RESEARCH ARTICLE

The Effect of Planting Orientation and Iron Ore Mining Substrates on the Survival and Growth of *Salix planifolia* Cuttings in a Greenhouse Experiment

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ABSTRACT

The restoration of sites disturbed by human activities often relies on the capacity of plants to propagate vegetatively. Willow cuttings are widely used in such projects. However, few studies have looked at the impact of planting orientation on the survival and growth of the cuttings. The objective of this study was to evaluate, in a greenhouse experiment, the performance (survival, shoot emergence, final number of shoots, biomass production) of *Salix planifolia* cuttings planted vertically or horizontally on peat moss (control) and on two substrates (overburden and waste rock) from an iron ore mine site in Schefferville (northern Québec, Canada). Overall, cutting performance (survival, shoot production and biomass) was greater on overburden and peat moss than on waste rock at the end of the seven week-long experiment. Horizontal cuttings had a greater survival rate than the vertical ones. However, cutting orientation impact on shoot production appears to vary according to substrate. Both orientations showed similar shoot production on overburden, but vertically planted cuttings produced a greater number of shoots than the horizontal ones on waste rock. These results suggest that vegetative propagation of *S. planifolia* has potential for the revegetation of abandoned iron mine sites.

Keywords: mining substrate reclamation, overburden, subarctic environments, waste rock, willow cuttings

Restoration Recap

- Canada is the 8th producer of iron ore worldwide. Most of this production (61%) occurs in Québec province.
- Iron ore extraction produces overburden (soil and rocks lying above the exploitable ore body) and waste rock (bedrock with low metal concentration) that need to be reclaimed, as they can contaminate the surrounding environment when exposed to wind and precipitation.
- Given their rapid growth rates and tolerance to heavy metals, willow cuttings are often used in reclamation projects. As their survival is closely associated with early rooting, we tested whether horizontal cuttings, which allow the entire length of the cuttings to contact the substrate, performed better than vertical ones.
- Horizontal cuttings had greater survival than vertical ones, although it did not translate into greater biomass. Since cutting performance was higher on overburden, our results suggest that it should be spread on top of waste rock before proceeding with horizontal cutting plantations.

Willow species are among the best candidate species for the implementation of a wide range of innovative environmental projects. This includes soil remediation projects for organic and heavy metal-contaminated sites (Mleczek et al. 2010, Grenier et al. 2015, Courchesne et al. 2017, Beauchamp et al. 2018), revegetation of mine overburden and waste rock (Bourret et al. 2009, Boyter et al. 2009), wetland and riparian restoration (Kuzovkina and Quigley 2005, Li et al. 2006, Kaczynski et al. 2018), and biomass production for energy purposes (Heller et al. 2004, Labrecque and Teodorescu 2004, Keoleian and Volk 2005). The preference of willow species for such projects is associated with their physiological and ecological adaptations that include a rapid growth rate, high shoot and biomass productions, high rooting capacity, high heavy

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metal uptake capacity, tolerance to low pH, and their ability to propagate vegetatively (Kuzovkina and Quigley 2005, Tharakan et al. 2005, Kuzovkina and Volk 2009).

Unrooted, dormant stem cuttings are commonly used in restoration projects. Such cuttings have a high rooting potential given the presence of root primordia formed during stem development (Nissim and Labrecque 2016). They also have large carbohydrate reserves that can be allocated to initial root and shoot production (Verwijst et al. 2012, Edelfeldt et al. 2015). However, their overall performance depends on their degree of rooting (Bourret et al. 2009). Traditionally, cuttings are planted vertically and, as a result, only their lower section is in contact with the substrate. However, successful vegetative propagation can also be achieved by planting them horizontally, an orientation that allows the entire length of the cuttings to be in contact with the substrate. As such, it reduces the physical distance between the axillary buds, from which roots and leaves will develop, and the substrate. Several studies have reported beneficial effects of horizontal planting for the stabilization of slopes or sediments and for riverbank restoration (Vervaeke et al. 2001, Cao et al. 2011, Edelfeldt et al. 2015).

Mining substrates such as overburden or waste rock represent a challenging environment for plant establishment, survival, and growth. Overburden consists of the superficial layer of soil and rocks that is removed at the beginning of the iron ore mining operations since it lies on top of the economically exploitable ore body. Waste rock consists mainly of the gangues that are removed from the iron ore during the concentration process and therefore contains higher concentrations of iron and manganese than overburden (Aebischer et al. 2015), two elements that are toxic for plant species and soil microorganisms. Both substrates are usually stored separately in dumps in the vicinity of the mining sites (Paradise 2017).

