TRANSLOCATION OF LABELLED ASSIMILATES IN THE SOYBEAN

III. TRANSLOCATION AND OTHER FACTORS AFFECTING LEAF GROWTH

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Summary

When a young, expanding leaf of soybean is darkened, translocation into it of labelled assimilate from the leaf below is depressed. The darkened leaf does not grow to full size and abscisses prematurely. The same effect is shown if the young leaf is developing in light but deprived of carbon dioxide. The growth rate of the next younger leaf developing at the apex is markedly increased in both cases.

The darkened leaf was given various treatments, and effects on longevity, final size, and growth rate were investigated.

Longevity could be increased when the following treatments were applied:

(1) the darkened leaf was fed with sucrose;

(2) the apex of the plant was cut off;

(3) the darkened leaf was sprayed with kinetin;

(4) the plant was decapitated and the roots cut off;

(5) the leaf was fed with sucrose and 3-indolylacetic acid;

(6) the plant was decapitated and the lower stem ringed.

Final size was increased by only two treatments, viz. decapitation and decapitation combined with ringing, the latter being the more effective. Growth rate of the darkened leaf was not increased by any treatment. Treatment with sucrose solution produced marked injection of the leaves. This is considered to be one reason for the poor growth shown by all leaves fed with sucrose. In comparison with leaves fed sucrose alone, those fed with sucrose and 3-indolylacetic acid showed significantly increased growth rate and final size.

I. INTRODUCTION

In the second paper in this series (Thrower 1962) it was shown that the expanding leaf of soybean imported assimilate from mature leaves below it on the stem. The importation of material followed a definite pattern, the amount rising to a maximum and falling to almost zero when the leaf had reached 50% of the adult area.

Having established this pattern, investigations were directed to discovering what treatments, if any, were able to change it. The present paper deals with the results of this study.

II. MATERIALS

Soybeans, *Glycine max* (L.) Merr., were grown in the greenhouse under conditions which have been described previously (Thrower 1962). The nutrient solution supplied the plants with nitrogen both as nitrate and ammonium ion. The plants used were between 60 and 100 days old and had from five to eight expanded trifoliate

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leaves, except where specifically stated otherwise. In all cases the treated leaf was the young expanding leaf at the apex.

III. Experimental and Results

The experimental work falls into three sections. In the first section [III(*a*)] young leaves were deprived of either light or carbon dioxide, and import of labelled assimilate from a source leaf below was measured at intervals. This experimental method suffered from the disadvantage that each time a measurement was made a plant had to be destroyed. A second method [used in Sections III(*b*) and III(*c*)] permitted continuous measurements to be made on the living plant. Estimates of leaf growth were based on daily measurements of length, and measurements of pre-abscission time were used as a measure of the amount and duration of import. This was based on the assumption that a young leaf in darkness depends for growth and survival on imported assimilate, mainly sucrose. As a test of this assumption a study was made of the effects of supplying the darkened young leaf with exogenous sucrose, with or without 3-indolylacetic acid (IAA) [cf. Section III(*b*)] and with kinetin, IAA, or gibberellic acid [cf. Section III(*c*)].

(*a*) *Import into Young Leaves Deprived of Light or Carbon Dioxide*

In this experiment 43 plants were used. Of these, 30 were treated by exposing the expanding leaf to dark conditions. This was achieved by inserting the young leaf (of 4–5 cm in length) into an erlenmeyer flask covered with opaque, black polythene sheeting (heavy grade, 0·008 in. thick, used double). The mouth of the flask was lightly plugged with cotton-wool. The flask was clamped in a tilted position so that any moisture condensing on the inner walls of the flask could escape.

The remaining 13 plants were treated by confining the expanding leaves in clear Perspex boxes through which was pumped a stream of humid, CO_2-free air. CO_2 was absorbed by bubbling through 20% KOH, after which the air was washed in water and drawn through the leaf boxes. Between each plant in the series, the air stream passed through a column of Carbasorb self-indicating granules which absorbed any respiratory CO_2 carried over from the previous leaf and gave an indication of the degree to which the system was free of CO_2.

The term "CO_2-free air" is not intended to indicate an absolute absence of CO_2. Numerous workers (e.g. Weigl, Warrington, and Calvin 1951; Krotkov, Runeckles, and Thimann 1958; Canny 1960, 1962; and Nishida 1962) have shown the possibility of photosynthetic reabsorption of respiratory CO_2 in a closed system; consequently it may be assumed that the leaves in the boxes reassimilated part of their respired CO_2, the rest of the gas being drawn out of the box with the air stream and absorbed in the Carbasorb column. The results showed that the method severely limited photosynthesis.

