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SHORT COMMUNICATION

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Short-term and long-term effects of weed control and fertilization on growth and wood anatomy of a *Populus deltoides* clone

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Aims of study: The short- and long-term effects of weed control and fertilization on growth and wood anatomy of 10-y-old *Populus deltoides* were investigated. Weed control and fertilization usually leads to an increase the growth rate of trees, and consequently, a possible modification in the quality of produced wood.

Area of study: We analyzed trees from an experimental plantation in Buenos Aires, Argentina (34° 50' S Lat; 60° 30' W Long).

Methods: 32 trees from three treatments: mechanical weed control (M), chemical and mechanical weed control (CHM) and fertilized plus chemical and mechanical weed control (CHM-F) were analyzed. Basal area, fibre morphology, cell wall area and vessel size were measured in the growth ring 1, 3 and 10.

Results: differences on wood anatomy among treatments were mainly observed at the third year (short-term effect). Long-term negative effects were not observed. Fertilized trees had greater proportion and quality of wood closer to pith.

Research highlights: fibre and vessel differences seen in CHM and CHM-F compared to controls in year 3 could be interpreted as evidence of maturation in cambial development (thicker, longer and wider fibres and greater vessels). The CHM-F treatment had a greater proportion of wood that showed characteristics of more mature wood.

Key words: silvicultural treatment; wood quality; fibre properties; vessels; poplar; cambial maturity.

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Introduction

Silvicultural practices such as weed control and fertilization usually lead to an increase in the growth rates in trees, thereby accelerating stand development (Bilodeau Gaurthier *et al.,* 2011). An increased growth rate during the early stages of development will necessarily modify the amount of wood in areas close to the pith. These changes may also strongly alter the quality of this stem, particularly in species with short production cycles (10 years) (De Bell *et al.,* 1998; Little *et al*., 2003; Efhami *et al.,* 2012). Studies in *Populus* have reported different stages of xylem maturation in cellular components of the same age, although this varies depending on site, genotype and growth rate (Matyas & Peszlen, 1997; De Bell *et al.,* 1998).

There are very few studies describing the effects of weed control and fertilization on wood quality. Literature review indicates that it is possible to obtain short-term responses in wood anatomy, although these vary depending on the specific silvicultural treatment used (dose, concentration, etc.) and the genotype (Little *et al*., 2003; Luo *et al.,* 2005; Pitre *et al.,* 2007; Efhami *et al*., 2012).

For poplars, there is a lack of information linking the long-term impacts of vegetation management and fertilization on wood characteristics. The aim of the

present study was to determine the short- and long-term effects of weed control and fertilization on growth and wood anatomy in a commercial clone of cottonwood for newsprint.

Materials and methods

Thirty-two 10-year-old *Populus deltoides* Bartr. ex Marsh. trees of the `Delta Gold´ clone were cut in an experimental plantation in Buenos Aires, Argentina (34° 50' S Lat; 60° 30' W Long; 55 m elevation). The trial evaluated 14 treatments, organized in a randomized complete block design with four replicates. We analyzed three treatments:

- **M,** mechanical weed control: mechanized cross-disking between trees, plus manual hoeing around each tree (over the first two growing seasons, twice per season).
- **CHM,** chemical and mechanical weed control: winter application of a pre-emergent herbicide (2.75 kg active ingredient/ha of simazine) in 1.3 m wide bands, complemented with a graminicide application (54 g active ingredient/ha of quizalofop-P-ethyl) in mid-November and January, plus disking between bands (over the first two growing seasons). Additional weed control with 0.75 kg active ingredient /ha of ammonium glufosinate was applied before the pre-emergent treatment in the second growing season in order to reduce weed coverage.
- **CHM-F,** fertilization plus chemical and mechanical weed control: same as CHM, plus two localized fertilization treatments with a total of 45 g of N / plant as urea, and 34 g of P / plant as triple superphosphate. Half of the N was applied at the beginning of the first growing season and the remainder at the beginning of the second growing season.
- **C,** weedy control: no weeding or fertilization after planting was applied.

8-10 trees were cut from each of the three treatments and control. A wood disc 5 cm thick was taken from the stem base of each tree. Three radii (from pith to cambium) were marked on each disc. Mean radial growth (MRG) was measured by averaging the three radii for each year and annual basal area (ABA) were calculated for years 1, 3 and 10. Cumulative basal area (CBA) was calculated for 3rd and 10th year.

The growth rings produced in years 1, 3 and 10 were sampled along the north-facing radius to determine both the short-term (years 1 and 3) and long-term (year 10) effects of the treatments on wood characteristics.

