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# Biomass allocation and photosynthetic capacity of willow (*Salix* spp.) bio-energy varieties

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## Abstract

Producing woody biomass for energy in short rotation coppice requires physiological understanding of willow growth. We tested growth parameters for a select group of 12 willow varieties grown from cuttings for 90 days in a field experiment with four replicate blocks in cool-humid climate. Varieties differed significantly ( $p < 0.05$ ) for leaf area, leaf chlorophyll concentration, biomass, and leaf allocation of biomass. Willow growth was functionally balanced as root biomass scaled positively with leaf area and the relationship between leaf chlorophyll concentration and leaf area remained non-significant. Mean plant biomass ranged from 3.8 g to 13.2 g for *Salix discolor* and the hybrid 'Fabius' (*Salix miyabeana* × *S. viminalis*), respectively. Plant biomass closely correlated with leaf area ( $R^2 = 0.83$ ;  $p < 0.05$ ). Accounting for leaf chlorophyll concentration slightly increased the predictive power of leaf area thus photosynthetic capacity. High photosynthetic capacity at low leaf allocation of biomass rendered 'Fabius' morphologically most suitable for producing woody biomass in willow short rotation coppices. The shoot-chlorophyll mass ratio is recommended for future screening of willow bio-energy species and hybrids.

**Key words:** bio-energy, *Salix*, leaf area, chlorophyll, biomass, leaf-mass ratio

## Introduction

Fast growth and easy vegetative propagation render species and hybrids of the genus *Salix* (L.) increasingly attractive for producing biomass-for-energy with renewable resources in short rotation coppice (SRC, Karp and Shield 2008). Willow SRC also represents an opportunity to put land back into production and revitalize rural economies in regions with a history of agricultural decline (Van Rees 2008). However, the large number of willow species and hybrids vary greatly in their ploidy level and come with a range of leaf size and shape as well as varying growth rate and habit from shrub to tree size. This variation poses a challenge for optimizing willow selection for SRC and more specifically for functional growth analysis which seeks to identify critical physiological and structural traits to predict willow biomass and biomass allocation (Robinson et al. 2004, Tharakan et al. 2005).

Biomass production is a function of leaf photosynthetic efficiency and plant photosynthetic capacity. The net biomass gain per unit leaf area and time thus foliar photosynthetic efficiency depends among other things on leaf chlorophyll and nitrogen (N) concentration. Plant photosynthetic capacity can then be defined as the product of foliar chlorophyll concentration and plant leaf area thus plant chlorophyll mass. Correlations between plant chlorophyll mass and plant biomass have long been used for functionally separating a range of plant species and communities (Bray 1960, Bokari 1983). On account of respiratory losses incurred in supportive tissues, photosynthetic capacity can also be expressed as the leaf area ratio (LAR) which means leaf area per unit plant mass. The LAR represents the product of a leaf-level and a plant-level structural property referred to as specific leaf area (SLA, area per unit leaf mass) and leaf-mass ratio (LMR, foliar biomass per unit total plant mass), respectively. Theoretically, increases in SLA (or LMR) may reduce leaf-level photosynthetic efficiency due to dilution of foliar nitrogen and chloro-

phyll. However, Osone et al. (2008) suggested that increases of root nitrogen absorption kept foliar N nearly constant with increasing SLA across a select group of herbaceous and woody species. Robinson et al. (2004) found leaf-level photosynthetic efficiency to differ significantly with concomitant variation of leaf anatomy and individual leaf area among willows. Morphological leaf traits were more useful than leaf-level physiological traits to differentiate biomass production in the genus *Populus* (Bunn et al. 2004). However, Aasamaa et al. (2010) found foliar chlorophyll concentration to be a strong indicator of woody biomass production in willows. Weih and Nordh (2005) identified leaf area and the total foliar N pool to be key determinants of plant biomass independent of coppice age and site conditions.

In a plot experiment, we tested plant functional and structural growth parameters for screening of potential willow bio-energy crops grown from cloned cuttings in cool-humid Cape Breton, Canada. On account of varying geographic origin and experimental hybridization, we expected a select group of seven willow species and five hybrids, collectively referred to as varieties for the purpose of this paper, to differ in their leaf area, leaf chlorophyll concentration, and biomass allocation thus in their inherent morphological and physiological potential for producing woody biomass in short rotation coppices.