As it was shown to impact root development on other substrates (Cao et al. 2011, Cao et al. 2012), we decided to evaluate if cutting orientation influences the early performance (survival, shoot production, biomass production) of *Salix planifolia* (Diamondleaf willow) cuttings on overburden and waste rock in a greenhouse experiment. We predicted that horizontal cuttings would have greater survival and produce more roots and shoots than vertical cuttings. Given the milder physico-chemical properties of overburden, we also predicted that cuttings would perform better on overburden than on waste rock. In fact, their performance on overburden should not be different than their performance on peat moss (used as a control substrate).

Methods

Schefferville (Québec Canada) is a mining town located in the forest tundra bioclimatic domain. The vegetation surrounding the mine is characterized by open forests and tundra ecosystems dominated by shrubs, grasses, lichens, and mosses. From 1971 to 2000, the average annual temperature was −5.3°C and average annual precipitation was 822.9 mm (rainfall: 408.1 mm, snow: 448 mm; Environment Canada 2018).

Table 1. Initial characterization of mine waste rock and overburden.

Physicochemical properties	Waste rock	Overburden
Sand (%)	49	41
Silt (%)	27	25
Clay (%)	2.46	7.50
Gravel (%)	21.60	27
pH_{H2O}	5.22	4.44
Water content (%)	0.42	4.21
Total C (mg/kg)	< 500	3600
N (%)	< 0.01	0.03
P (mg/kg)	71	320
K (mg/kg)	31	430
Na (mg/kg)	< 10	19
Ca (mg/kg)	38	240
Mg (mg/kg)	19	3700
Mn (mg/kg)	1090	940
Mo (mg/kg)	< 0.50	1.19
Fe (mg/kg)	118000	69000
Zn (mg/kg)	< 50	74
Pb (mg/kg)	4.46	13
Cu (mg/kg)	1.60	41
Cr (mg/kg)	3.43	19
Cd (mg/kg)	< 0.10	0.38
Ni (mg/kg)	1.43	25
Hg (mg/kg)	0.04	0.08

Overburden and waste rock were collected from the Timmins 4 site. Their physico-chemical properties are given in Table 1. We also used peat moss from Fafard et Frères (Québec, Canada) as a control substrate. It had a pH ranging from 3.1 to 3.9, a CEC from 60 to 80 meq/L, and a C/N ratio from 80 to 125 (Fafard 2016).

We harvested *S. planifolia* cuttings (1.5 to 2 cm in diameter) from the Schefferville area in October 2017. Once at Université Laval, cuttings were cut to a length of 25 cm and stored at 4°C in a cold room for four months.

Experimental description

The experiment was designed to evaluate the performance of two *S. planifolia* cutting orientations (horizontal and vertical) on three different substrates (overburden, waste rock and peat moss), for a total of 6 treatments (2 orientations × 3 substrates). It was therefore a 3 × 2 factorial experiment with each treatment replicated eight times (i.e., 8 blocks), for a total of 48 experimental units. We applied the treatments randomly within the different blocks.

Each experimental unit corresponded to a mesocosm (54 cm in length, 34 cm in width, 18 cm in depth) filled with 15 liters of substrate. At the experiment inception, four cuttings were planted either horizontally or vertically in each mesocosm, for a total of 192 cuttings (4 cuttings × 48 mesocosms). Vertical cuttings were planted at a depth of 5 cm and positioned 5 cm away from the mesocosm sides in each of the four corners. Cuttings planted horizontally were placed beside each other with the lower half of the cutting buried in the substrate.

The seven week-long experiment was conducted in a greenhouse at Université Laval. Growth conditions were: a temperature of 22.5°C, a minimum ambient humidity of 60%, a 16 h photoperiod, and a radiation intensity of 50 micromole/s/m^2 (in addition to daylight). At the beginning of the experiment, we watered the mesocosms every three days. Afterwards, we watered the mesocosms as needed.

Measurement and data analysis

Every week, we monitored cutting survival and shoot production. At the end of the experiment, we considered a cutting dead if it did not produce any shoot, had no axillary bud, and was desiccated. As four cuttings were planted in each experimental unit, we evaluated survival rates at the mesocosm level. As a consequence, survival rate for each mesocosm was assigned one of the following values: 0%, 25%, 50%, 75% or 100%. At the time of harvest, we removed the cuttings carefully from the mesocosms. We cut the shoots at their base. Roots were also harvested, washed with running water, rinsed with distilled water. All biomass was dried at 65°C for 48 h.

