

Nutrient Content of Artificially Defoliated Branches of *Betula papyrifera*

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ABSTRACT

Branches of *Betula papyrifera* were subjected to two classes of defoliation in the summer of 1968: Class I, removal of half-leaves; and Class II, removal of whole leaves. Groups of branches (three branches/group) of each class and undefoliated controls were analyzed for nitrogen, starch, hexose, hexose-pentose, and ether-soluble extractives. Starch and nitrogen were significantly reduced in defoliated

samples harvested during the subsequent dormant season and again in June 1969. A causal relationship apparently exists between the extent of defoliation and the observed syndrome involving subsequent amounts of nitrogen in branches, phenological responses, and death of branches. *Phytopathology* 61:631-635.

Additional key words: crown deterioration, dieback, Sephadex, absorbance, Kjeldahl.

Crown deterioration of white birch (*Betula papyrifera* Marsh.) has occurred throughout the range of the species in eastern Canada for many years. In 1967, Sippell et al. (14) reported crown deterioration of many hardwood species in Ontario, particularly white and yellow birch (*Betula alleghaniensis* Britt.). Portions of tree crowns which had produced heavy seed crops and had dwarfed or which otherwise lacked foliage died back in 1968, suggesting a depletion of food reserves. Other diseases that involve crown deterioration as part of their disease syndrome, e.g., birch dieback (1), ash dieback (13), and maple blight (4), also exhibit similar symptoms to those observed for birch in Ontario in 1967 to 1968.

Previous investigations dealing with tree tissues associated with crown deterioration involved the injection of chemicals or analyses of foliage. Morris (10) injected solutions of chemicals into the stems of *B. papyrifera* trees exhibiting the birch dieback syndrome, and concluded either that deficiencies of nitrogen, phosphorus, and potassium were not important factors in that disease, or that quantities of chemicals injected were too small to have a chemotherapeutic effect. Lortie & Laforte (8) analyzed the chemical composition of white birch leaves obtained from trees exhibiting dieback symptoms, and reported that amounts of nitrogen, calcium, phosphorus, magnesium, and potassium could not be correlated with dieback classes. Hepting (5) investigated carbohydrate levels in pine trees affected by "little leaf disease", and determined that reduction in carbohydrate content was characteristic of affected trees.

The investigative line followed here was determined primarily by the premise that crown deterioration was associated with depleted food reserves resulting from heavy seed production and the lack of foliage. The reduction in foliage area in 1967 was considered to have an effect on foliar patterns and food reserves the following year. The objective of our investigation was to determine whether or not artificial defoliation (reduction of foliage area) will cause significant reductions or variations in the quantities of nutrients in

branches, and if so, to correlate these reductions or variations with symptoms of crown deterioration.

MATERIALS AND METHODS.—Fifty groups of branches (three/group) were selected on 28 open-growing *B. papyrifera* trees, approx 30 years old, 10 to 20 m in height, and 15 to 25 cm in diam. Branches were approx the same size, and spaced along the bole or fork of the main bole within a vertical distance of 1 m and a 60 degree horizontal angle of exposure. Trees selected did not show signs of heavy seeding in 1967 to 1968 or crown deterioration.

The defoliation treatments, one/branch, are defined as follows: Class I, accomplished by cutting all leaves in half transversely; Class II, accomplished by cutting off all leaves at the distal end of the petiole; the control was an untreated branch (Fig. 1-3). Treatments were performed on all portions of a branch distal to the bole. Treated branches never comprised more than 5% of the crown. Groups of branches were defoliated in 1968 from 10 to 20 June. Leaves which developed subsequently were treated accordingly from 12 to 21 August. To avoid injuring branches, defoliation was performed from portable scaffolding. Thirty-two groups of branches were harvested 11 November 1968, and the phenology recorded. Eighteen groups of branches were left on the trees for purposes of studying the phenology of foliar development during the subsequent growing season and monitoring nutritional conditions. These were harvested 5 June 1969. Samples were taken from the main axis of the branch and stored at -20°C until processed. Lateral twigs and shoots were removed prior to processing. Treated branch samples are characterized by the following data (mean dimensions in cm): length 74.1 (SD = 15.9, SE = 2.7); diam large end 0.96 (SD = 0.15, SE = 0.03); and diam small end 0.39 (SD = 0.05, SE = 0.01). Groups of branches which contained one or more dead branches were omitted. Twelve groups from the November harvest and 12 from the June harvest were analyzed.