To determine fibre length, wood chips from the rings for years 1, 3 and 10 were macerated in Franklin´s solution (Franklin, 1945). To determine wood anatomy, cross-sections (20 μm) were cut from growth rings 1, 3 and 10 using a sliding microtome, then stained in safranin (1%), dehydrated and mounted in Entellan® for microscopic analysis. Images were captured with a digital camera (Olympus DP71) mounted on a research microscope (Olympus BX50, Japan).

Captured images were analyzed for the following parameters using image analysis software (ImagePro Plus, v. 6.3): fibre length (n = 240, sample size per year and treatment), fibre wall thickness (n = 400), fibre diameter (n = 400), vessel lumen diameter (n = 800), vessel frequency (n = 40 captured images) and percentage of cell wall area (n = 40 captured images). Each unit of area in the captured image was represented by 389376 Px = 1 mm^2.

Cell wall area (CWA) was calculated as follows:

$$CWA(\%) = \frac{TA - \left(VLA + FLA + RPA\right) \times 100}{TA}$$

CWA: cell wall area (%); TA: total area of the captured image; VLA: vessel lumen area; FLA: fibre lumen area; RPA: ray parenchyma area.

The weighted mean values (Pw) for all the anatomical variables were calculated as follows:

$$Pwi = \frac{Pi.Ai}{At}$$

Pwi: is the weighted mean value of the parameter in the cross section at radial position i; i: identifies the radial sampling position, i = 1, 2, 3 (1 = first growth ring, 2 = third growth ring, 3 = tenth growth ring); Pi: is the value of the parameter at radial position i; Ai: is the area of the ring contained in radial position i; At: is the total area of the section at rotation (10 years).

The mean (whole average) for each variable at rotation age (10 years) was calculated as the arithmetic mean of the weighted variables measured for rings 1, 3 and 10.

The three analysed treatments (M, CHM and CHM-F) and control (C), and ages (1, 3 and 10 years) were treated as Kruskal-Wallis ANOVA by Ranks (Non parametric analysis, Statistica V.7) because the variables did not comply with the assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene test) required by ANOVA (see supplementary information Suppl. Table S1: descriptive statistics [pdf on line]). Medians were compared using multiple comparisons z´values.

Results and discussion

Based on the Kruskal-Wallis ANOVA, the annual basal area (ABA) was significant for treatments only in the years 1 and 3 (Fig. 1, I; Suppl. Table S2 [pdf on line]). In the third year of analysis, ABA of CHM-F was 8 times higher than that of C. At rotation age (year 10), cumulative basal area (CBA) showed significant differences between treatments (Fig. 2, II), CBA was significantly higher in CHM-F relative to the other treatments. Through the third year of growth, C had the smallest value and differed from CHM and CHM-F (Fig. 2, II).

As was described elsewhere (Achinelli, 2007), mean radial growth and ABA analysis showed slight differences among treatments starting at year 5 (data not shown, Suppl. Fig. S1 [pdf on line]). However, differences in CBA achieved during establishment phase of a plantation could still be seen at rotation due to the effects seen for CHM-F (Fig. 2, II).

For wood anatomy, differences in weighted variables analysed were mainly observed in the third year after implantation (Fig 2). In first ring, only fibre length and vessel number in the treatments showed greater values compared to C. For values measured in ring 3, a trend for increasing size and frequency in all the anatomical parameters from C to CHM-F was observed; however, significant differences only were found between the CHM and CHM-F treatments and control. The fibres from CHM-F were almost five times larger, wider and thicker than those from C (Fig. 2 II, III, IV; Suppl. Table S2 [pdf on line]). CHM-F vessels were 4.8 times larger in diameter and were five times more frequent as compared to C (Fig. 2 V, VI; Suppl. Table S2 [pdf on line]). In ring 10, fibre and vessel diameter measurements were higher in control than in treatments but the medians were significantly different for CWA, fibre wall thickness, vessels diameter and number (Fig. 2 I to VI). The fibres from C were 1.3 times wider and thicker than those from CHM-F. C vessels were 1.4 larger in diameter and frequent as compared to CHM-F (Suppl. Table S2 [pdf on line]). These values were much lower magnitude than those reported in year 3.

A trend for increasing value in all the anatomical parameters from C to CHM-F was observed for the mean for each variable at rotation age (whole average), however no significant differences between treatments were found (Fig. 2 I to VI). Despite the fact that whole average for the anatomical measurements did not differ significantly at rotation, CHM-F had more proportion of wood with better features at year 3 (larger CBA, longer and wider fibres with thicker walls, increased number of vessels of greater diameter).