We conducted all statistical analyses in SAS 9.4 (SAS Institute 2012) and relied on data collected at the mesocosm level in order to avoid pseudo-replication problems. However, we divided shoot production and biomass data by four to represent the average performance of a single cutting. For the following variables: survival, final number of living shoots, aboveground biomass, root biomass, total biomass, and root: shoot ratio, ANOVAs were carried-out with the SAS PROC MIXED procedure with cutting orientation and substrate as fixed effects and block as a random effect. For shoot production over time, we conducted a repeated measures ANOVA to detect statistical differences between substrates or cutting orientation through time. Results were considered significant at $p < 0.05$. When ANOVAs were significant, we conducted a Tukey test to identify significant differences between treatments. We used the Shapiro-Wilk test to verify compliance with the assumptions of residual normality and variance homogeneity. Total biomass, aboveground biomass and root biomass as well as the final number of living shoots per cutting were log-transformed to meet these assumptions. However, we only present untransformed data to facilitate the interpretation of results.

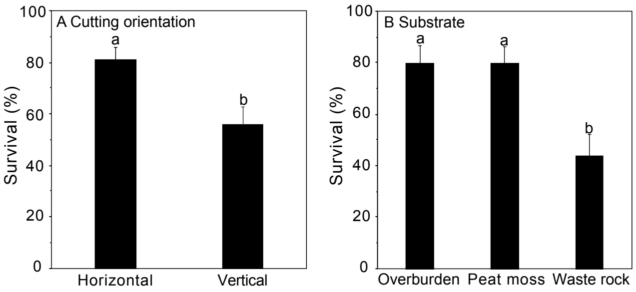

Figure 1. Survival rates of cuttings according to (A) cutting orientation (vertical and horizontal orientations) and (B) growth substrates (overburden, waste rock, and peat moss) (n = 8, mean ± standard error). Different lowercase letters indicate the presence of significant differences (Tukey test, $p < 0.05$).

Results

Survival

Survival data revealed that 130 of the 192 cuttings were still alive at the end of the experiment, for an overall survival rate of 67.7%. However, survival varied between the different treatments. Orientation influenced cutting survival ($F_{1,\,35} = 10.13$, $p = 0.003$, Figure 1A), as survival was significantly higher for horizontal cuttings (about 80%) than for vertical ones (about 55%). Substrate also influenced survival ($F_{2,\,35} = 9.30$, $p = 0.001$, Figure 1B) as it was higher on overburden and peat moss (both about 80%) than on waste rock (about 44%). There was no significant interaction between cutting orientation and substrate ($F_{2,\,35} = 0.05$, $p = 0.949$).

Shoot production through time

Overall, cutting orientation influenced shoot production through time, as revealed by the significant interaction between time and orientation ($F_{2,245} = 2.60$, $p = 0.026$, Figure 2A). While cuttings planted horizontally had more shoots than the vertical ones during the second and third weeks of the experiment, these differences disappeared afterwards, suggesting that shoot mortality was greater for horizontal cuttings during the fourth week of the experiment (Figure 2A).

By comparison, differences observed in shoot production between substrates were maintained throughout the experiment ($F_{2,245} = 73.36$, $p < 0.001$), resulting in a non-significant interaction between time and substrate ($F_{10,245} = 0.47$, $p = 0.908$). For each substrate, the number of shoots was maximal during the third week of the experiment before declining afterwards. Throughout the experiment, the number of shoots was significantly higher on overburden than on waste rock, while it was intermediate on peat moss and not significantly different from the two mining substrates (Figure 2B). Also, there was no significant three-way interaction between time, cutting orientation and substrate type ($F_{10,245} = 0.50$, $p = 0.887$).

