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Establishment of willows using the novel DeValix technique: ecological restoration mats designed for phytotechnologies

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ABSTRACT

Successful willow (*Salix* spp., hybrids and cultivars) establishment is a major determinant of their effectiveness when grown for phytotechnologies. Vertically-planted hardwood cuttings have been shown to produce adequate willow growth and survival, although site conditions at phytoremediation installations can make vertical planting methods unsuitable. The DeValix willow mat restoration technique was designed and tested as an alternative horizontal planting method that can be installed by hand in a variety of environmental applications. The DeValix technique was evaluated by testing five willow clones ("Millbrook"; "Sherburne"; "SX61"; "SX67"; "Tully Champion") grown at two phytoremediation sites (Ontonagon, MI; Manitowoc, WI) for the 2019 growing season. Differences in survival and growth were tested among sites, genotypes, and their interactions. Stem height, diameter, and number of stems per mat were compared to identify clones with greater establishment success and higher phytoremediation potential. Results demonstrated significant effects of site ($p < 0.0001$) and clone ($p < 0.0001$) on shoot number. Additionally, the site × clone interaction significantly affected stem height ($p = 0.0045$) and diameter ($p = 0.0166$). Stem density ranged from 95,000 to 212,000 stems per hectare, indicating the DeValix technique is a viable establishment method for environmental applications, including phytoremediation and shoreline stabilization.

Novelty statement

This research analyzes the establishment success of the DeValix technique, a novel horizontal planting method for willow cultivars, and evaluates the DeValix technique as an alternative to other horizontal techniques and traditional vertical planting methods currently used in environmental applications. Results from this study add to the current knowledge of planting techniques and assesses the use of the DeValix technique for planting willow cultivars that are currently being tested in several phytotechnology systems.

KEYWORDS
Biomass; horizontal planting; phytoremediation; *Salix*; Shoreline Stabilization; Short Rotation Willow

Introduction

Willows (*Salix* spp., hybrids and cultivars) have been recognized throughout the course of history for their ability to provide a variety of products and services. Romans used willows for numerous goods including fencing, medicine, baskets and framing for shields (Keoleian and Volk 2005). Native Americans harvested willows to craft arrows, baskets, furniture and sweat lodges, in addition to utilizing them to stabilize streambanks (Keoleian and Volk 2005). Today, although willows are still used to manufacture products such as baskets, wicker furniture and cricket bats (Balatinecz *et al.* 2014; Dickmann and Kuzovkina 2014), extensive research has focused on a wider range of applications. Biomass harvests of short rotation willow plantations provide feedstocks for bioenergy, biofuels and bioproducts (Abrahamson *et al.* 2002; Smart *et al.* 2005; Volk *et al.* 2006, 2016; Schroeder *et al.* 2009; Balatinecz *et al.* 2014; Murphy *et al.* 2021). Not only do such plantations provide biomass materials that serve as alternatives to traditional fossil fuel feedstocks, but they also help reduce greenhouse gas emissions by sequestering CO_2 throughout their life cycle (Keoleian and Volk 2005) and mitigate soil salinity on marginal lands (Huang *et al.* 2020).

Due to growing concerns of environmental degradation, willow genotypes that have demonstrated superior traits in biomass research have been increasingly used for environmental applications. Agroforestry windbreak systems take advantage of the rapid growth rate and planting density of willow trees to reduce wind speeds and drifting soil on agricultural land (Kuzovkina and Quigley 2005). Living snow fences of willows planted along highways act as windbreaks

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in the winter and reduce snow removal costs by trapping and preventing large amounts of snow from drifting onto the roadways (Heavey and Volk 2014). In addition, willows planted in riparian buffer systems reduce nonpoint source pollution from agricultural runoff (Da Ros *et al.* 2018). Willow buffers strips help to improve stream quality by reducing sediment load (Volk *et al.* 2006; Isebrands *et al.* 2014), stabilizing stream banks (Schultz *et al.* 1995; Kuzovkina and Quigley 2005) and removing nutrients and pesticides from agricultural runoff (Schultz *et al.* 1995; Volk *et al.* 2006). Willows' extensive fibrous root systems and ability to tolerate saturated soil are utilized to stabilize soils and prevent streambank erosion and periodic flooding (Kuzovkina and Quigley 2005; Kuzovkina and Volk 2009). Furthermore, willow root systems support diverse communities of microorganisms that degrade organic runoff chemicals (Volk *et al.* 2006). Agroforestry buffer systems that combine willows with grasses and shrubs maximize species' morphological and physiological traits to provide wildlife habitat, biomass feedstocks and pollution reduction (Schultz *et al.* 1995; Volk *et al.* 2006).

The study of willows for environmental applications has expanded in recent decades to include environmental restoration. Rapid growth, extensive rooting, elevated evapotranspiration, carbon sequestration and the ability to accumulate high levels of toxic chemicals make willow cultivars ideal candidates for environmental remediation (Kuzovkina and Quigley 2005; Kuzovkina and Volk 2009; Wani *et al.* 2011). These traits are maximized in phytoremediation systems, in which trees are implemented to clean up contaminated soil, surface water and groundwater through phytoextraction, phytodegradation, phytostabilization and hydraulic control (Isebrands *et al.* 2014; Mirck *et al.* 2005; Wani *et al.* 2011). Willows have been used to remediate dredging sludge (Vervaeke *et al.* 2003; Pilipović *et al.* 2019), mine tailings (Bourret *et al.* 2009), hydraulic fracturing wastewater (Bilek *et al.* 2020), sewage sludge (biosolids) (Laidlaw *et al.* 2012; Isebrands *et al.* 2014) and other brownfield sites (Guidi *et al.* 2012). Willow vegetation filters have also successfully been used to treat municipal wastewater (Perttu and Kowalik 1997) and landfill leachate (Godley *et al.* 2004; Zalesny and Bauer 2007a). Rising concerns of environmental pollution have expanded the use of willow phytotechnologies to include remediation of municipal landfills (Zalesny and Bauer 2007b). Phytoremediation buffer strips planted near landfills can intercept large volumes of groundwater due to high transpiration rates, and can remediate soils by (1) extracting heavy metals and other inorganic pollutants, and (2) degrading organic contaminants (Corseuil and Moreno 2001; Pulford *et al.* 2002; Vandecasteele *et al.* 2005; Guidi Nissim *et al.* 2014).

