BRIEFER ARTICLES

ON THE DEMONSTRATION OF THE FORMATION OF STARCH IN LEAVES

For a qualitative demonstration of photosynthesis in starch-forming leaves it is advantageous to know the time in darkness required for the disappearance of accumulated starch, and the time in light required for its subsequent demonstrable formation. No general rule can be given for either, since the time required varies widely for the different species. Therefore, in continuation of the series of studies carried on in the laboratory of plant physiology of Smith College on the physiological constants of the educationally useful plants,[1] I have tried to determine these data for such plants, and also, by comparison, the best plants for the purpose. Throughout this paper I have used the expression "disappearance of starch" rather than "translocation." In a general way the processes, of course, correspond, yet the term "translocation" implies the removal of the starch from the leaf, while here we are dealing only with its disappearance as starch.

The method employed in the present study was as follows: Five actively growing plants of each species, always after a bright day and between 4 and 5 o'clock, were put in a dark room having a steady temperature of 18°–22° C. as recorded by a thermograph. Twice a day, about 9 A. M. and 2 P. M., leaves were tested for starch by SACHS's iodine method, and the time when all starch had disappeared was noted. No attempt was made to find the exact hour when the leaves were empty. This would necessitate testing them every hour during the night as well as during the day, and for the purpose of the present study, the results would be of little value. In the following table, therefore, the time in darkness required to empty the leaves of starch is given in night and day periods rather than in hours. For the iodin test the leaves were first boiled 1 minute to swell the starch, were blanched in warm alcohol, were put in water a few minutes to remove the alcohol and soften the tissues, and were then immersed in a solution of iodin. The solution used was 5^{gm} potassium iodid, 1^{gm} iodin, 10^{cc} water, to which, when dissolved, water was added to make 1 liter of solution.

Thus was the time of disappearance of starch determined with sufficient accuracy for all practical purposes. To determine the time required

[1] BOT. GAZETTE **40**:302. 1905; **45**:50. 1908; **45**:254. 1908; **46**:50. 1908; **46**:221. 1908.

for starch formation, it was found best to use some type of screen such that a sharp contrast would show between the light and dark parts; and for this purpose light-screen boxes were used. These boxes have been fully described by Professor GANONG in the BOTANICAL GAZETTE.[2] Briefly, they are small boxes made of white paper blackened inside, with a network of threads across the top to support the leaf, and holes near the bottom to allow the air to pass through freely. A glass plate covered with tinfoil having a pattern cut in it is held closely against the network by a wire spring. When in use the leaf is held between the glass plate and the network. The principal advantage of these screens is that while excluding all light, they allow nearly the normal access of carbon dioxid to the leaf. The caution, by the way, against using screens which cut off all carbon dioxid as well as the light has been made several times in recent years, but some of the new elementary textbooks are still copying the old and erroneous method of putting cork or tinfoil on both sides of the leaf. Several light-screen boxes were attached to the leaves which had previously been emptied of starch, and the plants were placed in strong diffuse light. Leaves were then taken off and tested for starch at 10-minute intervals. In order to compensate the effects of individual peculiarities, 5 plants of each species were tested. The results given in the table are for full-grown (except in the three cases noted), but not mature, leaves on actively growing plants, in pots, in a greenhouse. In the first column is given the time in darkness required to empty the leaves of starch; in the second, the time in diffuse light required to make enough starch to show a pale but clearly defined figure with the iodin test; in the third, the time required to show a sharply defined, dark figure; while in the fourth is given the time required for the iodin to produce its full effect.

The best leaves, obviously, for this study are those in which photosynthesis is most active, from which starch disappears most rapidly in darkness, from which the chlorophyll can be extracted quickly leaving the leaf white, and which give the iodin reaction quickly. As shown by the accompanying table, these are *Pelargonium hortorum zonale*, *Fuchsia speciosa*, *Senecio mikanioides*, *Impatiens Sultani*, and young plants of *Helianthus annuus*, *Ricinus communis*, *Phaseolus vulgaris*, *Zea Mais*, and *Cucurbita Pepo*.