## Materials and Methods

The willow variety experiment for short rotation coppice was established on abandoned agricultural land near Sydney, Cape Breton, in early June 2010. The experimental site is situated in the Westbrook soil series with good drainage (Cann et al. 1963) and near the Atlantic shore with a mean daily temperature of 5.5 °C and 1505 mm annual precipitation (<http://climate.weatheroffice.gc.ca>). The cut-

Table 1. Species and hybrids of willow (*Salix* spp.) tested for bio-energy production near Sydney on Cape Breton Island (Canada) in 2010.

Code	Taxon	Variety name	Geographic Origin
SD	<i>S. discolor</i>		North America
SE	<i>S. eriocephala</i>		North America
SV1	<i>S. dasyclados</i>		Europe
SV	<i>S. viminalis</i>		Europe
SX64	<i>S. miyabeana</i>		East Asia
SX61	<i>S. sachalinensis</i>		East Asia
FC	<i>S. purpurea</i> 94006 x <i>S. purpurea</i> 94001	'Fish creek'	
FA	<i>S. miyabeana</i> x <i>S. viminalis</i>	'Fabius'	
OW	SX64 x SV7	'Owasco'	
OT	SX64 x SV2	'Otisco'	
SH	SX67 x SX61	'Sherburne'	
CA	SX64 x SX61	'Canastota'	

tings for a total of twelve willow varieties (Table 1) were supplied at a cutting length of 0.25 m by the willow breeding program at State University of New York. The field was ploughed prior to planting and weeds were controlled manually within rows and mechanically between rows throughout the growing season. Varieties were tested in a randomized block design with four replicate blocks each divided into 12 plots. Cuttings were inserted into the soil at a density of 22,000 plants per hectare in single rows 1.8 m apart and spaced 0.3 m apart within rows. There was no application of fertilizers and herbicides during willow SRC establishment in 2010.

Three willow plants were randomly selected and excavated to a depth of 0.3 m from each plot and wrapped in plastic bags on September 17, 2010. In the lab, one leaf was randomly plucked from the lower, middle and upper portion, respectively, of each plant to account for variation of leaf development (Verwijst and Wen 1996). The total of nine fresh turgid leaves per plot was photocopied and leaf photos were then clipped to determine leaf area gravimetrically. The dry weight of the nine leaves was determined on a precision balance following drying at 55 °C for 24 h. Chlorophyll index readings from two fresh leaves on each harvested plant were also taken using the CCM-200 plus v1.00 device (OPTI-SCIENCES Inc., Hudson, NH). The meter measures two wavelengths of light at 700 nm and 840 nm to determine plant chlorophyll content. Chlorophyll a absorbs 700 nm light, while 840 nm light is unaffected by the chlorophyll but indicates the reflectiveness of a leaf's physical surface. The meter compares the ratio of 700 nm and 840 nm available light to the ratio of the same wavelengths of reflected light, arriving at a chlorophyll index value (Van der Berg and Perkins 2004). The foliar chlorophyll index values were converted to leaf chlorophyll concentration according to Richardson et al. (2002). Root material was soaked for 24 h and repeatedly rinsed over a fine-meshed sieve. The plant biomass was separated into leaves, stems, and roots and determined following drying at 55 °C for 24 h. Moreover, the number of surviving plants per plot was recorded October 9, 2010. The same day, the number of stems and the height of the tallest stem per plant were recorded in the field for 10 plants per plot.

Plant leaf area (LA) was estimated by multiplying the leaf biomass of each excavated plant by the SLA determined separately for each plot. Multiplying foliar chlorophyll concentration (Chl) by LA provided an estimate of plant chlorophyll mass (PChl). The ratio of shoot biomass over PChl provided a measure of productivity. The Cohort<sup>®</sup> software package was used for analysis of variance (ANOVA) on a one-way randomized block design (n = 4). Least significant dif-

ferences (LSD) were calculated for comparison of means at a probability of less than 0.05. Linear regression analysis was used to explore relationships between growth parameters using variety means (n = 4).