Successful willow establishment is a significant determinant of the overall phytoremediation potential of willow plantations. Conventional willow establishment is performed using dormant hardwood cuttings planted vertically (Volk *et al.* 2016). Recently, the effect of alternative establishment methods on growth, survival, and cost effectiveness of willow plantations has been tested. Donnelly *et al.* (2019) demonstrated that 20-cm cuttings inserted diagonally into the soil, with half of the cutting grown aboveground, produced slightly higher stem yields than traditionally-planted vertical cuttings in a field setting, though the opposite was true in the greenhouse. Horizontal planting of willow cuttings has also been tested using various techniques. Cao *et al.* (2011) found similar stem biomass production between 25-cm cuttings planted horizontally and vertically in the greenhouse and field (Cao *et al.* 2012). An alternative horizontal planting method is the lay-flat system, in which long willow rods are placed into shallow trenches of various depths and covered with soil (McCracken *et al.* 2010). Some studies have shown the lay-flat technique to produce similar or greater yields compared to vertical planting methods (Lowthe-Thomas *et al.* 2010; McCracken *et al.* 2010; Larsen *et al.* 2014) while others found greater yields using vertical cuttings (Edelfeldt *et al.* 2015; Donnelly *et al.* 2019). One disadvantage with the lay-flat technique is the amount of planting material used. McCracken *et al.* (2010) reported that the lay-flat method can use over three times as much plant material as vertical cuttings, thereby reducing the cost-effectiveness of this strategy. Another method, billets, involves small lengths of willow that are harvested using a sugar cane harvester, buried horizontally in small trenches and covered with soil (Larsen *et al.* 2014; McCracken *et al.* 2010). Field trials using billet material generally report reduced yields compared to horizontal lay-flat rods and conventional 20-cm vertical cuttings (McCracken *et al.* 2010; Larsen *et al.* 2014; Edelfeldt *et al.* 2015). Microcuttings are another type of propagation material with smaller size than conventional cuttings. Compared to 20-cm cuttings, microcuttings (2- and 5-cm length) can reduce planting material costs by 16–60% (Guidi Nissim and Labrecque 2016), and show promise in field applications (Frenette-Dussault *et al.* 2019; Desrochers *et al.* 2020).

Although comparisons of horizontal and vertical planting methods show mixed results in biomass plantings, there is limited information about horizontal establishment techniques in environmental applications (Frenette-Dussault *et al.* 2019; Caterino *et al.* 2020; Desrochers *et al.* 2020). Some planting sites present unique situations that make establishment methods difficult. For example, the SALIMAT technique was developed in Belgium to establish willows on dredged sediment deposits that are often impossible to plant by hand or conventional planting machinery (De Vos 1994). Using the SALIMAT technique, 2-m long willow rods are wrapped around a central disposable tube and tied together using biodegradable string. A crane and dragline are used to horizontally unroll the tube for planting. The rods sink into the sediment under their own weight and are protected from desiccation by a water and silt layer (De Vos 1994; Stott *et al.* 1994; Vervaeke *et al.* 2001). The SALIMAT technique has exhibited successful establishment on dredged sediment and has potential for phytoremediation of contaminated soils (De Vos 1994; Stott *et al.* 1994; Vervaeke *et al.* 2001, 2003; Meers *et al.* 2005). However, several drawbacks limit the potential of the SALIMAT technique to be used across a range of phytotechnologies. Because the SALIMAT

Table 1. Characteristics of native soils at two phytoremediation sites where survival and growth of DeValix willow restoration mats were tested.

	Ontonagon, Michigan	Manitowoc, Wisconsin
Soil series	Oldman-Michigamme-Rock outcrop complex	Hochheim loam
Slope (%)	6 to 35	6 to 12
pH	4.6	7.4
Bulk density, 1/3 bar (g cm^{-3})	1.36	1.51
Organic matter (%)	6.41	1.25
Clay (%)	7.2	20.2
Sand (%)	51.3	45.4
Silt (%)	41.5	34.4
Texture class	Sandy loam	Clay loam
Drainage class	Moderately well drained	Well drained
Frost free days (#)	110	145

Data from Web Soil Survey. Available online at http://websoilsurvey.nrsc.usda.gov/. Accessed [April 21, 2020].

technique relies on a saturated substrate, the method is not suitable for use on sites with high sand or gravel content (De Vos 1994). Further, without proper sinking of the rods into the soil, soil erosion may hinder the success of SALIMAT rods during establishment of slope stabilization projects. The cost and accessibility of a crane is also prohibitive.

In the current study, the DeValix technique was designed and tested as an alternative horizontal planting method that not only can be implemented in a variety of environmental applications, but is also easily moved and planted by hand. Six willow rods of 91.4-cm length are placed in a "window" formation and tied together using biodegradable sisal twine to form a DeValix mat. Mats are placed on top of the substrate, covered with 5 cm of soil, and stabilized on sloped ground with 50.8-cm, genotype-specific cuttings used as stakes. Previous planting of vertical, 50-cm cuttings using a modified waterjet stinger (Hoag *et al.* 2001) has yielded successful willow establishment at one of the current test sites, but the jetting poses a risk of soil erosion on steep slopes where the high water pressure may destabilize the surrounding soil. Therefore, our objective was to evaluate the use of the DeValix technique as a willow establishment method for slope stabilization and phytoremediation applications where site conditions make vertical planting methods unsuitable. In particular, we established DeValix mats of five willow genotypes (*Salix purpurea* L. × *S. miyabeana* Seemen. "Millbrook"; *S. miyabeana* "Sherburne", "SX61", "SX67"; *S. viminalis* L. × *S. miyabeana* "Tully Champion") at two phytoremediation sites (Manitowoc, WI and Ontonagon, MI) and tested for differences in survival and growth across sites, genotypes and their interactions. We compared number of stems per DeValix mat along with height and diameter of each stem to identify clones with greater potential for successful establishment in slope stabilization and phytotechnologies systems. This information is useful for researchers and resource managers seeking to establish willows for environmental applications.

Materials and methods

Site description

This study was conducted at two phytoremediation sites located in the Midwestern United States. The first DeValix buffer, located adjacent to an active municipal landfill, was 94.8 m long and was established on land previously excavated for landfill cover soil in Ontonagon, Michigan (46.7844°N, −89.1335°W). The DeValix restoration mats were installed on the banks of a small intermittent stream that provides seasonally-variable water availability. The second DeValix buffer was 121.6 m long and was established at a former gravel pit where waste dumping occurred in Manitowoc, Wisconsin (44.0594°N, −87.7199°W). The DeValix mats were installed on the slopes of an engineered retention pond that were characterized by sandy soils with little to no vegetation and low water availability. Information on native soils of both sites is presented in Table 1. Precipitation, aboveground temperature and number of growing degree days (GDD) for the 2019 growing season are shown in Figure 1.

Plant material and DeValix mat development

Seven willow clones belonging to three diversity groups were selected based on their biomass productivity potential and use in similar environmental applications throughout North America. Five genotypes were grown at both sites and were included in our analyses: *Salix purpurea* L. × *S. miyabeana* Seemen. "Millbrook"; *S. miyabeana* "Sherburne", "SX61", "SX67"; *S. viminalis* L. × *S. miyabeana* "Tully Champion". The remaining two genotypes, whose data are reported for informational purposes only, were: *S. viminalis* × *S. miyabeana* "Fabius" grown at Manitowoc and *S. viminalis* × *S. miyabeana* "Preble" grown at Ontonagon. For all clones, dormant shoots were collected in February, 2019 from 2-year-old coppice plantings grown at landfill phytoremediation sites located in Whitelaw, Wisconsin (44.1770°N, −87.8411°W) and Menomonee Falls, Wisconsin (43.1806°N, −88.0681°W), brought to the USDA Forest Service, Institute for Applied Ecosystem Studies in Rhinelander, Wisconsin (45.6415°N, −89.4692°W), and processed into 91.4-cm rods ranging in diameter from 1.27 to 2.54 cm.