Thirteen plants in the first group and four in the second were grown until the leaves yellowed and abscissed. The darkened leaf on each plant in the first group and the young leaf above in both groups were measured daily, each measurement taking less than 20 sec. In the second group the final size at abscission was measured.

A check was made to see if the short daily exposure to light necessary for making measurements had any effect on growth. The expanding leaves of eight young plants were darkened. In four of these the young leaf was exposed to light for 20 sec daily, in the other four the leaf remained in darkness. After 11 days all leaves were measured and increases in area calculated. No significant difference in growth between the two groups was found.

The remainder of the plants (17 darkened; 9 in light and CO_2-free air) were, after various time intervals, allowed to assimilate $^{14}CO_2$ (1–4 plants being treated at each time). $^{14}CO_2$ was supplied to the expanded leaf immediately below the young treated leaf by the spot-feeding technique previously described (Thrower 1962). After a 2-hr period of $^{14}CO_2$ assimilation the plants were dissected, dried, and powdered. The activity of aliquots of the powder was determined by counting, using the methods described previously. The total activity of each plant was calculated together with the percentage of active material imported by the expanding leaf.

TABLE 1

EFFECT OF DARKENING AND OF DEPRIVATION OF CARBON DIOXIDE ON THE GROWTH AND LONGEVITY OF THE EXPANDING LEAF OF SOYBEAN

Treatment of Young Leaf	Days to Abscission (mean ± S.E.)	Size Attained* (mean ± S.E.)
Illuminated	Range 50–100	100†
Darkened	16 ± 0·2	48 ± 2‡
Deprived of carbon dioxide in the light	16 ± 0·6	55 ± 7‡

* Expressed as a percentage of the fully expanded leaf area, which is calculated in each case from measurements of the expanded leaf below the treated leaf.

† i.e. the leaf expands fully in light.

‡ Difference between means not significant.

Experimental results are shown in Table 1 and Figure 1. The values quoted for illuminated leaves in Table 1 were measurements made on 12 plants growing normally in the greenhouse and undergoing no treatment. The graph in Figure 1 showing import by the expanding leaf in the light is from a previous experiment (Thrower 1962) and is included for purposes of comparison. Both darkening and CO_2 deprivation in the light markedly shortened the life of the young leaf and prevented it from reaching full size. There is no significant difference in the extent to which this occurred in either darkened or CO_2-deprived leaves (see Table 1).

Figure 1 shows that the young leaf, either darkened or deprived of CO_2, continued to import labelled assimilate from the leaf below whilst it remained alive, irrespective of the size it had reached. This is in contrast to the behaviour of the young leaf in the light which, as Figure 1 shows, ceased to import at about 50% full size. It is also shown in this figure that under conditions of darkness or CO_2

deprivation the young leaf up to about 35% adult size imported considerably less assimilate than did the leaf in the light.

