CARBOHYDRATE DISTRIBUTION AS AFFECTED BY CALCIUM DEFICIENCY IN COTTON 1,2

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Carbohydrate accumulation in various plant organs has often been associated with calcium deficiency. Groom (7) in 1896 suggested one function of calcium was to combine with oxalate ions to form calcium oxalate. Groom concluded that under conditions of calcium deficiency potassium oxalate retarded the action of diastase and such plants tend to accumulate starch. Nightingale et al (11) present analyses of whole tomato stems which they claim demonstrate the accumulation of carbohydrates by calcium deficient plants. They conclude this accumulation of carbohydrates resulted from the failure of calcium deficient plants to absorb and assimilate nitrate. Gauch (5) states that failure of bean plants to absorb nitrate ions when grown on solutions deficient in calcium was due to an unbalanced condition of the nutrient solution. Using Nightingale's solution, Gauch showed that bean plants deficient in calcium could not absorb nitrate ions; however, in a second series employing a more dilute nutrient solution, Gauch demonstrated that calcium deficient bean plants were able to absorb nitrate ions. Gauch concludes that the difference in nitrate absorption by plants in the two series is associated with the level of magnesium in the solutions thus showing that cationic imbalance was a major factor in Nightingale's observations.

Eckerson (4) showed that calcium among other factors was essential for the normal nitrate reductase activity of plants. Skok (12) theorized that if minus calcium plants lose their capacity to reduce nitrates, they are essentially minus nitrogen as well as minus calcium plants. Utilizing urea and nitrate as sources of nitrogen, Skok noted that minus calcium bean plants made much better growth when the reduced form of nitrogen was supplied. Hibbard and Grigsby (8) found that bean plants grown in solutions deficient in calcium continued to synthesize sugars and proteins but in lesser amounts than were produced by plants grown in complete nutrient solutions. Darkes et al (2) reported liming of soil resulted in a 21% decrease in reducing and total sugars of tobacco. Thus existing reports on the role of calcium in the accumulation and distribution of carbohydrates in plants are in conflict. In light of those reports it seemed worthwhile to obtain more information concerning the influence of calcium deficiency on cotton.

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EXPERIMENTAL METHODS AND RESULTS

PLANT CULTURE: Cotton seeds, strains Texas S9 and S3-2-3 were planted in flats containing washed creek sand. Twenty-five days after planting the seedlings were transplanted to 5-gallon jars containing nutrient solution. Three seedlings of each variety were placed in each of 18 jars. During the course of the experiment a modification of Hoagland's solution was employed. Each nutrient treatments received solutions containing the following salts.

<table>
<thead>
<tr>
<th>SALTS</th>
<th>CONC, mM</th>
</tr>
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<tbody>
<tr>
<td>NH₄NO₃</td>
<td>5</td>
</tr>
<tr>
<td>KNO₃</td>
<td>5</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>2</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
</tr>
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</table>

In addition CaCl₂ was supplied to the three treatments as follows:

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CONC CaCl₂, mM</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>5.0</td>
</tr>
<tr>
<td>II</td>
<td>0.2</td>
</tr>
<tr>
<td>III</td>
<td>0.05</td>
</tr>
</tbody>
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The above calcium levels were 1, 0.04, and 0.01 times that of Hoagland's solution. Because of the general availability of sodium to plants and since the present author (9) has shown that under conditions similar to those of this investigation sodium may partially substitute for calcium in the nutrition of cotton, it was felt advisable to add sodium chloride to the culture solutions. Minor elements were added to insure a sufficient supply. Iron was added as Versenol iron chelate at the rate of 2 ppm iron to all solutions. The pH of each solution was checked daily, and control was maintained between limits of 5.0 to 6.3 by the addition of dilute HNO₃ or NH₄OH.

![Graph](fig1.png)

Fig. 1. The influence of substrate calcium on the growth of cotton as indicated by plant height.

![Graph](fig2.png)

Fig. 2. The influence of substrate calcium on flowering of cotton. Figures given are the number of flowers produced/3 plants over a period of three days.

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**TABLE I**

<table>
<thead>
<tr>
<th>PLANT PART</th>
<th>Ca LEVEL, 45-DAY HARVEST</th>
<th>Ca LEVEL, 85-DAY HARVEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>1.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Stem</td>
<td>4.96</td>
<td>0.63</td>
</tr>
<tr>
<td>Root</td>
<td>4.68</td>
<td>1.38</td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19.27</td>
<td>7.68**</td>
</tr>
</tbody>
</table>

* Figures given are average values for 6 plants.
** Difference between treatment and control (1) significant to 5% level.
*** Difference between treatment and control (1) significant to 1% level.
All jars were drained, washed, and filled with freshly prepared solution each week. Each jar was supplied with a porous clay aerator attached to the central air line and a pressure of 5 lbs/in.² was maintained during the experiment. The experimental design was a randomized block with 3 replications on the greenhouse bench. Between 10:00 A.M. and 12:00 noon at 45 and 85 days after transplanting, plants from each treatment were harvested for fresh and dry weight determinations and carbohydrate analyses. Because of the limited growth it was necessary to harvest at 45 days all plants in the 0.01 treatment to insure sufficient material for chemical analysis. Height measurements and flower counts were made during the experiment.