The DeValix technique was developed to provide a versatile planting method that is easily stored, handled and planted by hand. Mat design and willow rod length were chosen to maximize stem density and growth while minimizing intraspecific competition of willow stems within mats along with reducing interspecific competition with weed species between rods. To create each DeValix mat, four monoclonal 91.4-cm rods were placed together to form

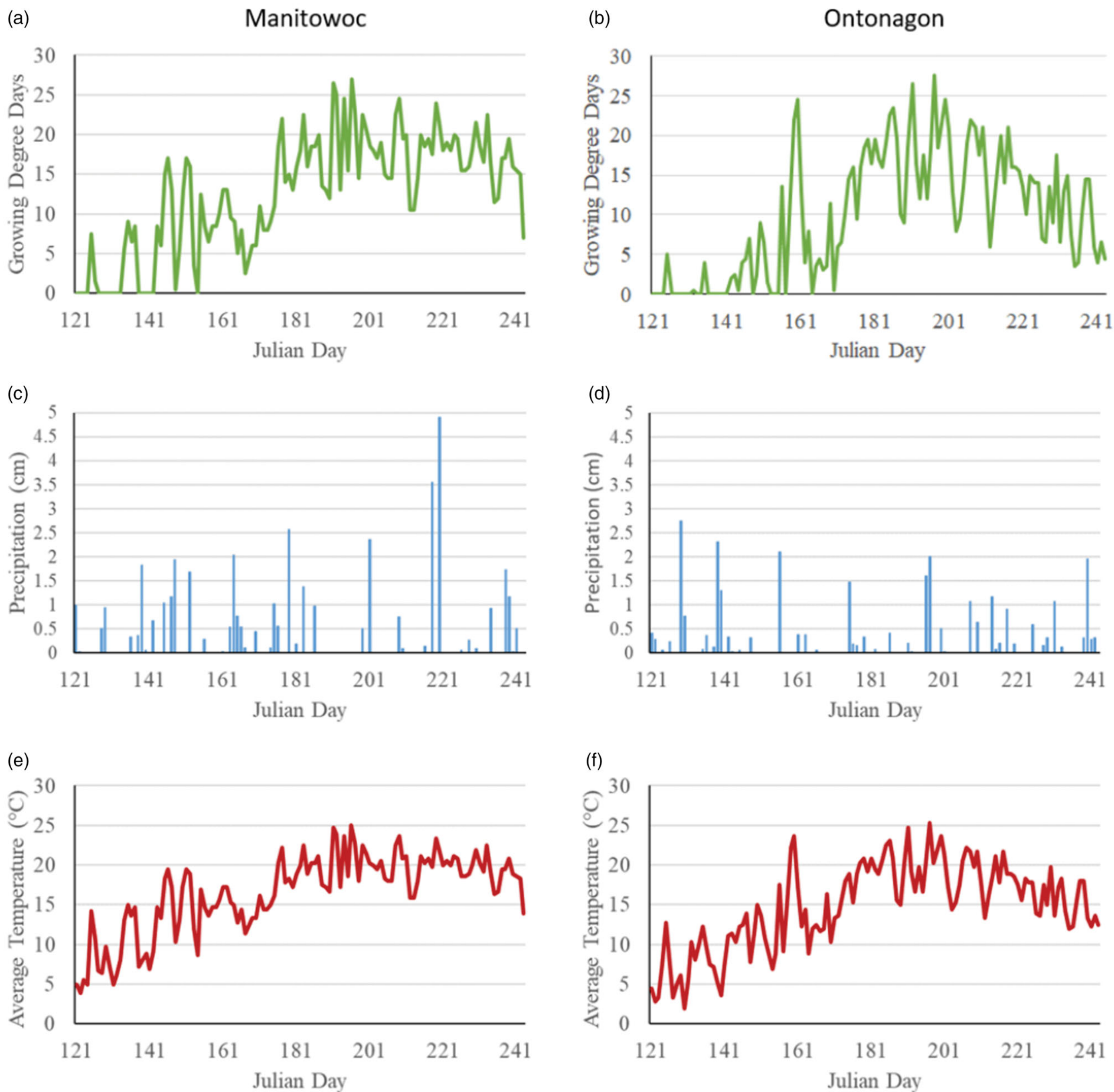

Figure 1. Growing degree days (GDD), precipitation, and aboveground temperature at two phytoremediation sites where survival and growth of DeValix willow restoration mats were tested during the 2019 growing season. Values represent 2019 growing season (May 1–August 31) data collected from the nearest weather station. Climate data were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center. Available online at http://www.ncdc.noaa.gov/oa/ncdc.html. Accessed [April 21, 2020].

a square shape with rods overlapping each other by 2 cm (Figure 2a–c). Two additional rods were placed in an equilateral cross shape in the center of the four previous rods to create a window formation (Figure 2d). Sisal twine, a biodegradable string, was used to tie overlapping rods together to finalize each mat (Figure 2e). Mats were bundled together according to clone and stored in the dark at 5 °C until planting (Figure 2f).

Experimental design and buffer establishment

In May 2019, DeValix mats were soaked in water for 72 hours to initiate root development before establishment. Prior to planting, large rocks that could inhibit mat establishment were removed, and the ground at both sites was scarified to loosen the soils (Figure 3a). The mats were planted at a spacing of 0.3 m between mats (Figure 3b) in a completely random design consisting of two sites (Ontonagon, Manitowoc), five clones ("Millbrook", "Sherburne", "SX61", "SX67", "Tully Champion"), and 24 mats per clone (with the exception of "Sherburne" at Ontonagon, which had 12 mats). At Ontonagon, buffers consisted of single rows of mats positioned parallel to the stream on both sides, including 12 additional mats each of "Millbrook" and "SX61", and 24 mats of "Preble" that were installed to ensure the buffer spanned the length of the stream. The planting at Manitowoc consisted of a double row of mats positioned parallel to the slope of the retention

