PHOTOSYNTHESIS IN ASPEN BARK [1]

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QUAKING ASPEN, *Populus tremuloides* Michx., is probably the most widely distributed tree in North America (Harlow and Harrar, 1941) and therefore is exposed to a wide variety of climatic conditions. Over much of its range, which is mainly north of latitude 30 degrees north, its forest associates are evergreens capable of photosynthesis during the cold months of the year, even at temperatures below the freezing point of water as demonstrated by Parker (1953). Some populations of aspen have bark of yellow-green color as contrasted with the more familiar powdery-white bark; Cottam (1954) has observed that the former are found at the highest elevations in Utah and neighboring states and as such must be especially well adapted to life in areas of long cold winters and short summers. Marr (1947) observed in Colorado that the aspens with greenish bark were much more winter hardy than the non-green individuals.

A yellow-green aspen which seems quite similar to those described by Marr and by Cottam is common in Minnesota. Sections of the bark taken at breast height from trees a foot in diameter and about 20 years old have invariably revealed a bright green zone close below the surface. The green pigment was found to be located in plastids when hand sections were examined microscopically. We are not aware of any published study of the nature and function of this bark pigment, although the opinion had been expressed orally by a pathologist that it was a green stain produced by a parasitic fungus.

These observations suggest the possibility that the green pigment might be chlorophyll in which case it could be capable of carrying on photosynthesis even in mature stems.

To test this hypothesis, three experiments were conducted during the spring and summer of 1957 at the University of Minnesota's Cedar Creek Forest Natural History Area in northern Anoka County, Minnesota, 30 miles north of Minneapolis. One of these experiments was designed to ascertain the nature of the green pigments in aspen bark and to measure the quantity of these pigments per unit area of bark. A second was a simple and rather crude test to measure differences in amount of starch present in normal light-exposed and in artificially darkened areas of bark. The third test was designed to give a more refined measure of photosynthate production, one which would have meaning even if negative results were obtained.

At the beginning of the observation period, April 1, neither flowering nor leaf expansion had begun. About April 16, the aspen reached full bloom. Leafing began May 5 on most of the trees although a few specimens growing on higher and sandier soil were about four days earlier than the others. By May 17 seed dispersal was complete. The leaves had reached maximum size by June 7 and at this time were a bright green color. On August 3 when the final observations were made the leaves had darkened to a deep almost blue-green color. At this time the bark also had changed color, being not as distinctly green as before on the south side of the stems.

Monthly maximum and minimum temperatures in the shade at the bark sampling height above the ground during the period of observation were 27° and minus 11°C. in April, 31° and minus 4° in May, 28° and 7° in June, and 33° and 12° in July.

CHLOROPHYLL DETERMINATIONS.—*Materials and Methods.*—Samples of bark were obtained by stamping them out with a steel die of 16 mm. diam. Twenty disks, each approximately 1 mm. thick, from bark of the south and of the north sides of the stem were collected. The outermost layer of bark was brown and on May 31 averaged 0.5 mm. in thickness on the north side but only 0.04 mm. on the south side. Beneath this was a green layer 0.20 mm. thick on the north side and 0.33 mm. thick on the south side. Thus all the green pigments were in the outer 1 mm. of bark from which the samples were taken. Twenty disks each were also taken from sun-exposed leaves on the south and from shade leaves on the north side of the crown; these averaged 0.18 mm. and 0.14 mm. thick respectively. All samples of bark and leaf were taken from a single pistillate tree which was 19 years old and 16 cm. in diameter at the two-meter level above ground where the bark samples were taken. The bark outside the true cambium averaged 8 mm. thick at this level.

To extract chlorophyll, two techniques were used. In the experiment of May 31, bark of a known surface area was placed in a Kenmore blender with a known quantity of 95 per cent ethanol and run at high speed for one minute. This mixture was then boiled for three minutes and stored in flasks covered with aluminum foil at 1°C. for three days until it was possible to examine with a spectrophotometer. At that time it was filtered and the volume of the green solution ascertained. Wave lengths of visible light at which maximum and minimum absorption occurred and also the amount of absorption of light at each of four different wave lengths were measured

