JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

DOI:10.1021/jf100887v

Factors Influencing the Variability of Antioxidative Phenolic Glycosides in *Salix* Species

NADJA FÖRSTER,*,† CHRISTIAN ULRICHS,† MATTHIAS ZANDER,† RALF KÄTZEL,§ AND INGA MEWIS#

†Division Urban Plant Ecophysiology, Faculty for Agriculture and Horticulture, Humboldt University of Berlin, Lentzeallee 75, 14195 Berlin, Germany, §Section of Forest Development/Monitoring, Landesforstanstalt Eberswalde, Alfred-Möller-Strasse 1, 16225 Eberswalde, Germany, and #Department Quality, Leibniz-Institute of Vegetable and Ornamental Crops, Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany

Phenolic glycosides, especially the salicylates, are important secondary metabolites in the bark of willows (*Salix* spp.). Because of their anti-inflammatory, analgesic, and fever-reducing properties, they are of particular interest to society. Compared to the fabrication of synthetic salicylacetylic acid, the commercial production of willow bark extracts with adequate amounts of salicylate is very difficult due to several biological and technical reasons. Therefore, one of the objectives was to identify salicylate-rich clones from three species, *Salix daphnoides*, *Salix purpurea*, and *Salix pentandra*, with potentially high amounts of phenolic glycosides. Three hundred different *Salix* clones were collected, and the chemical profiles of their bark were analyzed by HPLC. Overall, *S. daphnoides* clones showed the highest phenolic glycoside contents, followed by *S. purpurea* and *S. pentandra*. Second, seasonal changes of secondary compounds in willow bark were analyzed to determine the optimal harvesting time. The phenolic glycoside levels decreased over the growing season, with highest contents detected during plant dormancy. The effects of different cultivation conditions were also examined, and none of these treatments were found to have a significant effect on the phenolic glycoside content in willow bark. Biomass accumulation in the clones with grass competition was significantly lower than in the other three treatments.

KEYWORDS: Phenolic glycosides; salicin; salicylates; *Salix*; season variability; willow

INTRODUCTION

To avoid the harmful side effects of synthetic pharmaceuticals, many people prefer to use herbal medicines to relieve their symptoms and improve their health. A survey by Eisenberg et al. (*5*) in 1997 showed that 12.1% of adults in the United States had taken pharmaceuticals in the past 12 months. Medicinal herbs, such as willow bark extract, are among some of the earliest medicines used. Mahdi (*14*) mentioned the usage of willows as drug and nutrition in Mesopotamia 6000 years ago. Herbal medicine has recently gained a renewed interest from the general public as well as the scientific community. The ingredients of willow bark are known for their anti-inflammatory, analgesic, and fever-reducing effects. Certain phenolic glycosides of *Salix* bark, particularly salicin, and their esters (such as tremulacin or salicortin) have been shown to relieve rheumatic disturbances, infections, and headache (*1*−*3*, *13*, *19*). Because of high fabrication costs, sick people often buy the cheaper synthetic medicines such as non-steroid antirheumatics or acetylsalicylic acid (aspirin). With the development of asprin and its attribute as an over the counter medicine in 1900, the demand for this synthetic medicine increased. Whereas aspirin has healing properties similar to those of willow bark extracts, this synthetic drug can also have adverse side effects, including the aggregation of thrombocytes or local lesions of the gastric mucosa. These negative side effects are not found with the use of herbal preparations of willow bark. Furthermore, recent clinical trials confirm the effectiveness of willow bark as a therapeutic agent (*1*−*3*, *13*, *19*). These studies show the effectiveness of willow bark extract and helped revive interest in this herbal medicine at the end of the 20th century.

To date, the extraction, identification, and quantitative determination of biologically active phenolic compounds (e.g., salicylates) from *Salix* bark and usage in herbal medicines are of significant importance. The European Scientific Cooperative on Phytotherapy (ESCOP), an umbrella organization of the national organizations for phytotherapy, publishes monographs for the harmonization of phytopharmaca. Because of diverse claimed characteristics published in monographs by different organizations, this harmonization is very important. The ESCOP concluded that in order for the production of the extract to be economically and medically feasible, it must contain a minimum of 1.5% total salicin. Otherwise, the salicin content in the extract would be too small to induce a healing effect or excessive amounts of bark material would be needed to reach the necessary doses. During the manufacture of willow bark extracts the main part of the salicylates are converted to salicin. In the United States,

*Author to whom correspondence should be addressed (phone +49 30 31471351; fax +49 30 31471407; e-mail NADfoerster@aol.com).