## Results

Survival was good to very good for most varieties while intermediate for the Asian species SX61 and SX64 and poor for their hybrids SH and CA (Table 2). The number of stems per plant did not vary significantly with an average of 2.31 stems per plant across varieties. With a height of 0.94 m the hybrid FA was superior to its parents (i. e., SV and SX64) and all other varieties tested in the experiment.

The hybrid advantage of FA also became evident in the biomass

Table 2. Number of survived plants, number of shoots per plant, and maximum plant height of twelve willow varieties 90 days after planting of cuttings for short rotation coppice near Sydney on Cape Breton Island (Canada) in 2010.

Variety	Survived plants (%)	Shoot number (n plant <sup>-1</sup> )	Plant height (m)
SD	87	1.83	0.39
SE	92	2.35	0.32
SV1	94	2.13	0.39
SV	92	2.50	0.69
SX64	78	2.50	0.68
SX61	70	2.08	0.49
FC	80	2.08	0.67
FA	86	2.33	0.94
OW	86	2.68	0.59
OT	85	2.63	0.60
SH	63	2.45	0.66
CA	42	2.20	0.46
LSD <sub>p &lt; 0.05</sub>	15	n. s.	0.19

n. s. = no significant difference

Table 3. Biomass of plant parts and cuttings for twelve willow varieties 90 days after planting for short rotation coppice near Sydney on Cape Breton Island (Canada) in 2010.

Variety	Leaves	Shoot	Roots	Plant	Cutting
	----- (g plant <sup>-1</sup> ) -----				
SD	2.3	1.3	0.26	3.8	8.3
SE	2.2	1.9	0.33	4.4	7.2
SV1	2.7	3.0	0.50	6.2	7.1
SV	3.1	6.1	0.44	9.7	6.7
SX64	2.7	3.0	0.43	6.1	8.8
SX61	1.6	2.0	0.17	3.8	6.8
FC	1.6	3.2	0.31	5.1	5.6
FA	4.1	8.4	0.68	13.2	8.2
OW	5.1	7.1	0.84	12.9	8.5
OT	3.1	4.4	0.44	7.9	7.7
SH	2.4	2.6	0.50	5.6	5.9
CA	1.3	0.8	0.10	2.1	4.3
LSD <sub>p &lt; 0.05</sub>	1.8	3.4	0.40	5.4	2.6

results although this advantage could not be statistically ascertained against the parent SV (Table 3). Compared to shoot mass, differences among varieties were less pronounced for leaf and root mass. Mean plant mass amounted to 13.2 g for FA and 3.8 g for SD, respectively. Plant biomass did not closely correlate with height ( $R^2 = 0.43$ ) and cutting mass ( $R^2 = 0.25$ ), respectively.

As shown in Table 4, leaf area and chlorophyll differed significantly among varieties. Leaf area in itself a purely structural expression of photosynthetic capacity predicted reasonably well the biomass of roots (Figure 1) and plant biomass ( $R^2 = 0.83$ ;  $p < 0.05$ ). However, accounting for chlorophyll concentration (i. e., photosynthetic efficiency) slightly enhanced prediction of plant biomass (Figure 2;  $R^2 = 0.89$ ;  $p < 0.05$ ). The relationship between leaf chlorophyll concentra-

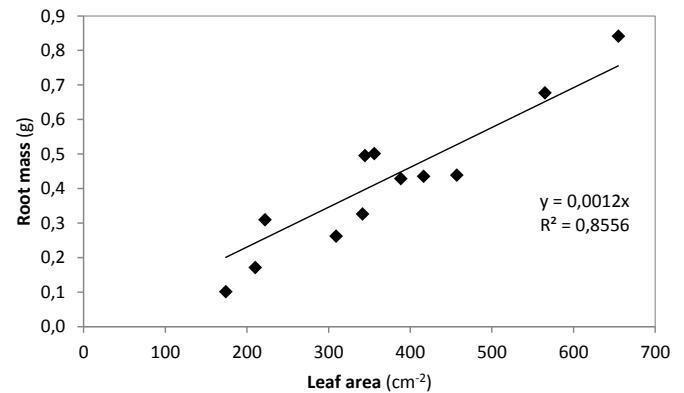


Fig. 1. Linear relationship between mean plant root mass versus mean plant leaf area ( $n = 4$ ) across twelve willow varieties 90 after planting of cuttings for short rotation coppice near Sydney on Cape Breton Island (Canada) in 2010.

