LEAF CHEMICAL COMPOSITION OF TWENTY-ONE *POPULUS* HYBRID CLONES GROWN UNDER INTENSIVE CULTURE

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ABSTRACT.--Leaf material from 21 nursery-grown *Populus* hybrid clones was analyzed for three nitrogen fractions (total N, soluble protein, and soluble amino acids) and three carbohydrate fractions (reducing sugars, total soluble sugars, and total nonstructural carbohydrates-TNC). In addition, nursery-grown green ash and silver maple, field-grown bigtooth and trembling aspen, and growth-room-reared eastern cottonwood were analyzed for comparison. The amounts of the various chemical fractions ranged widely among the *Populus* clones: total N from 2.14 to 3.86% dry weight, soluble protein 0.98to 5.89%, soluble AA .09 to .24%, reducing sugar 3.57 to 12.42%, total soluble sugar 9.61 to 21.43%, and TNC 16.02 to 26.49%. Percentage recoveries for the chemical fractions of comparison species fell within these ranges; the cottonwood leaf material, however, contained higher levels of total N and soluble AA than the other plant material. The high levels of N and carbohydrates in the leaf material indicate a potential for utilization as a high quality livestock feed supplement. Certain clones combined rapid growth rates with high N and carbohydrate levels in the leaves. If leaf utilization should become part of the total harvesting plan, these clones would make desirable selections for intensive culture plantations.

Additional keywords : Leaf nitrogen, protein, soluble carbohydrates, total nonstructural carbohydrates, muka production

INTRODUCTION

With present methods of whole tree utilization, leaves or leaves plus small branches and bark constitute a costly chip contaminant. Leaves and bark in chips usually decrease pulp yields and paper strength, and increase the chemicals used in pulping and bleaching (Crist 1976). Methods of separating such contaminants from wood chips are presently being developed (Arola, Sturos, and Mattson 1976).

Separating foliage and bark from chips would not only reduce the cost of pulp and paper production, but would also free this material for some potentially profitable uses. With the rising cost of oil, coal., and other energy sources, the utilization of leaves, bark, and other mill and forest residues for power production is not only increasing but it is expected to greatly expand in the future (Arola 1975, Neill 1976). Leaves could also be used (1) to produce chemicals such as essential oils, chlorophyll-carotene paste, and other pigment concentrates (Keays 1975); (2) to produce fuels, such as ethanol, methanol, and methane; (3) as a source of plant protein either for livestock or for human consumption (Stahmann 1968, Pirie 1971); and (4) to produce muka (Keays and Barton 1975).

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Muka is an animal feed and vitamin supplement made of finely ground leaves, bark, and small branches. Although not presently used in this country, current muka production in the USSR is approximately 100,000 tons per year (Keays 1975). Depending on the amount of branches and bark material in the mix and the tree species, muka can be a satisfactory substitute for an equal weight of alfalfa or clover meal.

The foliage biomass of a stand varies widely (from 5 to 25% of the dry weight of stem wood plus bark) depending on such factors as tree species, stand density, age, and season (Isebrands 1973, Keays 1975). Commercial foliage, a mixture of leaves, small branches, and bark, usually has a biomass double that of foliage alone (Keays 1975). However, all the commercial foliage on the site is not harvested-about 10% of the dry weight of stem wood plus bark is considered economically recoverable. Natural stands yield about 2.2 metric tons (mt)/ha/y of wood or chips when harvested with total tree harvesting methods, or about 0.2 mt/ha/y commercial foliage.

Hardwood plantations grown under short-rotation, intensive-culture systems can yield 11 or more mt/ha/y per year dry weight of wood (Saucier, Clark, and McAlpine 1972; Dawson, Isebrands, and Gordon 1975; DeBell 1975). Actual yield of commercial foliage in stands grown under intensive-culture methods would probably be much higher than that of natural stands because of the greater biomass in leaves and small branches; estimates go as high as 20% or 22 mt/ha after 10 years. In addition, such plantations may be more efficiently harvested than natural stands, thus increasing the yield of commercial foliage.