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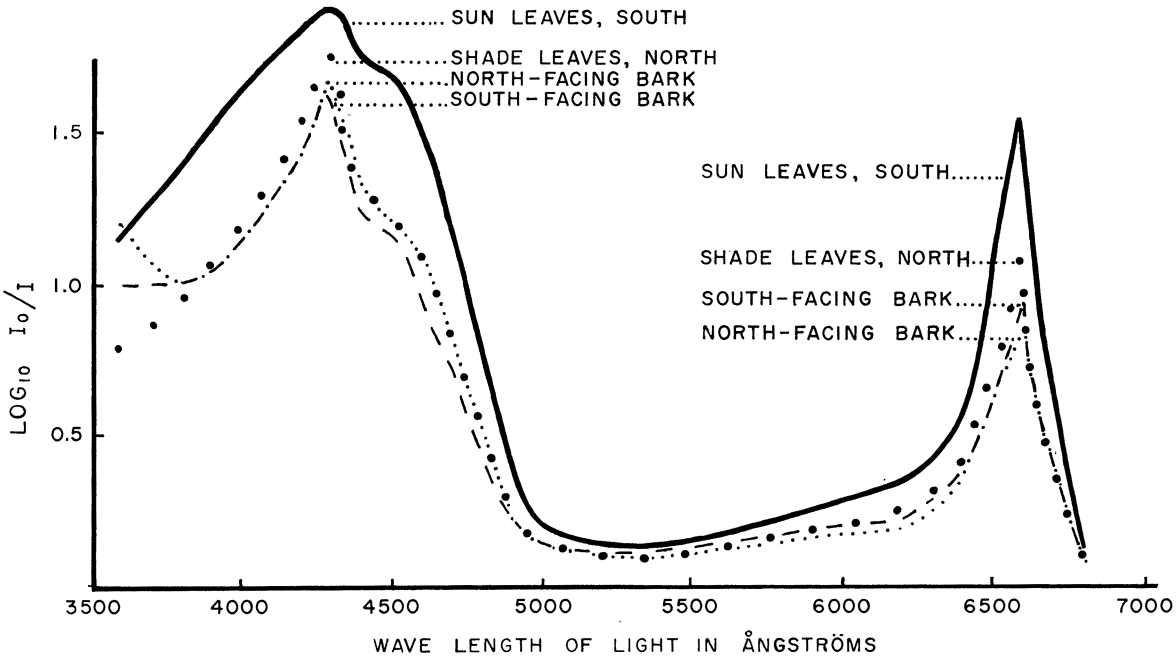

Fig. 1. Absorption spectra of pigments in aspen dissolved in ether. Data of August 3. Chlorophyll *a* measured at 6600 A.

with a Beckman Spectrophotometer Model B. The quantities of chlorophylls *a* and *b* present were estimated by comparison with absorption coefficients published by Zscheile and Comar (1941). Chlorophyll was also extracted from known areas of leaves of aspen by the same technique. In each case for this experiment bark or leaves totalling 40.20 cm.^{2} in area were mixed with 95 cc. of alcohol. An additional 15 cc. alcohol was used to rinse the containers. Samples were brought to the boil at the same rate as well as being boiled for the same length of time.

In the experiment of August 3 designed with the help of Dr. Frenkel, a sample of bark of known area— again 40.20 cm.^{2}—was placed in the Kenmore blender with approximately 50 cc. acetone plus about 2 g. powdered $CaCO_3$ and run at low speed for 3 minutes. The contents of the blender were then placed in a centrifuge; the blender was rinsed with an additional 25 to 30 cc. of acetone and this was added to the mixture in the centrifuge. The mixture was centrifuged for about three minutes until the chlorophyll solution was clear. The solution was poured into a separatory funnel containing about 50 cc. of ethyl ether. Water was added by applying small quantities along the side of the funnel and swirling carefully. When separation occurred, the heavier acetone and water mixture was drawn off and discarded. The chlorophyll-ether solution was washed with water several times until it appeared to be quite free of acetone, care being taken to avoid saponifying the chlorophyll by too direct application of the water. Ether was added to the resulting solution to bring the total volume to 100 cc.

Absorption was ascertained with the spectrophotometer at wave lengths of 4300, 4500, 4700, 6425, 6500, and 6600 A. The concentration of chlorophyll *a* was ascertained by measuring at 6600 A inasmuch as the absorption at this wave length due to carotinoids is negligible and that due to chlorophyll *b* less than 4 per cent of the total. The concentration of chlorophyll *b* was ascertained at 6425 A after calculating the absorption due to chlorophyll *a* at this wave length and subtracting that from the total optical density.