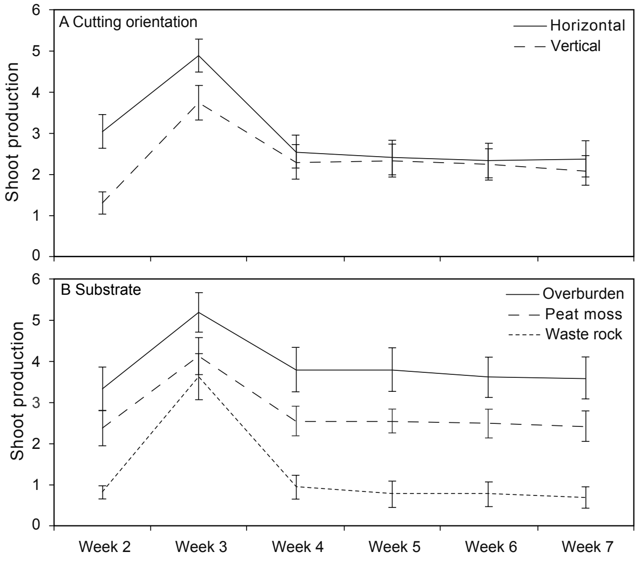

Figure 2. Shoot production over time according to (A) cutting orientation (vertical and horizontal orientations) and (B) growth substrates (overburden, waste rock, and peat moss) (n = 8, mean ± standard error).

A subsequent analysis on the final number of shoots (week 7) revealed a two-way interaction between cutting orientation and growth substrate ($F_{2,35} = 3.51$, $p = 0.041$, Figure 3). Cutting orientation had no effect on the number of shoots per cutting in the overburden. However, horizontal cuttings had a greater number of shoots on peat moss while vertical cuttings had a greater number of shoots on waste rock.

Biomass production

We observed significant or marginally significant two-way interactions between cutting orientation and growth substrates for total ($F_{2,35} = 3.27$, $p = 0.0439$); aboveground ($F_{2,35} = 2.98$, $p = 0.0637$) and root biomass ($F_{2,35} = 3.13$, $p = 0.0561$, Figures 4A–C). For all these variables, cutting orientation had no effect in the overburden but horizontal cuttings had greater biomass on peat moss while vertical cuttings had greater biomass on waste rock.

Neither cutting orientation nor growth substrates had a significant effect on the root to shoot ratio ($F_{1,35} = 0.65$, $p = 0.427$ and $F_{2,35} = 0.86$, $p = 0.430$, respectively). Moreover, there was no significant interaction between cutting orientation and growth substrate ($F_{2,35} = 1.34$, $p = 0.276$, Figure 4D).

Discussion

In this study, we assessed the influence of both mining substrates (overburden, waste rock) and cutting orientation (horizontal, vertical) on the performance of *S. planifolia* cuttings. Our results show that cutting survival and growth was significantly higher on overburden than on waste rock while horizontal cuttings had a greater survival than vertical ones.

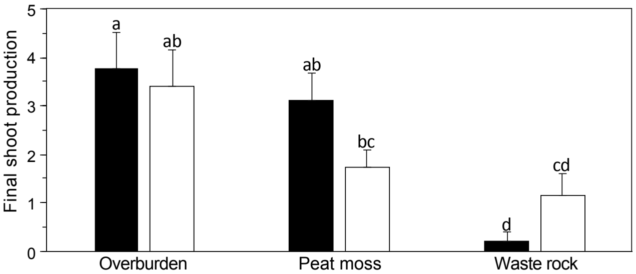

Figure 3. Final shoot production from cuttings planted vertically or horizontally in overburden, peat moss, and waste rock (n = 8, mean ± standard error). Black bars show horizontal cuttings; white bars show vertical cuttings. Different lowercase letters indicate the presence of significant differences (Tukey test, *p* < 0.05).

Influence of mining substrates

We compared the survival and growth of *S. planifolia* cuttings on two mining substrates with different physico-chemical properties. As predicted, overburden significantly improved cutting survival by about 36% compared to waste rock (Figure 1B). Waste rock offers extreme growth conditions for cutting development: high concentration of heavy metals, finer granulometry, high bulk density, and substrate compaction. Although some willow species are able to grow in stressful and highly contaminated environments (Landberg and Greger 1994, Bourret et al. 2009, Beauchamp et al. 2018), the physico-chemicals properties of waste rock from the iron ore extraction in the Schefferville area are likely at or over their tolerance threshold. Interestingly, cuttings survival rate did not differ significantly between overburden and peat moss, a growth substrate commonly used in agriculture and horticulture. As such, it appears that overburden does not inhibit cutting survival, suggesting that it could be used to facilitate the revegetation of disturbed sites.