As part of the information base necessary for the utilization of foliage from trees grown under intensive culture, we determined the major chemical constituents of leaves from 21 *Populus* hybrid clones. In addition, we analyzed the chemical constituents of leaves from bigtooth aspen, trembling aspen, silver maple, and cottonwood for comparison.

METHODS

Plant Material

The 21 *Populus* hybrid clones, green ash, (*Fraxinus pennsylvanica* Marsh.), and silver maple (*Acer saccharinum* L.) were grown in the nursery at Rhinelander, Wisconsin. These 3-year-old trees were part of the maximum fiber yield clonal stocks and spacing trials and were grown under near-optimum fertilizer and water regimes. These clones (table 1) were selected for fast growth, disease resistance, or some other criteria (see Dawson 1974 for the clonal origin). They were derived from parents from several taxonomic sections (Aegeiros, Tacamahaca, Leuce) within the genus *Populus* (Bogdanov 1968, Muhle Larsen 1970). The bigtooth aspen (*Populus grandidentata* (Michx.) and trembling aspen (*Populus tremuloides* Michx. were 2-year-old root sprouts from natural stands. The cottonwood (*Populus deltoides* Bartr.) was grown from seed in a growth room in sand-nutrient culture (see Dickson and Larson 1975 for a description of the growing conditions). Recently matured leaves of all trees were collected in July and freeze-dried. The midveins and petioles were removed, and the remainder was ground to 20 mesh in a Wiley mill and stored in a freezer at -20°C until analyzed.

Table 1.--Source numbering code and description of parentage for the 21 Populus hybrid clones examined in this study

Source number	Parentages
4877	*Populus alba* L.
4879	*Populus* x *euramericana* cv. I-476
4881	*Populus* (unknown)
5258	*Populus* (unknown)
5260	*P. tristis* Fisch. x *P. balsamifera* L. cv. Tristis 1.
5261	*P. deltoides* Bartr. x *P. balsamifera* 'Northwest'
5262	*P.* cv. *Candicans* x *P.* cv. *Berolinensis*
5263	*P.* cv. *Candicans* x *P.* cv. *Berolinensis*
5264	*P.* cv. *Angulata* x *P.* cv. *Plantierensis*
5265	*P.* cv. *Angulata* x *P. trichocarpa* Torr. & Gray
5266	*P.* cv. *Angulata* x *P. trichocarpa*
5267	*P. deltoides* x *P.* cv. *Caudina*
5271	*P.* cv. *Charkowiensis* x *P. deltoides*
5272	*P. nigra* L. x *P. laurifolia* Ledeb.
5273	*P. deltoides*
5324	*Populus* x *euramericana* cv. B-56
5328	*Populus* x *euramericana* cv. I-45/51
5331	*P.* cv. *Betulifolia* x *P. trichocarpa*
5334	*P.* cv. *Angulata* x *P. trichocarpa*
5351	*Populus* spp. (unknown - similar to Tristis)
5377	*Populus* x *euramericana* cv. Wisconsin 5

Laboratory Procedures

Fifty-mg samples of the ground leaf material were analyzed for total N by the Dumas method. Crude protein values were calculated from total N (N x 6.25). Soluble protein was extracted from the leaf material (25 mg) with 0.05 M Tris buffer pH 7.5, precipitated by 10% TCA (equal volumes of Tris extract and 20% trichloroacetic acid), and determined by the Lowry method (Lowry *et al*. 1951). The amino acids and soluble carbohydrates were extracted from the leaf material (50 mg) with methanol:chloroform: water mix (M:C:W, 12:5:3) (Bieleski and Turner 1966) and determined colorimetrically. The amino acids were determined colorimetrically with ninhydrin (Lee and Takahashi 1966); reducing sugars were determined with 3,5-dinitrosalicylic acid (DNS) (Clark 1963); and total soluble sugars were determined with DNS after hydrolysis with 0.1N HCl at 100°C. Total nonstructural carbohydrates were determined with DNS after extraction and hydrolysis with Clarase (R)2/(Smith 1969).