On the other hand, some leaves are not good for this study. *Begonia palmata*, *Oxalis Bowiei*, and *Pelargonium peltatum*, when boiled to swell the starch, partially disintegrate, so that the figure does not show clearly with the iodin test. It is possible, of course, to apply the iodin test without previous boiling of the leaf, but it takes 24 to 48 hours according to the

[2] BOT. GAZETTE 43:277. 1907.

age of the leaf; besides, these leaves turn brownish yellow on the application of iodin and therefore do not show clearly the reaction with starch. *Ficus elastica* requires more than one day to make a perceptible amount. Only very young leaves of *Primula obconica*, *Primula sinensis*, and *Cineraria cruenta* can be emptied of starch; being stemless plants, they probably use the older leaves for its storage. Young plants of *Pelargonium domesticum* empty their leaves of starch in about 40 hours, but older plants are unsatisfactory, because, even after 4 days in darkness, there are still spots of starch in the mesophyll. All these plants, except *Oxalis Bowiei*, and the young plants of *Pelargonium domesticum*, require 3 to 5 days in darkness —too long a time for the subsequent good of the plant.

Name of plant	Disappearance of starch in darkness (T. 18°–22° C.)		Formation of starch in light (T. 20°–25°C.)		Iodin test
			Perceptible fig.	Good fig.	
	nights	and days	minutes	minutes	minutes
Abutilon	2	1	30	120	5–10
Begonia coccinea	3	3	60	240	10
Brassica oleracea	1	0	20	50	5
Cineraria cruenta (young ls)	5	4	45	180	30
Coleus Blumei	2	1	30	50	3–8
Cucurbita Pepo	1	0	15	50	4–15
Euphorbia pulcherrima	2	1	60	240	20
Fuchsia speciosa	1	0	45	90	15–25
Helianthus annuus	1	0	30	120	5
Heliotropium peruvianum	1	0	45	120	5–10
Impatiens Sultani	1	0	30	120	5
Lupinus albus	1	0	60	240	3
Oxalis Bowiei	1	0	45	240	10–30
Pelargonium domesticum	2	1	50	240	40
Pelargonium peltatum	3	3	50	270	60
Pelargonium hortorum × zonale	2	1	20	50	10–15
Phaseolus vulgaris	1	0	20	90	5
Primula obconica (young ls)	5	4	120	240	5–10
Primula sinensis (young ls)	4	3	45	120	10
Raphanus sativus	1	0	35	60	3
Ricinus communis	1	0	20	60	5–15
Salvia involucrata	2	1	90	120	4
Salvia splendens	3	2	30	60	1–5
Senecio mikanioides	1	0	20	50	5
Senecio Petasitis	3	2	30	180	25
Tropaeolum majus	2	1	50	90	1
Vicia Faba	1	0	60	240	10
Zea Mais	3	2	30	120	5

Prolonged darkness produces two distinct deleterious results. First, some plants, as Heliotropium and Impatiens, after 3 or 4 days in darkness, drop their leaves, owing to some derangement of their mechanism, the cause of which I have not investigated. Also, Tropaeolum leaves soon turn yellow in darkness. The second injurious result, and the most important in this study, occurs in nearly all plants. Some of the photosynthate is of course being constantly used in growth or for storage in the stem, and since the plant can make no more in darkness, the percentage of sugar in the cell-sap decreases more and more by diffusion into the stem. Then, as PFEFFER has shown,[3] when the plant is brought into the light no starch is deposited until this percentage of sugar is repaired. The following example is typical: Two plants of *Fuchsia speciosa*, apparently alike, were put in the light at the same time, after having been in darkness, the one 64 hours, the other 16 hours. Halves of leaves tested showed no starch. After 2 hours in the light, the other halves on the first plant showed much less starch than those on the second plant. To obtain the most rapid formation of starch, therefore, it is important that the plant should be kept in darkness only long enough just to cause the disappearance of starch.

