CHEMICAL COMPOSITION, CRYSTALLINITY AND CRYSTALLITE CELLULOSE SIZE IN *POPULUS* HYBRIDS AND ASPEN

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The chemical composition (holocellulose, lignin, extractives and ash) of four *Populus* hybrid clones and an aspen sample was investigated. Statistical analyses indicated that no significant differences existed among the four *Populus* hybrid clones, although a notable discrepancy regarding the holocellulose content was found between aspen and the other four *Populus* hybrid clones. X-ray was used to study the crystallinity and the average thickness of cellulose crystallites in the four *Populus* hybrids and in aspen; the crystallinity index (Ic) ranged between **3**6.1 and 51.5%, and a negative correlation was found between the diameter breast height (DBH) and Ic (-0.85); thickness of the crystallite cellulose ranged from 5.67 to 7.09 nm, with no significant difference in the tree samples. No definite tendency was noticed, which is an obvious effect of wood ash application on DBH and Ic, and also on the thickness of crystallite cellulose.

Keywords: *Populus* hybrid, aspen, cellulose, crystallinity

INTRODUCTION

Populus hybrids occur naturally throughout the US and Canada.[1-3] Historically, the silvicultural activities have been focused on increasing the growth rate of the trees rather than on increasing the quality of the wood they produce. Recently, a shift in the research focus has occurred towards the effect of forest management and genetics, wood quality being targeted,[4] mainly for plantation grown species, the *Populus* ones included.

Four taxa of *Populus* hybrids, namely, NE-222 (*P. delitoids x P. nigra* var. *Caudina*), DN-34 (*P. x euramericana*, cv. "Eugenei"), DN-5 (*P. x euramericana*, cv. "Gelrica"), NM-6 (*P. nigra x P. maximowicaii*), and a seed lot of Aspen were planted in 1998. Prior to planting, the site was treated to kill weeds and fertilized with wood ash at a rate of 4 and 8 tons per acre, or without ash treatment in a randomized block design. The trees were planted at a uniform density spacing of 8×8 feet. Weed control was conducted by cultivation by mechanical means and by broadcast herbicide application. The trees were pruned in the fourth and fifth year, to reduce them to single stems. Fertilizers were applied in the spring of the third, fourth and fifth year.[5]

After six growth seasons, the fastest growing clone with the maximum diameter and height, known as NM-6, experienced the problem of frequent stem breakage, the reasons or causes of which, for this particular clone, were unknown. Two hypotheses were put forward regarding the effect of 1) the chemical composition, and 2) the crystallinity of cellulose. The wood chemical composition includes mainly cellulose, hemicelluloses, lignin, extractives and inorganic components.

Cellulose occurs in the helically wound reinforcing microfibrils. Lignin and hemicelluloses serve as a gluing and stiffening matrix.[6-8] Hemicelluloses and lignin are amorphous, while cellulose consists of two regions – the crystalline and the amorphous one. The relative quantity of these two regions is defined as the degree of crystallinity, which is highly variable, depending upon the genetic and environmental factors, tree species, growing conditions and method of evaluation. The degree of crystallinity is one of the most important factors affecting the physical and chemical properties of cellulose.[9] Young's modulus, tensile strength, alpha-cellulose content, dimensional stability, density and hardness increase with increasing crystallinity. While water uptake, dye sorption, chemical reactivity, swelling and flexibility decrease.[9,10] Previous studies on wood crystallinity have used a variety of techniques, such as X-ray diffraction (XRD), infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and dynamic mechanical spectroscopy.[11] In the present study, XRD was used to investigate the crystallinity and crystalline size of cellulose in the four *Populus* hybrids and in aspen.

The breakage of the NM-6 clone was suspected to result from the influence of the fast growth rates, while the chemical composition and cellulose crystallinity may be also involved. The objectives of this study were to 1) analyze the variation in the content of lignin, holocellulose, extractives and ash for these trees, and 2) estimate the wood crystallinity and crystalline size of the cellulose in these *Populus* hybrids and in aspen.

EXPERIMENTAL

Materials

Fifteen trees (each clone including 3 trees) were selected for bole straightness and absence of decay symptoms. The samples were taken at breast height (1.2 m) from each clone. The bark was removed and the wood samples were taken and ground to pass through a 40-mesh sieve. Wood powder was used for chemical analysis and X-ray measurement. Prior to crystallinity measurements, the samples were air-dried, then stored under normal room conditions for 3 months.

Methods

Evaluation of chemical composition

Chemical composition was evaluated by the following procedures: ASTM D1105-96 for the extractive content; ASTM D 1102-84 for ash content; ASTM D 1106-96 for Klason lignin content; GB/T2677 10-1995 (Chinese standard methods for the fibrous raw material) for the holocellulose content.[12-13] Six replicates were used for assessing the content of extractives, Klason lignin and holocellulose, and three replicates for the ash content.