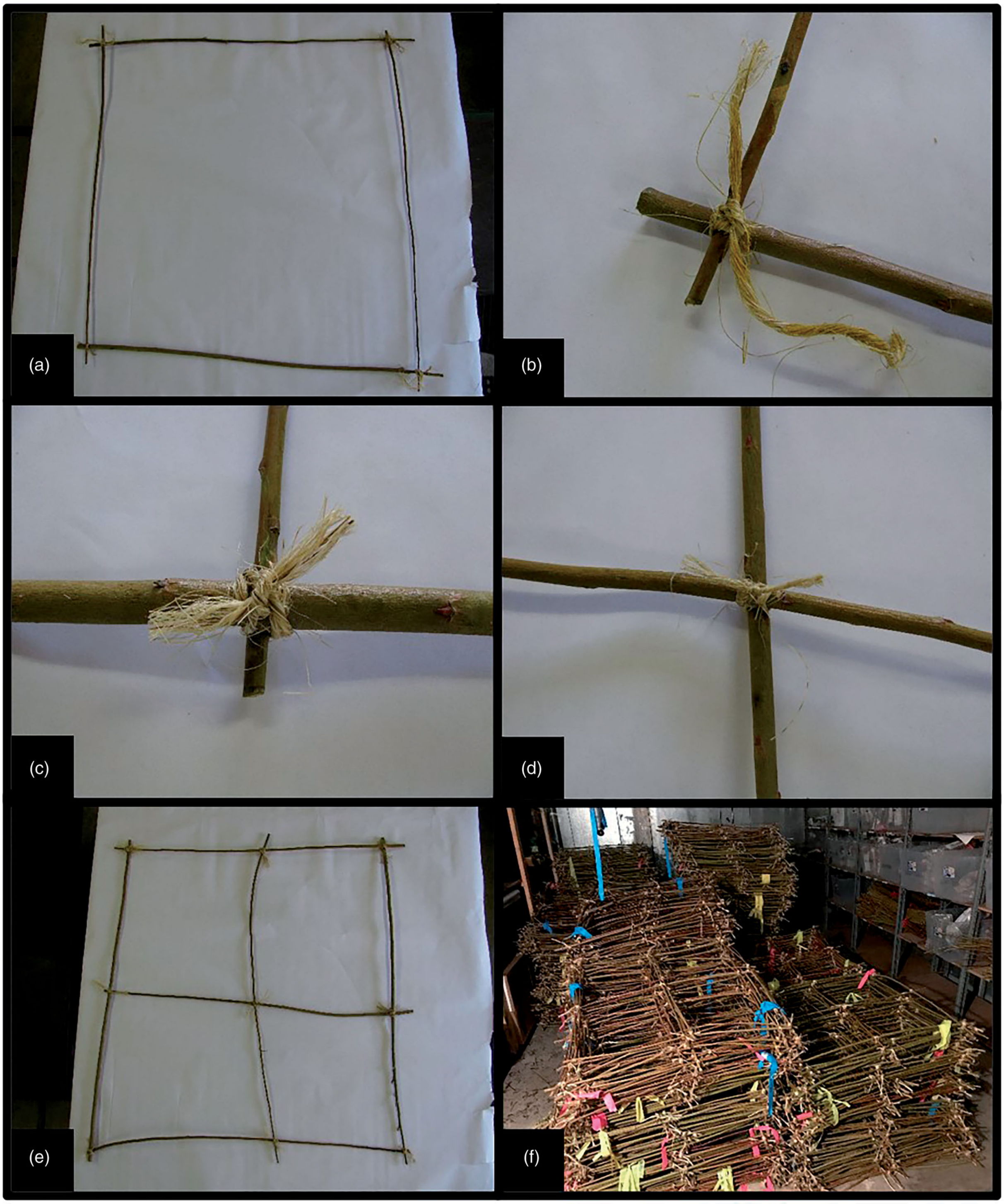

Figure 2. DeValix mat design, construction, and final product. (a) Four monoclonal 91.4-cm rods placed together to form a square shape with rods overlapping each other by 2 cm. (b) The four rods tied together using sisal twine, a biodegradable string. (c) Two additional cuttings placed in an equilateral cross shape in the center of the four previous cuttings to create a window formation and tied together to the existing rods and (d) at the center of the mat. (e) Final DeValix mat product. (f) Mats bundled together according to clone and stored at 5 °C until planting.

pond, and also included 24 mats of "Fabius". In addition, 32 mats of "SX61" were installed at the eastern pond edge (in double rows) and 35 mats on the western side (in triple rows) of the DeValix buffer, respectively (Figure 4).

During planting, two genotype-specific, 50.8-cm rods were vertically planted 15 cm into the ground in the topside corners of each DeValix mat to help stabilize and prevent mat movement. Mat stakes were not soaked before planting, nor was their growth measured at the end of the growing season, although some stakes grew. Each mat was then covered with 5 cm of soil using the surrounding scarified soil (Figure 3c). Promatrix™ Engineered Fiber Matrix™ (EFM™) (Profile Products LLC, Buffalo Grove, Illinois) was mixed with water and sprayed over the top of each mat at both sites using a T60 HydroSeeder® (Finn Corporation, Fairfield, Ohio) to prevent potential soil erosion and mat

Figure 3. Installation and growth of willow DeValix mat phytoremediation systems grown in Manitowoc, Wisconsin, United States. (a) Soil scarification site preparation using a tractor. (b) Planted DeValix mats before soil cover. (c) Planted DeValix mats following soil cover. (d) Application of Promatrix^{TM} Engineered Fiber Matrix^{TM} (EFM^{TM}) (Profile Products LLC, Buffalo Grove, Illinois) via T60 HydroSeeder$^{®}$ (Finn Corporation, Fairfield, Ohio) on top of newly planted DeValix mats. (e) Completed installation of DeValix mats with Promatrix^{TM} Engineered Fiber Matrix^{TM} cover. (f) Growth of DeValix mats, during time of measurements, after one growing season.

movement (Figure 3d,e). Given the high planting density and erosion potential, weed removal was not performed. Also, irrigation of buffers was not possible.

Sampling and analysis

Height and diameter of all shoots for each DeValix mat were measured during August 2019. Tree height measurements to the nearest cm are from ground level to apical tip of each shoot with the aid of a meter stick. Digital calipers were used to measure the diameter of each shoot just above the ground level to the nearest 0.01 mm. The number of shoots measured during height and diameter data collection were then used to determine the number of shoots per individual mat. Survival of DeValix mats was determined by evaluating individual mats on a holistic basis. A mat with at least one living shoot at the time of measurement was deemed “alive”, while a mat with no living shoots was deemed “dead”.

Height, diameter, and number of shoots data were averaged per mat and analyzed using analyses of means and