We believe that, by rotation age, CHM-F caused a developmental advance of CBA relative to the growth observed in C. At the same time, from ABA analysis, we discovered that this advance occurred primarily during the first three years of growth in the plantation (establishment phase). As a result, the weighted wood anatomy variables were directly affected by this difference in the proportion of wood accumulated close to pith. Differences in fibres and vessels between treated and control trees at year 3 could be interpreted as evidence of maturation in cambial development (short-time effects). The CHM and CHM-F cambium produced thicker, longer and wider fibres and larger vessels than C trees, possibly as a result of an advanced stage of maturity of the cambium.

Publications in *Populus* have reported different stages of maturity in wood elements of the same age,

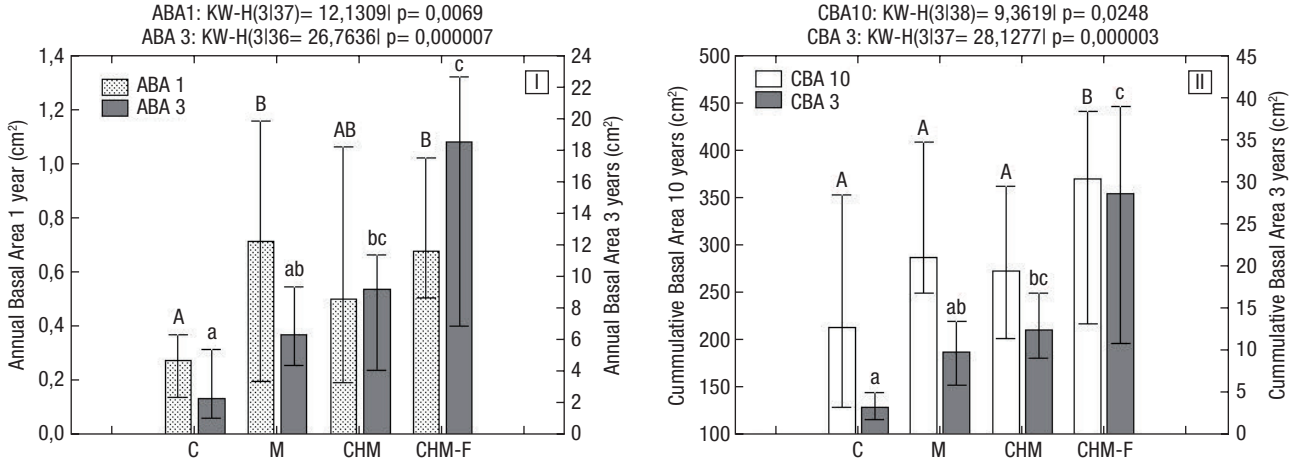

Figure 1. I: Annual basal area (ABA) for years 1 and 3. II: Cumulative basal area (CBA) at years 3 and 10. Treatments: M: mechanical weed control; CHM: chemical and mechanical weed control; CHM-F: chemical and mechanical weed control plus fertilization; C: control. Median, whisker: non-outliers range. Statistics of Kruskal-Wallis ANOVA. Different letters indicate significant differences between treatments of the same age.

