Industrial Crops and Products 97 (2017) 525–535

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Extractable phenolic yield variation in five cultivars of mature short rotation coppice willow from four plantations in Quebec

Nicholas J.B. Brereton[a,*], Nicolas Berthod[a], Benoit Lafleur[b], Karine Pedneault[c], Frederic E. Pitre[a,d], Michel Labrecque[a,d]

[a] Institut de recherche en biologie végétale (IRBV), University of Montreal, 4101 Sherbrooke E, Montreal, QC, H1X 2B2, Canada
[b] Institut de recherche sur les forêts, Université du Québec en Abitibi-Témiscamingue 445, boul. de l'Université Rouyn-Noranda, QC, J9X 5E4, Canada
[c] Department of Science, Université Sainte-Anne, Church Point, NS, Canada
[d] Montreal Botanical Garden, 4101 Sherbrooke E, Montreal, QC, H1X 2B2, Canada

ARTICLE INFO

Article history:
Received 28 August 2016
Received in revised form
23 December 2016
Accepted 27 December 2016
Available online 9 January 2017

Keywords:
Salix cultivars
Phenolics
Green chemicals
Biomass

ABSTRACT

Short rotation coppice willow (*Salix* sp.) is well established as an effective phytoremediation crop and is also emerging as an attractive lignocellulosic bioenergy option. The prospect of integrating value-added renewable chemicals as a supplementary component of the crop's value is explored here in terms of absolute phenolic yields, extractable from field cultivated mature biomass. Five willow cultivars, selected as leading biomass yielding cultivars, were cultivated at four field sites in Quebec, Canada: *Salix x dasyclados* 'SV1', *Salix viminalis* 'SV5027', *Salix miyabeana* 'SX61', 'SX64', 'SX67'. Substantial and significant cultivar variation was observed in groups of compounds including hydroxycinnamic acids and derivatives, benzoic acids derivatives, flavonols and condensed tannins in concentration per ton of biomass and/or yield per hectare per year. The highest phenolic yields were produced by *Salix miyabeana* 'SX67' cultivated at St-Roch at 5.43 (±0.60) kg ha^{-1} yr^{-1}, which also produced 35.54 (±4.10) kg ha^{-1} yr^{-1} condensed tannins. These phenolic yields suggest further exploration of renewable chemicals production as a supplement to other biomass end-uses could be worthwhile. Such flexibility would help provide important advantages for weathering renewables policy and market uncertainty as well as improve the feasibility and competitiveness of sustainable biomass production as part of an integrated green technology platform.

1. Introduction

Willow is an important dedicated bioenergy crop in temperate regions (Smart et al., 2007) due to fast biomass accumulation from low agricultural inputs as well as having the potential for naturally high accessibility cell walls (to deconstruction enzymes) at maturity (Serapiglia et al., 2013).

Cultivation of biomass is often seen as the most substantial hurdle for economically feasible bioenergy production (Gnansounou and Dauriat, 2010). One way to reduce, or abolish, such cultivation costs is to produce biomass on marginal, degraded or even polluted land (phytoremediation) (Labrecque and Teodorescu, 2005). In this manner, cultivation can serve as a positive value-stream, in terms of the financial profit as well as a clear local environmental benefit (FCM, 2009), helpful for relieving at least some of the concerns over the environmentally positive contribution of biofuels within the renewable energy landscape. Specifically in crops such as willow, secondary metabolite induction by abiotic stress has been extensively researched (Tegelberg and Julkunen-Tiitto, 2002) and one of the reasons willow is thought to be able to tolerate highly challenging growth conditions is due to highly intricate secondary metabolite production (de Jong et al., 2015).

Over 8000 phenolic compounds can be produced by plants (Bravo, 1998) to perform a wide range of functions including abiotic and biotic resistance (Dai and Mumper, 2010). In terms of value, compounds such as condensed tannin can be used as green alternatives to synthetic compounds used in adhesive production (Ping et al., 2011) as well as environmentally friendly bioflocculants and biocoagulants (Beltran-Heredia and Sanchez-Martin, 2009). Lignans are currently extracted primarily from crops such as flax and are thought to be effective in mammals *in vivo* as antioxidants, having the potential as a cancer chemopreventative as well as anti-inflammatory activity (During et al., 2012). Hydroxycinnamic acids and derivatives as well as benzoic acids are produced throughout the plant world and there is an extensive amount of research describing anti-inflammatory, anti-tumoural, antioxidant,

* Corresponding author.
E-mail address: nicholas.brereton@umontreal.ca (N.J.B. Brereton).

http://dx.doi.org/10.1016/j.indcrop.2016.12.049
0926-6690/

antimicrobial, antimutagenic and anti-melanogenic function (Zhao and Moghadasian, 2010). Importantly, in the context of a potential added-value stream for an integrated bioenergy process, the botanicals commodity industry (often also termed nutraceuticals or chemoceuticals) extensively uses plant flavonoids as product "bio-" additives. For example: the flavan-3-ols catechin and epicatechin are present in high levels in black, green and white tea (Rusak et al., 2008) and are often sold with claims to reduce human DNA mutation rates although little *in vivo* human evidence is available. Similarly for flavonols, compounds such as quercetin, which is present in garlic, leeks and onions in high levels, are thought could have positive impacts on the human immune system and prevent cancer through *in vivo* antioxidant action (Shan et al., 2009) although only a small amount of evidence is available suggesting that human consumption of food rich in quercetin necessarily reflects the purified compound activity (Knekt et al., 2002). Another flavonol, kaempferol, is present in high concentrations in many common berry varieties and could reduce cancer cell proliferation (although, again, little convincing evidence of human *in vivo* activity from berry consumption is available) (Duthie, 2007). Whilst many of these claims are lacking evidence, certainly for the highly complex activities often claimed in humans, it is clear that the nutraceutical industry itself is enormous with some market research (published by Global Industry Analysts, Inc. April 1, 2014) estimating the value of the industry at $ 165.62 billion in 2014 and set to grow by over 7% a year until 2021. Given the scale of this industry, the potential of phenolic compound extraction as a supplementary value stream to improve economic competitiveness of environmentally sustainable bioenergy cannot be ignored.