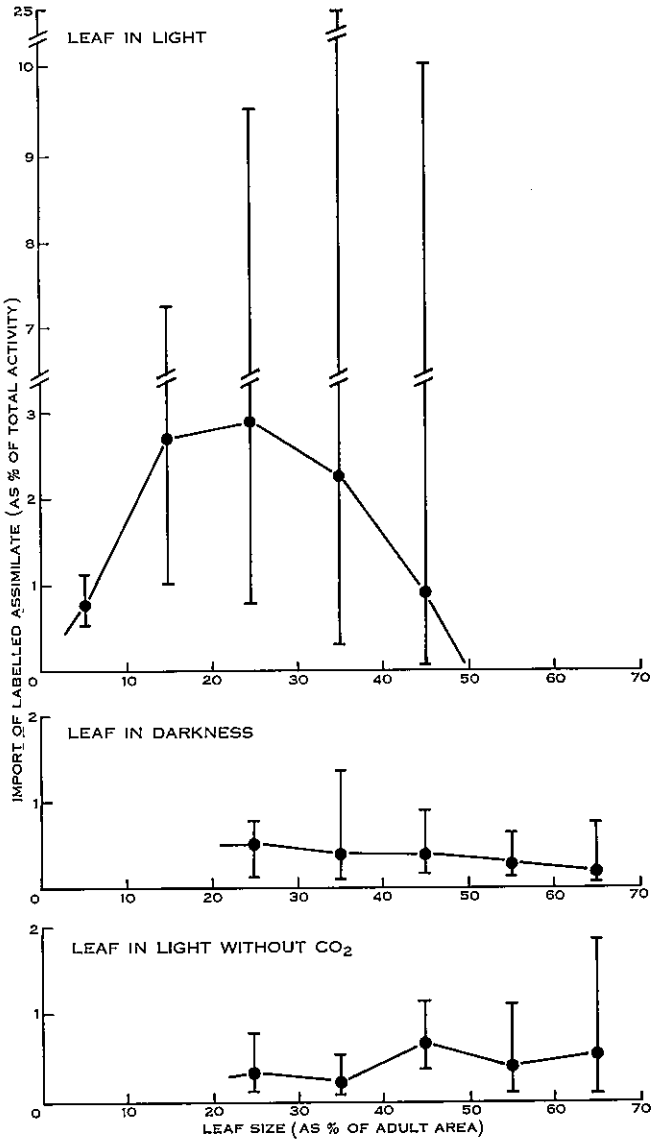

Fig. 1.—Import of labelled assimilate by the expanding soybean leaf under conditions of light, of darkness, and of CO_2 deprivation. 95% confidence limits are shown. The upper curve ("leaf in light") is from a previous experiment (Thrower 1962).

Whilst the continued growth and survival of the young leaf in the dark is presumably dependent on import of material, there is the possibility of utilization of starch stored in the leaf. A series of young leaves of 4–5 cm length was therefore

tested with iodine solution after 0, 1, 2, and 3 days in darkness. Leaves harvested at 3.30 p.m. after a bright day showed the presence of abundant starch. After 24 hr of darkness this was markedly diminished, after 48 hr starch was scarcely perceptible, and after 72 hr had completely disappeared. Replication showed this result to be typical of such leaves.

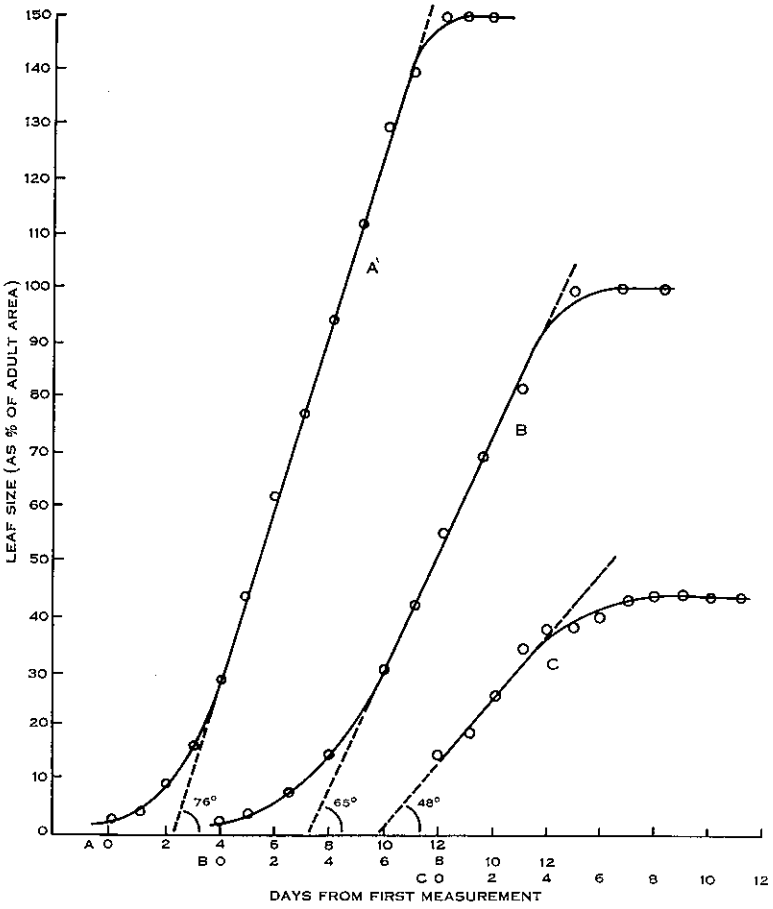

Fig. 2.—Typical growth curves for the young expanding soybean leaf under three different conditions: *A*, young leaf developing in the light whilst the leaf below it on the stem is in darkness; *B*, young leaf developing in light; *C*, young leaf developing in darkness. The angle measuring growth rate is shown for each curve.

Clor, Crafts, and Yamaguchi (1963) have demonstrated that when a whole plant is under conditions of high humidity, translocation from leaves is markedly increased. Placing the expanding soybean leaf under conditions where it was possible for high humidity to develop introduced a further factor having possible effects on translocation from this leaf. The remainder of the leaves on these plants were under normal greenhouse conditions and the fact that the darkened leaf grew showed that

import of assimilate occurred rather than export. However, growth was shown to be markedly reduced and the possibility exists that humid conditions may have played some part in this reduction of growth.

