Diagnostic tools to evaluate the foliar nutrition and growth of hybrid poplars

Arlette Lteif, Joann K. Whalen, Robert L. Bradley, and Claude Camiré

Abstract: This 2-year study examined the effect of fertilizers on tree growth and foliar nutrition in a *Populus trichocarpa* Torr. & A. Gray × *Populus deltoides* Bartr. ex Marsh. plantation located in southwestern Québec. The treatments included a control that did not receive N or P fertilizer, inorganic NP fertilizers, organic fertilizers applied at 65–70 kg N·ha^{-1}, and organic fertilizers applied at 130–140 kg N·ha^{-1}. Fertilized trees were taller and had larger diameters than control trees. Three methods were used to diagnose limiting nutrients and nutrient imbalances, and compare the nutrient supply from different fertilizer sources. The critical value approach and the compositional nutrient diagnosis methods found below-optimum N and P concentrations, sufficient K and Mg concentrations, and an excessive Ca concentration in foliage. Vector analysis compared the N nutrition in foliage from fertilized trees and the control trees. The compositional nutrient diagnosis r^2 (nutrient imbalance index) was negatively correlated with annual tree growth in height (r = –0.46, P < 0.05) and diameter (r = –0.59, P < 0.05), meaning that trees with a greater nutrient imbalance grew less in height and diameter than trees with balanced foliar nutrition. Of these diagnostic methods, compositional nutrient diagnosis holds promise for identifying nutrient limitations and predicting growth responses to fertilization in hybrid poplar plantations.

Résumé : Cette étude, d'une durée de 2 ans, a porté sur l'effet des fertilisants sur la croissance des arbres et la nutrition foliaire dans une plantation de *Populus trichocarpa* Torr. & A. Gray × *Populus deltoides* Bartr. ex Marsh. située dans le sud-ouest du Québec. Les traitements incluaient un témoin qui n'a pas reçu de fertilisant contenant du N ou du P, des fertilisants inorganiques à base de N et P, des fertilisants organiques appliqués à raison de 65–70 kg N·ha^{-1} et de 130–140 kg N·ha^{-1} Les arbres fertilisés étaient plus hauts et avaient un plus gros diamètre que les arbres témoins. Trois méthodes ont été utilisées pour diagnostiquer les carences et les déséquilibres nutritifs et comparer l'apport de nutriments provenant de différentes sources de fertilisant. Selon l'approche de la valeur critique et la méthode du diagnostic de composition nutritive, les concentrations de N et P étaient sous-optimales, les concentrations de K et Mg étaient adéquates et la concentration de Ca était en excès dans le feuillage. L'analyse vectorielle a été utilisée pour comparer la nutrition foliaire en N des arbres fertilisés et des arbres témoins. Le r^2 du diagnostic de composition nutritive (indice de déséquilibre nutritif) était négativement corrélé avec la croissance annuelle en hauteur (r = –0,46, P < 0,05) et en diamètre (r = –0,59, P < 0,05), ce qui signifie que les arbres avec un plus grand déséquilibre nutritif croissaient moins en hauteur et en diamètre que les arbres avec une nutrition foliaire équilibrée. Parmi ces méthodes diagnostiques, le diagnostic de composition nutritive est une méthode prometteuse pour identifier les carences nutritives et prédire les réactions en croissance à la fertilisation dans les plantations de peupliers hybrides.

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Introduction

In Canada, 14 300 ha of short-rotation forests have been planted with hybrid poplar, a fast-growing species that is harvested for bioenergy, fiber, and wood products. More than 20% of these hybrid poplar plantations are in Quebec, replanted in forest clearcuts and established on marginal agricultural land (Labrecque and Teodorescu 2003). Sites with low inherent soil fertility require fertilization, especially with N, to achieve optimal hybrid poplar growth (Heilman et al. 1996; Dickmann et al. 2001). Although the growth response of hybrid poplar to inorganic fertilizers (IF) is well known (Brown and van den Driessche 2002; Cooke et al. 2005), there is less information on using organic fertilizers (OF) to supply N and other nutrients for hybrid poplars.

Our previous work (Lteif et al. 2007) demonstrated that a hybrid poplar plantation established on marginal agricultural land in Quebec responded favorably to applications of papermill biosolids and liquid pig slurry, with a greater gain in biomass when these OFs were applied together rather than separately. Hybrid poplars produced between 1 and 4 m^3·ha^{-1}·year^{-1} of leafless aboveground biomass at this site, which is lower than the expected biomass gain of 8–15 m^3·ha^{-1}·year^{-1} expected for hybrid poplars growing in Quebec (Réseau Ligniculture Québec 2007). This could be related to the growth potential of the clone as well as edaphic and climatic stresses at the study site (Van Oosten 2000), but a greater biomass increment in fertilized than in

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Table 1. Estimated N, P_2O_5, and K_2O inputs and fresh mass of papermill biosolids and liquid pig slurry applied to a hybrid poplar plantation (St. Camille, Quebec) in May 2004 and May 2005.

Treatment	Abbreviation	Estimated $N:P_2O_5:K_2O$ input (kg nutrient·ha^{-1}·year^{-1})*	Biosolids (Mg·ha^{-1})	Pig slurry (Mg·ha^{-1})
Unfertilized control	CTRL	0:0:30	0	0
Inorganic fertilizer†	IF	35:30:30	0	0
100% biosolids‡	B100 (1×)	65:17:26	39	0
66% biosolids, 33% pig slurry	B66P33 (1×)	66:12:30	26	7
33% biosolids, 66% pig slurry	B33P66 (1×)	66:7:33	12	14
100% pig slurry§	P100 (1×)	70:3:37	0	21
100% biosolids	B100 (2×)	130:33:53	78	0
66% biosolids, 33% pig slurry	B66P33 (2×)	132:24:59	52	14
33% biosolids, 66% pig slurry	B33P66 (2×)	132:15:65	24	28
100% pig slurry	P100 (2×)	140:7:73	0	42

*Estimated N, P_2O_5, and K_2O input from each treatment based on the inorganic fertilizer equivalency (IFE) of each fertilizer source (CPVQ 2000; CRAAQ 2003).