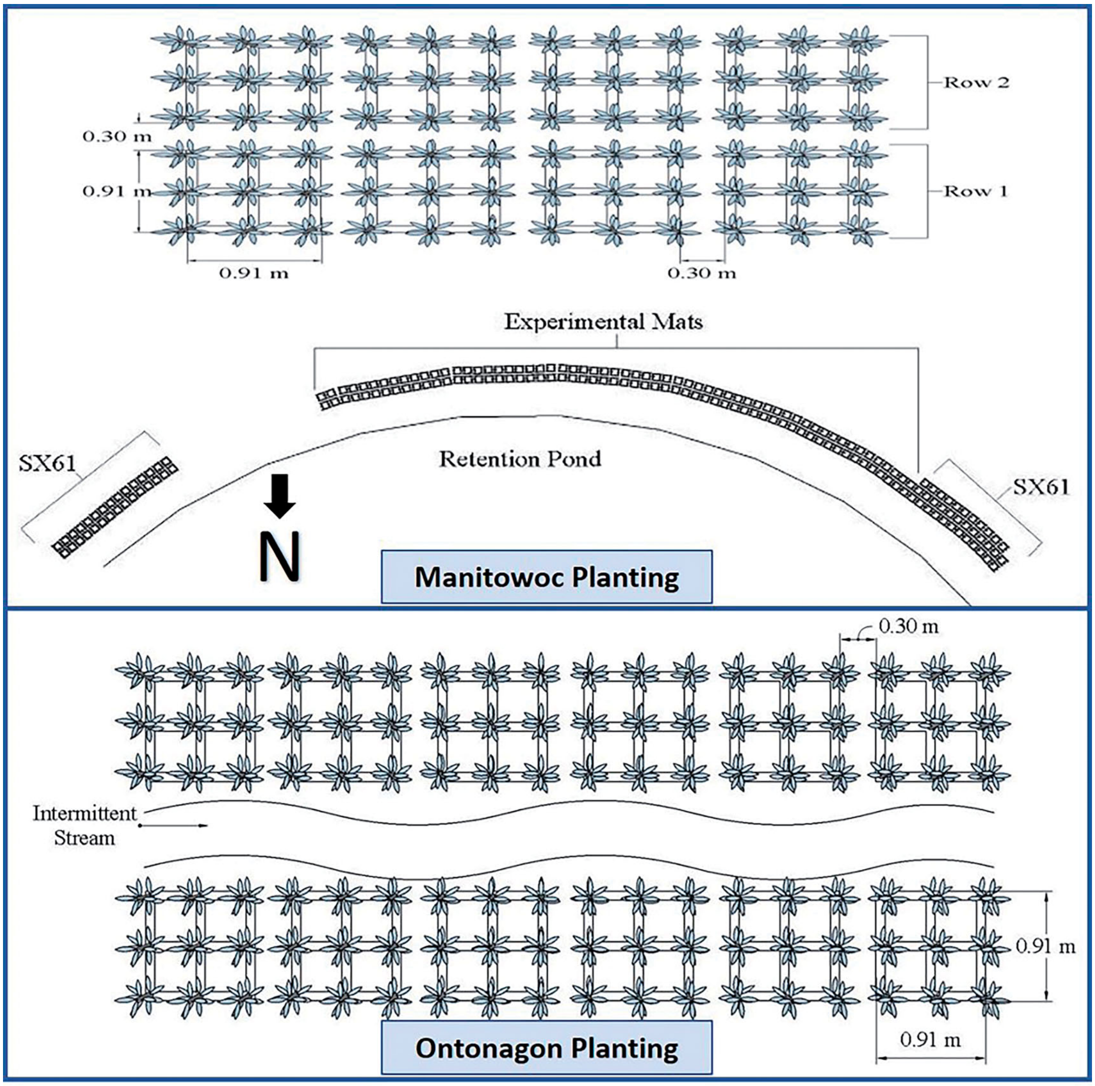

Figure 4. Schematic showing the planting layout of DeValix mat phytoremediation buffers at Manitowoc, WI and Ontonagon, MI. Additional "SX61" plantings at Manitowoc were measured but not analyzed in this study.

two-way analyses of variance according to SAS® (PROC ANOM; PROC GLM; SAS Institute, Inc., Cary, North Carolina, USA). All data were analyzed using a completely randomized design with two sites, five clones, and 24 mats per clone (12 mats for "Sherburne"). Site and clone main effects and the site × clone interaction were considered significantly different at $p < 0.05$ according to Fisher's protected least significant difference (LSD).

A summation index was created to classify clones as generalists and specialists based on their performance across sites. Clones were ranked for height, diameter and number of shoots at both sites, the absolute value of the difference in these ranks for Ontonagon versus Manitowoc was calculated, and the differences for all three parameters were summed (with equal weight given to each parameter) to determine the overall index value for each clone. The summation index values were then compared among clones to classify the genotypes as generalists or specialists. Generalists were clones that demonstrated similar growth performance across both sites for all parameters, and therefore had a notably lower summation index value in comparison to other clones. Specialists were clones that demonstrated variable growth performance across both sites for all parameters, and therefore had a markedly higher summation index value in comparison to other clones.

Results

Survival

At Manitowoc, mat survival was 100%, with every mat producing shoots that were alive at the end of the growing season. Mats at Ontonagon experienced variable mortality across clones. "Tully Champion" was the only clone to have 100% survival, while "SX67" demonstrated the lowest percent survival (79.2%), as 5 of the 24 mats were dead at the time of measurements. "SX61" (91.7%), "Sherburne" (83.3%), and "Millbrook" (95.8%) had mortalities of two, two, and one mat, respectively. Overall, "Tully Champion" was the only clone with 100% survival across both sites. Survival across sites for the remaining clones was: "Millbrook" (97.9%), "SX61" (95.8%), "Sherburne" (94.4%), "SX67" (89.6%).

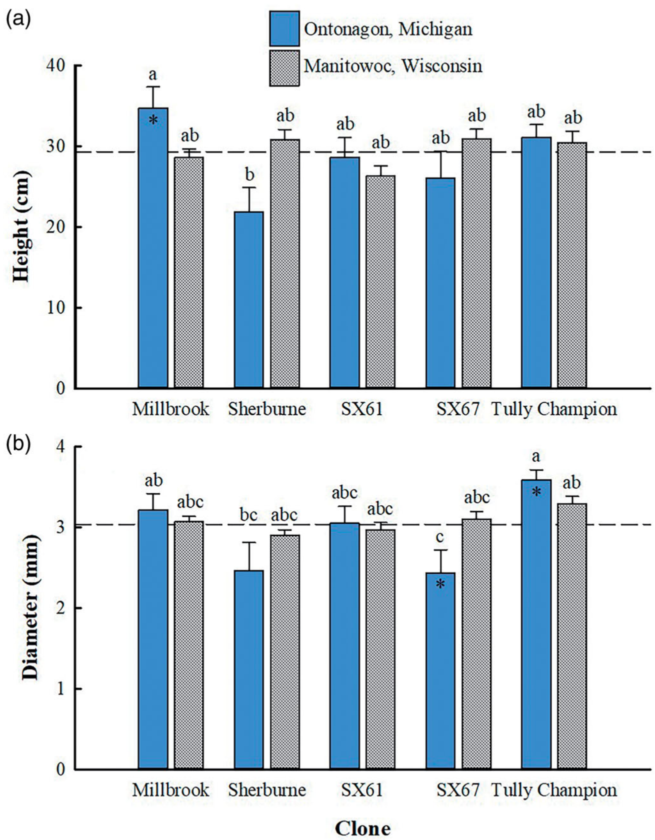

Figure 5. Height (a) and diameter (b) (± one standard error) for significant site × clone interactions of five clones established with DeValix willow restoration mats and tested at two phytoremediation sites for the 2019 growing season. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $p < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $p < 0.05$. Twenty-four mats were tested for all clones, except "Sherburne" at Ontonagon, where $n = 12$.

Height

Neither the site ($p = 0.4659$) nor clone ($p = 0.0608$) main effects were significant for height, but the site × clone interaction was significant ($p = 0.0045$). Mean height ranged from 21.8 ± 3.0 cm ("Sherburne" at Ontonagon) to 34.7 ± 2.6 cm ("Millbrook" at Ontonagon) with an overall mean of 29.3 ± 0.7 cm. Further, only "Sherburne" × Ontonagon and "Millbrook" × Ontonagon were significantly different from each other, and only "Millbrook" × Ontonagon was significantly different than the overall mean height (Figure 5A). None of the clones differed significantly from the overall mean height, although "Millbrook" shoots were 15% taller than "SX61" shoots. Changes in height ranks were observed among clones and varied across sites. "Millbrook" (ranking 1st at Ontonagon and 4th at Manitowoc), "Sherburne" (5th, 2nd) and "SX67" (4th, 1st) varied widely in height ranking, whereas "SX61" had moderately similar rankings (3rd, 5th) and "Tully Champion" (2nd, 3rd) produced a similar clonal ranking at both sites (Table 2).