 Published on Web 07/01/2010

herbal pharmaceuticals are classified as dietary supplements and, therefore, they are not under the control of the Food and Drug Administration (FDA). The manufacturer is responsible for the quality of the produced drug but, regardless, there is an increased demand for effective herbal preparations.

Differences of the chemical phenolic glycoside profiles among *Salix* species have been reported by Julkunen-Tiitto (*9*), Meier et al. (*16*), and Thieme (*22*, *23*). Furthermore, single compounds can differ among species and clones, and it is reported that the salicylate and phenolic glycoside contents vary by season (*6*, *15*, *16*, *23*, *24*). However, the seasonality of these secondary metabolites is not well understood, and reports about the yearly changes give contradictory results (*15*, *24*). Therefore, one of the research objectives was to analyze constitutive salicylate and phenolic glycoside contents in the bark of three *Salix* species (*Salix daphnoides*, *Salix purpurea*, and *Salix pentandra*) to identify salicylate-rich clones. These clones were then used to study the influence of season on secondary metabolite levels. Furthermore, in the literature a lot of contradictory findings are written about the impact of growth conditions on secondary metabolite concentration (*12*, *17*). In addition to genetic factors, environmental properties play an important role in the content of secondary metabolites. For this reason, effects of different cultivation conditions on the salicylate and phenolic glycoside contents were analyzed last. Because a high biomass accumulation per hectare is necessary to gain a high salicylate harvest (*18*), growth parameters such as shoot length and shoot number were determined.

MATERIALS AND METHODS

Seasonal Analyses. Osier stacks of different willow clones from *S. daphnoides* Vill., *S. purpurea* L., and *S. pentandra* L. were collected in northeastern Germany (eastern Brandenburg, Baltic Sea coast) and northwestern Poland (Baltic Sea coast) between April and May 2006 and stock planted in Zepernick (Berlin). The phenolic glycoside content of all clones was analyzed.

On December 2006, bark samples from 140 clones were collected and analyzed. According to their chemical profiles seven independent clones with high phenolic glycoside content of *S. daphnoides* (DA7, DA40, DA52, DA56, DA58, DA61, DA64) and *S. purpurea* (PU9, PU10, PU11, PU12, PU15, PU24, PU25) as well as four clones of *S. pentandra* (PE11, PE12, PE13, PE23) were selected for further studies. Willow bark from each clone was collected for chemical analysis in March, June, July, August, October, and December 2007 as well as April 2008 ($n = 5$) to determine changes within phenolic glycosides over the year.

Cultivation Analyses. Three different clones from each *Salix* species (*S. daphnoides*, *S. pentandra*, and *S. purpurea*) were cultivated in Wriezen (in northeastern Berlin, Germany) under different growing conditions. Four treatments were tested: dry position, damp position, grass competition, and narrow planting. Three clones from each species (*S. daphnoides*, S48, S66, DA83; *S. pentandra*, PE11, PE12, PE13; *S. purpurea*, PU1, PU68, ZA1) were analyzed ($n = 3$). For the two moisture treatments, the sample areas had a natural water gradient based on different soil textures. On the dry position the clay-silt fraction was 10% and on the damp position it was 25%. On the area with the lesser clay-silt fraction, the water-holding capacity (WHC) is much lower, and water quickly drains away, whereas the area with the high amount of clay-silt fraction is affected by bank water for a long period of time during the year. Consequently, because of the different soil textures the WHC differs very clearly on the sample area, and different levels of plant-available water can be found. Normally (dry and damp condition, grass treatment), the osier stacks were planted 50 cm apart, but in the narrow planting treatment this distance was reduced to 25 cm. For the grass competition treatment, the willow clones grew with perennial ryegrass (*Lolium perenne*), Kentucky bluegrass (*Poa pratensis*), and creeping fescue (*Festuca rubra*) and, therefore, had to compete for water and nutrients. The phenolic glycoside content, biomass accumulation, survival shoot length, and shoot number of the clones in the four cultivation treatments were analyzed.