†Recommended application rate for field-grown deciduous trees in Quebec (CPVQ 2000).

‡On a wet mass basis, biosolids contained 5.7 g N·kg^{-1} (30% IFE), 0.55 g P_2O_5·kg^{-1} (80% IFE), and 0.70 g K_2O·kg^{-1} (100% IFE).

§On a wet mass basis, pig slurry contained 3.2 g N·kg^{-1} (60% IFE), 0.20 g P_2O_5·kg^{-1} (80% IFE), and 2.1 g K_2O·kg^{-1} (100% IFE).

unfertilized plots indicated that nutrient supply was an important factor controlling tree growth (Lteif et al. 2007). Interpretation of foliar analysis could indicate whether nutrient concentrations were sufficient for metabolic requirements, diagnose limiting nutrients and nutrient imbalances, and compare the nutrient supply from different fertilizer sources.

There are several diagnostic tools that can be used to interpret foliar analysis. The critical value approach (CVA) provides information on the deficient, sufficient, and excessive nutrient concentrations in foliage at specific growth stages. The CVA method can verify the accuracy of fertilization practices, detect nutrient deficiencies before visual symptoms are observed, and indicate the yield potential of agricultural crops and fruit trees (Ulrich and Hills 1967; Benton Jones et al. 1991). Sufficient and optimal foliar nutrient concentrations for *Populus* spp. and hybrid poplar clones were proposed by Camiré and Brazeau (1998) based on a review of the scientific literature. Another diagnostic tool is compositional nutrient diagnosis (CND), a multivariate method that calculates the optimum foliar nutrient balance and ranks nutrients from most to least limiting of plant performance. The CND method has been used to diagnose nutrient limitations in agricultural crops (Aitchison 1986; Parent and Dafir 1992) and a few tree species (Parent et al. 1995; Schleppi et al. 2000). The CND indices for hybrid poplar were calculated by W. René et al. (unpublished data) using foliar analyses and growth of high-performing clones planted in southern Quebec. Finally, vector analysis (VA) is a method that evaluates foliar nutrition of trees in response to experimental treatments, such as fertilizer application, and tracks nutrient uptake in foliage during tree development (Timmer 1985; Teng and Timmer 1990). As far as we know, the foliar N status of hybrid poplars has not been evaluated using the VA method. Each of these diagnostic tools provides slightly different information, but we hypothesized that CND method would most clearly diagnose nutrient limitations and predict tree growth in response to OF applications because it calculates multivariate nutrient ratios and represents the overall nutrient balance in foliage (Aitchison 1986).

The objective of this study was to evaluate three foliar diagnostic tools that could provide an indication of the foliar nutrition and growth of hybrid poplar.

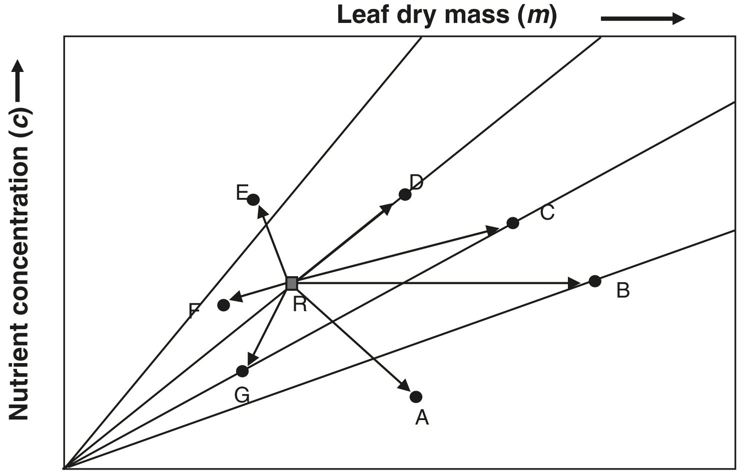
Materials and methods

Study site and experimental design

The study site and experimental design were described by Lteif et al. (2007). Briefly, the site was a hybrid poplar plantation (*Populus trichocarpa* Torr. & A. Gray × *Populus deltoides* Bartr. ex Marsh. clone 3225) established in May 2001 by planting 1-year-old bare root seedlings in an unimproved hayfield near the town of St. Camille, Quebec (45°40′36″N, 71°44′13″W). Average daily temperature at the nearby St. Camille climate station ranges from –13.4 °C in January to 17.2 °C in July, with mean annual precipitation of 1191 mm. Trees were planted in rows at 3 m intervals. During this study, vegetation in the 3 m space between tree rows was controlled by mowing twice each summer. The site was virtually flat (slope of less than 1%) and soils were fairly homogeneous based on analysis of 40 soil samples collected from a 0.5 ha area at the site prior to this experiment. The soil was a clayey-loam, imperfectly drained Magog stony loam (Orthic Gleysol) containing 39% sand, 32% silt, and 29% clay. On average (±SD), soils had 35 ± 1 g organic C·kg^{-1}, 2.5 ± 0.1 g total N·kg^{-1}, and pH 5.6 ± 0.1 in the surface 0–15 cm. The Mehlich-3 extractable nutrient concentrations were 19 ± 1 mg P·kg^{-1} and 45 ± 1 mg K·kg^{-1}, indicating low soil fertility.

