amounts of auxin rather than absolute ones are being considered the auxin amounts may be more conveniently expressed in arbitrary units.

The above-described method has been used for the determination of the distribution of the auxin concentrations in maize, Avena and pea seedlings and also by Yin (unpublished as yet) to study the auxin distribution of Papaya leaves, and has proved to be reliable and to give reproducible results (Avena experiments of table 1). Figure 1 shows the auxin distribution in gammas hetero-auxin equivalents per liter. Note the high auxin concentration in the basal part of the primary leaves of Avena and maize and also the relatively high concentration in the basal parts of those seedlings. The concentration in the lateral buds of the pea seedling is higher than that in the adjacent stem tissue. A detailed paper discussing the relation between growth and the auxin distribution in maize and Avena seedlings and also the relation between auxin concentration and bud inhibition in the pea seedlings will be submitted to *The Botanical Gazette*.


**LIGHT INTENSITY AND THE NITROGEN HUNGER PERIOD IN THE MANCHU SOYBEAN**

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Leguminous plants grown on a nitrogen-poor substrate and dependent on the fixation of atmospheric nitrogen for their supply of this element frequently exhibit during their development a "period of nitrogen hunger." This period occurs fairly early in the growth of the plant, when the stores of nitrogen in the seed have been exhausted and before the centers of fixation, the nodules, have developed sufficiently to meet the ever-increasing demands of the plant for nitrogen. As would be expected the phenomenon usually occurs under environmental conditions which favor photosynthesis,
and the usual evidence of carbohydrate excess are apparent. The plants are stalky with yellow leaves and woody tissues. Ordinarily the period lasts for less than a week, after which the nitrogen fixation process is initiated at a rate that adequately supplies the requirements of the plant for nitrogen. The leaves turn green, and the tissues become more succulent. Thereafter, the rate of carbohydrate synthesis rather than that of nitrogen fixation may become the limiting factor in the development of the plant.

In the summer of 1932 studies were begun at this station for the purpose of comparing the nitrogen metabolism of soybeans which were fixing nitrogen with that of plants which were supplied combined nitrogen. The first experiment was started out-of-doors early in June, just as the prolonged drought of that summer began. The weather during the period immediately following the planting of the soybeans was characterized by sunlight of high intensity and by hot, dry winds. The response of the nodulated soybeans to these rather extreme conditions was most unexpected—they entered the nitrogen hunger period and remained there. Plants supplied NH₄NO₃ developed normally. This difference in the response of the soybean plants to the environmental conditions suggested that the effect was concerned specifically with the nitrogen fixation process. Since the roots of the plants suffering from nitrogen hunger possessed numerous and well-developed nodules, it was concluded that the effect was primarily on the actual fixation of nitrogen. It occurred to us that perhaps the carbohydrate-nitrogen balance in the plant had become so excessive that assimilation of free nitrogen was inhibited. To test this hypothesis, part of the nodulated plants which were in the nitrogen hunger stage were shaded for one week in order to decrease carbohydrate formation and to increase the soluble forms of nitrogen in the plant. The response was clear-cut; in a few days nitrogen fixation had begun in the shaded plants, and at the end of a week, the leaves of these plants had become dark green. Analyses for nitrogen confirmed the observation that the shaded plants were markedly superior to the unshaded controls.

As has been indicated in our previous reports,¹²³ these results have important implications for several aspects of the mechanism of symbiotic nitrogen fixation. Among these may be mentioned: (a) the influence of the carbohydrate-nitrogen balance in the host plant on the process, and (b) the relative efficiency of free and combined nitrogen in the nutrition of the soybean. Because of these implications confirmation and extension of the observations are desirable. It should be noted that repetition of this type of work is not entirely in the hands of the experimenter as the requisite first stage, the inhibition of the fixation process, cannot be readily controlled. The weather must be consistently "bright and hot" during the first few weeks after planting in order that the nitrogen hunger stage will be prolonged to a point where inhibition of fixation will obtain. If at the
FIGURE 1
(See facing page)
critical stage in the growth of the plant, namely, when the nitrogen in the
seed has been utilized, there are a few cool, cloudy days, nitrogen fixation
will be initiated, and the further development of the plants is normal. At-
ttempts to duplicate the phenomenon under the more easily controlled en-
vironment of the greenhouse with artificial illumination have met with little
success, probably because of the low intensity of light available.

To increase the probability of securing plants in which the process of
nitrogen fixation has been inhibited, experiments were made during the
past two summers in which several jars of soybeans were planted every
two weeks during June, July and early August. In 1936 inhibition was
observed on two separate occasions, and this inhibition was overcome not
only by shading as in our earlier experiments,\(^1\) but also by addition of com-
bined nitrogen. Support was thus obtained for the hypothesis that the
inhibition was connected with an excessive carbohydrate-nitrogen relation
in the plant. Unfortunately, no analyses were made of these plants, but
the experiments were duplicated in 1937 as described in this paper.