(b) Growth and Longevity of Young Leaves under Various Conditions

The midrib length of the leaves used in these experiments averaged 4 (range 3·6–5·0) cm at the time of darkening. Such leaves are known to import assimilate in both light and darkness. Lengths of the midrib were measured daily and the number of days from darkening to abscission was noted, together with any morphological changes induced by the treatment. The size reached was calculated from midrib length (Thrower 1962) and expressed as a percentage of normal adult area. When plotted against time the percentage areas gave sigmoid curves (Fig. 2). The section of the curve representing the "grand" period of growth was approximately linear. The angle made by the tangent with the horizontal axis was used as a measure of maximum growth rate. Such rates were also measured for the younger leaves developing next above the treated leaves.

Figure 2 illustrates the differences in growth curves for expanding young leaves under three different conditions. *B* is the control, the leaf developing normally in the light with no treatment. *A* and *C* are from two consecutive leaves on one plant: *C* is the lower of the two and has developed in darkness; *A*, the upper leaf, shows an enhanced growth rate correlated with this. The curves in Figure 2 have been constructed from measurements on individual leaves. When the mean growth rates for leaves in each group are calculated they are found to be significantly different at the 1% level. In both this experiment and that reported in the following section the growth rate of the young leaf developing above a darkened leaf (when present) was consistently enhanced.

A total of 29 plants was used (four in each treatment and 13 in control); all were initially treated by darkening the expanding leaf [as in Section III(*a*)]. Subsequent treatments were as follows:

(1) The attached expanding leaf was floated on a 5% sucrose solution which was renewed twice weekly.

(2) The stem apex was decapitated above the node of the darkened leaf.

(3) As in (2) but in addition the plants were removed from the pots and the roots gently washed free of vermiculite and placed in darkened flasks of a nutrient solution which was renewed daily. The lateral roots were clipped off over a 3-day period to minimize shock to the plant, but the stump of the tap root remained.

(4) As in (2) but in addition the stem was ringed below the lowest leaves and the ring covered with grafting wax. The amount of secondary growth in the lower stem made ringing relatively easy. A new ring was cut every week as vascular bridges grew out very rapidly. Adventitious roots were clipped off as they appeared.

(5) Plants intact, no treatment of the expanding leaf other than darkening (control plants).

The effects of these treatments on the longevity of the darkened leaf are shown in Figure 3(*a*). All treatments have significantly delayed abscission and extended the life of the leaf, the most effective being treatment (4). The leaves in this group had not abscissed when harvested at 40 days. The maximum sizes reached by the darkened leaf as a consequence of these treatments are shown in Figure 3(*b*). Two groups, corresponding to treatments (2) and (4), showed significantly greater growth than the controls, one leaf in the latter group reaching 100% mature size. The two groups corresponding to treatments (1) and (3) showed less growth than the controls. The leaves in the treatment (1) groups became turgid after some days floating on the sucrose solution and there was considerable injection of intercellular spaces.

Figure 3(*c*) shows growth rates of the darkened young leaves receiving the four separate treatments. In comparison with the control only treatment (1) has significantly altered the growth rate and in this case it has been reduced.

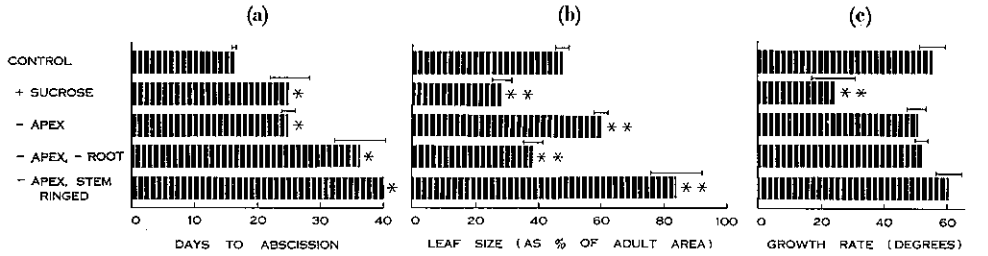

Fig. 3.—Mean values for number of days to abscission (*a*), leaf size (*b*), and growth rate (*c*) of darkened young soybean leaves receiving different treatments. Standard errors are shown. *, ** indicates significant difference from control at 1% and 2% levels, respectively.