The experiment used a randomized complete block design (four blocks), with 10 plots per block, for a total of 40 experimental plots. Each plot (100 m^2) contained 16 trees. There were 10 treatments including a control (i.e., no fertilizer N or P applied) and one IF treatment and eight OF treatments consisting of papermill biosolids and pig slurry applied singly or in combination. As seen in Table 1, the IF treatment was applied at the rate recommended for field-grown deciduous trees (Conseil des productions végétales du Québec (CPVQ) 2000) and the N, P_2O_5, and K_2O input from OFs was estimated from the IF equivalency factors

Fig. 1. Interpretation of directional shifts in nutrient concentration, nutrient content, and dry mass (adapted from Imo and Timmer 1997).



Vector direction	Change in relative			Nutritional effect	Possible diagnosis
	m	*c*	*n*		
A	+	–	+	Dilution	Growth dilution
B	+	0	+	Sufficiency	Steady-state
C	+	+	+	Deficiency	Limiting
D	0	+	+	Luxury consumption	Accumulation
E	–	++	+/–	Excess	Toxic accumulation
F	–	–	–	Excess	Antagonistic
G	0/+	–	–	Depletion	Retranslocation

and loss coefficients used in Quebec's agricultural soils (CPVQ 2000; Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ) 2003). One of the challenges in using OFs to fertilize hybrid poplars and other plants is that nutrient concentrations can vary substantially from published values depending on storage and handling methods. In this study, we had a precise analysis of the N, P_2O_5, and K_2O concentrations in OFs at the time these materials were spread on the field. All plots received potash fertilizer at a rate of 30 kg $K_2O \cdot ha^{-1} \cdot year^{-1}$ due to the low extractable K concentration at the site. Fertilizers were applied by hand around the four trees in the middle of the plot (<1 m from the trunk) in mid-May 2004, when trees were 4 years old, and mid-May 2005 (trees were 5 years old). In both years, fertilizers were left on the soil surface (unincorporated) to avoid damaging tree roots with tillage implements.

Tree measurements and foliar analysis

Tree measurements taken in spring 2004 at leaf emergence, before fertilizer applications, represented the size of 3-year-old hybrid poplars (F0 year). The four trees in the middle of each experimental plot were also measured before leaf senescence in September 2004 (F1 year) and October 2005 (F2 year). Tree height (*H*, m) was measured with an 8 m digital measuring rod and diameter (*D*, cm) at breast height (1.3 m from the ground) was measured with a diameter tape. The total volume outside bark (TVOB, cm^3) was calculated according to Krinard (1988):

$$[1] \qquad \mathrm{TVOB} = 1700 + (3.15 \times 10^{-3} \times D^2 \times H)$$

Annual growth increments (AI) in height ($cm \cdot year^{-1}$) and diameter ($cm \cdot year^{-1}$) were calculated between the F0 and F1 years and between the F1 and F2 years.

Foliage samples taken prior to leaf senescence in October 2003 (F0 year) revealed that poplar nutrition was homogeneous at the site prior to the application of fertilizers. On average (±SD), leaves contained 1.3 ± 0.07% N, 0.2 ± 0.01% P, 0.5 ± 0.04% K, 2.2 ± 0.18% Ca, and 0.5 ± 0.03% Mg. For consistency, we collected leaves in September 2004 (F1 year) and October 2005 (F2 year). All foliage samples included 10 leaves with petioles collected from two opposing branches (five leaves per branch) at the apex from each of the four trees in the middle of the experimental plot. Leaves were rinsed in distilled water, oven dried (60 °C for

Table 2. Growth parameters for 3-year-old hybrid poplars before fertilization (F0 year), 4-year-old hybrid poplars receiving one fertilizer application (F1 year), and 5-year-old hybrid poplars receiving two annual fertilizer applications (F2 year).

		Height (cm)		Diameter at breast height (cm)		Volume (cm^3)	
Year	Plots	Mean ± SE	Range (max.–min.)	Mean ± SE	Range (max.–min.)	Mean ± SE	Range (max.–min.)
F0	All	206±11	247–156	1.5±0.1	2.1–0.9	1862±35	2020–1740
F1	Control	299±31	415–182	2.0±0.4	3.8–0.8	2165±224	3591–1740
	Fertilized	361±24	466–104	2.8±0.3	5.1–1.0	2690±282	5435–1767
F2	Control	390±42	532–216	3.1±0.6	5.9–1.1	3156±673	7545–1781
	Fertilized	500±31	647–284	4.8±0.6	8.3–1.8	5934±1122	15771–1990

Note: The hybrid poplar plantation was located in St. Camille, Quebec. Values are the means ± SE and maximum and minimum of four trees measured from each plot (all, n = 160), four trees from each of the control plots (n = 16), and four trees from each plot receiving fertilizer (n = 154).

Table 3. Annual increment in hybrid poplar growth between the F0 and F1 years and the F1 to F2 years.