CHEMICAL METHODS: Tissue which had been dried at 60°C to a constant weight in a forced draft oven was ground to pass an 80-mesh screen and, after extraction with 80% alcohol, sugars and starch were determined by a modification of the Wildman and Hansen (14) method as employed by Eaton and Joham (3).

PLANT GROWTH RESPONSE TO VARIOUS CALCIUM LEVELS: Both strains, Texas S9 and S3-2-3, responded in like manner to variations in substrate calcium. Height, number of flowers, and dry weight were directly related to substrate calcium (figs 1, 2 and table 1). The number of days from planting to appearance of the first flower was inversely related to substrate calcium. This relation is particularly noticeable in strain S3-2-3 (fig. 2). Substrate calcium had little or no influence on the date of maximum flower production; however, a marked influence of calcium on boll set was noted since at the second harvest no fruit were set on the 0.04 plants (table 1).

INFLUENCE OF SUBSTRATE CALCIUM ON CARBOHYDRATE DISTRIBUTION: In calcium deficient plants carbohydrates accumulated in the leaves while extremely low levels were noted in the stems and roots, and in some instances were not detectable by the analytical methods employed. Again, as in growth, both strains responded in a like manner thus only the data for Texas S9 are presented in figures 3, 4, and 5. Reducing sugars, sucrose, and starch of the leaves were inversely related to substrate calcium at both harvest dates while a direct relation was observed in the stems and roots (figs 3, 4, 5).

At 45 days reducing sugars and sucrose in the organs of the high calcium treated plants increased in the following order: leaves, stems, roots. The inverse of the above order tended to be the case in the 0.04 and 0.01 calcium treated plants with the leaves always containing the highest concentrations of reducing sugars, sucrose and starch. Little trend was noted for starch concentration in the plant parts of the high calcium treated plants.

Figs. 3 to 5. The concentration of carbohydrates in various parts of the cotton plant as influenced by substrate calcium. Fig. 3 (top). Reducing sugars. Fig. 4 (middle). Sucrose. Fig. 5 (bottom). Starch.
From 45 to 85 days a marked reduction in carbohydrates of the leaves occurred in all treatments, although the relation between calcium deficiency and carbohydrate accumulation in the leaves was still apparent. During the period from 45 to 85 days carbohydrates in the stems and roots of the high calcium treated plants decreased while in the same period an increase was noted for reducing sugars and starch in the same organs of the 0.04 plants. During that period the high calcium plants set a large boll crop while no fruit were set by the 0.04 plants.

**Discussion**

Carbohydrate accumulation is often associated with limitation of growth by nutrient deficiencies provided photosynthesis has not been impaired. Growth limiting levels of calcium supplied to cotton resulted in increased carbohydrate concentrations in the leaves and decreased levels in the stems and roots. Thus, calcium deficiency differs widely from the effect of nitrogen deficiency on carbohydrate distribution in cotton. In the latter case, according to Wadleigh (15), carbohydrates accumulate in all tissues of the cotton plant. The initial influence of nitrogen deficiency is a limitation of growth resulting from a reduction in protein synthesis. Synthesis and translocation of carbohydrates are not greatly influenced by nitrogen deficient levels above those producing chlorosis. Calcium deficiency, on the other hand, is expressed in a different manner. From data presented here it seems likely that one factor associated with the limitation of growth by calcium deficiency is the failure of normal carbohydrate transport. The marked accumulation of carbohydrates in the leaves indicates synthesis of these materials continues under calcium deficient conditions which severely limit growth.

On first analysis of the paper by Nightingale et al (11) and the data presented here, it would seem that tomato and cotton respond differently to calcium deficiency as far as the distribution of carbohydrates is concerned. They (11) stated that carbohydrates accumulated in the stems of calcium-deficient tomato plants. Examination of their data makes their conclusion questionable. In their experiment, they employed tomato plants which had grown for several weeks in four-inch pots, and at the time of treatment application the plants were already high in carbohydrates. During the course of the experiment little or no change occurred in the stem carbohydrates of the minus calcium plants as shown by their initial and final analyses. Considering that very little growth took place it seems likely that their data do not represent an accumulation of carbohydrates in the stems of the minus-calcium plants but rather a failure of carbohydrate translocation and utilization under calcium deficiency. Thus, the present data on cotton and those presented by Nightingale et al on tomato may actually be in close agreement.