Table 1. Mean Salicylate (SC) and Phenolic Glycoside (PGC) Contents of the 15 Top Clones of Each *Salix* Species in December 2006 and 2007

	S. daphnoides		*S. purpurea*		*S. pentandra*	
	2006	2007	2006	2007	2006	2007
SC (%)	6.5 ± 1.0	8.9 ± 1.8	4.0 ± 0.5	6.7 ± 1.4	3.1 ± 0.6	3.6 ± 0.9
mean	7.7 ± 1.6		5.3 ± 1.2		3.3 ± 0.7	
PGC (%)	8.3 ± 1.0	11.5 ± 2.2	5.2 ± 0.6	9.0 ± 1.6	4.0 ± 0.6	4.8 ± 1.3
mean	9.9 ± 1.8		7.0 ± 1.7		4.4 ± 0.9	

Sample Preparation. The peeled bark was lyophilized until dryness and milled, and 50 mg was used for the extraction of phenolic glycosides. For the first extraction, 750 μL of 80% methanol was used with 150 μL of standard solution (5 mM resorcinol). The samples were kept in an ultrasonic bath on ice for 10 min and then centrifuged at 6000 rpm for 5 min to collect the supernatant. The pellet was used for re-extraction with 500 μL of 80% methanol twice more. The combined supernatants were concentrated in a vacuum concentrator until 250 μL of liquid was left and filled to 1 mL with ultrapure water. After filtration, the extracts were analyzed by HPLC. The willow bark extracts (10 μL) were injected in a Dionex P680A HPLC system equipped with an ASI-100 autosampler and a PDA-100 photodiode array detector. Phenolic glycosides were separated on a narrow-bore column (Acclaim Polar Advantage C16, 3 μm, 120 Å, 2.1 × 150 mm, Dionex). Eluents used for HPLC analysis were (A) 2% tetrahydrofuran and 0.5% phosphoric acid and (B) 100% methanol. The eluent flow program was as follows: 0% B (0−5 min), 15% B (10 min), 25% B (20 min), 35% B (30 min), 50% B (35 min), 100% B (40−42 min), and 0% B (44−49 min). The flow rate was 0.35 mL/min, and the eluent was monitored at 270 nm. Qualitative identification of the phenolic glycosides was done by using standards and via specific UV spectra (*20*). The quantitative analysis was based on the peak area relative to the standard resorcinol by using respective response factors. For the peak evaluation the software Chromeleon version 6.0 was used.

Statistical Analysis. Data were analyzed for significant differences using analysis of variance following the mean comparison test Tukey's HSD for honest significant difference with SYSTAT 12.0.

RESULTS

The quantitative analysis of the phenolic glycoside content of each species indicates that *S. daphnoides* usually contained the highest mean amounts of salicylates and phenolic glycosides, followed by *S. purpurea* and *S. pentandra*. Of about 270 analyzed *Salix* species the average contents in the bark of the 15 best clones of each *Salix* species in December 2006 and 2007 were as follows: *S. daphnoides* had mean salicylate and phenolic glycoside contents of 7.7 and 9.9%, *S. purpurea* 5.3 and 7.0%, and *S. pentandra* 3.3 and 4.4% (**Table 1**). The contents of salicylate and phenolic glycosides in willow bark were higher in 2007 than in 2006 (**Table 1**). The calculated standard variation shows that the interspecific variability is lower in 2006 than in 2007. On average, the variability of the secondary metabolite content in each species varies between 11 and 25%.

Furthermore, the salicylate and phenolic glycoside contents varied significantly between the clones of each species (**Table 2**). The intraspecific variability (s%) of the salicylate content was highest in *S. pentandra*. *S. purpurea* showed the highest variability between the different clones in phenolic glycoside content (**Table 2**). In *S. daphnoides* the highest phenolic glycoside and salicylate contents were found in April 2008. Clone DA64 had significantly lower amounts of salicylate and phenolic glycoside than many other clones from that species (**Table 2**).

On the basis of the clone screening from December 2006, *Salix* clones with the highest phenolic glycoside contents were selected to examine the seasonal variability of secondary metabolites in willow bark. In all three species salicylate and phenolic glycoside maxima were generally found from fall until spring, whereas

Table 2. Significant Differences in the Salicylate (SC) and Phenolic Glycoside (PGC) Contents in the Three *Salix* Species at Peak Concentration[a]