From a 30 cc. sample of the ether-chlorophyll solution, chlorophylls *a* and *b* were removed by saponifying with a solution of 30 per cent KOH in methanol and washing with water. The heavier chlorophyll complex was drawn off the bottom of a separatory funnel; the remaining pigments, a mixture of carotinoids, were studied with the spectrophotometer at several wave lengths including 4300, 4500, and 4700 A and the optical densities recorded. The resulting values gave a comparison of the relative concentration of carotinoids in each of the four tissues studied.

Experimental results.—The absorption spectra for aspen bark extract and for aspen leaf extract are very similar as shown in fig. 1, having maxima near 6610 A and 4300 A and a minimum at about 5400 A. In the original alcohol and acetone solutions, the bark extract showed an additional maximum at 3860 A not found in the leaf extracts. This is not apparent in the ether solution from which the curves

TABLE 1. *Milligrams of chlorophylls a and b per square meter of aspen tissue. Values of May 31 from alcohol solution, those of August 3 from ether*

	Date	Leaves Sun	Leaves Shade	Bark Sun	Bark Shade
Chlorophyll *a*	May 31	202	147	258	233
	Aug. 3	300	250	195	193
Chlorophyll *b*	May 31	156	116	241	246
	Aug. 3	190	130	115	100

in fig. 1 were drawn. A water soluble brown pigment was washed out along with the acetone in preparing the ether solutions of the bark extract; this pigment was probably responsible for the increased absorption at 3860 A noted in the alcohol extraction of May 31.

In table 1 are shown estimates of the quantity of chlorophylls *a* and *b* for each of the four tissues and at each of two dates, the May 31 data from alcohol extracts, those of August 3 from ether. By calculating the optical density at each of three wave lengths for the carotinoid solutions and subtracting these values from the total values at the same wave lengths for the chlorophyll solutions (which included carotinoids) additional values were obtained which could be used as checks on the accuracy of the original calculations. This procedure indicated that the values shown for chlorophyll *a* are accurate within about 5 mg; the values for chlorophyll *b*, however, are somewhat less accurate.

When the carotinoid spectra of the four tissues were compared (not shown graphically in fig. 1) they were seen to be similar with maxima at 4200 A, 4400 A, and 4700 A and minima at 4300 A and 4600 A. Comparison of these spectra with the absorption spectrum for β-carotene published by Cooley and Koehn (1950) suggests that β-carotene is not the most abundant of carotinoids present in these tissues. Judging from the total absorption at 4700 A, sun and shade leaves contained approximately equal quantities of carotinoids, south-facing bark about one and one-half times as much carotinoid pigment, and north-facing bark about twice as much carotinoid as leaves. This relationship held true at both dates. The proportions were in the same order but of somewhat different magnitudes at 4300 A and 4500 A indicating the same carotinoids were not present in the same proportions in all four tissues. The absorption spectra for α-and β-carotenes, xanthophyll, and lycopene published by Miller (1934) failed to account for the spectral pattern obtained from the aspen tissues.

FIRST PHOTOSYNTHATE MEASUREMENTS.—*Materials and methods.*—To test for starch, samples of bark were boiled in alcohol for a few minutes, dipped in hot water to soften, then removed and laid on a table top, and to the inner cut surfaces was added a solution of I · KI. The intensity of the resulting blue color was compared with that of a sample of wheat or potato starch, which had been treated with the same solution, and scored as a per cent of the latter to give a measure of starch present. Because of the possibility that this type of subjective measurement might want in precision, randomization and replication procedures were followed, each treatment being replicated four times. The randomization completely removed all human bias from the measurements.

On April 12, before the leaves appeared, masks made of four thicknesses of paper picnic plates were attached securely by four cup-hooks to the bark of south and north sides of two aspens, one of them the same tree from which the chlorophyll samples were taken, the other smaller. The following week a layer of aluminum foil was placed under each mask to cut off completely the passage of light.

The masks were left in place for three weeks after which at weekly intervals samples of bark were taken from under them and tested for starch along with samples of light-exposed bark. From each treatment (combination of exposed vs. darkened bark, south vs. north-facing bark, and trees of different sizes) four samples were taken and tested. These samples were about one mm. thick; they were removed with a sharp knife after which a randomized number was placed on the under surface of each sample with a lead pencil.