The limitation of amylase activity with calcium deficiency as suggested by Groom (7) does not seem to be a factor in the failure of carbohydrate transport or starch accumulation by calcium deficient cotton plants. Marked increases in reducing sugars and sucrose in leaves of calcium deficient plants and deficiencies of these materials in stems and roots indicates factors other than amylase activity are responsible for inhibition of the normal translocation mechanism.

The influence of calcium deficiency on carbohydrate distribution in cotton is very similar to the effect of boron deficiency in tomato plants (10). Gauch and Dugger (6) suggested that calcium also could affect the translocation of carbohydrates through its relation to boron as reported by Brennan et al (1). In the latter publication it was noted that increased substrate calcium accentuated boron deficiency. Thus according to the reasoning of Gauch and Dugger, high levels of substrate calcium in the presence of low levels of boron would cause boron deficiency and thus influence the carbohydrate distribution. In the present paper calcium deficiency was noted to limit carbohydrate translocation, therefore, it seems likely that the role of calcium in transport of carbohydrates may be independent of boron.

**Summary**

Growth and fruiting of cotton as measured by height, dry weight, number of flowers produced, weight of bolls and earliness to flower were directly related to substrate calcium. An inverse relation was noted between leaf carbohydrates and calcium availability while carbohydrate levels of stems and roots tended to be directly dependent on substrate calcium. Such a distribution pattern was considered to be a result of the failure in carbohydrate translocation due to calcium deficiency. A comparison was made between the results of calcium and nitrogen deficiency on cotton. Both calcium and boron deficiencies were noted to exert similar influences on the distribution of carbohydrates.

**Literature Cited**


The biological activity of 6-(2-furfuryl)aminopurine (kinetin), a substance which has been found to effect increased cell division in tobacco “callus” tissue (8), has led to the synthesis and biological study of a number of 6-(substituted)purine derivatives. Several of these compounds have recently been found to have pronounced biological activity in both plant and animal systems. For example, in the moss Tortella caespitosa, 6-(2-thienyl)aminopurine, 6-benzylaminopurine and 6-n-pentylaminopurine have been found to have effects equal to or slightly greater than that of kinetin on the development of gametophores (5). Further, some of the purine analogues such as 6-(ω-phenylehtyl)aminopurine were found to be several thousand fold more active than adenine and several hundred fold more active than kinetin in retarding tentacle regeneration in hydra (6, 10).

The use of various organic compounds (especially auxins) in an attempt to increase the rate of germination of seeds has been studied for many years (1); however, only limited success has been obtained (3). In view of the biological activity of these purine derivatives, a study was made of their effects on seed germination.

The effect of several compounds, especially thiourea, upon lettuce seed germination has been reported by Thompson and Kosar (13), and during the final stages of preparation of this manuscript an article appeared (7) which presented some data on stimulation of lettuce seed germination by 6-(2-furfuryl)-aminopurine as well as some other 6-(substituted)-aminopurines. These latter results were interrelated to the red light effect previously observed as a requirement for lettuce seed germination (2). We have also observed such an effect with 6-(substituted)thiopurines (12).

**EFFECT OF 6-(SUBSTITUTED)THIO- AND AMINO-PURINES ON GERMINATION OF LETTUCE SEED**

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Materials and Methods

Several types of field variety seed were initially studied in an effort to determine the effect of pre-soaking in 6-(substituted)purine solutions on their germination. In several instances enhanced rates of germination were noted; however, the most readily adaptable seed found for this study was a variety of lettuce (Early Curled Simpson).

Most of the compounds studied are relatively new, and their method of synthesis are reported elsewhere (4, 6, 9 to 12).

Unless otherwise noted, 100 or more seeds were pre-soaked in 100 ml of each purine solution, made up in distilled water at a concentration of 10 μgm/ml, for a period of 8 hours. In order to minimize light effects, the soaking period was uniformly overnight, and a water control was always included under identical experimental conditions as a standard. The seeds were drained of excess solution on filter paper, and finally placed on filter paper, wet with the corresponding purine solution, in Petri dishes and allowed to germinate in a dark room at 30° C. The same relative results were obtained whether the initial manipulation of seeds was carried out in diffuse light or in blue light (fluorescent light filtered through four layers of du Pont 300 MSC dark blue cellophane). The temperature of 30° C was chosen because these lettuce seed, soaked in water alone, germinate very slowly at this temperature in the dark. In order to show that the inactive compounds were not inhibiting germination, a corresponding experiment was performed so that germination was allowed to proceed in the presence of continuous fluorescent light at 25° C.

The light and temperature requirements for this variety of lettuce seed, with respect to rate of germination using several of the more “active” compounds, was studied. The seeds were pre-soaked in water, or