	S. daphnoides April 2008			*S. pentandra* December 2007			*S. purpurea* October 2007	
	SC (%)	PGC (%)		SC (%)	PGC (%)		SC (%)	PGC (%)
DA7	5.04 ab	6.99 AB	PE11	1.87 b	2.70 A	PU9	5.06 ab	6.00 AB
DA40	5.81 a	7.80 A	PE12	2.73 a	3.45 A	PU10	5.53 a	7.08 A
DA52	5.03 ab	7.39 A	PE13	1.90 b	2.66 A	PU11	4.57 bc	5.59 BC
DA56	5.44 a	7.62 A	PE23	2.64 ab	3.24 A	PU12	3.15 d	4.45 C
DA58	5.30 a	7.47 A				PU15	3.91 cd	4.99 BC
DA61	5.05 ab	7.57 A				PU24	4.21 bc	5.40 BC
DA64	4.17 b	5.55 B				PU25	4.72 abc	6.41 AB
mean	5.12	7.20	mean	2.29	3.01	mean	4.45	5.70
s[b]	0.51	0.77	*s*	0.46	0.39	*s*	0.78	0.88
s%[c]	9.87	10.68	*s*%	20.28	13.07	*s*%	17.56	15.47

[a] Different lower case letters indicate significant differences of salicylate contents within the species and upper case letters indicate differences within levels of phenolic glycosides, Tukey's HSD test, $p < 0.05$. [b] Standard deviation. [c] Percentage standard deviation.

phytochemical minima were occurring during the summer. In general, the phenolic glycoside and salicylate contents decreased over the year from March to June 2007, from June to July 2007, and further from July to August 2007 (**Figure 1**). There was one exception; the phenolic glycoside content of *S. pentandra* did not change from June to July. Overall, the decrease in secondary metabolite contents from March to June was greater than from June to July and from July to August. In general, the decrease in the phenolic glycoside content from March to June was significant ($p < 0.05$). The decrease in the salicylate content was significant from March to June as well as from June to August in *S. daphnoides* clones, from March to August in *S. pentandra* clones, and from March to July in *S. purpurea* clones. After the clones of all *Salix* species reached their minimum secondary metabolite concentrations in August 2007, an increase of the phenolic glycosides took place until the end of the year during plant dormancy. This increase was significant for the salicylate of *S. daphnoides* and *S. purpurea* from August to October ($p < 0.05$). An increase of the phenolic glycoside content was determined from August to October in *S. daphnoides* and *S. purpurea* clones and from August to December in *S. pentandra* clones.

The different cultivation treatments had no significant effect on salicylate and phenolic glycoside contents (**Table 3**). However, the treatments did affect growth characteristics of the *Salix* clones (**Table 4**). Willow clones grown with grass competition had a significantly lower shoot length (1.52 m) than clones grown in the dry position (2.31 m) or the narrow planting (2.44 m) ($p < 0.05$). Biomass accumulation in the clones with grass competition was significantly lower (0.29 kg) than in the other three treatments, with 0.99 kg on average ($p < 0.05$). The shoot number and survival were not significantly different among the cultivation treatments.

DISCUSSION

In addition to interspecific variability, the obtained data reveal that it is very important to know the exact salicylate and phenolic glycoside contents of each clone in the species, due to an intraspecific (**Tables 1** and **2**) and clone variability. Because of clone variabiities, up to 20% (data not shown) high intraspecific variabilities of the willow species were not unexpected. Therefore, the right clone selection seems to be a very important factor to get plant material with highest contents of salicylates.

The results concerning the seasonal changes of phenolic glycosides reveal significant seasonal variations of secondary metabolite concentrations in willow bark. In general, the findings described in this paper are consistent with the trend documented by Thieme (*23*, *24*). He observed the highest phenolic glycoside content in willows (for example, *S. purpurea* and *S. pentandra*) during March. During the growing season, he reported a decrease of secondary metabolite contents in bark with its minimum occurring during September/October. He hypothesized that these compounds accumulated in the bark during the autumn and winter. The trend of decreasing phenolic glycoside concentration over the summer shown in this paper was also consistent with Thieme's work (*23*, *24*). The result of analysis in this paper showed the lowest concentration of the phenolic glycosides in August. A detected compound concentration minimum in October shown by Thieme could be excluded on the basis of the described data. By evaluating the received results an accumulation of phenolic glycosides took place starting in August. Contrary to this, Meier et al. (*15*, *16*) reported that the salicylate and phenolic glycoside contents from the species *S. daphnoides* and *S. purpurea* decreased from winter to summer.