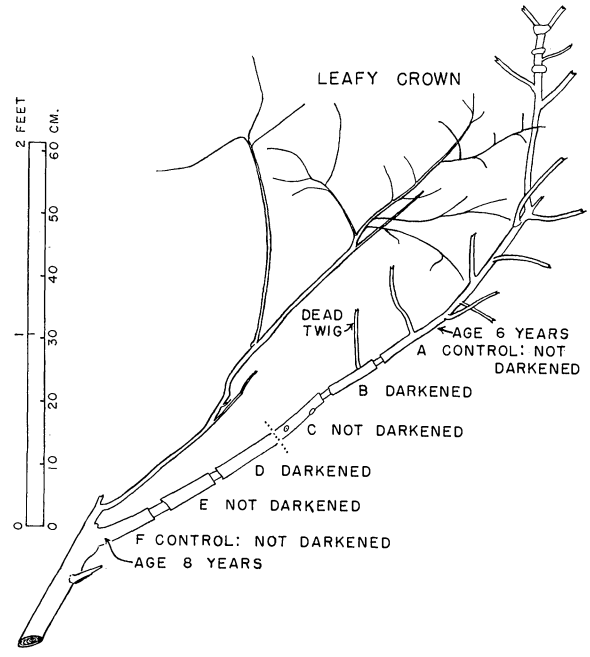

Fig. 2. Sketch of the aspen branch girdled in four places to prevent translocation of foods to the intergirdle regions, labeled to indicate treatments during first week of experimentation. Regions *B* and *D* were covered with aluminum foil over black paper to exclude light; *C* and *E* were exposed to light.

Experimental results.—Starch was present in all of the bark samples and it was impossible to detect any consistent differences among them. The experiment indicated either that the starch occurring there was not produced in the bark as a result of photosynthesis or else that the starch produced in the exposed bark was translocated into areas of darkened bark relatively rapidly; the weakness of this experiment was that these two possible effects could not be separated. Therefore a more complex experiment involving removal of phloem tissue by girdling to prevent translocation of foods was devised.

SECOND PHOTOSYNTHATE MEASUREMENTS.—*Materials and methods.*—On May 31 a branch of an aspen tree was girdled in four places along a leafless portion as shown in fig. 2. Assuming that translocation of foods takes place via the phloem, each of the three intergirdle regions as well as the leafy distal region was thus dependent on its own photosynthetic activities for its food, at least after stored food had been used up in respiration. The xylem was not damaged; thus an abundant supply of water and mineral nutrients was assured each region. While there were no leaves present on any of the three intergirdle regions, the distal region bore a normal supply of photosynthetic leaves.

One intergirdle part of the branch (Region *B* in fig. 2) was completely covered with black paper and this in turn was covered with aluminum foil which was held in place with plastic electrician's tape. Another region (*C-D*) was partly (*D*) covered with black paper and aluminum foil and partly (*C*) exposed, whereas a third (Region *E*) was left entirely exposed to light. After one week, samples of bark were removed from each section and tested for starch; the black paper and aluminum foil were then replaced but with Region *E* covered and Region *B* exposed. Samples were taken again the following week, but it was noted that by that time much of the tissue was dead and it was therefore felt that little reliance could be placed on the results of the second sampling. In this experiment as before randomization and replication procedures were followed.

Experimental results.—Differences were very pronounced among the samples collected at the end of the first week following girdling. Among the group of samples taken the second week, little starch was present in any of the intergirdle regions and the variation among treatments (other than the controls) could all be attributed to chance variation. The day after the sample collection was made at the end of the second week, the girdled branch showed marked wilting and leaf discoloration indicative of the extent to which necrosis had progressed. The averages of the four samples of each treatment taken the first week are shown in table 2.

Because the treatments were randomized and replicated, it is possible to test the differences among treatment averages in table 2 with Fisher's LSD (least significant difference) test. All differences except those between the two controls (treatments 1 and 2) exceed the LSD in value. In other words, the chances are less than one in 20 that even the smallest differences shown in table 2 are due to chance variation among the samples tested and we can therefore feel confident that the treatment differences are real differences—that the chlorophyll in aspen bark is photosynthetically active.