	F0 to F1		F1 to F2	
Treatment	Height (cm)	Diameter (cm)	Height (cm)	Diameter (cm)
CTRL (0×)	91.1±19d	0.6±0.2d	91.2±10b	1.1±0.2c
IF (1×)	130±10cd	0.9±0.1cd	126±6.1ab	1.6±0.1bc
B100 (1×)	165±9.2abc	1.2±0.1abc	117±7.7ab	1.9±0.1ab
B66P33 (1×)	164±6.8abc	1.4±0.1abc	143±5.2a	2.2±0.1ab
B33P66 (1×)	162±11abc	1.3±0.2abc	148±13a	2.1±0.2ab
P100 (1×)	136±5.4bcd	1.0±0.1bcd	139±10a	1.8±0.1abc
B100 (2×)	151±12abc	1.4±0.1ab	123±2.5ab	1.9±0.2ab
B66P33 (2×)	181±6.2ab	1.7±0.1a	155±9.2a	2.5±0.1a
B33P66 (2×)	192±8.7a	1.7±0.1a	155±6.7a	2.7±0.2a
P100 (2×)	142±8.6bc	1.2±0.2abc	141±14a	2.2±0.3ab
Contrast analysis				
CTRL vs. OF	$P < 0.001$	$P < 0.001$	<0.001	$P < 0.001$
IF vs. OF (1×)	ns	$P = 0.018$	ns	ns
IF vs. OF (2×)	$P = 0.023$	$P < 0.001$	ns	$P = 0.003$
Mix vs. single[a]	$P = 0.004$	$P = 0.038$	$P = 0.021$	$P = 0.014$

Note: The hybrid poplar plantation was located in St. Camille, Quebec. Treatments are described in Table 1. Values are the mean ± SE of four experimental plots. Within a column, significantly different treatment means are indicated by different letters (Scheffe test, $P < 0.05$); ns, not significant ($P > 0.05$).

[a]Mix, combinations of biosolids and pig slurry; single, biosolids or pig slurry alone.

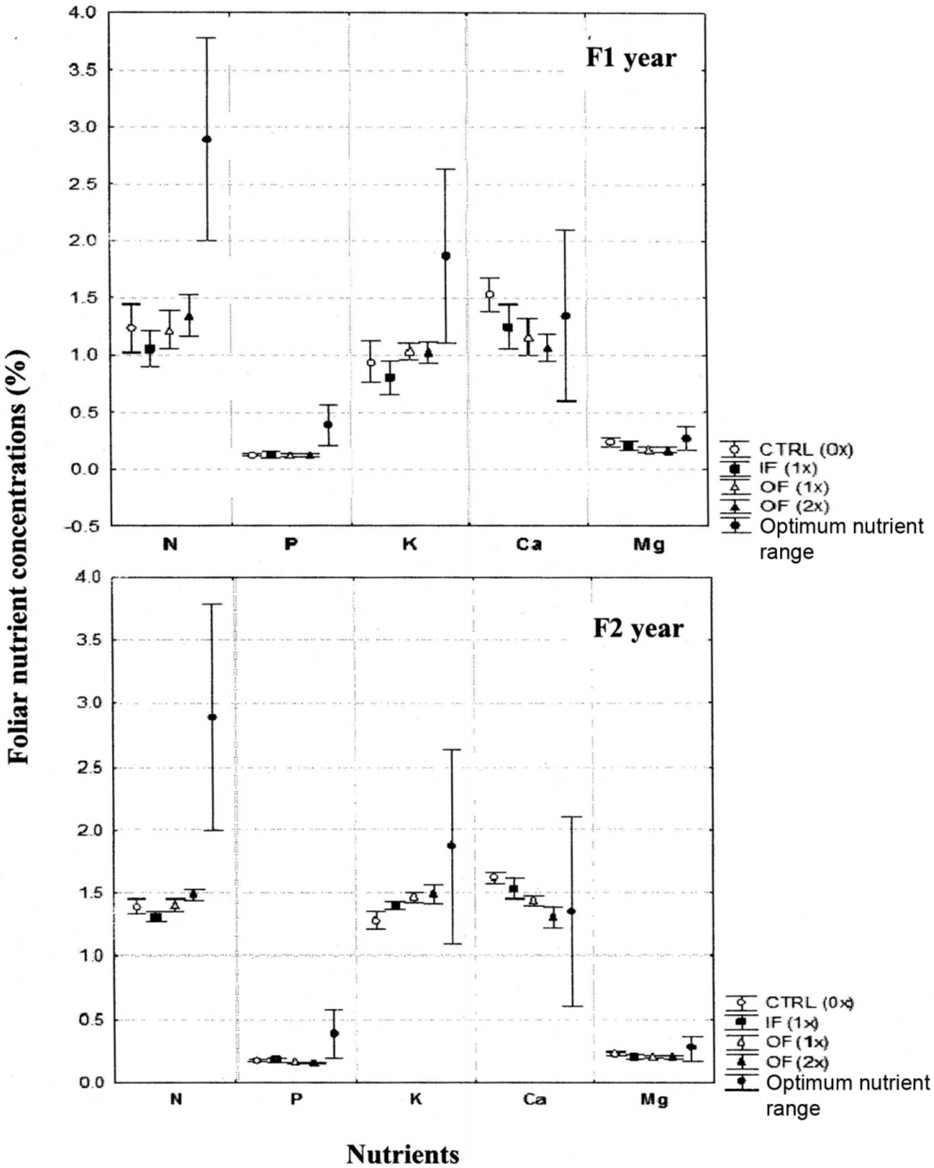
48 h), and weighed individually and then the mean leaf biomass for each plot was calculated (n = 40). Leaves were then combined and ground (<1 mm mesh) to make one composite sample per plot and digested with H_2SO_4 and H_2O_2 following the method of Parkinson and Allen (1975). Digests were analyzed colorimetrically for N and P using flow-injection analysis (Quick-Chem 4000, Lachat Instruments, Milwaukee, Wisconsin) and for K, Ca, and Mg using atomic absorption spectrometry. Foliar N content (mg) in each treatment was calculated as N concentration ($mg \cdot g^{-1}$) in composite sample × leaf dry mass (g).