X-ray diffraction measurements

Measurements were performed with Cu Kα radiation (λ = 1.541 Å) selected by a ground and bent graphite monochromator. The diffractometer was powered by a Rigaku 200B rotating anode generator (45 KV, 100 mA). The scattered photos were detected by a scintillation counter. The goniometer radius was of 180 mm. The instrumental broadening was measured as the full width at half maximum (FWHM) of a standard material 660 (lanthanum hexaboride) from NIST.

To determine wood crystallinity, a diffractometer was used in the symmetrical reflection mode. The divergence and receiving slits were of 0.5 degrees and 0.3 mm, respectively. Intensity was measured as a function of the scattering angle 2θ by θ -2 θ scan. The angle ranged from 10 to 40 degrees at 0.2 degrees per step. The scan speed was of 1 degree/minute.

RESULTS AND DISCUSSION

Comparison of chemical composition

The average properties of the tested samples are listed in Table 1, including Duncan's mean letters, associated with the levels of significance.

The analysis of the Klason lignin fraction indicated some differences, although not great, among the clones. The lignin values ranged from 20.39 to 22.55% of the oven-dried wood powder, which is similar to the results reported by Blankenhorn *et al*.,[14] according to whom the Klason lignin content for 7 poplar hybrid clones of six-year-old trees ranged between 17.26 and 25.70%.

The difference in the holocellulose values for the four *Populus* hybrids was not obvious, except for aspen. The holocellulose content ranged between 79.58 and 80.18%, which was less than 87.6% holocellulose estimated

by Murphey *et al*.[15] for a 4-year-old *Populus* hybrid. This is partly due to the continuation of the treatment for only 3.5 h while, in the present study, the procedure was extended to 6 h, so that a larger lignin fraction was dissolved. This result was similar to those reported by Olson *et al*.[16] The average holocellulose content of 75 *Populus* clones of 3 years old was of 80.2%.

Table 1
Average lignin, holocellulose, extractives and ash content in *Populus* hybrid and aspen samples

Clone	Klason lignin	Holocellulose	Extractives	Ash
NM-6	20.39c	80.05a	4.16b	1.00 a
DN-34	21.60b	79.58a	3.98b	0.97ab
NE-222	22.55a	80.18a	4.00b	0.69c
DN-5	21.70b	79.71a	4.62b	0.89ab
Aspen	21.48b	77.23b	6.28a	0.82bc

Clonal means are for N=6 except for ash content which is for N=3, means with the same letters are not significantly different at the 0.05 level as determined by Duncan's mean separation procedure

The extractive contents were not significantly different among the four *Populus* hybrids, ranging from 3.98 to 4.62%, similarly to previously reported results,[17] while the extractive content for *Populus* hybrid NE-388 (4 years old) ranged between 4.2 and 5.2%. Aspen contained more extractives than the *Populus* hybrids, which may be partly due to the fact that the breast diameter (DBH) of aspen is lower than that of the poplar hybrids, wherefrom the samples were taken, and also to the fact that it contained more heartwood. Therefore, the extractive content is higher than that from the other samples.

Ash contents showed some notable differences among the clones, ranging between 0.69 and 1.00%, which is similar to the results reported by Blankenhorn *et al*.[14] The ash content for 7 poplar hybrid clones of a 6 year-old tree ranged from 0.68 to 1.13%.

A comparison between the clone of NM-6 and that of the other *Populus* hybrids shows no significant difference in the content of holocellulose, extractives and ash, except for the Klason lignin, although the difference was not considerable. Therefore, further study is needed to explore the reason of stem breakage in the NM-6 clone.

Evaluation of crystallinity

The crystallinity index (Ic) was determined with eq. (1):

$$I_C = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the intensity of the 002 diffraction peak at 2θ angle close to 22°, representing the crystalline region of the material, and I_{am} is the intensity of the peak at 2θ angle close to 16º, representing the amorphous region of the material in cellulose fibres.[11,18,19]

The average size of crystallites, B_{hkl}, was estimated from the widths of reflection *hkl*, using the well-known formula of Scherrer:

$$B_{hkl} = \frac{0.9\lambda}{\Delta 2\theta \cos\theta} \quad (2)$$

where Δ 2θ is the FWHM of reflection in radians, and the effect of instrumental broadening on the width of reflections was included by assuming that the shapes of both reflection and instrumental functions were approximately Gaussian, a value of Δ 2θ being calculated with the following formula:

$$\Delta 2\theta = (b_s^{\,2} - b_i^{\,2})^{1/2} \quad (3)$$

where b_s is the measured FWHM of reflection 002 or 004, and b_i is instrumental broadening.[20-23]

The average thickness of the cellulose crystallites was determined by measuring the FWHM of reflection 002 in the pattern measured in the symmetrical reflection mode. Instrumental broadening at reflection

002 (2θ ≈22.4º) was estimated at 0.144 degrees.