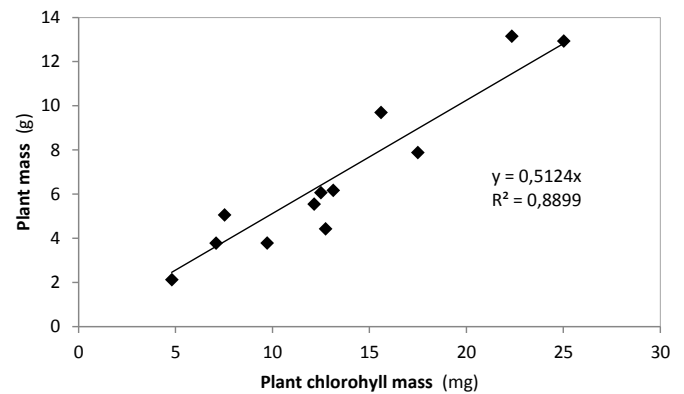


Fig. 2. Linear relationship between mean plant mass versus mean plant chlorophyll mass ( $n = 4$ ) across twelve willow varieties 90 after planting of cuttings for short rotation coppice near Sydney on Cape Breton Island (Canada) in 2010.

Table 4. Functional growth parameters for twelve willow varieties 90 days after planting for short rotation coppice near Sydney on Cape Breton Island (Canada) in 2010.

Variety	LA (m <sup>2</sup> )	Chl (g m <sup>-2</sup> )	PChl (mg)	NAR (g m <sup>-2</sup> yr <sup>-1</sup> )	LMR (g g <sup>-1</sup> )	SLA (m <sup>2</sup> kg <sup>-1</sup> )	LAR (m <sup>2</sup> kg <sup>-1</sup> )
SD	0.030	0.314	10	120	0.66	13.7	8.4
SE	0.034	0.374	13	140	0.54	15.1	7.5
SV1	0.034	0.383	13	180	0.50	12.8	5.9
SV	0.042	0.364	16	220	0.36	13.3	4.5
SX64	0.039	0.319	12	150	0.49	14.7	6.8
SX61	0.021	0.331	7	180	0.44	13.7	5.7
FC	0.022	0.349	8	230	0.34	14.3	4.5
FA	0.057	0.394	22	250	0.32	13.7	4.2
OW	0.066	0.383	25	200	0.44	12.7	5.2
OT	0.046	0.383	17	170	0.43	14.9	6.2
SH	0.036	0.333	12	160	0.50	14.7	6.7
CA	0.017	0.266	5	120	0.61	13.9	8.1
LSD <sub>p &lt; 0.05</sub>	0.025	0.053	10	50	0.08	n. s.	1.6

LA = plant leaf area, Chl = leaf chlorophyll concentration, PChl = plant chlorophyll mass, NAR = net assimilation rate, LMR = leaf mass ratio, SLA = specific leaf area, LAR = leaf area ratio, n. s. = no significant difference

tion and LA (and SLA) was weak ( $R^2 = 0.16$ ). The high-performing hybrid FA produced biomass at high chlorophyll concentration relative to low leaf allocation of plant biomass (i. e., LMR) while the inverse was true for the low-performing SD. Thus, high leaf allocation did not fully compensate for loss of leaf-level efficiency due to low chlorophyll concentration in SD. Conversely, the LMR differed significantly among varieties ranging from a low of 0.32 for the most productive FA to a high value of 0.64 for SD among the least productive varieties. Variation of SLA values remained statistically non-significant. Significant differences of LAR (i. e., the product of SLA x LMR) among varieties occurred largely owing to varying LMR thus specific patterns of biomass allocation, respectively. In the same vein, the ratio of shoot biomass to plant chlorophyll mass revealed significant differences and identified SV, FA, and FC as the most efficient bio-energy producers (Figure 3). The ratio was however, much more variable for the hybrids than for the parent species SV.