Foliar nutrition diagnoses with CVA, CND, and VA methods

The CVA method was used to compare the mean nutrient concentrations in foliage from each treatment (F1 and F2 years) with literature values. The optimal nutrient ranges for N, P, K, Ca, and Mg in hybrid poplar foliage came from values reported for clone D38 of *P. deltoides* (Leech and Kim 1981) and for 1- to 10-year-old plantations of various *Populus* spp. (Bonneau 1988; Ericsson et al. 1992).

Mean nutrient concentrations (N, P, K, Ca, and Mg) in foliage from each treatment (F1 and F2 years) were used to calculate CND nutrient indices (I_N, I_P, I_K, I_{Ca}, and I_{Mg}) and CND nutrient imbalance index (CND r^2). The CND method described by Parent and Dafir (1992) was calibrated for hybrid poplar by W. René et al. (unpublished data) using foliar nutrient concentrations from high-yielding subpopulations of *Populus nigra* L. × *Populus maximowiczii* Henry clone 3729, *P. maximowiczii* × *Populus balsamifera* L. clone 915303, and *Populus euramericana* (Dode) Guinier × *P. maximowiczii* clone 915508 hybrid poplar plantations in southern Quebec. The CND nutrient indices reveal the order of limiting nutrients and the magnitude of nutrient limitation in each treatment relative to the optimum range. Negative CND indices represent nutrient limitation and the most negative value indicates the most limiting nutrient. Positive CND indices are an indication of nutrient excess and the largest value indicates the nutrient present in greatest excess. The CND r^2 is calculated from the multivariate analysis of nutrient ratios compared with the optimum nutrient ratios. When the CND r^2 is close to zero, nutrient requirements are balanced and maximum plant growth is expected (Aitchison 1986; Parent et al. 1994).

Fig. 2. Nutrient concentrations in foliage collected from 4-year-old hybrid poplars (F1) and 5-year-old hybrid poplars receiving fertilizer treatments in St. Camille, Quebec, compared with the optimum nutrient range for *Populus* spp. Data points are the mean and SE of the control (CTRL) treatment, the inorganic fertilizer (IF) treatment, the pooled organic fertilizer (OF) treatments at the 1× rate, and the pooled OF treatments at the 2× rate described in Table 1.



The VA method is a comparative technique and diagnosis is independent of preestablished optimal ranges or ratios (Timmer 1991; Swift and Brockley 1994). Briefly, three variables (nutrient concentration, nutrient content, and biomass of leaves or other plant components) are used as the axes for the vector diagram (Haase and Rose 1995). Relative values are standardized in relation to a control, and vectors are drawn to join the normalized control to data points representing experimental treatments. One limitation of the VA method is that foliar nutrition will be affected by the number of leaves produced during a growing season. Since fertilization in the F1 year could affect the number of leaves produced in the second year of the study, the VA method was used to examine the foliar N response to fertilization in the F1 year only. Foliage biomass, N content, and N concentration were expressed relative to the control and then relative values were standardized to 100 and plotted along with the normalized control (Timmer 1991; Haase and Rose 1995). Nutritional diagnosis was determined from the arrow direction and magnitude of each vector based on the vector interpretation diagram (Fig. 1; adapted from Imo and Timmer 1997). The VA method can thus distinguish nutrient dilution effects from nutrient deficiencies and sufficient or excessive nutrient supplies (Fig. 1).

Statistical analyses

Prior to analysis, the data were tested for normality using the Kolmogorov–Smirnov test and were subsequently $\log_e$ or square root transformed when required to adjust for normality and stabilize variance. To remove the confounding effect of preexisting growth differences among the experimental trees, data were analyzed by one-way ANCOVA in a gen-

Table 4. Nutrient indices and the nutrient imbalance index (CND r^2) for a hybrid plantation in St. Camille, Quebec, compared with the CND norms calculated for hybrid poplar clones grown in southern Quebec (W. René et al., unpublished data).

	CND nutrient index						
Treatment	I_N	I_P	I_K	I_{Ca}	I_{Mg}	CND r^2	Order of nutrient limitations
F1 year							
CTRL (0×)	–3.41	–2.72	–0.39	4.76	0.50	43.4	N < P < K < Mg < Ca
IF (1×)	–3.68	–2.10	–0.66	4.26	0.43	43.3	N < P < K < Mg < Ca
B100 (1×)	–2.57	–2.44	0.64	3.93	–0.69	33.7	N < P < Mg < K < Ca
B66P33 (1×)	–2.85	–2.38	–0.01	4.17	–0.19	35.3	N < P < Mg < K < Ca
B33P66 (1×)	–3.10	–2.06	0.54	3.52	–0.16	30.6	N < P < Mg < K < Ca
P100 (1×)	–3.43	–2.92	0.92	3.62	–0.01	43.2	N < P < Mg < K < Ca
B100 (2×)	–1.87	–2.03	0.58	3.00	–0.60	20.8	P < N < Mg < K < Ca
B66P33 (2×)	–2.07	–1.86	–0.13	3.52	–0.39	23.1	N < P < Mg < K < Ca
B33P66 (2×)	–2.69	–2.13	0.53	3.25	–0.38	29.9	N < P < Mg < K < Ca
P100 (2×)	–3.29	–3.40	0.98	3.77	0.03	47.0	P < N < Mg < K < Ca
F2 year							
CTRL (0×)	–3.44	–1.71	0.54	4.42	–0.08	34.9	N < P < Mg < K < Ca
IF (1×)	–3.68	–1.49	1.07	4.22	–0.38	35.0	N < P < Mg < K < Ca
B100 (1×)	–3.01	–2.32	1.16	4.25	–0.42	34.1	N < P < Mg < K < Ca
B66P33 (1×)	–3.66	–1.99	1.24	4.27	–0.30	37.2	N < P < Mg < K < Ca
B33P66 (1×)	–3.06	–1.85	1.37	3.53	–0.25	27.3	N < P < Mg < K < Ca
P100 (1×)	–3.18	–2.38	1.74	3.85	–0.45	33.8	N < P < Mg < K < Ca
B100 (2x)	–2.90	–2.41	1.29	3.51	–0.12	28.4	N < P < Mg < K < Ca
B66P33 (2×)	–2.81	–2.21	1.08	4.02	–0.45	30.3	N < P < Mg < K < Ca
B33P66 (2×)	–2.71	–2.06	1.66	3.38	–0.55	26.1	N < P < Mg < K < Ca
P100 (2×)	–2.92	–2.03	1.95	3.26	–0.43	27.4	N < P < Mg < K < Ca
Optimum range	–1.26 to 1.26	–1.82 to 1.03	–1.73 to 1.60	–0.80 to 1.64	–2.67 to 0.50		