Tserki *et al.*[24] reported that flax fibres exhibit four well defined peaks at 15.1°, 16.8°, 22.0° and 34.4°, the values of 15.1° and 16.8° for the 2 θ reflection, corresponding to the 1$\bar{1}$0 and 110 crystallographic planes, respectively. The other two peaks at 22.0 and 34.4 correspond to the 002 and 004 planes. In high cellulose content materials, such as flax fibres and cotton, at around 16°, one may observe two peaks in the lower cellulose content of the material, the fibre containing high amounts of amorphous materials, such as lignin, hemicelluloses and amorphous cellulose; similarly with the wood fibres, these two peaks are smeared, thus appearing as one broad peak. In the samples of the four *Populus* hybrids and the aspen clone, three peaks appeared around 15.5°, 22.0° and 34.4 °, corresponding to the 1$\bar{1}$0, 002 and 004 planes, respectively (Fig. 1), which is due to the large amount of amorphous regions present in cellulose, and also to the presence of amorphous lignin and hemicelluloses, which agrees with the results of Tserki.[24] Figure 2 shows that the Ic of the samples ranged between 36.1 and 51.5%. The Ic of aspen (the lowest DBH) ranged from 47.9 to 51.5%

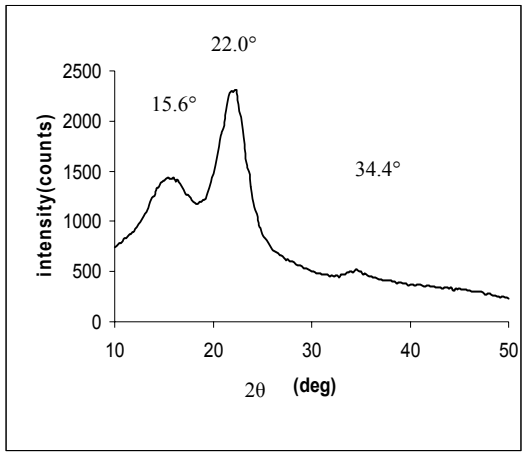

Figure 1: Diffraction patterns of a *Populus* hybrid sample, measured in the reflection mode

The Ic of the fastest growing clone (NM-6) ranged between 36.7-38.7%. Figure 2 also evidences a negative correlation (-0.85) between Ic and the stem diameter at breast height level. The negative correlation between the diameters at breast height of the sample trees and the Ic suggested that the percentage of crystalline cellulose decreases with an increasing diameter.