Diameter

Differences in diameter were significant for clone main effects ($p = < 0.0001$) and negligible for site main effects ($p = 0.2806$), though site × clone interaction governed diameter ($P = 0.0166$). Overall, the mean diameter was 3.04 ± 0.06 mm, ranging from 2.44 ± 0.28 mm ("SX67" at Ontonagon) to 3.59 ± 0.12 mm ("Tully Champion" at Ontonagon), both of which were significantly different than the overall mean (Figure 5b). "Tully Champion" mats planted at Ontonagon grew shoots that had, on average, 18% significantly larger diameters than the overall mean. Conversely, the mean shoot diameter of "SX67" mats planted at Ontonagon was 20% significantly lower than the overall mean. There was also a significant difference between these two clones at Ontonagon, with "Tully Champion" having a 47% greater mean diameter than "SX67". Moreover, "Tully Champion" mats planted at Ontonagon produced shoots with a 46% significantly higher mean diameter than "Sherburne". Additionally, when comparing other site × clone interactions, "Tully Champion" mats at Manitowoc and "Millbrook" mats at Ontonagon had significantly larger mean diameters than "SX67" at Ontonagon. On an individual-clone basis, "Tully Champion" produced shoots with the largest mean diameter ($p < 0.0001$), which was 13% significantly greater than the overall mean. In addition, the mean diameter of "Tully Champion" shoots were significantly greater than that of "SX61", "SX67" and "Sherburne" by 14, 24, and 25%, respectively. Millbrook also produced shoots that were 13% greater in diameter than "SX67" and 14% significantly greater than "Sherburne". Although the mean diameter of shoots from "Sherburne" mats was the lowest of any clone, this value was not significantly lower than the overall mean, which can be attributed to its smaller sample size and subsequent wider range between the upper and lower detection limits for significance. On the other hand, the mean diameter of "SX67", the second lowest producing clone in terms of diameter, was 9% significantly lower than the overall mean.

Similar to height responses, clonal ranks for diameter varied across sites, except for "Tully Champion", which produced the largest mean diameter among all clones at both sites (Table 2). "Millbrook" (ranking 2nd at Ontonagon and 3rd at Manitowoc) and "SX61" (3rd, 4th) also demonstrated stable diameter values across sites, as did "Sherburne" (4th, 5th). In contrast, "SX67" exhibited considerably different performance in diameter between the two sites (Figure 5B). While "SX67" mats planted at Manitowoc were ranked second among clones in mean diameter, mats at Ontonagon ranked the lowest among all clones, with a mean diameter that was significantly lower than the overall mean.

Number of shoots

Number of shoots per mat was governed by site ($p < 0.0001$) and clone ($p < 0.0001$) main effects, while site × clone interaction was not significant ($p = 0.2614$). DeValix mats at Manitowoc produced an overall average of 24 ± 1 shoots per mat, which was 118% more than that of Ontonagon (11 ± 1 shoots per mat). On an individual-clone basis, mean shoots per mat ranged from 12 ± 1 ("SX67") to 21 ± 2 ("Sherburne"), with an overall mean of 17 ± 1 shoots per

Table 2. Rank in height, diameter, and number of shoots of five willow clones established with DeValix willow restoration mats at two phytoremediation sites for the 2019 growing season.

	Site	
Clone	Ontonagon, Michigan	Manitowoc, Wisconsin
	Height	
Millbrook	1	4
Sherburne	5	2
SX61	3	5
SX67	4	1
Tully Champion	2	3
	Diameter	
Millbrook	2	3
Sherburne	4	5
SX61	3	4
SX67	5	2
Tully Champion	1	1
	Number of shoots	
Millbrook	4	4
Sherburne	2	2
SX61	1	1
SX67	5	5
Tully Champion	3	3

mat. “SX61” and “Sherburne”, the two clones producing the most shoots per mat, had significantly greater mean shoot numbers than “Millbrook” and “SX67”. Both clones, “SX61” and “Sherburne”, produced 31% more shoots than “Millbrook” and 75% more shoots than “SX67” (Figure 6). “SX61” and “Sherburne” also produced, on average, 24% more shoots per mat than the overall mean, which was significant. Meanwhile, the mean number of shoots per mat for “Millbrook” was significantly higher than “SX67”, the clone with the lowest mean shoot number. “Millbrook” produced 33% more shoots per mat than “SX67”, and “SX67” mats had 29% significantly less shoots than the overall mean across clones (Figure 6). Although the site × clone interaction was not significant, several clones performed significantly different than the overall mean when planted at specific sites. The mean number of shoots for “SX61”, “Sherburne”, “Tully Champion”, and “Millbrook” planted at Manitowoc were 57, 37, 37, 36% greater than the overall mean, respectively. On the other hand, mats of “SX67”, “Millbrook” and “Tully Champion” planted at Ontonagon produced 72, 56, and 24% fewer shoots than the overall mean. Furthermore, consistent clonal rankings across sites were evident; at both sites, “SX61” produced the greatest average number of shoots per mat, followed by “Sherburne”, “Tully Champion”, “Millbrook” and “SX67” (Table 2).

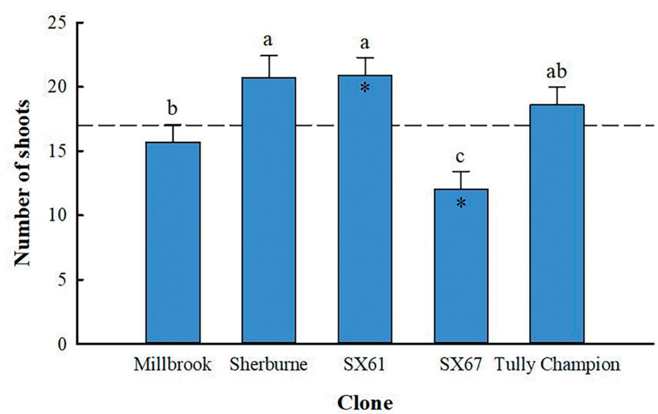

Figure 6. Number of shoots per mat (± one standard error) across two phytoremediation sites where five clones were established with DeValix willow restoration mats and tested for the 2019 growing season. The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $p < 0.05$. Bars with the same letters were not different according to Fisher’s protected LSD at $p < 0.05$. Forty-eight mats were tested for all clones, except “Sherburne” with $n = 36$ because only 12 mats (rather than 24) were grown at Ontonagon.

Generalists and specialists

Clonal performance rankings among the three parameters varied across sites (Table 2). While clones exhibited the same rankings at Ontonagon and Manitowoc for number of shoots, rankings fluctuated across sites for height and diameter. Based on summation indices, “Tully Champion” was classified as a generalist (i.e. had stable rankings) across both sites for all three parameters. Specifically, “Tully Champion” demonstrated the greatest consistency across sites when considering height (ranking 2nd at Ontonagon and 3rd at Manitowoc), diameter (1st, 1st) and number of shoots per mat (3rd, 3rd). Its summation index value of 1 reflects this consistency. On the other hand, “SX67” had a calculated index value of 6, which was notably higher than any other clone. This is demonstrated by its variable performance between the two sites (Table 2). “SX67” produced noticeably greater height (ranked 1st) and diameter (ranked 2nd) at Manitowoc, while at Ontonagon, it was among the lowest performing clones for height and diameter (rankings of 4th and 5th, respectively). Also, the mean diameter for “SX67” was significantly lower than the overall mean across clones. Due to its large summation index value, “SX67” was classified as a specialist. Based on the index values calculated, the three remaining clones were not distinctly classified as generalists nor specialists.