Note: Nutrient indices were calculated for 4-year-old hybrid poplars that received one fertilizer application (F1) and 5-year-old hybrid poplars that received two annual fertilizer applications (F2).

eral linear model. The effect of fertilizer N treatments on AI in tree height and diameter were evaluated using a Scheffe multiple comparisons test. Preplanned comparisons between fertilizer treatments (control versus IF and OF sources, mixed OF versus single OF sources) were evaluated with single degree of freedom orthogonal contrasts. Pearson coefficients were used to measure the correlation between the CND r^2 and the AI in tree growth. All statistical tests were performed using SAS statistical software (version 9.1) (SAS Institute Inc., Cary, North Carolina) and an alpha level of 0.05. Values in tables and graphs are untransformed means ± SEs.

Results

Hybrid poplar growth

Table 2 summarizes tree growth parameters of 3- to 5-year-old hybrid poplars, which were not reported previously by Lteif et al. (2007). One-year-old bare root seedlings were about 1.5 m tall at planting and grew to 2.1 m, on average, by the F0 year (Table 2). Five-year-old trees that were not fertilized reached 3.9 m, while fertilized trees were about 5.0 m tall by the F2 year (Table 2). On average, fertilized trees were taller with a larger diameter at breast height and volume than unfertilized trees in the F1 and F2 years (Table 2).

Examining the fertilizer sources applied to hybrid poplars revealed that the AI in height and diameter were significantly ($P < 0.05$) greater in plots that received the OF treatments than in the control (Table 3). Orthogonal contrasts showed that AI in diameter was greater in the OF (1×) treatment than in the IF treatment in the F1 year and greater in the OF (2×) treatment than in the IF treatment in both years (Table 3). The AIs in height and diameter were always greater when trees received a mixture of biosolids and pig slurry than when trees were fertilized with either biosolids or pig slurry alone (Table 3).

Foliar nutrient diagnosis

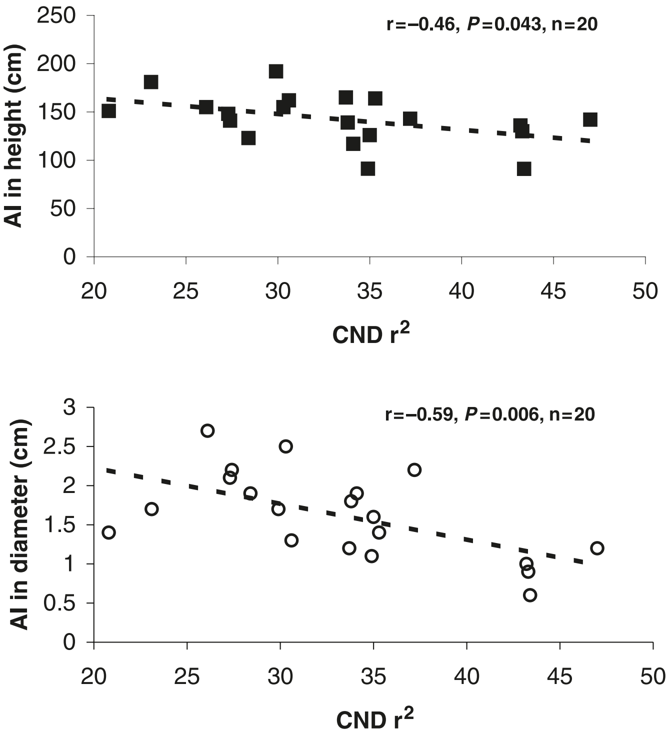
CVA method

There was no difference ($P > 0.05$) in the nutrient concentration of foliage from trees that received OF (1×) rates of 65–70 kg N·ha^{-1}·year^{-1} or among the OF (2×) treatments applied at 130–140 kg N·ha^{-1}·year^{-1}, so data were pooled and presented as the mean of the OF (1×) and the OF (2×) treatments for simplicity (Fig. 2). Foliar N and P concentrations in all treatments were below the optimal ranges for *Populus* spp. during the F1 and F2 years (Fig. 2). In the F1 year, the foliar K concentration was less than the optimal range in the IF and control treatments (Fig. 2). However, the foliar K concentration in the F2 year and the foliar Ca and Mg concentrations in both years were within the optimal range (Fig. 2).