In a further experiment an attempt was made to overcome the disdavantage due to injection of leaves floated on sucrose. These leaves were not floated on sucrose solution but the leaf tips were cut off and the cut end dipped into sucrose solution. Further, as it has been shown by Said and Naguib (1952) that IAA stimulates uptake of sucrose by carrot disks and it has also been shown [Section III (*c*)] that IAA alone causes growth of darkened soybean leaf vascular tissue, both sucrose and sucrose plus IAA solutions were used.

The terminal leaflet on each of eight plants was used and 0·5 cm of the tip was cut with a razor-blade under water. The leaflet was then quickly transferred to a tube containing sucrose solution and arranged so that the cut end dipped into the solution. Sulphanilimide (0·25%) was added to the solution to inhibit the growth of bacteria. IAA solution (concn. 1 mg/l, 0·3 ml) was added to give a final concentration of 0·1 mg/l. This was renewed daily. Darkening and daily measurement of these leaves was carried out as for the previous experiment.

As the tip of the treated leaflet was cut off, calculation of the area of these leaflets was no longer possible from a simple measurement of the midrib length. Two groups of four control plants were therefore included to give information which would enable this calculation to be made. In one group of plants the tip of the terminal

leaflet was cut off to find whether this had any effect on the growth of the remainder of the leaflet. In the second group the distal 0·5 cm was marked with a droplet of ink and measurements made of the growth of the tip and the rest of the leaf. It was found that cutting off the tip did not affect the growth of the remainder of the leaflet and the ratio of final length to initial length was the same for both tip and remainder of the leaf. These ratios were calculated for darkened leaves and used in calculations of leaf areas.

Results for the sucrose and IAA treatments are shown in Figure 4. As this experiment was performed in winter (June) these results are not strictly comparable with those of the previous experiment, which was performed in summer (January–February). Despite the fact that only the distal part of these leaves was dipping into the solution the leaves were observed to suffer again from considerable injection.

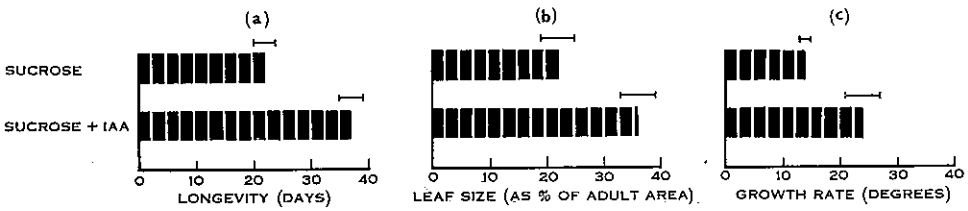

Fig. 4.—Comparison of longevity (*a*), maximum size (*b*), and growth rate (*c*) for darkened young leaves receiving sucrose with and without added IAA. In each case the difference between the two values is significant at the 5% level. Standard errors are shown for each mean.

From Figure 4 it can be seen that growth rate, longevity, and size are all low for the treated leaves but that increased growth and longevity have followed the addition of IAA. The leaves showed no "frilling" with IAA treatment, but the midribs were noted as being slightly enlarged.

(*c*) *Effects of IAA, Kinetin, and Gibberellic Acid on the Longevity, Leaf Size, and Growth Rate of the Darkened Young Leaf*

Darkened expanding leaves (one per plant) were sprayed daily with the solutions shown in the following tabulation:

Treatment No.	Substance Applied	Concentration (mg/l)	No. of Plants
1 (control)	None	—	13
2	Gibberellic acid	100	4
3	Kinetin	10	5
4	Kinetin+IAA	10+1000	5
5	IAA	1000	4
6	IAA	0·01, 0·1, 1·0, 10, and 100	20 (4 each concn.)

The concentrations were chosen as likely to be effective after examination of the results of Addicott and Lynch (1951), Marth, Audia, and Mitchell (1956), and Osborn and McCalla (1961). The leaves were measured throughout the experiment as described previously.

The effects of the application of gibberellic acid, kinetin, and IAA (at a concn. of 1000 mg/l), either singly or in combination, on the longevity of the darkened leaf are summarized in Figure 5(*a*). The application of kinetin significantly lengthened the life of the leaf whereas the application of IAA or gibberellic acid decreased it. It is notable that the application of kinetin together with IAA counteracted the adverse effect of IAA alone on the leaf, extending its life to equal the control. When concentrations of IAA lower than 1000 mg/l were used the length of life was variable and no significant differences from the control were found. The leaves in the first treatment group showed gross distortion and curling of the leaves. This effect diminished with decreased concentrations of IAA and no distortion was shown at concentrations below 1 mg/l.

It was noted that the leaves sprayed with kinetin remained green markedly longer than did control leaves or leaves receiving other treatment. Petioles of leaves receiving gibberellic acid showed considerable elongation.