When processed, samples were taken from cold storage, raised to room temperature, ground in a Wiley mill to pass a 20-mesh screen, and mixed. Seven-, 3.0-

and 1.0-g portions were removed from the ground samples. The 3.0- and 1.0-g portions were refrozen (-20°C) for subsequent holocellulose and nitrogen analyses, respectively. For ether-soluble extractives, the 7.0-g samples were placed in 43×123 -mm double thickness Whatman extraction thimbles and extracted with ether in Soxhlet columns for 24 hr. Empty thimbles were used as controls. Ether was removed from all extracts by using a Rinco rotating evaporator in conjunction with a filter pump. Weights of control residues were subtracted from those of ether-soluble residues and recorded as the "ether-soluble extract".

After ether extraction, the control thimble and thimbles with samples were extracted with 95% ethanol in Soxhlet columns for 24 hr to remove soluble sugars. The ethanol extracts were reduced to a volume of 25 to 30 ml at 50°C . Extracts were then centrifuged for 0.5 hr at approx 30,000 *g*. Supernatants were placed in 100-ml volumetric flasks. Centrifuged pellets were resuspended and centrifuged under the above conditions, and the resulting supernatants decanted into appropriate volumetric flasks. These were then raised volumetrically to 100 ml by adding distilled water [hereafter referred to as raw extract I (RE I)]. One ml of each of the RE I solutions was diluted with distilled water volumetrically to 50 ml [hereafter referred to as raw extract II (RE II)].

Combined hexose-pentose of RE II was measured with the orcinol test for carbohydrates following the procedures outlined by Jensen & Ashton (7) and Jensen (6), with minor modifications in volumes and concentrations of reagents. Analytical grade glucose was tested at six concentrations ranging from 10 to 100 $\mu\text{g}/\text{ml}$ to determine a standard curve ($Y = 59.0 X + 4.3$, $r = .996$, $n = 18$). One-half ml of RE II was treated and the absorbance ($425 \text{ m}\mu$) interpreted with a proper dilution factor to give the glucose equivalent (hereafter referred to as GE) of the amount of hexose-pentose present in 1.0 g oven-dry wt (hereafter referred to as odw) of each of the samples.

One ml of RE I was applied to a 28-cm column of Sephadex gel beads (column K15/30, Sephadex G-10; Pharmacia, Uppsala, Sweden); a flow rate of 1.0 ml/10 min was maintained. A fraction was collected free of high mol wt substances (referred to hereafter as Sephadex extract, SEF) and contained low mol wt carbohydrates. This fraction was diluted to 50 ml, volumetrically, with distilled water. One-half ml of SEF was treated with orcinol as described above.

One ml of SEF was treated with cysteine for measuring hexose as outlined by Jensen & Ashton (7) and Jensen (6). Analytical grade glucose was tested at 10 concentrations ranging from 25 to 300 $\mu\text{g}/\text{ml}$ to determine a standard curve ($Y = 316.6 X - 2.8$, $r = .994$, $n = 29$). The difference in absorbance at 380 $\text{m}\mu$ and 415 $\text{m}\mu$, interpreted with the proper dilution factor, indicated the amount of hexose present in 1.0 g odw of each of the samples expressed in terms of GE.

The methods of McCready et al. (9), with minor modifications made in apparatus, were used to analyze samples for starch. Analytical grade glucose at six concentrations ranging from 20 to 125 $\mu\text{g}/\text{ml}$ was treated

by using the anthrone test (absorbance at 630 $\text{m}\mu$) to determine a standard curve ($Y = 108.3 X + 2.1$, $r = .999$, $n = 24$). One g of each ether-ethanol extracted sample was used to determine the amount of starch present, in GE, in 1.0 g odw sample. It was determined the 1.0 g odw sample lost 0.108 g ($\text{sd} = 0.018$, $\text{se} = 0.0046$) through extraction by ether and ethanol. Starch values were adjusted by a constant based on this loss of wt in order to present values based on the original odw.

Nitrogen contents were calculated based on the digestion of 250 mg samples by using the analytical methods of Rennie (12) in conjunction with Kjeldahl distillation, and are expressed as mg of nitrogen per 100 g odw (% odw) with respect to ammonium ferrous sulphate in standard solution.

Holocellulose contents are expressed as a percentage of 3.0 g odw sample, and were obtained by using the methods of Wise et al. (17), with minor modifications regarding the kinds of equipment used and the volume of wood analyzed.