Discussion

Tree establishment is the most important component influencing the success of willow restoration systems. Survival and early growth parameters such as height, diameter and number of shoots are useful indicators in evaluating establishment techniques. Not only do these early growth parameters aid in selection of superior genotypes, but they also influence belowground growth, which ultimately effects the phytoremediation potential of willow plantings (Rogers *et al.* 2019). In the current study, the DeValix mat technique, a novel horizontal planting method, was tested at two sites in order to: (1) evaluate its efficacy as a willow establishment method for phytotechnologies and restoration applications, (2) identify superior cultivars, and (3) classify clones as generalists and specialists.

Assessment of DeValix mat survival across sites and clones revealed a contrast in survival between sites. While survival among all clones was 100% at Manitowoc, mats at Ontonagon experienced varying survival ranging from 79.2% ("SX67") to 100% ("Tully Champion"). Mat survival was determined on a holistic basis; presence of shoots on any of the six rods of a mat was deemed "alive". This method did not account for individual rod mortality within mats, so therefore not all mortality that existed at the sites was taken into account. Differential survival of mats between sites may be due to both soil characteristics and variable soil cover atop each of the mats. Soil from Ontonagon was acidic (pH = 4.6) compared to Manitowoc"s more neutral pH (pH = 7.4), which could have made it a less favorable environment for selected cultivars (Abrahamson *et al.* 2002; Kuzovkina *et al.* 2018). While water availability may have been a factor in decreased survival of Ontonagon mats, it is not likely due to the availability of nearby water (i.e. less than 3 m from the mats) flowing in an intermittent stream during the initial stages of establishment. Despite efforts to evenly cover mats with soil, it is possible that erosional processes exposed some of the planting material to desiccation, while others experienced greater depth of soil cover that prevented stems from reaching the surface (Edelfeldt *et al.* 2015). The need to cover each mat with soil was exhibited by a separate trial of 48 and 36 DeValix mats established at Manitowoc and Ontonagon, respectively. Intended as an investigation into the need for soil cover during DeValix mat establishment, these mats (established in the same manner as all other mats in the current study) were not covered with soil or fiber matrix. After several weeks, these mats were removed as they showed signs of severe desiccation without any growth. This trial therefore demonstrated the need for proper soil cover for horizontal planting methods.

Results of the current study showed that both height and diameter were governed by significant site $\times$ clone interactions. Previous studies assessing the viability of different horizontal establishment techniques have compared planting methods using biomass yields after one or more growing seasons (Lowthe-Thomas *et al.* 2010; McCracken *et al.* 2010; Donnelly *et al.* 2019), while others have reported growth measurement values for principal stems (Edelfeldt *et al.* 2015; Donnelly *et al.* 2019). Given the effect of establishment success on willow development in subsequent years after planting, it is important to evaluate growth and survival of willow plantings following the first growing season. Therefore, all stems in the current study were measured after one growing season at each of the sites and the mean values of height and diameter were calculated for each mat. Though this makes direct comparisons with previously-mentioned studies challenging, other studies have also tested willow performance after the first growing season. For example, Larsen *et al.* (2014) compared performance of four different establishment methods (vertically-planted, 0.2-m cuttings; horizontally-planted, 0.1- and 0.2-m billets; horizontally-planted, 1.8-m rods) after one growing season in Denmark. Higher mean shoot height and diameter were reported for all establishment techniques than the DeValix mats of the current study. This elevated growth can be partially attributed to climatic variation between the two studies but is more likely the result of differences in soil properties as well as weed treatments. Soils on which DeValix mats were grown in the present study were highly disturbed via soil removal and compaction by heavy machinery. In contrast, plantings in Denmark were established on former agricultural plots, suggesting more suitable conditions for biomass production. Furthermore, no weed removal was performed throughout the duration of the current DeValix mat study, unlike the mechanical or chemical treatments administered by Larsen *et al.* (2014). Weeds reduce willow yields (Albertsson *et al.* 2014; Welc *et al.* 2017), especially during the establishment phase when willow are the most susceptible to competition.

In addition to height and diameter parameters, shoot density plays a crucial role in the success of willow buffer systems, particularly for phytotechnology applications (Isebrands *et al.* 2014). Shoot number was governed by both clone and site main effects in the current study. "Tully Champion" produced the highest number of shoots across both sites. Along with "Sherburne", "Tully Champion" mats developed a significantly higher number of shoots than the mean of all clones. On the other hand, "SX67" consistently had the lowest shoot number of any clone, producing significantly less shoots than the mean of all clones. This is in contrast to Labrecque and Teodorescu (2005) who reported a higher shoot number for "SX67" after the first growing season, though establishment method may be the cause of this discrepancy. DeValix mats at Manitowoc produced 118% more shoots per mat than the Ontonagon site, indicating favorable site conditions for shoot development at Manitowoc. One factor that likely contributed to an elevated shoot number at Manitowoc is the differing level of weed competition; there was notably less weed presence and density at Manitowoc at the time of measurements. This was predicted due to a lack of weed presence prior to site preparation. These results highlight the importance of mechanical and/or chemical weed management during the establishment phase of willow. Additionally, such findings point to a potential drawback in the current DeValix design; the spacing between rods of the mats made weed management difficult. Shorter rods to construct the mats may be necessary to reduce the effect of weed competition in future applications, though such an adjustment must also consider changes to intraspecific competition.

Site conditions (i.e. climate and soil composition) likely also influenced shoot number in the current study. Mean aboveground temperature, total precipitation and growing degree days were all higher at Manitowoc than Ontonagon across the entire growing season (Figure 1). These climatic conditions could have promoted shoot development by providing adequate water availability and suitable temperatures for more optimal growth (Lafleur *et al.* 2017). Further, soils with a higher rock composition at Ontonagon may have affected shoot emergence, thereby affecting shoot growth. Horizontal planting techniques can lead to delayed shoot bud burst through the soil compared to vertical cuttings and have led to shoot death prior to new shoot emergence (Edelfeldt *et al.* 2015). Therefore, delayed shoot emergence coupled with unfavorable soil conditions may have hindered development of new shoots above the soil surface at Ontonagon. Depth of soil cover was another determining factor in mat shoot number. Mats that experienced a large amount of soil erosion and had rods that were exposed to the air exhibited lower shoot numbers than those mats that remained fully covered throughout the growing season. This is likely due to desiccation of rods and the inability of rods to produce roots without soil contact. Although erosion was apparent in several locations throughout both study sites, the severity and extent of erosion was notably less than that experienced using previous methods of jetting vertical cuttings at Manitowoc (data not shown).