2/ (R) Miles Laboratory Inc., Elkhart, Indiana. The use of trade names is for the information of the reader and does not constitute an official endorsement by the U.S. Department of Agriculture.

Statistical Analysis

All analyses were run in duplicate to detect any errors in sampling or technique. No statistical analyses were run comparing differences between the individual clones or among the clones. The growth-room cottonwood leaf material was used as a standard and was analyzed periodically with the clonal material. Four replications of the cottonwood leaf material for each of the analyses, along with the standard error of the mean, are given in Table 2 to indicate the reliability of the methods. The confidence interval for the analysis of the chemical fractions of cottonwood leaf indicates the sampling error. We would expect similar errors in the analysis of the clones. For example, the 95% confidence interval for the total N determination is 0.84%. This value indicates that total N mean would fall between 4.00-4.84, 95% of the time. Thus, if the total N for two clones differ by more than 0.84% there is probably a real difference in the N content. The error values were determined on four samples from a single day's run. Day-to-day variation would increase the error. However, even a doubling of the given error value would still yield significant differences between many of the clones for all chemical fractions.

Table 2.--Chemical fractions of cottonwood leaf: reproducibility of the analyses and potential error

(In % dry wt.)

Sample No.	Total N	Soluble protein	Soluble amino acids	Reducing sugar	Total soluble sugar	Total nonstructural carbohydrate
1	4.44[a/]	5.53	.333	6.39	12.43	21.37
2	4.65	4.95	.337	6.52	11.91	21.00
3	4.04	5.38	.336	6.60	12.89	21.25
4	4.55	5.92	.337	6.37	12.34	21.26
Mean	4.42	5.44	.336	6.47	12.39	21.22
$s_{\bar{x}}$	.134	.200	.009	.055	.201	.078
($t_{.05}\ s_{\bar{x}}$) (95% confidence interval)	.84	1.28	.06	.34	1.28	.50

a/ Leaf material from plants raised in a growth room under high nitrogen conditions in nutrient culture. The four samples were analyzed as a group. Day-to-day variation in laboratory procedures would increase the standard error somewhat but probably not more than 50%.

RESULTS AND DISCUSSION

Nitrogen Fractions

Total N in the leaves of the *Populus* clones (table 3) ranged from 2.14 to 3.86% dry weight. The average value of total N for the clones (3.12%) was higher than for the aspen sprouts (table 4) and probably reflects the frequent application of fertilizer in the nursery. In trembling aspen the low leaf N concentration (2.01%) indicates a potential N deficiency in the natural stands. Leaves of the silver maple and green ash also contained

Table 3.--Major chemical fractions from leaves of 21 Populus hybrid clones

(In % dry wt.)

