calcium has an important function in the utilization of nitrogen. The evidence is in agreement with Eckerson's findings that calcium deficient plants have a lowered reductase activity since the calcium deficient plants receiving urea, which is a reduced form of nitrogen, make much better growth than do those receiving nitrates. The calcium-deficiency symptoms of the plants receiving urea, then, are really truer symptoms which can be directly assigned to the lack of calcium.

Since other elements, namely potassium, phosphorus, and sulphur, were also found to be necessary for normal reductase activity by Eckerson (3), it may be entirely possibly that their deficiency symptoms may also be lessened in severity with the use of urea.

Calcium, of course, is also a building element and therefore cannot be eliminated entirely and still retain good growth. From evidence of an early preliminary experiment it seems that plants receiving urea may be able to carry on good growth with only small amounts of calcium. The plants in this preliminary experiment were grown in used sand which evidently contained small amounts of calcium, (the actual amount being unknown). The -Ca, +CO(NH₂)₂ plants grown to maturity produced almost as much fruit as the +Ca, +NO₃ plants. This fact together with the results obtained in experiments I and II points to the possibility that with urea the usual amount of calcium used may be in excess and it may be the factor which causes poorer growth with a +Ca, +CO(NH₂)₂ solution than with a +Ca,

Fig. 3. Plants grown in used sand at 39 days.
The plants shown in figure 3 were grown in used sand. They were photographed 39 days after planting. When they were 45 days old, the \(-\text{Ca, } +\text{CO(NH}_2\text{)}_2\) plants had produced 25 per cent. more total growth than the \(+\text{Ca, } +\text{CO(NH}_2\text{)}_2\) plants. At maturity (53 days after planting) those receiving no calcium had produced 2.7 times as much fruit as those to which calcium had been added.

More conclusive experiments are needed to elucidate the relationship of calcium to nitrogen metabolism. The minimum amount of calcium required for normal growth with nitrate and with urea should be known. The determination of the reductase activity of plus- and minus-calcium plants grown with urea and with nitrate might throw some light upon its relation to protein synthesis in the two different nitrogen series. Determinations of assimilated nitrogen also should be made for actual comparisons of protein synthesis. Finally, histological examinations would show the condition of the cell wall in each group and aid in determining the minimum amount of calcium needed for normal cell wall development.

**Summary**

1. Calcium is known to be one of the most important mineral elements needed for normal plant growth. Among other things it is closely associated with nitrogen metabolism. Eckerson found that it is needed for normal nitrate reduction in the synthesis of proteins. In these experiments the bean plant was grown with and without calcium using nitrate nitrogen and urea, a reduced form of nitrogen.

2. With calcium present nitrate nitrogen produced better growth than urea.

3. In the absence of calcium much better growth was made with urea than with nitrates. With urea the calcium deficiency symptoms were much delayed, and when they became evident they were very much less severe.

4. The calcium-deficient urea plants produced about 60 per cent. more growth than the calcium-deficient nitrate plants and all of them flowered. Twenty-five per cent. of the calcium-deficient urea plants produced fruit, whereas none of the calcium-deficient nitrate plants produced fruit, and only a few flowered. The roots of the calcium-deficient urea plants did not exhibit the usual calcium-deficiency symptoms and they produced twice as much growth as those of the calcium-deficient nitrate plants.

The writer wishes to express his sincere thanks and appreciation to Professor C. A. Shull for his interest and valuable suggestions made during the course of this work.

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