CND method

The negative I_N and I_P indices indicated that N and P

Fig. 3. Pearson correlation coefficients (r) for the average nutrient imbalance index (CND r^2) and the average annual increment (AI) in height and diameter of 4- and 5-year-old hybrid poplars (F1 and F2 years, n = 20) in St. Camille, Quebec. Theoretically, tree nutrition is balanced when the CND r^2 value equals zero. The correlation is illustrated with a dashed trendline.



were limiting nutrients in all treatments (Table 4). In contrast, the I_K and I_{Mg} indices were close to zero, which suggests sufficient K and Mg concentrations in foliage, and I_{Ca} indicates a Ca concentration in excess of the optimal range (Table 4). The CND r^2 was greater than zero in all treatments and both study years, indicating a nutrient imbalance in foliage collected from hybrid poplars at the study site (Table 4). Treatments with values closest to zero were the B100 (2×) treatment in the F1 year and the B33P66 (2×) treatment in the F2 year (Table 4). The CND r^2 values were negatively correlated with the AI in height and diameter for each treatment, indicating that hybrid poplars were taller and had a larger diameter when the CND r^2 was closer to zero, i.e., nutrient requirements were balanced (Fig. 3).

VA method

Vectors from the reference point (control) to the other points represent the changes in N concentration, N content, and foliage dry mass after 1 year (F1) of fertilization. The vector orientation showed an increase in N content and foliar dry mass of fertilized trees compared with the control trees (Fig. 4). The B33P66 (2×) treatment had sufficient N nutrition, while N dilution was observed in the following treatments: IF, P100 (1×), P100 (2×), and B33P66 (1×) (Fig. 4). Foliage from the control trees was N deficient compared with trees receiving the 1× and 2× rates of B66P33 and B100, and the control trees were more N deficient than the 2× treatments, as indicated by the vector magnitude (Fig. 4). The B66P33 and B100 treatments produced more foliar biomass and had a greater foliar N content than treatments that were diagnosed as having N dilution (Fig. 4).

Discussion

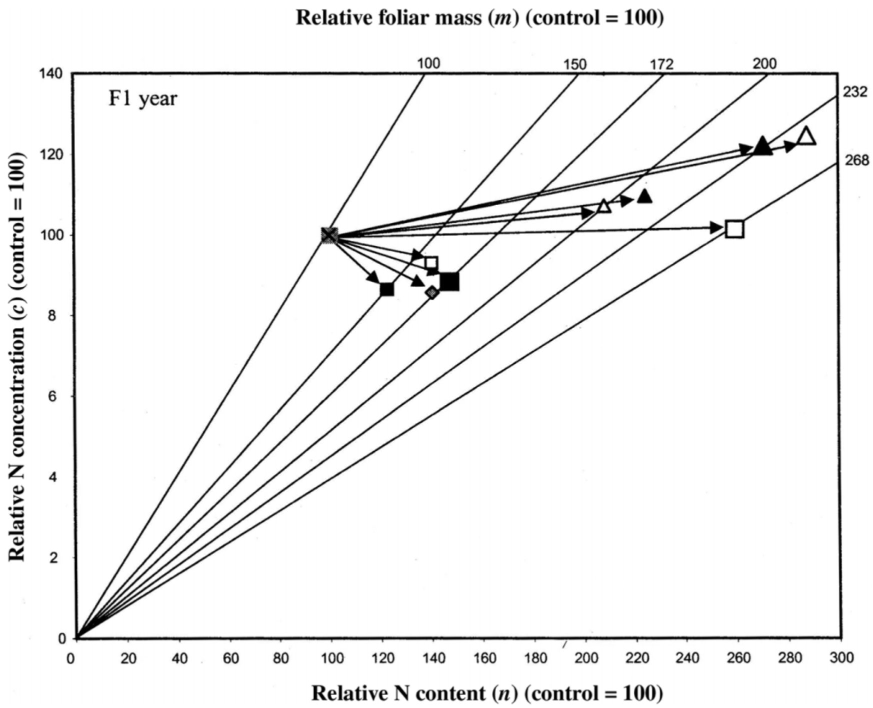
Lteif et al. (2007) reported that the leafless aboveground biomass of hybrid poplars was greater in plots that received OF than the IF and control treatments and that mixed OF produced greater biomass than single OF sources. While OF applications increased the incremental gain in height and diameter of hybrid poplars, these were less than the incremental gains reported in other fertilized *P. trichocarpa* × *P. deltoides* hybrid poplars (Heilman and Xie 1994) and expected for hybrid poplar plantations in Quebec (Réseau Ligniculture Québec 2007). Foliar analysis is a means of assessing whether the apparent suboptimal growth in our plantation was related to nutritional deficiencies that persisted even after fertilization.

CVA method

The optimal nutrient range for N, P, K, Ca, and Mg in foliage from hybrid poplars was based on published values for *Populus* spp. Regardless of the fertilizer treatment, foliar N concentrations were below the optimal range and were also lower than the N concentrations reported for *P. trichocarpa* × *P. deltoides* and *P. trichocarpa* × *P. euramericana* hybrid poplar clones by Heilman and Xie (1993) and Ripullone et al. (2004). One factor that may have contributed to the relatively low foliar N concentrations is the relatively late sampling date brought on by logistical constraints. Up to 70% of leaf N can be reabsorbed from the senescing leaves of hardwoods to be transported and stored in the secondary woody tissues (stem and roots) prior to leaf abscission (Drossopoulos et al. 1996). For example, Heilman (1985) noted a decline in the foliar N concentration of *P. trichocarpa* and *P. trichocarpa* × *P. deltoides* hybrid poplars after mid-August. We found that foliar P concentrations at the study site were also below the expected range. In walnut trees, Drossopoulos et al. (1996) found that leaf P concentrations do not decline as rapidly as leaf N concentrations at abscission; we assume that hybrid poplars display a similar trend. Therefore, the P input from fertilizer treatments (3–33 kg P_2O_5·ha^{-1}·year^{-1}) seems insufficient to meet the requirements of hybrid poplar according to the CVA results. The foliar K concentration was below the expected range in some treatments at the end of the first year, but not the second year, suggesting that the application of 30 kg K_2O·ha^{-1}·year^{-1} to all treatments was sufficient for this hybrid poplar plantation. Foliar Mg and Ca concentrations were within the expected ranges, so fertilization with Mg and Ca would not be recommended at this particular site.