TABLE 2. *Relative starch content of lighted and darkened aspen bark one week after girdling. Values are given as the color intensity of the samples expressed as a per cent of the intensity of a pure starch-iodine complex*

Treatment	Avg of 4 samples
1) Control. Proximal region of branch (Region *F*)	100%
2) Control. Distal region (Region *A*)	93%
3) Girdle-isolated region completely exposed to light (Region *E*)	68%
4) Covered portion (*D*) of the partly exposed girdle-isolated region (*C-D*)	38%
5) Girdle-isolated region completely covered (Region *B*)	5%
Least significant difference, 5% probability	28%

DISCUSSION.—Assuming that the production of chlorophyll in a plant tissue is more or less proportional to the intensity of the light falling on that particular tissue, it would be expected that plant organs exposed to the sun would contain more chlorophyll per unit area than similar organs kept in the shade. This proved to be the case in aspen, both for leaves and for bark. Also in support of this assumption was the observation that the chlorophyll content of the leaves increased over the period May 31 to August 3 whereas the chlorophyll content of bark decreased during the same period. In fact, the difference in chlorophyll content between south and north-facing bark which was noted in May, when the difference in light intensity was also great, had apparently been erased by August along with the difference in light intensity, an expected result. However, bark—even from the north side of the tree—proved to contain more chlorophyll per unit area of surface[3] on May 31 than any of the leaves. To the writers, this was a rather unexpected finding, although it is certainly not in disagreement with the assumption that more chlorophyll is produced in tissues exposed to abundant light than in darkened tissues. On May 31 bark could well be expected to be rich in chlorophyll produced prior to leafing, possibly during the winter months, whereas the leaves had not yet had time to accumulate a large store of these pigments.

The photosynthates produced in aspen bark are apparently translocated quite readily from one

[3] Leaf surface area is the value for one side of the leaf only.

part of the plant to another through the phloem. That translocation of foods in aspen bark occurs was indicated in the earlier test in which there were no differences between covered and exposed bark in the amount of starch present. Removal of the phloem between the different regions under observation in the final test, however, resulted in the appearance among the samples of differences in starch content. Comparison of treatments 4 and 5 in table 2 illustrates this further. These two treatments were identical in every respect except that in treatment 5 (Region *B*) there was no phloem contact between the darkened bark and either leaves or bark that were exposed to light, while in treatment 4 the darkened bark (Region *D*) was in direct contact with bark that was exposed to light (Region *C*). The bark under both treatments was connected by undamaged xylem to the rest of the tree. In each case, the bark from which the samples were taken was covered to exclude all light; also in each case the sampled region was part of an intergirdle section. The differences between these treatments, therefore, must be due to the phloem contact which Region *D* (treatment 4) had with a light exposed portion of bark, Region *C*. As a result of this contact, the four samples of bark taken from Region *D* all contained starch whereas no starch at all could be observed in three of the four samples from Region *B* and only a small amount in the fourth.

Failure to find differences among the samples taken from areas of different treatments the day before the girdled branch began to wilt points up the necessity in experiments of this type, in which drastic treatments are imposed upon plants otherwise in their native habitat and under natural conditions, to complete such experiments as rapidly as possible.

These experiments give support to the hypothesis that the green pigment in aspen bark is chlorophyll and that aspen bark of mature stems is capable of carrying on photosynthesis, at least under the conditions in Minnesota in the spring of 1957. There is a possibility that the results reported here may bear a relationship to conditions in the Rocky Mountains as described by Marr (1947), by Cottam (1954), and others mentioned by him. However, similar experiments would need to be conducted in the Inter-mountain area to verify these assumptions. There is also a possibility that these results obtained in the late spring may bear a relationship to winter conditions and an experiment to measure photosynthesis in aspen bark at temperatures below 0°C. is planned.

SUMMARY

By use of the spectrophotometer, the green pigments in aspen bark were shown to be chlorophyll; it was also ascertained that these pigments are more abundant in bark than in leaves of aspen per unit area during the early part of the growing season, but more abundant in leaves later in the summer. By use of the starch-iodine test, it was ascertained that the chlorophyll in aspen bark is photosynthetically active. It is suggested that this ability of aspen to carry on photosynthesis in its bark might partly account for the ability of this species to thrive in what is otherwise mainly an evergreen conifer environment.

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