## Discussion

Our results for plant biomass, leaf area, SLA, and leaf chlorophyll concentration fell within the range of previously published SRC willow data (Robinson et al. 2004, Tharakan et al. 2005, Weih and Nordh 2005, Aasamaa et al. 2010, Hangs et al. 2011). We are in agreement with Weih and Nordh (2005) and Tharakan et al. (2008) that plant leaf area and chlorophyll mass represent key determinants of plant biomass. We were able to reliably predict plant biomass by structural up-scaling from leaf-level to plant-level and by correcting plant leaf area for leaf chlorophyll concentration. The predictive power of plant chlorophyll mass is important in light of the work by Weih and Nordh (2005) on *S. viminalis* and *S. dasyclados*. The authors demonstrated that the relationship between plant biomass and foliar N pool in pot-grown willows could be used to reliably predict long-term biomass production and final yields across some Swedish willow SRC sites. However, our experiment revealed the importance of LMR and identified the shoot-chlorophyll mass ratio to be useful for separating the morphological suitability of willow varieties for bio-energy production. Lack of any significant correlation between cutting mass and willow growth had previously been reported by Hangs et al. (2011) suggesting that cutting size was not critical for

successful willow establishment and growth.

Our results support the proposition of compensatory root absorption capacity by Osonne et al. (2008) as increases of LA and SLA did not reduce leaf-level efficiency and root biomass closely correlated with LA. Portsmouth and Niinemets (2007) also found biomass production in woody species to be more strongly associated with leaf-level efficiency than with structural traits. Significant biomass differences among varieties in our experiment suggested strong genotypic control of willow productivity. Leaf chlorophyll concentration has been shown to relate closely to DNA loading in tree species (c. f., Mathura et al. 2006). Biomass differences may have also been related to phenological variation of seasonal leaf development in our willow varieties originating from three different geographic regions (Weih 2009). Although there may be room for increasing plant leaf area and biomass production through fertilizer use, fertilizer does not lineally increase leaf chlorophyll concentration (Merilo et al. 2006). Increasing nutrient availability mainly enhanced LAR but only moderately leaf-level efficiency in the genus *Betula* (Portsmouth and Niinemets 2006).

## Conclusions

High photosynthetic capacity at low leaf allocation is a desirable trait combination for producing biomass for energy in willow short rotation coppice. Screening of seven species and five hybrids of willow for the growth parameters leaf area, leaf chlorophyll, and LMR rendered the hybrid 'Fabius' physiologically as well as morphologically most suitable for short rotation coppice. The quantity of shoot biomass produced per unit of plant chlorophyll mass, in short the shoot-chlorophyll mass ratio, proved to be a distinctive criterion effectively ranking the morphological suitability of willow varieties as bio-energy crops. The shoot-chlorophyll mass ratio is recommended for future screening of willow bio-energy species and hybrids. Our experiment has also shown that it has become feasible to increase data capture in willow selection trials and assess the productive potential of willows on a physiological basis with the availability of cost-efficient and rapid methods for determining leaf area and chlorophyll. This should expedite willow selection trials in a new SRC region like the Maritime Provinces of Canada towards the aims of establishing sustainable bio-energy production on abandoned farmland and reducing clear-cutting of natural broad-leaved forests for non-sustainable production of bio-energy.

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## References

- Aasamaa K., Heinsoo K., Hom B. 2010. Biomass production, water use and photosynthesis of *Salix* clones grown in a wastewater purification system. *Biomass Bioenergy* 34, 897-905
- Bokari U.G. 1983. Chlorophyll, dry matter, and photosynthetic conversion efficiency relationships in warm-season grasses. *J. Range Management* 36, 431-434
- Bray J.R. 1960. The chlorophyll content of some native and managed plant communities in central Minnesota. *Can. J. Bot.* 38, 313-333
- Bunn S.M., Rae, C.S., Herbert C.S., Taylor G. 2004. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. *Forestry* 77, 307-323