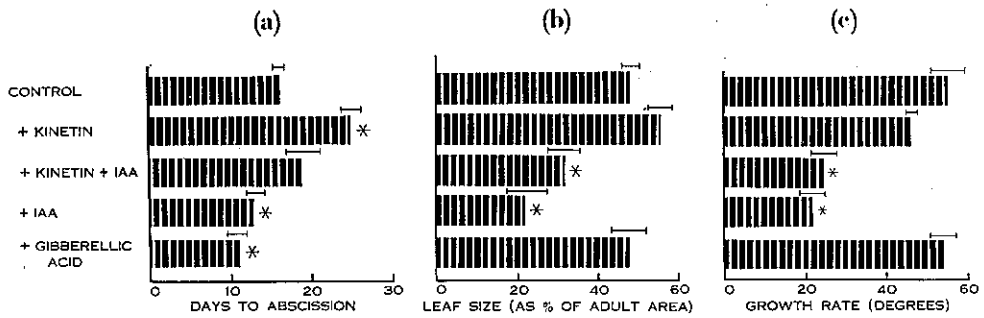

Fig. 5.—Mean values for number of days to abscission (*a*), maximum leaf size (*b*), and growth rate (*c*) of darkened expanding soybean leaves receiving different treatments. * indicates significant difference from control at 1% level.

The effects of the above applications of gibberellic acid, kinetin, and IAA on leaf size were determined. Neither kinetin nor gibberellic acid had any significant effect on the size reached by the darkened expanding leaf [Fig. 5(*b*)], but IAA (at a concn. of 1000 mg/l) significantly decreased the final size reached. Figure 6(*a*) shows that final leaf size decreased as the concentration of IAA increased.

Darkening a young leaf always depressed the growth rate but, as before, this was accompanied by more rapid growth of the leaf above. This increased growth rate was constant throughout and did not vary with differing treatments received by the darkened leaf. Figure 5(*c*) shows the growth rates of the darkened leaf under the five treatments. The results here run parallel to those illustrated in Figure 5(*b*)—treatment with kinetin and with gibberellic acid produced no effect, treatment with IAA at 1000 mg/l produced a significant reduction of growth rate. Kinetin in conjunction with IAA had no "protective" effect on growth rate comparable to its effect on longevity.

Figure 6(*b*) shows the effect of a range of IAA concentrations on the growth rates of darkened young leaves. Whereas for the control darkened leaf the growth curve was approximately linear over the first 7–9 days, in the IAA-treated plants the

curve flattened out after 2 days. In Figure 6(*b*), the upper curve shows the growth rate represented by the angle made by the tangent to the curve and the x-axis for the first 2 days of growth, the lower curve shows the growth rate as represented by the angle made by the tangent to the more flattened curve obtaining after the first 2 days. It has been shown previously that stored starch is present in the leaf for the first 2–3 days of darkness. The depression of the growth rate by the IAA-treatment only becomes apparent when the stored metabolite has been exhausted. The growth rate after 2 days is slower at higher concentrations of IAA. A similar trend was noted with respect to distortion of the leaves which was increasingly severe as the concentration was increased, and was not evident below 1 mg/l. Swelling of the pulvinus and petiole was observed at 0·1 mg/l and higher concentrations but not at 0·01 mg/l.

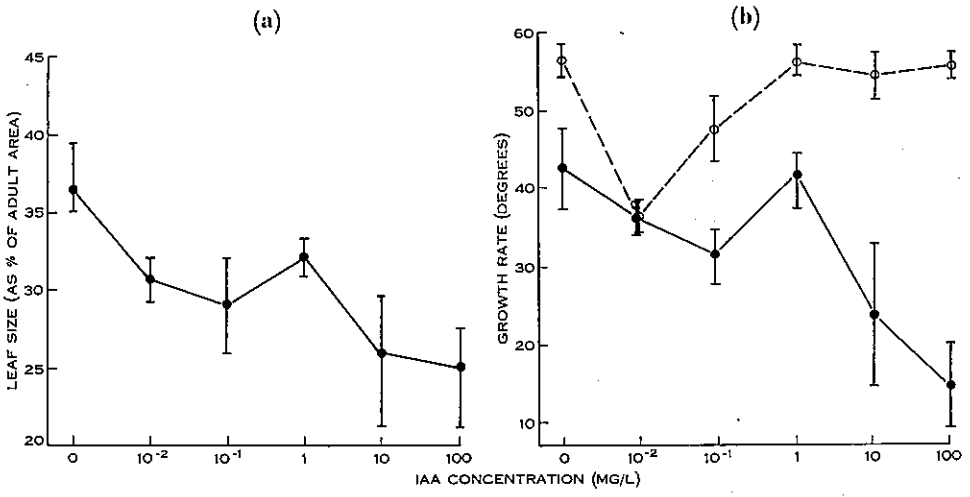

Fig. 6.—Effect of different concentrations of IAA on (*a*) the amount of growth and (*b*) the initial growth rate, i.e. over first 2 days (○ – – – ○) and growth rate subsequent to the first 2 days (● ——— ●) of darkened young soybean leaves. Standard errors for each mean value are indicated.