The highest phenolic glycoside contents of the species were found from autumn to spring. Consequently, an accumulation of these special secondary metabolites took place during plant dormancy, when the plant had already lost its leaves after the first frost. *S. daphnoides* showed the maximum secondary metabolite concentration in spring (March/April), *S. pentandra* in winter (December), and *S. purpurea* in autumn (October). There seems to be no connection between the phenolic glycoside content and the duration of plant dormancy. *S. daphnoides* and *S. daphnoides* are early-flowering species compared to *S. pentandra*, which is a very late flowering *Salix* species. The salicylates and phenolic glycoside contents of all three species decreased over spring and summer and reached a minimum in August.

Although the importance of cultivation in the literature is frequently cited, we did not find a significant effect of the four cultivation treatments (wet, dry, grass competition, and limited space) on the phenolic glycosides of willow bark. It was expected that a narrow planting as well as growth in a grassy area would increase the competition for nutrients and water with a subsequent decrease in secondary metabolite content. Coley et al. (*4*) mentioned a trade-off between growth and secondary metabolite accumulation in plants. The soil−water availability should affect the growth of the willow species on damp site adapted plants in a positive way and therefore the phenolic glycoside concentration negatively. However, no differences in secondary metabolite concentrations were found among variants, but growth and biomass accumulation in some of the variants were affected by treatment. On the basis of the described analysis the willow growth was not affected by narrow planting or under the different water status positions. However, willow clones growing in the grass treatment had depressed biomass accumulation. For this treatment, willow plants had to compete with the different grasses for water and nutrients.

In an analysis by Paunonen et al. (*17*), mulching had a positive effect on the plant growth of *Salix myrsinifolia* because of less weed competition. In addition, the water availability is increased, the soil temperatures are stabilized, and water erosion is reduced. The plant growth and thereby the yield can be increased. Because of a higher competition for water and nutrients the depressed biomass accumulation on the grass sowing area in our analysis could be explained. As well, Heiska et al. (*7*) showed that with the use of black polythene mulch the hectare yield of salicylates in *S. myrsinifolia* could be enhanced through a greater biomass accumulation.

The fabrication of willow bark extracts is very expensive. Due to this, using willow species and clones with high amounts of