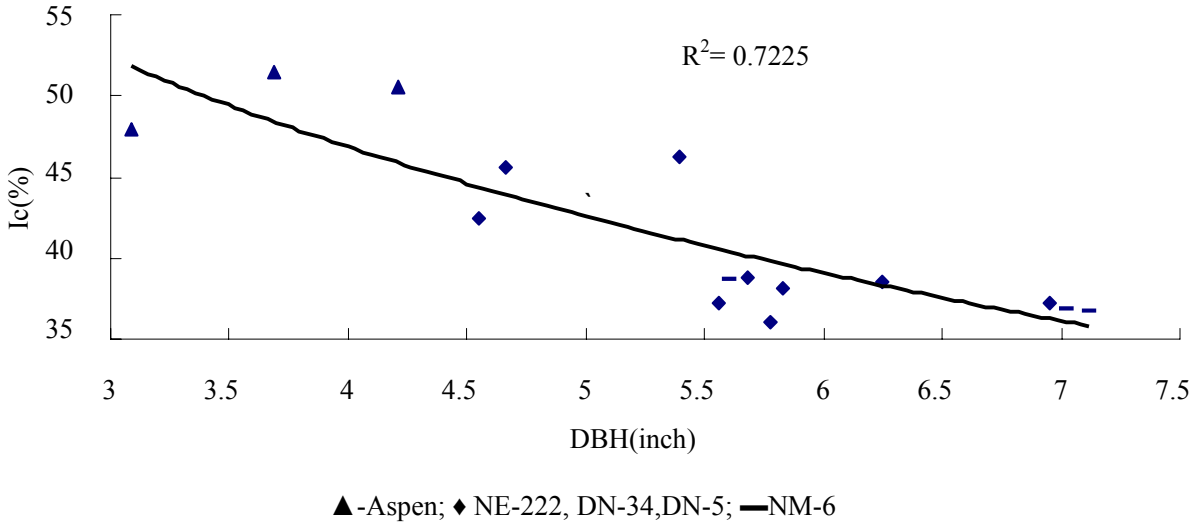

Figure 2: Crystallinity index of samples with different DBH values

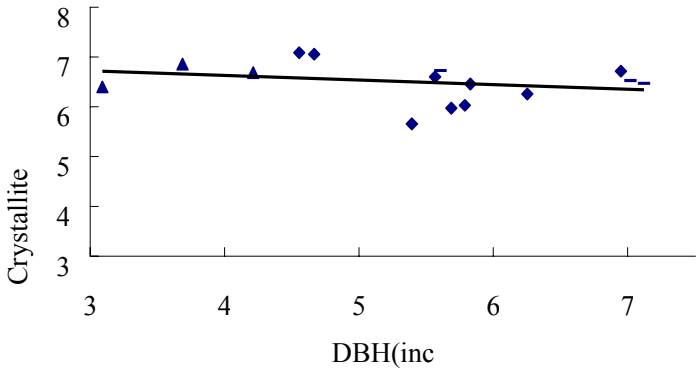

Figure 3: Crystallite thickness of samples with different DBH values

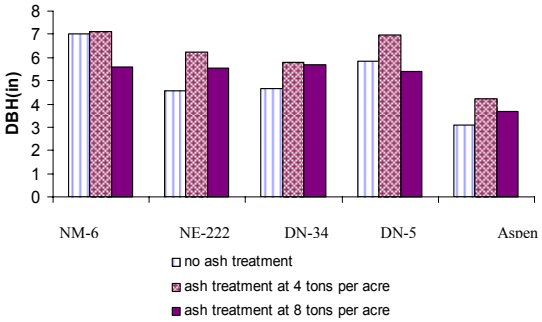

Figure 4: Relationship between ash treatment and DBH

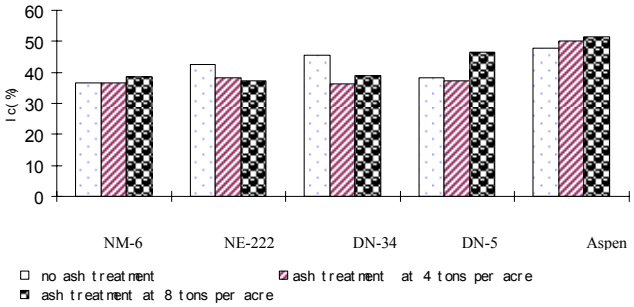

Figure 5: Crystallinity index of clone samples treated with different ash content

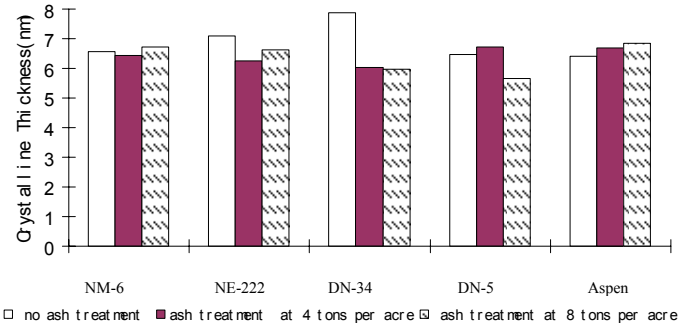

Figure 6: Crystalline thickness values of clone samples treated with different ash content

It can be therefore concluded that the wood samples from the fast growing *Populus* hybrid (NM-6) contain more amorphous and less crystalline cellulose,

which decreases the strength of the trees, resulting in tree breakage. The average size in the 004 plane (Fig. 3) showed that crystallite thickness ranged from 5.67 to 7.09 nm. No correlation was observed between crystallite thickness and diameter at breast height.

Wood ash could be used to reduce the negative soil effects of short-rotation harvesting practices. Wood ash application (10-20 mg ha^{-1}/treated plots) may increase soil pH from 6.1 to 7.1, as well as soil extractable phosphorus, potassium, calcium and magnesium, and also the stem size.[25] However, Figure 4 shows no predictable relation between ash application and the diameter at breast height of the sample trees. Figures 5 and 6 evidence no definite relationship between wood ash application and the Ic and thickness of crystalline cellulose.

CONCLUSIONS

1. No significant chemical differences appear in the composition of the four *Populus* hybrid clones, although a notable discrepancy was observed between the content of holocellulose of aspen and those of the other four *Populus* hybrid clones.
2. A negative correlation was found between diameter breast height (DBH) and Ic (-0.85). The problem of breakage in clone NM-6 may be due to the fast growing speed, resulting in lower cellulose crystallinity.
3. Crystallite cellulose thickness ranged between 5.67 and 7.09 nm, no significant differences being observed between the tree samples from the aspen and the four *Populus* hybrid clones.
4. No definite tendency can be predicted as to the relationship between ash application, Ic and thickness of crystallite cellulose.

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