(*d*) *Movement of* ^{14}C *in Kinetin-treated Leaves*

A different type of experiment was devised following Mothes and Englebrecht's (1961) demonstration that labelled amino acids will move towards a kinetin-treated area. Four expanded soybean leaves were used, and in three of these one lateral leaflet and the distal half of the terminal leaflet were sprayed daily with kinetin solution (10 mg/l). After 4 days all the leaves were darkened. Each leaf then received 5 μc of $^{14}CO_2$ by spot-feeding over a 1 cm^2 area on the undersurface of the proximal part of the terminal leaflet. The upper surface over the application area was illuminated. The leaves were harvested after 2 hr, dissected, dried, and radioautographed using the method described previously (Thaine, Ovenden, and Turner 1959).

Radioautographs of the dissected leaves showed labelled material in the kinetin-sprayed leaf tip and both laterals of all three treated leaves. The amount in the kinetin-treated lateral leaf was slightly the greater. The control leaf showed no activity in either lateral leaflet and very slight activity in the terminal leaflet tip.

It therefore appears that the application of kinetin to a growing darkened leaf caused translocation of assimilate into the treated part. This effect extended to the opposite leaflet to that sprayed, which also imported more than the normal leaf—but it is not sure that this was not due to some spray having been carried over from the treated leaflet. Accumulation of ^{14}C-labelled assimilate mainly as starch around droplets of kinetin on clover leaflets (*Trifolium subterraneum*) has been demonstrated by L. B. Thrower (1963).

IV. Discussion

The initial aim of this study was to discover how the translocation pattern of the expanding leaf was altered by various treatments. Direct measurements of translocation with labelled carbon showed that when the young expanding leaf was deprived of either light or of carbon dioxide, there was a reduced import of assimilate. This was accompanied by a reduction in leaf growth rate, in final leaf size, and in longevity. Thus when the leaf is unable to carry out photosynthesis its growth is seriously impaired; it cannot, in the otherwise normal plant, make up for the deficiency by increasing its imports from other parts of the plant; on the contrary the rate of import is reduced although its duration is prolonged.

The longevity of the darkened leaf was increased by the following treatments:

(1) the darkened leaf was fed with sucrose;

(2) the apex of the plant was cut off;

(3) the darkened leaf was sprayed with kinetin;

(4) the plant was decapitated and the roots cut off;

(5) the leaf was fed with sucrose and IAA;

(6) the plant was decapitated and the lower stem ringed.

The effects caused by these treatments [except treatment (3)] may be interpreted most simply as being due to an increased supply of carbohydrates, resulting in the maintenance of normal respiration and delayed senescence.

The role played by auxin in retarding abscission has been studied extensively in *Coleus* (Jacobs 1962) and in *Phaseolus* explants (Addicott and Lynch 1955; Biggs and Leopold 1957; Rubenstein and Leopold 1963) but not to date in intact soybean plants.

Auxin in low concentration had no effect on the longevity of darkened leaves, in high concentration it accelerated abscission. When applied in low concentration with sucrose solution, the longevity of the leaf was markedly increased. If auxin had retarded abscission, the darkened leaf would be connected to the source of supply for longer and could import more. Alternatively, there is some evidence (Said and Naguib 1952) that auxin may increase uptake of sucrose from solution by plant cells. In this case the increased sucrose supply would prolong the life of the leaf, and incidentally, delay abscission. It is not possible to tell from the results how important is auxin's role in the abscission process under these circumstances.

Removal of the stem and root apices and stem ringing will decrease competition for assimilates by these active sinks, allowing more assimilate to move to the darkened leaf.

Kinetin appears to increase the sink capacity of the darkened leaf itself; it increased import but not sufficiently to allow increased growth. The mechanism of this process remains obscure.

The effects of the various treatments of the leaf on growth rate and final size are more complex and we have not sufficient data to attempt a thorough analysis. The present results, however, do add something to our knowledge of leaf growth physiology, which is still in a disjointed and fragmentary state (Jacobs 1962; Humphries and Wheeler 1963).