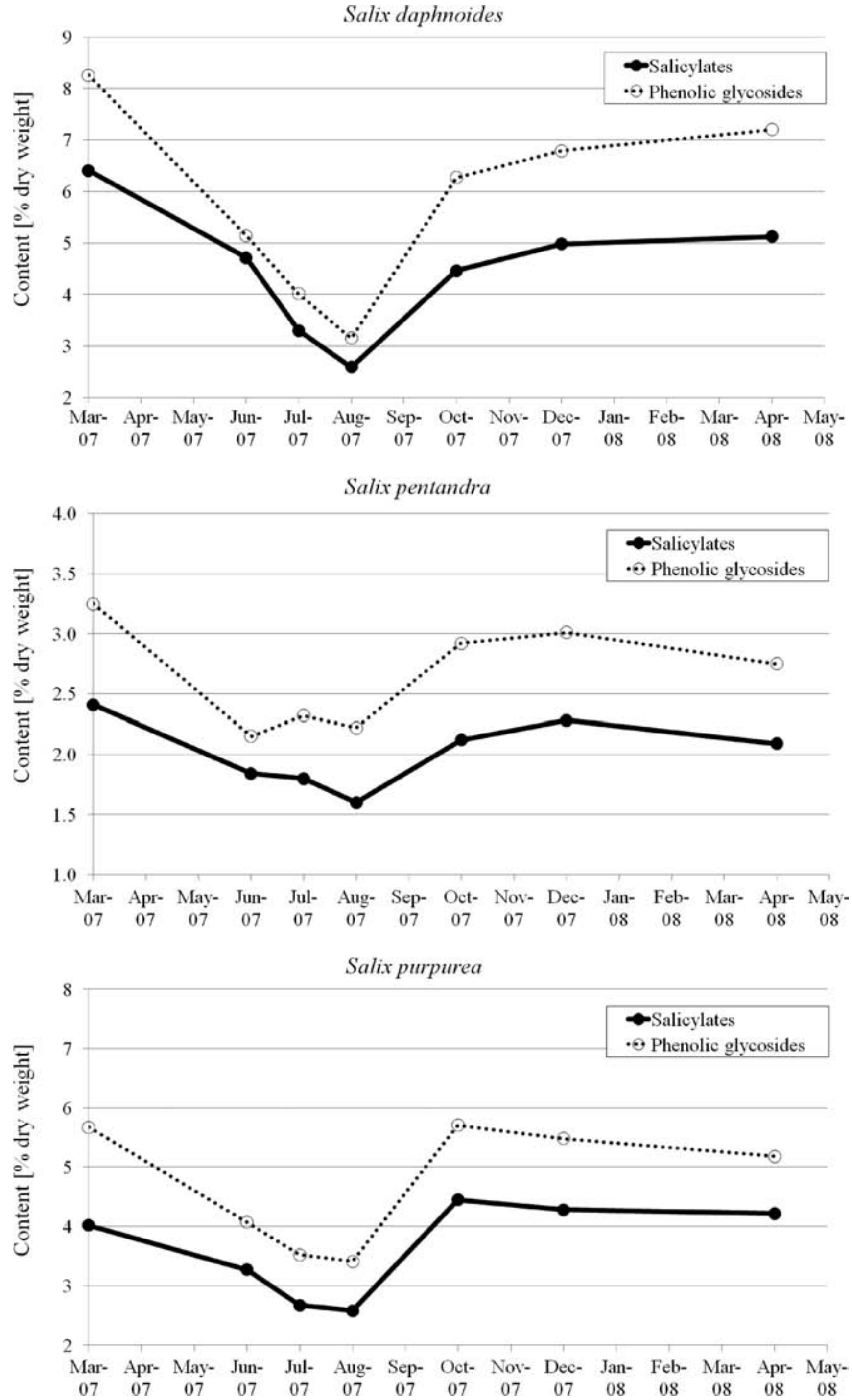

Figure 1. Seasonal variability of the salicylate and phenolic glycoside contents of the three *Salix* species.

salicylates is obligatory. However, identification and maintenance of salicylate-rich species is very difficult because of high interspecific (*9*, *16*, *22*, *23*) and intraspecific variation (**Tables 1** and **2**) of secondary metabolite content. In addition, to have maximum harvest per hectare, individual clones need to have high growth performance and a large number of shoots (*18*).

In addition to the utilization of the bark, other plant organs of willow, such as the leaves, contain phenolic glycosides (*9*, *11*, *22*) and could be used as base material for phytopharmaca production. Thieme (*24*) found that not all *Salix* species have the ability to synthesize salicylates in their leaves. Thieme (*22*, *24*) and Hiller and Melzig (*8*) reported that *S. purpurea* offers high salicylate concentrations with up to 6%. *S. pentandra* showed about only 2% minor salicylate contents. For *S. daphnoides* no examination has been done. Investigation of the secondary metabolite contents of the leaves is the object of our further studies to find out whether an additional use of the leaves for fabrication of willow bark extracts is beneficial. Because of an exclusion of a relocation of phenolic glycosides from the leaves in the bark before leaf abscission in the fall (*11*), the use of bark and leaf material of some species is imaginable.

Besides the willow species and clone variability (*6*, *9*), many other parameters influence the secondary metabolite content of willows. These include climate (*10*, *16*), physiological age (*9*, *21*, *24*), time of day when the bark harvest took place (*24*), and gender (*22*). The higher phenolic glycoside concentration of all three species in 2007

Table 3. Salicylate (SC) and Phenolic Glycoside (PGC) Contents of the Three *Salix* Species under the Four Different Cultivation Treatments

	S48		S66		DA83	
S. daphnoides	SC (%)	PGC (%)	SC (%)	PGC (%)	SC (%)	PGC (%)
narrow planting	4.07	4.98	5.51	6.65	7.26	8.45
damp position	4.28	5.15	4.83	6.23	7.96	9.62
grass sowing variant	3.64	4.34	5.83	7.48	7.78	9.04
dry position	4.22	5.02	6.44	7.80	7.29	8.54
S. purpurea	**PU1**		**PU68**		**ZA1**	
	SC (%)	PGC (%)	SC (%)	PGC (%)	SC (%)	PGC (%)
narrow planting	3.33	4.05	3.11	4.36	3.84	4.55
damp position	3.13	3.70	3.10	4.89	4.04	5.30
grass sowing variant	2.58	3.43	3.01	5.03	5.30	5.38
dry position	2.92	3.69	2.73	3.95	5.16	6.06
S. pentandra	**PE11**		**PE12**		**PE13**	
	SC (%)	PGC (%)	SC (%)	PGC (%)	SC (%)	PGC (%)
narrow planting	2.35	3.14	3.50	4.01	2.22	2.75
damp position	2.57	3.30	2.50	3.06	1.79	2.33
grass sowing variant	2.24	3.10	2.10	2.61	1.82	2.73
dry position	2.76	3.55	3.30	3.77	2.56	3.08