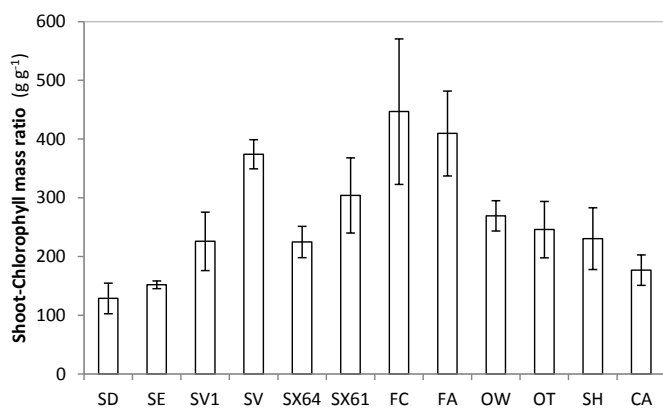


Fig. 3. Shoot-chlorophyll mass ratio for twelve willow varieties 90 after planting of cuttings for short rotation coppice near Sydney on Cape Breton Island (Canada) in 2010. Vertical bars denote SE ( $n = 4$ ).

- Cann D.B., MacDougall J.I., Hilchey J.D. 1963. Soil survey of Cape Breton Island. Report No. 12 Nova Scotia Soil Survey. Truro, NS, Canada
- Hangs R.D., Schoenau J.J., Van Rees K.C.J., Steppuhn, H. 2011. Examining the salt tolerance of willow (*Salix* spp.) bioenergy species for use on salt-affected agricultural lands. *Can. J. Plant Sci.* 91, 509-517
- Karp A., Shield I. 2008. Bioenergy from plants and the sustainable yield challenge. *New Phytologist* 179, 15-32
- Mathura S., Fossey A., Beck S.L. 2006. Comparative study of chlorophyll content in diploid and tetraploid black wattle (*Acacia mearnsii*). *Forestry* 79, 381-388
- Merilo E., Heinsoo K., Kull O., Söderbergh I., Lundmark T., Koppel A. 2006. Leaf photosynthetic properties in a willow (*Salix viminalis* and *Salix dasyclados*) plantation in response to fertilization. *Eur. J. Forest Res.* 125, 93-100
- Osone Y., Ishida A., Tateno M. 2008. Correlation between relative growth rate and specific leaf area requires associations of specific leaf area with nitrogen absorption rate of roots. *New Phytologist* 179, 417-427
- Portsmouth A., Niinemets U. 2006. Interacting controls by light availability and nutrient supply on biomass allocation and growth of *Betula pendula* and *B. pubescens* seedlings. *For. Ecol. Management* 227, 122-134
- Portsmouth A., Niinemets U. 2007. Structural and physiological plasticity in response to light and nutrients in five temperate deciduous woody species of contrasting shade tolerance. *Functional Ecol.* 21, 61-77
- Richardson A.D., Duigan S.P., Berlyn G.P. 2002. An evaluation of non-invasive methods to estimate foliar chlorophyll content. *New Phytologist* 153, 185-194
- Robinson K.M., Karp A., Taylor G. 2004. Defining leaf traits linked to yield in short-rotation coppice *Salix*. *Biomass Bioenergy* 26, 417-431
- Tharakan P.J., Volk T.A., Nowak C.A., Abrahamson L.P. 2005. Morphological traits of 30 willow clones and their relationship to biomass production. *Can. J. For. Res.* 35, 421-431
- Tharakan P.J., Volk T.A., Nowak C.A., Ofezu G.J. 2008. Assessment of canopy structure, light interception, and light-use efficiency of first-year regrowth of shrub willow (*Salix* sp.). *Bioenergy Res.* 1, 229-238
- Van der Berg A.K., Perkins T.D. 2004. Evaluation of a portable chlorophyll meter to estimate chlorophyll and nitrogen contents in sugar maple (*Acer saccharum* Marsh.) leaves. *For. Ecol. Management* 200, 113-117
- Van Rees, K.C.J. 2008. Developing a national agroforestry and afforestation network for Canada. *Policy Options* 29, 54-57
- Verwijst T., Wen D.-Z. 1996. Leaf allometry of *Salix viminalis* during the first growing season. *Tree Physiology* 16, 655-660
- Weih M. 2009. Genetic and environmental variation in spring and autumn phenology of biomass willows (*Salix* spp.): effects on shoot growth and nitrogen economy. *Tree Physiology* 29, 1479-1490
- Weih M., Nordh N.-E. 2005. Determinants of biomass production in hybrid willows and prediction of field performance from pot studies. *Tree Physiology* 25, 1197-1206