Overall, the DeValix technique produced a dense stand of willow with an overall mean stem density of 95,000 to 212,000 stems per hectare. These results corroborated previous studies that evaluated shoot density of horizontal planting techniques after establishment year. Larsen *et al.* (2014) reported shoot densities of 38,000 stems per hectare for 20-cm billets and 52,000 stems per hectare for both 10-cm billets and 1.8-m horizontal rods. This is compared to the 29,000 stems per hectare produced by traditional 20-cm, vertically-planted cuttings in the same study. Studies of the SALIMAT technique show shoot density to range from 230,000 to 755,000 shoots per hectare after the first growing season (Vervaeke *et al.* 2001, 2003). Although stem density of the DeValix method is slightly lower, shoot density of SALIMAT plantings had reduced to 110,000 stems per hectare after three growing seasons (Vervaeke *et al.* 2001), suggesting a self-thinning process over time that may not impact the DeValix technique to the same extent, given the wider spacing between rods. Horizontal planting methods have also demonstrated apical dominance (the region of the rod near the tip of the original willow whip produces a higher number of shoots as a majority of the dormant buds are vegetative in nature), which can impact the number and distribution of shoots (Edelfeldt *et al.* 2015). Buds near the basal region of the original plant material may exhibit delayed emergence and therefore produce stems later, leading to uneven establishment and impacts from weed competition. When collecting measurements of shoot density there was no consideration to differentiate shoot density based on original stem location. However, apical dominance was not observed at either Manitowoc or Ontonagon, as a relatively even distribution of shoots was experienced within mats across sites and clones.

Table 3. Mean (± one standard error) height, diameter, and number of shoots per DeValix willow restoration mat grown at two phytoremediation sites for the 2019 growing season.

	Height[a] (cm)		Diameter[a] (cm)		Number of shoots[b]	
Ontonagon, Michigan						
Millbrook	26.6 ± 1.4	(77)	3.10 ± 0.11	(77)	6 ± 1	(12)
Preble	26.3 ± 0.6	(501)	3.35 ± 0.05	(501)	21 ± 2	(24)
SX61	40.9 ± 1.6	(207)	3.68 ± 0.09	(207)	17 ± 2	(12)
Manitowoc, Wisconsin						
Fabius	28.6 ± 0.8	(547)	2.99 ± 0.06	(547)	23 ± 1	(24)
SX61	27.4 ± 0.4	(1,861)	3.01 ± 0.03	(1,861)	28 ± 1	(67)

Information for these clones and mats was not included in the statistical analyses because "Preble" and "Fabius" were each only grown at one site, and these "Millbrook" and "SX61" mats were established in addition to those analyzed. See Materials and Methods for more information.
[a]The number of shoots measured is in parentheses.
[b]The number of mats measured is in parentheses.

In addition to the five experimental clones analyzed within this study, two other clones were planted at the test sites: "Preble" at Ontonagon and "Fabius" at Manitowoc. Though these clones were not included in our analysis because they were only grown at one site, they did demonstrate adequate growth that was comparable to other clones tested in this study (Table 3). Further investigation of these clones across multiple sites is needed to properly analyze their growth, yet this demonstrates the applicability of the DeValix technique across multiple clones and highlights the need for future research into performance of different clones and sites using this technique.

Our results indicated that the DeValix method could be a viable establishment method for phytotechnologies. However, a few drawbacks to the technique must be addressed before there can be a more widescale implementation of DeValix mats. In addition to the previously-mentioned difficulty in performing weed control, the current process of forming the mats by tying together rods with sisal twine is cost-prohibitive for commercial-scale installations. The average cost per mat was $13.17 US, which included planting material, mat construction labor, hydroseeder rental and fiber matrix costs, but excluded installation labor and site preparation costs. Cost per shoot produced from the DeValix mats ranged from $0.48 to $2.72 US, depending upon clone and site. The overall mean cost was $0.71 US for each shoot produced. On the other hand, the average cost for 25.4-cm cuttings used at other phytoremediation buffer systems within the same testing network as the current study was $0.25 US per cutting (also excluding installation labor and site preparation costs). Accounting for 82.6% survival across five sites within this network, the cost increased to $0.30 US per cutting (i.e. per shoot during the establishment year). Developing a mechanized process or using different material to more efficiently tie rods together may reduce DeValix mat costs and make establishment costs more comparable to conventional vertical establishment. Further testing of such processes and materials is warranted.

Selection of willow that are suitable for growth in desired environments is important for maximizing biomass production and phytoremediation potential. Identifying species that perform well across a wide range of site conditions (i.e.

generalists) and those that demonstrate optimal growth and physiology within certain site conditions (i.e. specialists) is useful for site managers in selecting cultivars for establishment (Rogers *et al.* 2019; Pilipović *et al.* 2021; Zalesny *et al.* 2021). In the current study, based upon clonal rankings for growth and survival parameters among all cultivars between both sites, "Tully Champion" was classified as a generalist while "SX67" was identified as a specialist. All other clones ("Millbrook", Sherburne" and "SX61") demonstrated intermediate variation in growth and survival metrics and were therefore neither generalists nor specialists. "Tully Champion" performed consistently well at both sites, producing the highest number of shoots and survival of all clones across sites in addition to relatively stable height and diameter measurements at both Manitowoc and Ontonagon. Conversely, "SX67" mats grew preferentially well at Manitowoc. Despite having shoots among the tallest and widest of any clone at Manitowoc, "SX67" was among the lowest performing clones at Ontonagon in terms of height and diameter. Furthermore, "SX67" shoot density and survival were the lowest among clones tested. In an investigation analyzing the effect of genotype × environment interaction on the yield of 16 genotypes across 10 field trials, including all clones but "SX67" from the current study, Fabio *et al.* (2017) found no significant difference of individual cultivar yield across all sites. Of the 16 clones tested, "SX61" was among the top 3 clones with the most stable yields, suggesting that it is a generalist. "Tully Champion", on the other hand, showed a strong genotype × environment interaction; it grew exceptionally well in sites with the lowest mean yields, indicating a potential tendency toward being a specialist. "Millbrook" and "Sherburne" demonstrated similar trends as in the current study, exhibiting an intermediate level of genotype × environment interaction. Meanwhile, Liu (2013) reported stable yields of "SX61" and "Millbrook" across 16 sites in the US whereas "Tully Champion" and "Sherburne" varied significantly across sites. Unlike the current study, "SX67" also demonstrated stable yields across sites, which may indicate that weeds had a larger impact on growth than soil or climate differences between sites in the current study. Furthermore, in a study of first- and second-rotation yields of willow across six sites in Michigan, Miller (2016) designated "Millbrook", "SX61" and "Tully Champion" as "general performers" due to their good performance across sites. Such results highlight the need to test clones across multiple sites with varying climatic and soil characteristics in order to determine suitability of cultivars for specific sites.

Conclusion

This study has shown the DeValix technique to be a viable alternative to conventional vertical methods of willow establishment. After one growing season, a density of 95,000 to 212,000 stems per hectare was achieved, which is adequate as an alternative to vertical and other horizontal planting methods currently tested. Improvements to the DeValix mat technique are necessary to make it economically viable, including finding ways to reduce mat assembly time and altering the design to reduce weed competition and/or promote easier weed control. "Tully Champion" and "SX67" were classified as generalist and specialist cultivars, respectively, suggesting a need to select clones that are suitable for growth under specific site conditions. Further testing of the DeValix technique should include new willow cultivars and additional study sites along with alterations in rod spacing and size to study these effects on biomass production and survival.

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