Source number	Nitrogen fractions: Total N	Nitrogen fractions: Calculated crude protein a/	Nitrogen fractions: Soluble protein	Nitrogen fractions: Soluble amino acids	Carbohydrate fractions: Reducing sugar	Carbohydrate fractions: Total soluble sugar	Carbohydrate fractions: Total nonstructural carbohydrate
4877	3.06	19.1	4.91	.190	3.57	10.17	16.08
4879	3.07	19.2	3.83	.143	6.59	15.94	21.11
4881	3.00	18.8	3.31	.151	6.26	9.61	16.02
5258	2.93	18.3	3.61	.147	11.55	18.78	23.74
5260	3.59	22.4	2.71	.134	9.91	15.18	21.23
5261	3.80	23.8	4.56	.192	8.39	17.14	20.60
5262	2.91	18.2	4.04	.120	9.43	15.95	22.76
5263	3.86	24.1	3.53	.134	11.07	19.64	24.03
5264	3.55	22.2	4.49	.152	8.34	16.39	23.22
5265	2.66	16.6	2.71	.090	5.64	14.94	20.45
5266	3.00	18.8	2.81	.125	9.15	18.18	24.88
5267	3.74	23.4	2.37	.136	9.15	11.95	17.63
5271	2.41	15.1	0.98	.227	5.42	15.67	19.03
5272	2.91	18.2	2.48	.127	10.74	18.30	24.79
5273	2.73	17.1	3.10	.143	12.27	21.06	26.49
5324	2.14	13.4	2.77	.162	11.40	18.34	25.43
5328	2.46	15.4	2.54	.119	12.42	21.43	25.72
5331	3.77	23.6	4.56	.243	6.60	20.22	23.12
5334	3.03	18.9	3.06	.156	9.53	16.68	22.24
5351	3.44	21.5	4.10	.171	8.71	13.37	17.36
5377	3.49	21.8	5.89	.211	6.79	16.45	21.98
Average	3.12	19.5	3.25	.156	8.71	16.45	21.79

a/ Crude protein, an estimation of total protein in the tissue calculated as %N x 6.25.
Numbers underlined define the range in experimentally determined values.

less total N than the average Populus clone. The low level of N in these fertilized trees may reflect a species difference. However, there were a few Populus clones with lower N content than the maple and ash.

Soluble protein and soluble amino acids also varied among the Populus clones and other trees. These fractions were usually directly correlated with total N in the leaves. For example, the leaves of Clone 5374 contained high levels of total N, soluble protein, aid soluble amino acids. In contrast, Clone 5271 contained low levels of total N and extracted protein but one of the highest levels of soluble amino acids. Many factors, such as stage of maturity, post-harvest treatment, extracting solution composition, temperature, or pH can influence the extraction of leaf protein (Betschart and Kinsella 1973) . Unless the tissue is young and tender, much of the protein in leaf tissue is not extracted with simple buffer solutions. Thus, the calculation of crude protein (%N x 6.25) is perhaps a better indication of the total protein present in the leaf tissue and of the potential for muka production than extracted protein. The average crude protein value for leaves from the Populus clones was 19.5% of dry weight. This value approximates that found in alfalfa, one of the major forage crops produced in the United States. Chopped and dried alfalfa contains about 20% protein, whereas ground and roll-pressed alfalfa meal (some protein and pigments are removed to make a high protein feed supplement) contains about 19% protein based on dry weight of the meal (Kohler and Bickoff 1971). The best Populus clone contained 24% crude protein. Thus there is a possibility of improving the feed value of leaf material through clonal selection.

Carbohydrate Fractions

There were also wide differences in carbohydrate contents among the Populus clones (table 3). In the foliage examined in this study, soluble and storage carbohydrates made up a large part of the total leaf dry weight. Total nonstructural carbohydrate (soluble sugar plus starch) averaged about 22% of the leaf dry weight in the Populus clones. Soluble sugar was the major carbohydrate fraction of the total nonstructural carbohydrates.

In the soluble sugar fraction, reducing sugars and sucrose (the major non-reducing sugar in Populus leaves) were present in about equal proportions. Average concentrations in the clones were 8.7% reducing sugar and 7.7% sucrose (total soluble sugars minus reducing sugars). The estimated starch content was low compared to the other carbohydrate fractions. A low starch content can be expected in Populus because trees of this genus store large amounts of lipids in addition to starch as reserve foods (Zimmermann and Brown 1971).

The percentages of sugars and other carbohydrates in aspen and cottonwood foliage were similar to those in the Populus clonal foliage (table 4); however, the estimated percentage of starch was relatively low in bigtooth aspen and high in cottonwood. The carbohydrate fractions in silver maple were different from those of Populus. In silver maple there was no difference in the analysis for reducing sugar and total soluble sugar; thus all the soluble sugars present were reducing sugars. Silver maple foliage also contained the highest estimated level of starch among the trees analyzed.