Limited root development in waste rock likely triggered shoot mortality. The finer granulometry and higher density of waste rock likely hampered root penetration, limiting root development to the upper layer of the substrate. Such superficial root colonization would prevent cuttings from taking advantage of the available water deeper in the soil, a phenomenon that might have induced hydric stress. In fact, deep rooting allows plants to use the water stored at greater depth (Eapen et al. 2005, Jung et al. 2014). Moreover, several studies have shown that some *Salix* species depend on groundwater for their survival (Kuzovkina and Volk 2009, Martino et al. 2019). In addition to limited water absorption, shallower root development has a negative impact on the ability of the cuttings to extract nutrients (Jung et al. 2014). This could explain why we observed a significant reduction of shoot survival and growth in waste rock.

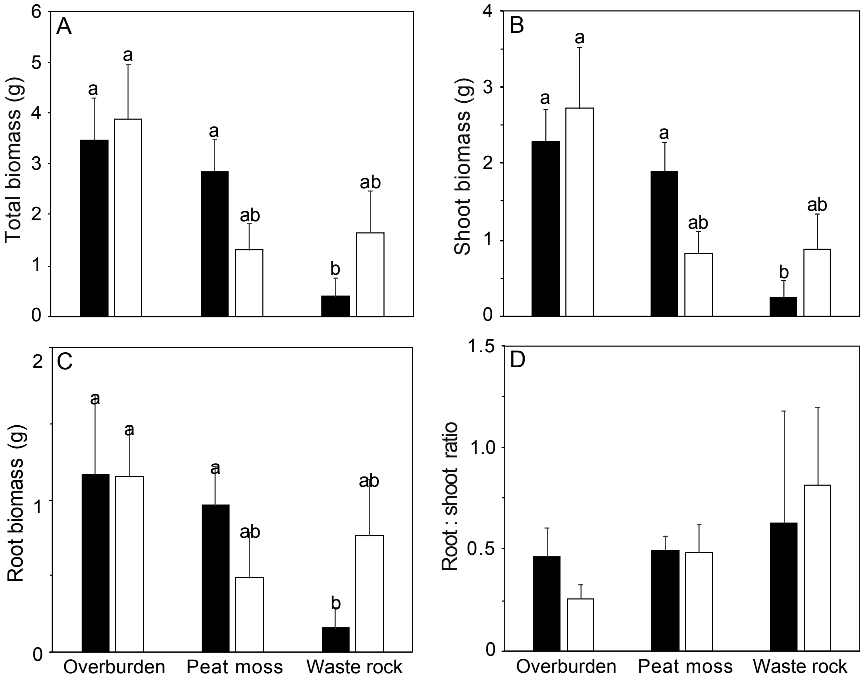

Figure 4. A) Total biomass, B) shoot biomass, C) root biomass, and D) root: shoot ratio from cuttings planted vertically or horizontally in overburden, waste rock and peat moss (*n* = 8, mean ± standard error). Black bars: horizontal cuttings; white bars: vertical cuttings. Different lowercase letters indicate the presence of significant differences (Tukey test, $p < 0.05$).

As expected, biomass production was higher in the overburden than in the waste rock, regardless of the biomass parameter considered (aboveground biomass, root biomass and total biomass). These results corroborate other studies that compared the performance of different willow cultivars in contaminated versus non-contaminated soils (Grenier et al. 2015, Beauchamp et al. 2018), and agricultural land versus tailings from fluvial deposition (Boyter et al. 2009). In general, these studies showed a reduction in the productivity of cultivars with an increase in metal accumulation, although the effect varies according to the genotype (Grenier et al. 2015, Beauchamp et al. 2018). In addition, it has been shown that the production of aboveground biomass by willow species is generally associated with the number of shoots, the diameter and the height of the shoots (Tharakan et al. 2005, Kuzovkina and Volk 2009). As cuttings on overburden produced around five times more shoots than the ones on waste rock, our results are in accordance with these studies. The overall performance of *S. planifolia* cuttings on overburden strongly suggests that this species could be used for the reclamation of the overburden substrate in the Schefferville region.