Table 4. Influence of Cultivation Treatment on Willow Growth

	growth parameters of willow clones[a]			
cultivation variant	deficit (%)	shoot number (*n*)	shoot length (m)	biomass accumulation (kg)
narrow planting	25.70 a	10.00 a	2.44 a	0.95 a
damp position	18.98 a	7.78 a	2.29 ab	1.06 a
grass sowing variant	40.28 a	5.89 a	1.52 b	0.29 b
dry position	39.35 a	10.78 a	2.31 a	0.95 a

[a] Different letters indicate significant differences within one growth parameter between the cultivation variants, Tukey's HSD test, $p < 0.05$.

(**Table 1**) could be linked to a different climate in 2006 and 2007. Through a long-lasting warm climate in 2006 the growth period as compared to 2007 was extended. Expecting a trade-off between biomass accumulation and plant growth, it is assumed that the longer growth period was used for greater biomass accumulation, and therefore lower levels of phenolic glycosides were determined. The variability of the secondary metabolites cannot be linked to environmental factors only. Genetic properties play an important role as well.

All parameters (plant physiological and economical factors) need to be considered for effective commercial recovery of willow bark extracts. For this reason the bark usage of *S. daphnoides* and *S. purpurea*, as species with high biomass accumulation, is recommendable. In contrast to a bark harvest in October from clones of *S. purpurea*, the bark of *S. daphnoides* should be harvested in March.

LITERATURE CITED

(1) Biegert, C.; Wagner, I.; Lüdtke, R.; Kötter, R.; Lohmüller, C.; Günaydin, I.; Taxis, K.; Heide, L. Efficacy and safety of willow bark extract in the treatment of osteoarthritis and rheumatoid arthritis: results of 2 randomized double-blind controlled trials. *J. Rheumatol.* **2004**, *31*, 2121–2130.

(2) Chrubasik, S.; Eisenberg, E.; Balan, E.; Weinberger, T.; Luzzati, R.; Conradt, C. Treatment of low back pain exacerbations with willow bark extract: a randomized double- blind study. *Am. J. Med.* **2000**, *109*, 9–14.

(3) Chrubasik, S.; Künzel, O.; Black, A.; Conradt, C.; Kerschbaumer, F. Potential economic impact of using a proprietary willow bark extract in outpatient treatment of low back pain: an open non-randomized study. *Phytomedicine* **2001**, *8*, 241–251.

(4) Coley, P. D.; Bryant, J. P.; Chapin, F. S. Resource availability and plant antiherbivore defense. *Science* **1985**, *230*, 895–899.

(5) Eisenberg, D. M.; Davis, R. B.; Ettner, S. L.; Appel, S.; Wilkey, S.; Van Rompay, M.; Kessler, R. C. Trends in alternative medicine use in the United States, 1990−1997: results of a follow-up national survey. *JAMA, J. Am. Med. Assoc.* **1998**, *280*, 1569–1575.

(6) Förster, N.; Ulrichs, C.; Zander, M.; Kätzel, R.; Mewis, I. Influence of the season on the salicylate and phenolic glycoside contents in the bark of *Salix daphnoides*, *Salix pentandra*, and *Salix purpurea*. *J. Appl. Bot. Food Qual.* **2008**, *82*, 99–102.

(7) Heiska, S.; Rousi, M.; Turtola, S.; Meier, B.; Tirkkonen, V.; Julkunen-Tiitto, R. The effect of genotype and cultivation method on the total salicylates yield of dark-leaved willows (*Salix myrsinifolia*). *Planta Med.* **2005**, *71*, 1134–1139.

(8) Hiller, K.; Melzig, M. In *Lexikon der Arzneimittelpflanzen und Drogen. Erster Band A bis K*; Spektrum Akademischer Verlag: Heidelberg, Germany, 2006.

(9) Julkunen-Tiitto, R. Analyse und Verteilung phenolischer Glycoside (Salicinderivate) in Weiden (*Salicaceae*). *Die Holzzucht*. **1992**, *46*, 2–5.