There are two difficulties in diagnosing foliar nutrition using the CVA method. First, this univariate approach does not consider nutrient interactions. For instance, N deficiency may reduce the uptake and translocation of some, but not all, nutrients during the growing season (Benton Jones et al. 1991). Second, it was not clear from the literature when foliage should be sampled from *Populus* spp., although the foliar nutrient concentrations from experimental trees and optimal nutrient ranges should be compared at the same

Fig. 4. Relative mean responses in N and foliar dry mass of 4-year-old hybrid poplars in St. Camille, Quebec, receiving inorganic and organic fertilizers (treatments described in Table 1). Small symbols represent the 1× rate and large symbols represent the 2× rate. The control was normalized to 100, and arrow direction reflects change in foliar nutrition due to fertilizer treatments.Open triangles, B100; solid triangles, B66P33; open squares, B33P66; solid squares, P100; diamonds, IF.



growth stage (Sumner 1977). Thus, it is important to report the sampling time or growth stage when foliage is collected.

CND method

Nutrient limitations revealed by the CND method were consistent with results from the CVA method. The I_N and I_P indices were below the optimum range, while the I_K and I_{Mg} indices were within the optimum range. Although the foliar Ca concentrations were within the optimal range for *Populus* spp. according to the CVA method, the I_{Ca} index exceeded the optimum Ca range for hybrid poplars reported by W. René et al. (unpublished data). A larger data set of foliar nutrient concentrations for hybrid poplars would help to resolve this discrepancy. The nutrient imbalances that we observed seemed to be related to a limited supply of N and P as well as an excess of Ca in foliage. The theoretical basis of the CND method is that plants with balanced foliar nutrition (i.e., CND r^2 close to zero) will have a greater biomass and yield than plants with a foliar nutrient imbalance (Aitchison 1986; Parent et al. 1994). We found that the CND r^2 values were negatively correlated with the mean AI in height (r = –0.46, P = 0.0432) and the mean AI in diameter (r = –0.59, P = 0.0061) during the study. This indicates that hybrid poplars were smaller in height and diameter as the foliar nutrient imbalance increased. We suggest, therefore, that CND is a robust method for making predictions about the growth of hybrid poplars based on foliar nutrition, but further work is needed to validate these findings.

VA method

The advantage of the VA method, compared with the CVA and CND methods, is that it permits site-specific comparisons of treatments with a control. The VA method is best prescribed for the first year after treatment using trees with determinate growth, such as conifers (Haase and Rose 1995). Although poplars do not have such determinate growth, the VA method has still been applied to this species for the first year after treatment with some degree of success (Timmer 1985). Here, we assumed a priori that fertilizer applications would have a lesser effect on the number of leaves produced in the F1 year than in the F2 year, and the VA method was therefore used to analyze the foliar response of treatments in the F1 year only.

Average leaf mass was as least two times greater in the B33P66 (2×), B66P33 (1× and 2×), and B100 (1× and 2×) treatments than in the control. This suggests that hybrid poplars can increase specific leaf area during a growing season when OFs are added, which increases the photosynthetic capacity of the trees. The control was N deficient compared with the B66P33 and B100 treatments, which suggests that they supplied more plant-available N than the control and hence could be the most efficient fertilizer sources. The IF, P100 (1× and 2×), and B33P66 (1×) treatments produced foliage that had more biomass and a lower foliar N concentration than the control, which indicates growth dilution (i.e., nutrient uptake is slower than biomass accumulation) and suggests that the N contained in foliage was suboptimal but not limiting to hybrid poplar growth (Imo and Timmer 1997). These fertilizer sources possess a pool of readily available N (Lteif et al. 2007), but it may not be enough to sustain foliage N requirements during the growing season. Only in the B33P66 (2×) treatment was steady-state nutrition achieved (i.e., nutrient uptake was proportional to biomass accumulation), perhaps because there was a balance between the N released from the B33P66 fertilizer and N uptake and translocation to foliage. Although hybrid poplar does not have a determinate growth habit, which would normally preclude the use of the VA method to diagnose nutrient deficiencies, it does provide insight into the amount of N released from fertilizer sources during the growing season as well as the physiological processes (N deficiency, growth dilution) that may limit hybrid poplar growth when no N fertilizer is applied. The VA method does not permit ready comparisons among sites or among clones, so the CVA and CND methods may be more robust indicators of foliar nutrition in hybrid poplar.

Conclusions

The CVA and CND methods provide similar diagnoses of the nutritional status in foliage from hybrid poplars compared with optimum ranges and indicated that a lack of plant-available N and P probably limited hybrid poplar growth in the plantation under study. The nutrient imbalance index (CND r^2) was negatively correlated with tree growth, indicating that trees would be taller and have a larger diameter and greater biomass as the nutrient imbalance decreased. This finding points to the power of the CND method to diagnose foliar nutrition and predict hybrid poplar production. The VA method indicated the fertilizer sources that provided a continuous supply of plant-available N (B66P33 and B100 treatments) or a periodic supply of plant-available N (IF, B33P66, and P100 treatments), relative to the control, during the first season that hybrid poplars were fertilized. Interpretation of foliar analysis with the CVA and CND methods would be diagnostic of nutrient sufficiency in hybrid poplar plantations relative to the optimal nutrient concentrations or nutrient indices for high-yielding clones and sites. However, the nutrient supply from fertilizer sources and physiological processes that may limit hybrid poplar growth could be inferred with the VA method.

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