Methods.—The methods used were those described previously.\(^1\) Briefly,
they consist of growing Manchurian soybeans inoculated with an efficient
strain of *Rhizobium japonicum* in two-gallon jars on a nitrogen-poor pit
sand to which has been added an adequate supply of all plant nutrients
except nitrogen. The plants are kept out-of-doors, protected from rain
whenever necessary, and are watered daily with nitrogen-free tap water.

*Experiment I.*—On July 1, 1937, eight jars were planted and inoculated.
The first signs of nitrogen hunger were noted about July 15; on July 28 the
plants were still in the nitrogen hunger stage and were exhibiting pronounced
signs of carbohydrate excess. On this date treatment was begun as fol-
 lows: two jars were retained as controls; two jars were removed to a
shaded cold-frame in which the light intensity was about one-fifth that of
the cold-frame in the open; combined nitrogen was added to the remaining
six jars. The plants which were shaded or to which combined nitrogen
was added soon responded to these treatments. At the end of a week, their

**Explanation of Figure**

The effect of shading and of combined nitrogen on nodulated soybeans in which
an excessive carbohydrate-nitrogen balance had inhibited the nitrogen fixation
process.

A—Experiment I.  

Control: no treatment; to others combined nitrogen added,
as Ca(NO\(_3\))\(_2\), 33 days before harvest.

B—Experiment I.  

Control: no treatment

1: in shade continuously for 33 days before harvest.

2: in shade for 12 days, returned to sun for 21 days.

3: in shade continuously for 15 days before harvest.

C—Experiment II.  

Control: no treatment

Shade: in shade continuously for 19 days before harvest.

50 mg. N: added as Ca(NO\(_3\))\(_2\), 19 days before harvest.
color had changed from yellow to green, and they had increased in size. After 12 days one of the jars that had been kept in the shade was returned to the sun, inasmuch as the plants were showing the ill effects caused by low light intensity. The two controls in the sun remained yellow and showed no increase in size; on August 15 one of these controls was transferred to the shade. The response of the plants of this control was much slower than that of the first plants transferred, but eventually they also began to turn green and were definitely superior in general appearance to those of the remaining control at harvest on August 30.

Experiment II.—Six jars were planted and inoculated on July 10, 1937. Nitrogen hunger was first apparent at the end of July and continued until August 11, at which time treatments were started. Two jars were removed to the shade; 50 mgm. nitrogen as Ca(NO₃)₂ was added to two other jars; and the two remaining jars were retained as controls. These plants were harvested with those of Experiment I on August 30. Since duplicates of the same treatments were identical so far as general appearance was concerned, the plants of only one jar of each treatment were analyzed and the remaining jar after photographing, was used in other work.

Discussion.—Analytical data from the two experiments are summarized in table 1, and the appearance of the plants at harvest is shown in figure 1. The data of table 1 confirm and extend the observations previously reported. They demonstrate that it is unnecessary to remove the plants

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
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<tbody>
<tr>
<td><strong>Effect of Shading and of Adding Combined Nitrogen on Nitrogen Fixation by Nodulated Soybeans Suffering from a Prolonged Nitrogen Hunger Period</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>NUMBER OF PLANTS</th>
<th>DRY WEIGHT GM.</th>
<th>PER CENT</th>
<th>TOTAL MGM.</th>
<th>FIXED NITROGEN PER PLANT MGM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodulated control kept in sun</td>
<td>5</td>
<td>4.2</td>
<td>1.14</td>
<td>47.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Same + 5 mgm. N as Ca(NO₃)₂</td>
<td>6</td>
<td>20.7</td>
<td>1.26</td>
<td>260.5</td>
<td>35.6</td>
</tr>
<tr>
<td>Same + 10 mgm. N as Ca(NO₃)₂</td>
<td>7</td>
<td>28.5</td>
<td>1.28</td>
<td>364.8</td>
<td>43.7</td>
</tr>
<tr>
<td>Same + 25 mgm. N as Ca(NO₃)₂</td>
<td>6</td>
<td>30.8</td>
<td>1.45</td>
<td>446.9</td>
<td>63.3</td>
</tr>
<tr>
<td>Same + 50 mgm. N as Ca(NO₃)₂</td>
<td>8</td>
<td>37.7</td>
<td>1.72</td>
<td>648.1</td>
<td>67.7</td>
</tr>
<tr>
<td>In shade continuously for 33 days</td>
<td>6</td>
<td>8.9</td>
<td>2.20</td>
<td>195.8</td>
<td>25.6</td>
</tr>
<tr>
<td>In shade for 12 days, returned to sun</td>
<td>6</td>
<td>18.7</td>
<td>1.98</td>
<td>370.0</td>
<td>54.7</td>
</tr>
<tr>
<td>In shade for 15 days before harvest</td>
<td>6</td>
<td>6.4</td>
<td>1.08</td>
<td>69.2</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Experiment II