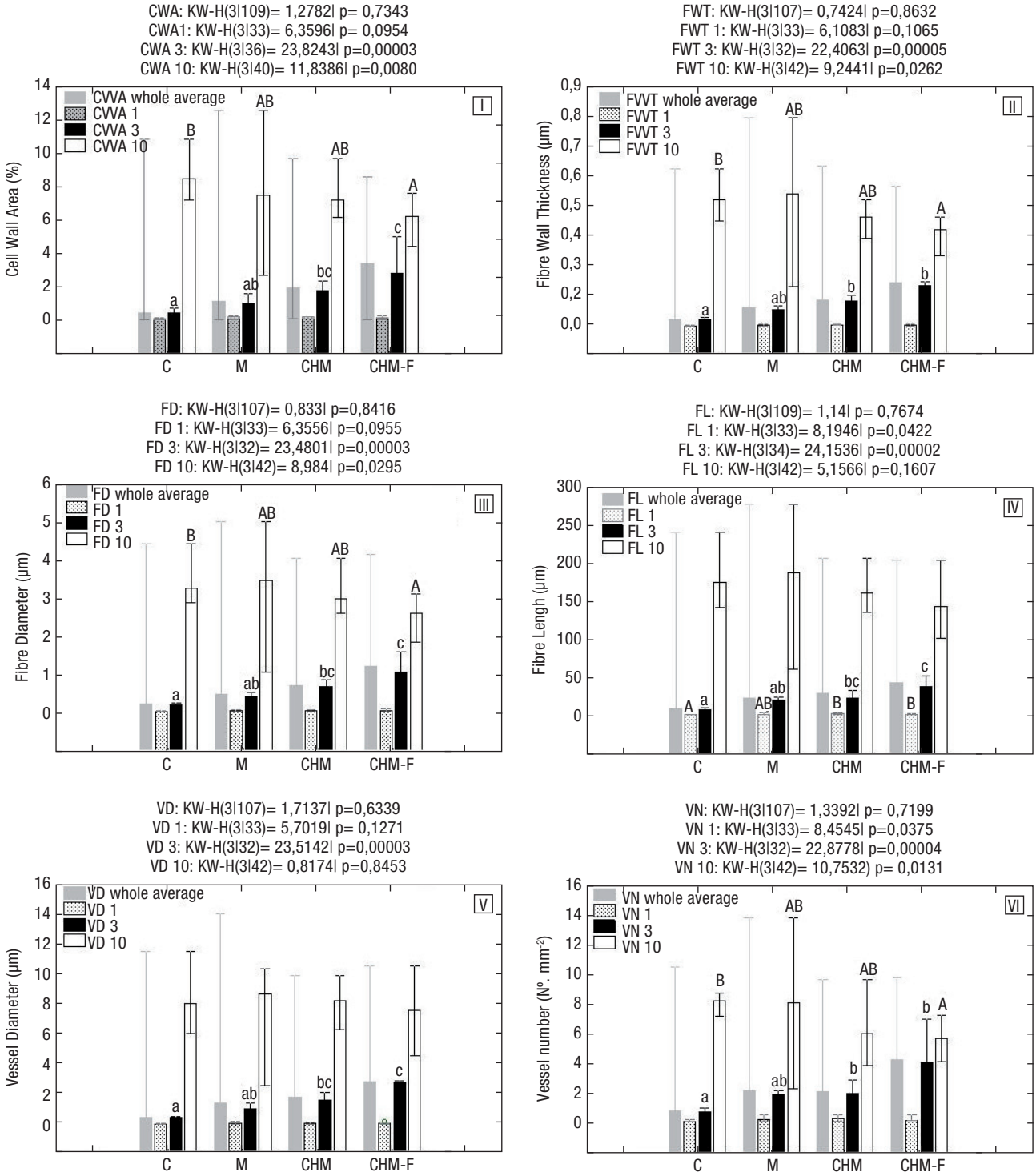

Figure 2. Wood anatomy for the three growth rings analyzed at years 1, 3 and 10 and whole average. Treatments: M: mechanical weed control; CHM: chemical and mechanical weed control; CHM-F: chemical and mechanical weed control plus fertilization; C: control. Median, whisker: non-outliers range. Statistics of Kruskal-Wallis ANOVA. Different letters indicate significant differences between treatments of the same age.

depending on the site, genotype and growth rate. As reported in Matyas & Peszlen (1997), a low-quality site for poplar growth (based on edaphic and climatic conditions) was associated with the formation of fibres and vessels that showed juvenile characteristics for longer time, which resulted in a delay of two to four years in the age mature wood was produced. Under more appropriate site conditions, Matyas & Peszlen (1997) found that maturation was accelerated, but the level of maturity for certain

anatomical properties were reduced. The size of the cells produced by division of the cambial initials depends mainly on the size of those cells. If the growth rate is fast, it is possible that mother cells may receive stimuli for further division before they can grow larger. Another explanation suggest that the effect of growth rate on shortening the cambial initials would be overshadowed by the subsequently greater elongation of daughter cells in the fast-growing trees (Efhami *et al.,* 2012).

According to the literature, there are important structural changes that occur in the very short term in wood cells that form under different fertilizer concentrations (Luo *et al.,* 2005; Pitre *et al.,* 2007). However, it is important to note the importance that these changes will be present when wood is harvested at rotation age (long-term effect). Changes that occur during the first years of growth involve very little of the mean basal area relative to the total for the 10 years. Based on our results, CHM and CHM-F treatments reflect the large contribution of ring 3 on the whole average when weighting the anatomical variables, with the highest expression observed with CHM-F treatment. Therefore, we can speculate that treatments do not have any negative effects on the quality of the wood produced at rotation age (year 10) for all wood anatomy parameters.

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