Influence of cutting orientation

Our results suggest that cutting orientation did not systematically influence the performance of *S. planifolia* cuttings in overburden and waste rock. Survival and early shoot production (second week) were the only two variables for which horizontal cuttings performed significantly better than vertical ones. This may be explained by the fact that horizontal cuttings had a larger contact area with the substrate, which would increase the probability of root development during the early stages of the experiment. Indeed, previous studies have shown that early rooting strongly influences the survival of willow cuttings (Li et al. 2006, Bourret et al. 2009). As for early shoot production, we hypothesize that this result is also related to early root development, although we have no data to test this hypothesis. For horizontal cuttings, the physical distance between roots and shoots is smaller and might trigger faster shoot production when compared to vertical cuttings. It is also possible that the substrate was too compact at the lower extremity of the vertical cuttings to allow root development. The absence of significant differences after the third week of the experiment suggests that vertical cuttings were able to produce as many shoots as the horizontal ones once the roots were initiated. This result corroborates studies conducted on another willow species (*Salix schwerinii*), for which no significant difference was observed for cuttings planted horizontally or vertically (Cao et al. 2011, 2012).

It is also worth noting the lack of statistical difference between horizontal and vertical cuttings for biomass production. Since we observed the same number of shoots per cutting at the end of the experiment for both cutting orientations, this result was expected.

Implications for revegetation of abandoned iron mine sites

Our results showed that cuttings of *S. planifolia* can grow on both overburden and waste rock, although they performed significantly better on the former. From an ecological perspective, willows are a suitable choice for mining substrate reclamation since they show high biomass and shoot production, good rooting capacity, and high heavy metal tolerance (Kuzovkina and Volk 2009). Beyond these characteristics, the use of *S. planifolia,* a native species, is a sound restoration practice.

Based on our results, overburden appears to be a less challenging substrate for in situ revegetation than waste rock. As the two substrates are usually stored separately near the mine, we suggest spreading overburden on top of waste rock in order to facilitate the reclamation of the latter. Once covered with overburden, we believe that horizontal planting may be the option to prioritize in a region with harsh climatic conditions. In such regions, cutting survival and early shoot production will be important drivers of the fate of any reclamation project. Horizontal planting should allow for early rooting and faster growth and would require less plant material for large-scale revegetation projects.

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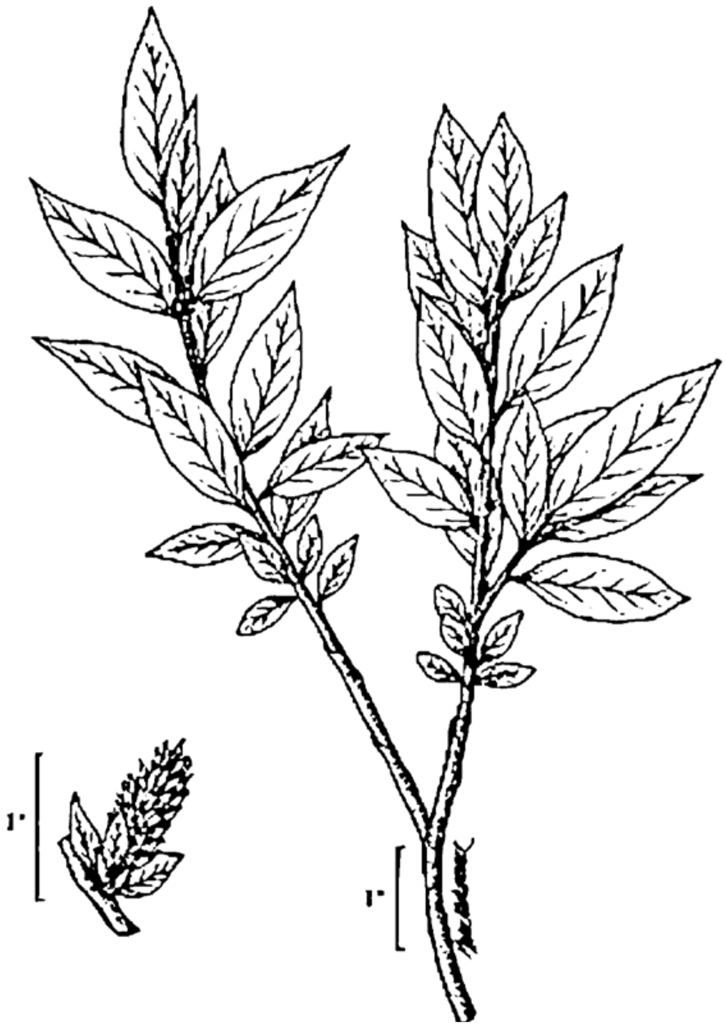

***Salix planifolia*. USDA-NRCS PLANTS Database. USDA NRCS. *Wetland Flora: Field Office Illustrated Guide to Plant Species*. USDA Natural Resources Conservation Service.**