| Nodulated control kept in sun | 7 | 9.0 | 1.00 | 90.0 | 5.9 |
| Same + 50 mgm. N as Ca(NO₃)₂ | 7 | 20.0 | 1.60 | 320.0 | 31.6 |
| In shade continuously for 19 days | 8 | 18.0 | 1.21 | 217.8 | 20.2 |
from the high light intensity in order to initiate the nitrogen fixation process. Thus they lend support to the hypothesis that an excessive carbohydrate balance in the plant is the inhibitory factor rather than light itself. Furthermore, the breaking of the nitrogen hunger period by use of combined nitrogen indicates that the effect did not arise as a result of lowering the temperature concurrently with the reduction in light intensity. However, the excessive carbohydrate condition which apparently inhibits the nitrogen fixation process may arise in part from high temperatures, as well as from high intensity of light, since it is usually encountered only after periods of hot dry weather. Examination of the nodules of the plants kept in the sun confirmed the observation previously made,\(^1\) namely, that the nodules were well developed and in no way resembled these on plants inoculated with a poor strain of bacteria.

Another point of interest is the difference between the controls (nodulated plants kept in the light with no addition of combined nitrogen) in the two experiments. At the harvest the control plants of Experiment II, although 10 days younger than those of Experiment I, were definitely larger and, in general appearance, were superior. Although the plants of the control jars of Experiment II were still quite yellow in color when harvested, there were some indications that the nitrogen hunger period was being broken by the cloudy weather just preceding the harvest. In contrast the control plants of Experiment I showed no signs of coming out of the nitrogen hunger stage even though they had been exposed to the same weather conditions. The quantitative difference in the behavior of the controls in the two experiments suggests that if the carbohydrate excess is not corrected early, it becomes increasingly difficult to overcome the inhibition. This view received confirmation by the response of those control plants in Experiment I which were transferred to the shade 15 days prior to the harvest. Although at harvest there were definite signs that nitrogen fixation had started in these plants, the inhibition was overcome much more slowly than in the plants which were moved to the shade soon after the nitrogen hunger period had begun.

As previously noted,\(^1,3\) the percentage nitrogen in the plants is only a crude measure of the effective carbohydrate-nitrogen relation in these experiments. Both the plants which were shaded for 15 days in Experiment I and the control plants of Experiment II had a lower percentage nitrogen than did the control plants of Experiment I, in spite of the fact that the development of the latter plants was markedly inferior. The other treatments used, however, caused a definite reduction in the carbohydrate-nitrogen relation, as measured by an increase in the percentage nitrogen, coincident with overcoming the inhibition of the nitrogen fixation process.

Shading is extremely effective in breaking the nitrogen hunger period induced by excessive carbohydrates, but if the plants are kept in the shade,
they eventually become fragile and twiny, the usual symptoms in the soybean of inadequate illumination. Once the inhibition is overcome, reduced carbohydrate synthesis causes decreased fixation of nitrogen. If plants are returned to full sunlight after initiation of nitrogen fixation through shading development is normal, and excellent growth and fixation are obtained.

Finally, the quantitative aspects of the combined nitrogen treatments should be emphasized. The greatest fixation of elemental nitrogen was obtained with those plants supplied the largest quantities of combined nitrogen, a rather unusual finding, since combined nitrogen is commonly supposed to inhibit the fixation process.

In our other communications we have emphasized primarily the significance of these findings for theoretical phases of symbiotic nitrogen fixation, but their practical application to problems of agriculture should not be overlooked. Not infrequently experiment stations receive reports of crop failures with soybeans and other leguminous plants which are most puzzling, since apparently all cultural practices known to influence the crop yield, e.g., inoculation of the seed with bacteria of known efficiency, have been properly performed. It is suggested that leguminous plants, when seeded in late spring or early summer, may encounter local environmental conditions which will cause an excessive carbohydrate balance in the host plant and result in inhibition of nitrogen fixation. Our experiments indicate that all the necessary conditions will obtain only on soils of low fertility, especially low with respect to presence of soluble forms of combined nitrogen. Investigation of local weather conditions after seeding of crop may throw light on some of these apparently inexplicable failures.

Summary. When nodulated soybeans are grown under sunlight of high intensity, fixation of atmospheric nitrogen is inhibited, and the nitrogen hunger stage in the plants is unduly prolonged. The inhibition appears to be associated with an excessive carbohydrate-nitrogen balance in the plant, probably with the relation of soluble forms of carbohydrate and nitrogen. Reduction of this excessive carbohydrate-nitrogen relationship either by shading (decrease in photosynthesis and hydrolysis of protein) or by addition of soluble forms of combined nitrogen is accompanied by initiation of the nitrogen fixation process, followed by a normal development of the plant.

* Herman Frasch Foundation in Agricultural Chemistry, Paper No. 146.