Few studies have directly determined yields of phenolic compounds in willow from mature stem biomass when extracted and quantified in absolute amounts, as they could be as part of a green chemicals platform integrated with bioenergy. Here, highly abundant phenolics are assessed and compared between five leading (in terms of high biomass yields) North American willow cultivars grown at four field sites across Quebec, Canada (Fig. 1). Yields are compared to other phytochemical production crops in terms of tissue concentrations as well as yields per hectare in order to establish if green chemical production is high enough to contribute to a sustainable bioenergy production system.

2. Methods

2.1. Study sites

The study was conducted on four sites representing a relatively broad climatic gradient. These sites are located throughout Quebec and present marked differences regarding regional climatic (Fig. 1) and soil conditions (Table 1). According to the nearest weather station for each site (EnvironmentCanada, 2015), from 2012 to 2013 average annual temperatures varied between 4.8 °C (St-Siméon) and 7.3 °C (Beloeil and St-Roch), whereas total annual precipitation varied between 776 mm (La Pocatière) and 1010 mm (St-Roch). During the growing season (i.e. May to September), average temperatures varied between 14.5 °C (St-Siméon) and 17.9 °C (Beloeil and St-Roch), whereas total precipitation varied between 423 mm (La Pocatière) and 599 mm (St-Roch). Finally, growing degree-days >5 °C vary between 1504 (St-Siméon) and 2166 (St-Roch). P, K, Ca, and Mg were extracted by Mehlich-3 digestion and determined using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS). Soil texture was determined by granulometric analysis (Soil Classification Working Group/Groupe de travail sur la classification des, 1998)

2.2. Cultivars and experimental design

The experiment was a randomized complete block design, where five willow cultivars (i.e. *Salix* × *dasyclados* 'SV1', *S. viminalis* 'SV5027', and *S. miyabeana* 'SX61', 'SX64' and 'SX67') were evaluated. Construction of confident *Salix* phylogeny is confounded by polyploidy and can be obscure, including important cultivars such as 'SV1' (Lauron-Moreau et al., 2015) where pedigree is disputed (could potentially be an *S. viminalis*, *S. caprea* and *S. cinerea* hybrid). The three cultivars ('SX61', 'SX64' and 'SX67') are all *S. miyabeana*, a promising biomass yielding species which has also been shown can be distinctly high yielding within lignocellulosic biofuel processing (Ray et al., 2012).

All treatments were replicated four times for a total of 20 plots per site. Prior to the establishment of the experimental plantations, each site was plowed in fall 2010, and harrowed two times the following spring prior to planting. In spring 2011, cuttings were planted 0.3 m apart along five rows spaced by 1.80 m. Each row consisted of 20 cuttings, for a total of 100 cuttings per cultivar per block (i.e. 400 cuttings per cultivar per site, or 18 500 cuttings ha^{-1}). In compliance with common willow cultivation practices, stems were coppiced (cut back) in fall 2011, and sites were fertilized in spring 2012. Fertilization was provided using a granular mineral fertilizer at a dose of 100 kg N ha^{-1}, 100 kg P ha^{-1} and 150 kg K ha^{-1}. In plantations where soil analyses performed prior to plantation showed a minimum of 100 kg P ha^{-1} and 150 kg K ha^{-1}, however, only N fertilization was conducted.

2.3. Field sampling

In early November 2013, six trees (coppiced willows are often also termed shrubs due to their multi-stem morphology) per cultivar per block were randomly collected from the three middle rows and weigh on the field to estimate fresh weight biomass yield produced during the growing season. These plants were subsampled and oven dried at 70 °C to constant weight in order to determine stem moisture content, which was then used to determine plant dry weight. These results were further used to estimate cultivar yield (oven dry ton) on a per hectare per year basis.

Furthermore, for one tree per cultivar (x5) per block (x4) per site (x4) stem wood subsamples were collected (making 80 trees in total) to determine their phenolics compound concentrations. Stem samples (of 10 cm length) were collected for extraction at mid-point of the tallest stem and ground using a Wiley mill. Particle size was standardised by meshing through a 40 mesh (525 μm sieve). Phenolic concentrations were further combined to biomass estimations to estimate phenolics yields on a per hectare per year basis.

2.4. Phenolics extraction and analysis

Willow tissues typically contain a large variety of phenolic compounds, 36 of which were targeted for concentration and yield analyses due to their abundance within mass spectrometry detectable limits (analyses described below, presented in full in supplementary file 1). In order to estimate expected yields from mature biomass with relevance to biorefinery, compounds are grouped for presentation as: shikimic acid, hydroxycinnamic acid and derivatives (*p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, ferulic acid, caffeic acid, caffeoyl glucoside, coumaroyl glucoside and 5-caffeoylquinic acid), benzoic acid derivatives (vanillic acid, gallic acid, 3,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, hydroxybenzoic acid and salicylic acid), flavonols and their derivatives (quercetin, quercetin glucoside/galactoside, quercetin rhamnoside, quercetin 3-arabinoside, quercetin diglucoside, kaempferol