Table 4.--A comparison of the major chemical fractions from leaves of Populus hybrid clones and other trees of potential value for intensive culture plantations

(In % dry wt.)

Chemical fractions	Populus clones	Trembling aspen	Bigtooth aspen	Silver maple	Green ash	Eastern cottonwood
Total N	3.12[a]	2.01[b]	2.56	2.43	2.78	4.42
Crude protein (N x 6.25)	19.5	12.6	16.0	15.2	17.4	27.6
Soluble protein	3.25	2.07	3.28	1.36	2.21	5.44
Soluble amino acids	.156	.118	.212	.205	.053	.336
Reducing sugar	8.71	6.03	10.8	10.9	7.76	6.47
Total soluble sugar	16.4	20.0	22.4	10.6	11.5	12.4
Total nonstructural carbohydrates	21.8	-	23.8	20.7	19.0	21.2
Starch (TNC-total sugar)	5.36	-	1.37	10.2	7.47	8.83

a/ Values are the average for all 21 Populus hybrid clones.
b/ Trembling aspen was not analyzed for nonstructural carbohydrates.

CONCLUSIONS

As whole-tree harvesting of natural stands becomes more widespread and the establishment of intensive-culture plantations increases, large volumes of leaves, small branches, and bark will become available. If separations are made in the field, it might be preferable to utilize this material rather than simply returning it to the harvesting site. If nutrient depeletion becomes a problem, it can easily be corrected with fertilization. The chemical analysis of Populus foliage has shown that the nitrogen and nonstructural carbohydrate content is more than adequate for the production of muka, a high quality animal feed supplement. Although not determined in these analyses on Populus foliage, the content of pigments, vitamins, and mineral nutrients would probably also be adequate for muka production or for the production of leaf chemical products. The use of foliage in the production of muka in the tonnages necessary to make unit costs competitive with present day forage crops could release millions of acres of productive farmland to grow food for man. Assuming that the substitution ratio is 1:1, every ton of muka fed to animals could release a ton of grain.

Chemical properties of foliage such as nitrogen content can be expected to vary depending on fertility of the site and other environmental factors. Nevertheless, certain hybrids examined in this study combined a variety of good clonal characteristics for fiber production with leaf chemical characteristics desirable for muka production (table 5). For example, the P. nigra hybrid (5331) combined rapid growth rate, high specific gravity, low lignin, and low extractive content of the wood (Dickson, Larson, and Isebrands 1974) with high leaf rust resistance (Dawson 1974). In addition, the foliage contained high levels of nitrogen, protein, and total nonstructural carbohydrates. Clones used for intensive-culture plantations must be selected initially for rapid growth rate and disease resistance. As more information becomes available, clones can be further improved by either selecting or breeding for desirable wood and leaf chemical characteristics. In this way, a given clone could be tailored for specific end products at harvest. Such clones could help make intensive-culture plantations and complete tree utilization methods even more profitable.

Table 5.--Leaf chemical fractions of four Populus hybrid clones with potential for "muka" production

(In % dry wt.)

Chemical fractions	P. Candicans x P. berolinensis (5263)	P. Betulifolia x P. trichocarpa (5331)	P. euramericana (cv. Wisconsin 5) (5377)	Unknown[a] (5351)
Total N	3.9	3.8	3.5	3.4
Crude protein (N x 6.25)	24.1	23.6	21.8	21.5
Extracted protein	3.5	4.6	5.9	4.1
Soluble amino acids	0.13	0.24	0.21	0.17
Reducing sugars	11.1	6.6	6.8	8.7
Total soluble sugar	19.6	20.2	16.4	13.4
Total nonstructural carbohydrates (TNC)	24.0	23.1	22.0	17.4
Starch (TNC-total sugar)	4.4	2.9	5.5	4.0

a/ Populus sp. Parentage unknown, but it contains some P. balsamifera.

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