As a background to further discussion we make the following assumptions:

(1) that leaf imports and exports via the phloem certainly include sucrose and may include organic nitrogenous compounds and hormones.

(2) that if growth hormones affect translocation (Curtis and Clarke 1950; Booth *et al.* 1962), they do so indirectly by altering "sink power" — presumably by modifying growth rate and so changing the nutrient concentrations.

(3) that IAA may control leaf cell division and expansion although when applied externally it mainly affects elongation of vascular tissue (see above, and also Went 1938; Thimann 1957; Loomis 1958; Wheeler 1958, 1960). In very young leaves the auxin content is at first low; it rises early in expansion to a maximum and falls with age (Avery 1935; Shoji, Addicott, and Swets 1951; Loomis 1958).

The importance of competition between the active "sinks" for nutrients in the plant is indicated by the observation that the leaf immediately above a darkened leaf grows faster. Such leaves presumably contain sufficient growth substances and obtain a better nutrient supply when the darkened leaves import less.

When most of the competing sinks are removed (decapitation plus stem ringing) there is no effect on the growth rate of the darkened leaf, but it lives longer and grows to a greater size — in one case such a leaf reached the normal full size. The increased gradient of nutrients apparently permitted enough import to extend the duration of growth but not to increase its rate.

When darkened or deprived of carbon dioxide the young leaf might be expected to become a stronger sink for sugar (and other nutrients) but on the contrary its "sink power" is reduced, for import is decreased. Several questions arise: is the reduced import due to reduced growth and, if so, is the reduced growth due to a lack of photosynthate or to a reduction in the amount of growth substance? We suggest that both nutrients and auxins may be concerned in this most complex system. Suppose that, as the young leaf unfolds, photosynthesis increases the nutrient concentration, and accelerates cell division; the dividing cells produce more auxins which stimulate further growth. If the early photosynthetic rate cannot match this a "sink" will develop and nutrients will be imported. With further growth and development photosynthesis supplies a surplus of sugar and import is replaced by export. At this stage we may suppose that the net production of growth substances

in the leaf falls off, so that leaf growth eventually ceases, even in the light. Possibly this fall is connected with the increasing preponderance of cell expansion over cell division. The abolition of photosynthesis in the cells of a young expanding leaf will deprive the growing cells of a substantial part of their nutrient supply. This could explain the fall in growth rate (in spite of some buffering by starch hydrolysis) but we must also assume a fall in growth substance such that imported material can no longer be used rapidly in growth. Instead it accumulates and so reduces the gradient along the phloem pathway, which in turn will reduce the rate of import.

This hypothesis would be strengthened if we could show that by applying a concentration of growth substances, the darkened leaf would begin growth again and obtain its own sugar supply via the phloem. We have not been able to do this. Application of IAA alone to a darkened leaf caused leaf distortion due to disproportionate growth between veins and mesophyll. Kinetin alone did not increase growth rate or final leaf size, although it did increase import of labelled assimilate. Added sucrose caused inhibition of growth but this was thought to be due to the accompanying injection of the leaf. The inhibition was still present but smaller if the sucrose was given simultaneously with IAA, and under these conditions there was no distortion of the leaf. Schneider (1938), using the much simpler system of *Avena* coleoptiles, was able to demonstrate that whilst either auxin or sucrose alone increased growth, auxin and sucrose together had approximately three times the effect of either alone. From his results he concluded that growth could be limited by deficiency of either auxin or sucrose.

We have assumed that growth substances concerned in leaf growth are synthesized and destroyed within the leaf itself — i.e. that there is no import of leaf growth substances. However, one rather tempting suggestion (so far without foundation) is that during its importing stage the leaf imports growth hormones along with the nutrients; then when import is succeeded by export of assimilate, hormone content diminishes. However, in only one of our experiments was there some evidence which might be interpreted in terms of an exogenous supply of growth substances other than nutrients. Whilst ringing of the lower stem coupled with decapitation increased leaf size, cutting off the roots decreased leaf size (but had no effect on growth rate). Such root removal should (like ringing) increase carbohydrate supply to the darkened leaf. The result obtained could mean that the root normally supplies something via the xylem which cannot be supplied through a cut stem dipping into the nutrient solution. This factor limiting growth could be an elaborated nitrogen compound or a hormone. The idea, which was put forward by Went (1938), that roots produced a substance essential for leaf growth has never been completely explored. Kulaeva (1962) indicates that there is considerable evidence for the existence of such a substance.

V. Acknowledgment

The author is indebted to Professor J. S. Turner for advice and assistance in the preparation of this paper.

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