(10) Julkunen-Tiitto, R. Chemotaxonomical screening of phenolic glycosides in northern willow twigs by capillary gas chromatography. *J. Chromatogr.* **1985**, *324*, 129–139.

(11) Julkunen-Tiitto, R.; Sorsa, S. Phytochemical changes in senescent *Salix myrsinifolia* leaves. *Planta Med.* **1990**, *56*, 565.

(12) Julkunen-Tiitto, R.; Tahvanainen, J.; Silvola, J. Increased CO_2 and nutrient status changes affect phytomass and the production of plant defensive secondary chemicals in *Salix myrsinifolia* (Salisb.). *Oecologia* **1993**, *95*, 495–498.

(13) Lardos, A.; Schmidlin, C. B.; Fischer, M.; Ferlas-Chlodny, E.; Loniewski, I.; Samochowiec, L.; Musial, H. D. Wirksamkeit und Verträglichkeit eines wässrig ausgezogenen Weidenrindenextraktes bei Patienten mit Hüft- und Kniearthrose. *Z. Phytother.* **2004**, *25*, 275–281.

(14) Mahdi, J. G.; Mahdi, A. J.; Bowen, I. D. The historical analysis of aspirin discovery, its relation to the willow tree and antiproliferative and anticancer potential. *Cell Proliferation* **2006**, *39*, 147–155.

(15) Meier, B.; Lehmann, D.; Sticher, O.; Bettschart, A. Identifikation und Bestimmung von je acht Phenolglykosiden in *Salix purpurea* und *Salix daphnoides* mit moderner HPLC. *Pharm. Acta Helv.* **1985**, *60*, 269–275.

(16) Meier, B.; Sticher, O.; Bettschart, A. Weidenrinden-Qualität. Gesamtsalicinbestimmung in Weidenrinden und Weidenpräparaten mit HPLC. *Dtsch. Apoth. Z.* **1985**, *125*, 341–347.

(17) Paunonen, R.; Julkunen-Tiitto, R.; Tegelberg, R.; Rousi, M.; Heiska, S. Salicylate and biomass yield, and leaf phenolics of dark-leaved willow (*Salix myrsinifolia* Salisb.) clones under different cultivation methods after the second cultivation cycle. *Ind. Crops Prod.* **2008**, *29*, 261–268.

(18) Riipi, M.; Ossipov, V.; Lempa, K.; Haukioja, E.; Koricheva, J.; Ossipova, S.; Pihlaja, K. Seasonal changes in birch leaf chemistry: Are there trade-offs between leaf growth and accumulation of phenolics? *Oecologia* **2002**, *130*, 380–390.

(19) Schmid, B.; Lüdtke, R.; Selbmann, H. K.; Kötter, I.; Tschirdewahn, B.; Schaffner, W.; Heide, L. Wirksamkeit und Verträglichkeit eines standardisierten Weidenrindenextraktes bei Arthrose-Patienten: Randomisierte, Placebo-kontrollierte Doppelblindstudie. *Z. Rheumatol.* **2000**, *59*, 314–320.

(20) Shao, Y. Phytochemischer Atlas der Schweizer Weiden; Zürich 1991. *Diss. Naturwiss. ETH Zürich* 1991, *No. 9532*, DOI: 10.3929/ethz-a-000614751.

(21) Sulima, P.; Przyborowski, J. A.; Wiwart, M. Willow bark − herbal raw material harvested from plants cultivated on arable lands. *Herba Pol.* **2006**, *52*, 18–25.
(22) Thieme, H. Die Phenolglykoside der Salicaceen 5. Mitteilung: Untersuchungen über die Glykosidspektren und den Glykosidgehalt der mitteldeutschen Salixarten. *Pharmazie* **1965**, *20*, 570–574.
(23) Thieme, H. Die Phenolglykoside der Salicaceen. 6. Mitteilung: Untersuchungen über die jahreszeitlich bedingten Veränderungen der Glykosidkonzentrationen, über die Abhängigkeit des Glykosidgehaltes von der Tageszeit und vom Alter der Pflanzenorgane. *Pharmazie* **1965**, *20*, 688–691.
(24) Thieme, H. Vorkommen und Verbreitung von Phenolglucosiden in der Familie der Salicaceen. *Herba Pol.* **1971**, *17*, 248–257.

Received for review March 9, 2010. Revised manuscript received June 17, 2010. Accepted June 21, 2010. The project was funded by the BLE (Bundesanstalt für Landwirtschaft und Ernährung).