Absorbance readings, relative to carbohydrate analyses, were made on a Beckman DU spectrophotometer. A Unicam model SP-700 spectrophotometer was utilized during the initial stages of the investigation in order to examine the range and characteristics of absorbance of extracts.

The presence of significant differences in amounts of nutritional materials present in each sample was tested, by using the F test, for tree-to-tree and treatment differences. If significant differences were present among treatments, the Scheffé S test (15) was used to determine the significance of differences between treatments.

RESULTS.—Observations on the phenology of treated groups of branches are presented in Tables 1 and 2, and Fig. 4. Analytical results derived from nutrient analyses are given in Tables 3 and 4. No significant differences among treatments were present in quantities of hexose, hexose-pentose, holocellulose, and ether-soluble extractives for the samples harvested in November. Analyses of these materials were not performed for the June 1969 samples. Quantities of nitrogen and starch for the November samples (Table 3) were significantly less for the Class II defoliation compared to controls and Class I defoliation. In the June sample, significant reductions in nitrogen (Table 4) of Class I and Class II when compared to the controls were observed, and the quantity of starch present in Class II was significantly more than Class I and control.

DISCUSSION.—Groups of branches harvested during the dormant period were analyzed, and significant differences were observed for starch and nitrogen. Eighteen additional groups of branches were harvested early in the growing season of the following year, 12 of which were analyzed to further investigate the levels of starch and nitrogen for purposes of relating phenology (Fig. 4) of branches to reduction of starch and nitrogen.

A reduction in nitrogen content was detected from dormancy to a point early in the growing season in the control and both classes, and was probably due to re-foliation. Class II defoliation was associated with reduced nitrogen content. The evidence for reduced