The disappearance of starch is not always even. In Coleus, *Primula verticillata*, *Primula obconica*, and *Fuchsia speciosa*, the base of the leaf is emptied of starch before the tip. This agrees with what SACHS found in some other leaves.[4] So far as I have tested them, I have found this to be true only of ovate or oblong leaves. This may be correlated with the greater abundance of stomata near the base of the leaf. In round leaves like *Pelargonium zonale* and Tropaeolum, the starch seems to disappear evenly from all parts. The starch disappears from the young leaves on a plant before it does from those which are mature.

The effects of temperature on the amount of starch present are especially important. In several of my experiments, leaves of *Fuchsia speciosa*, *Euphorbia pulcherrima*, and *Pelargonium zonale* which were kept in direct sunlight 4 hours showed very little starch, while leaves on plants in diffuse light, at the end of 4 hours were full of starch. *Fuchsia speciosa* after being in diffuse light at 28°–31° C. for 3 hours showed only a trace of starch, while other leaves in 3 hours at 18°–20° C. appeared black with starch, with the iodin test. A comparison of these two sets of experiments shows that the small amount of starch present in leaves in direct sunlight is undoubtedly connected with their high temperature. In order to get the best results, therefore, from experiments in the formation of starch in the leaves of potted greenhouse plants, it is necessary to keep the plants at a temperature not exceeding 22° C., and to insure this it is well to keep them in diffuse rather than in direct sunlight. It will be of interest to compare these results with those obtained by SACHS for outdoor plants, as described in his classical

3 Physiology 1:321.

4 Ges. Abhandl. 360.

paper.[5] He found that the rate of the disappearance of starch from the leaves increases with the temperature. When the nights were very warm, some plants (Nicotiana, Phaseolus, Juglans, and others) completely emptied their leaves of starch in one night. But after cool nights, 6°–9° C., there was no perceptible loss of starch in some, and the disappearance was incomplete in others. SACHS found also that the amount of starch present at any time of the day is affected by temperature. At a temperature of 20°–25° C. the quantity of starch in the leaves increased steadily from morning until evening. But on hot afternoons at a temperature of 30°–35° C. the leaves of Helianthus contained less starch than in the morning at 8 o'clock. The reason for this phenomenon is found in the fact that translocation from the leaf into the stem increases with rising temperature more rapidly than photosynthesis. All of these considerations emphasize the important precaution that to obtain the best starch formation in leaves, the temperature should not be permitted to rise above 20°–22° C., which is apparently the optimum for this process, as it is for the best general health of such plants as are used in these studies.

Also it must be remembered that plants cannot give good results in this or any physiological experiment, when suffering from previous starvation, as in the case of pot-bound plants or those which have been kept in darkness for a long time; when suffering from over-stimulation from high feeding, or from being kept at too high temperatures; and when they have passed their grand period of growth. As a rule plants, and particularly annuals, are in their best condition just before flowering.—SOPHIA ECKERSON, *Smith College.*

THE MORPHOLOGY OF *RUPPIA MARITIMA*—A CRITICISM[6]

In section D, "Function of the root," GRAVES (p. 113) has wandered beyond the natural limits of his paper as a morphological study, and while the propriety of this is perhaps questionable, the basis for this criticism is that this section D contains a statement which I think is an unwarranted misinterpretation of some of my own writings, and also a statement that would leave most readers misinformed. I wish first to consider the following: "On the other hand, POND's experiments[7] fail to show conclusively whether or not water and dissolved salts are absorbed by the part of the plant above

[5] Arbeit. Bot. Inst. Würzburg **3**: 1 ff., as cited in Ges. Abhandl. 354–387.

[6] GRAVES, A. B., The morphology of *Ruppia maritima*. Trans. Conn. Acad. Arts & Sciences **14**: 59–170. 1908.

[7] POND, RAYMOND H., The biological relation of aquatic plants to the substratum. U. S. Fish Commission Report **1903**: 483–526. 1905.