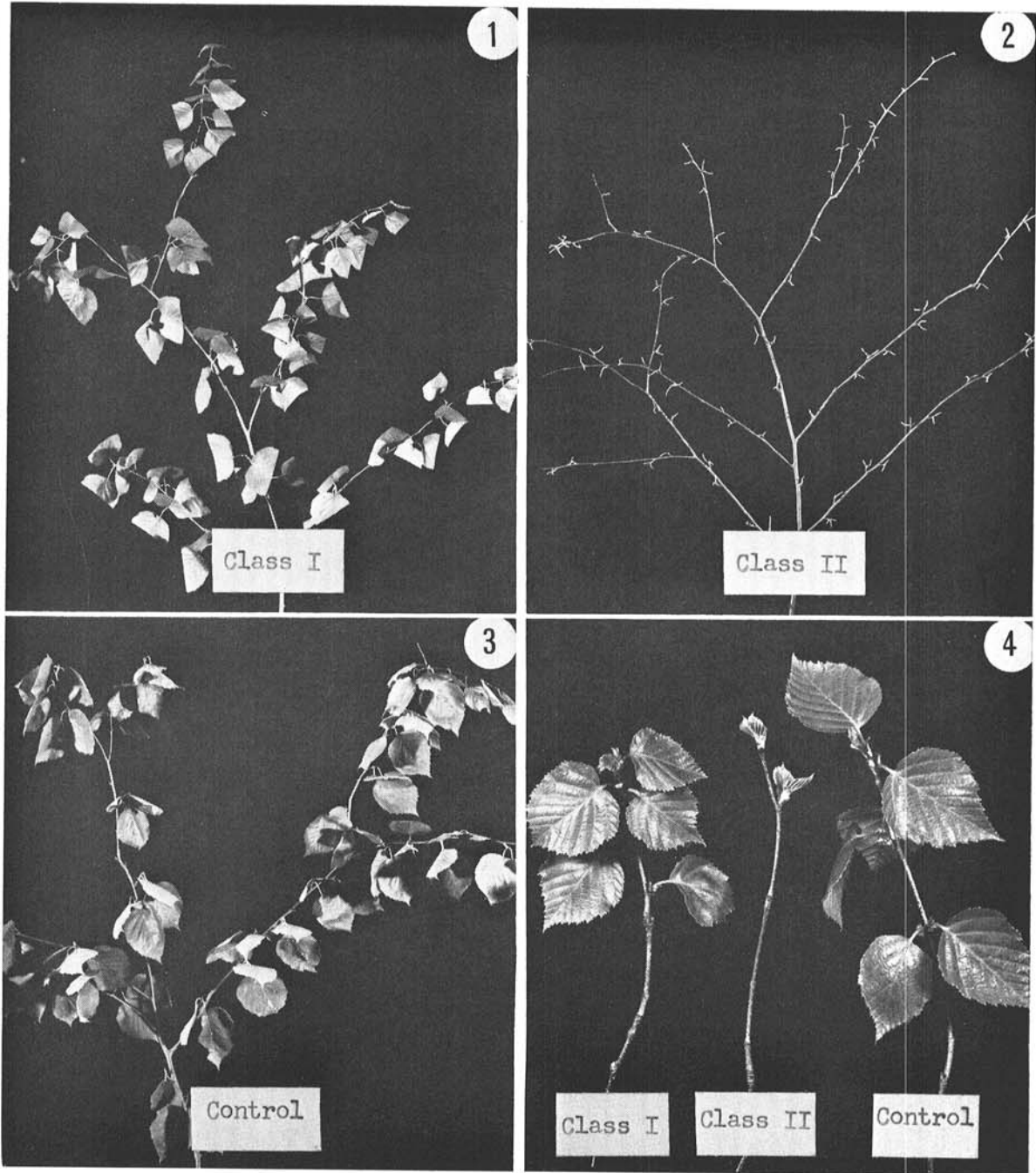


Fig. 1-4. 1) Defoliation Class I; all leaves on a branch of *Betula papyrifera* cut in half transversely. 2) Defoliation Class II; all leaves on a branch of *Betula papyrifera* cut off at the distal end of the petiole. 3) Control; untreated branch of *Betula papyrifera*. 4) Phenological response of branches of *Betula papyrifera* 5 June 1969 to defoliation 10 to 20 June and 12 to 21 August 1968.

nitrogen content associated with Class I defoliation was not as conclusive. A significant reduction was not apparent in the November sample, and refoliation patterns (Fig. 4) in June indicate that sufficient nitrogen existed in Class I branches to support near normal refoliation up to that time. It is notable that six of the 18 branches in Class II maintained for subsequent

phenological observations were dead in June. There was also a marked over-all similarity of the phenology of experimentally defoliated branches to those under natural forest conditions that have exhibited dieback symptoms.

The nitrogen measured in this experiment represents total nitrogen, and does not truly reflect the nitrogen

TABLE 1. The condition of defoliated^a groups of *Betula papyrifera* branches in November 1968

Defoliation treatment ^b	No. branches observed	Branches appearing normal	Branches dead	Branches with shoot mortality	
				Moderate (11-30%)	High (31%+)
Class I	32	9 (91%)	2 (6%)	1 (3%)	0 (0%)
Class II	32	14 (43%)	7 (22%)	4 (13%)	7 (22%)
Control	32	31 (97%)	0 (0%)	1 (3%)	0 (0%)

^a Groups of branches were defoliated during 10 to 20 June, and again during 12 to 21 August.

^b Class I, all leaves on a given branch cut in half transversely; Class II, all leaves on a given branch cut off at the distal end of the petiole; Control, an untreated branch.

TABLE 2. The condition of defoliated^a groups of *Betula papyrifera* branches on 5 June 1969

Defoliation treatment ^b	No. branches observed	Branches appearing normal	Branches dead	Foliage dwarfed
Class I	18	18 (100%)	0 (0%)	0 (0%)
Class II	18	0 (0%)	6 (33%)	12 (67%)
Control	18	18 (100%)	0 (0%)	0 (0%)

^a Groups of branches were defoliated during 10 to 20 June, and again during 12 to 21 August.

^b Class I, all leaves on a given branch cut in half transversely; Class II, all leaves on a given branch cut off at the distal end of the petiole; Control, an untreated branch.

forms available for growth. There is also a strong implication that defoliation of the kind described here results in two categories of nitrogen loss: (i) a physical loss, or that quantity which is lost at a given point in time when the leaf or part of the leaf is removed; and (ii) reduction of physiological potential by reducing the size of the foliar apparatus. The removal of the leaf in total or in part is the apparent cause for the reduced nitrogen observed. The classes of defoliation used here, in terms of physiological damage, are not

wholly unlike those caused by insects, fungi, fume damage, drought, frost, and hail injury.

Combes (2, 3) after analyzing leaves, stems, and roots of young forest trees, noted that nitrogen was removed from leaves to twigs in a period of 8 weeks, while leaves were yellowing, and that *Fagus* sp. leaves lost 40% of their nitrogen, *Castanea dentata* lost 50%, and *Aesculus hippocastanum* lost 65%. Tamm (16) reported this phenomenon in *Betula* sp. when monitoring the nitrogen content of leaves from July to September. His findings indicate that approximately one-third to one-half of the leaf nitrogen was removed in a 2-week period. Our data, based on controls (Tables 3, 4), can be interpreted to indicate that approx one-third of the nitrogen goes from branches to leaves in the spring or sometime in the growing season. Nitrogen movements per se were not monitored in the experiment presented here, but the data derived from it support those of Combes (2, 3) and Tamm (16): If certain quantities of nitrogen containing compounds move, in the fall, from whole leaves to branches, then correspondingly lesser amounts move from partially or totally excised leaves. From this, it is reasonable to assume that two different, but closely related, damaging influences are involved relative to the nutritional status of associated

TABLE 3. Quantities of materials^a present in treated branches of *Betula papyrifera* in November 1968

Branch constituent analyzed	Unit of measure	Groups ^b of branches				Standard error of mean values
			Class I ^c	Class II	Control	
Nitrogen	% odw ^f	10 ^h	<u>0.61</u>	<u>0.36</u>	0.65	.024
**Starch	GE ^g /g odw	12	<u>12,160</u>	<u>10,880</u>	12,860	370
**Hexose (SEF ^d)	GE/g odw	10	<u>66,070</u>	<u>73,400</u>	77,100	500
Hexose-pentose (RE ^e II)	GE/g odw	10	106,400	104,300	109,600	550
Hexose-pentose (SEF)	GE/g odw	10	89,400	87,000	90,700	240
Holocellulose	% odw	10	69.5	68.5	68.2	1.7
*Ether-soluble extract	mg/g odw	10	53	56	57	5

^a Underlined values are significantly different at $P = .01$ (F test and S test). Prefix before material analyzed indicates significant tree-to-tree variation, * $P = .05$; ** $P = .01$ (F test).

^b Three branches/group.

^c Class I, all leaves on a given branch cut in half transversely; Class II, all leaves on a given branch cut off at the distal end of the petiole; Control, an untreated branch.

^d Sephadex extract.

^e Raw extract.

^f Oven-dry weight.

^g Glucose equivalents.

^h Two groups of branches were lost during experimental procedures where 10 groups of branches appear.

TABLE 4. Quantities of materials^a present in treated branches of *Betula papyrifera* in early June 1969

Branch constituent analyzed	Unit of measure	Groups ^b of branches	Class I ^c	Class II	Control	Standard error of mean values
*Nitrogen	% odw ^d	12	<u>0.34</u>	<u>0.27</u>	<u>0.43</u>	.018
Starch	GE ^e /gm odw	12	<u>6,920</u>	<u>10,420</u>	<u>6,820</u>	700

^a Underlined values are significantly different at $P = .01$ (F test and S test). Prefix before material analyzed indicates significant tree-to-tree variation, * $P = .05$ (F test).

^b Three branches/group.

^c Class I, all leaves on a given branch cut in half transversely; Class II, all leaves on a given branch cut off at the distal end of the petiole; Control, an untreated branch.

^d Oven-dry weight.

^e Glucose equivalents.

branches: (i) an initial nitrogen loss in the fall because of prior defoliation affecting branch survival during the dormant season; and (ii) the resulting low nitrogen levels present at the break of dormancy which limit foliage production, thus compounding existing injury as time progresses.

Ovington & Madgwick (11) reported that the nitrogen content of birch branches was 0.60% odw during the growing season. Our data for control branches may be comparable to this figure, but a comparison related to sampling times cannot be made. The June figure of 0.43% odw is probably near the low point in nitrogen content on an annual basis, and 0.65% odw near the high point.

Significant differences among treatments were present in quantities of starch in the November and June samples. In November, both Class I and control possessed greater quantities of starch when compared to Class II (Table 3). Within the precision of the experiment, starch contents of Class II branches were approx the same during both sampling periods. In June, starch content of Class I and Control branches was significantly less than Class II (Table 4), indicating that starch was available in Class II branches, and not utilized to the same extent as the starch in Class I and Control. The foregoing suggests that the inhibition of starch utilization, associated with Class II defoliation, was an effect of low nitrogen levels.

There is evidently a close relationship between defoliation and reduced nitrogen contents. Reduced nitrogen contents were closely associated with the phenotypic responses recorded in Tables 1 and 2, and Fig. 4. Evidence is also provided that starch accumulation in twigs may be a function of the foliar area present during the growing season.

It is our opinion that investigations into crown deterioration through the study of nitrogen nutrition appear to be a promising means of understanding, in part, a malady that is common to many hardwood species.

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