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Wood-bark-cambium relations during willow cloning and the mechanism of rooting in *Salix discolor*

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Abstract

The process of pussy willow *(Salix discolor)* propagation, or cloning, is an important biological process, which allows the increase of the amount of plantlets with the properties of the mother plants. Pussy willow has very weak dormancy. Therefore, the process of rooting of stem cuttings may proceed ten months in a year except September and October. We investigated the localization of the rooting process on the stem, performing surgery in the form of girdling of bark and partial stripping of bark with cambium from the stem. The process of rooting was investigated. We observed that roots are formed only on the bark with cambium. If we strip the bark, no roots on the wood were observed. Rooted cuttings were subjected to preparation and we observed that roots were formed only on the bark-cambium complex. During the preparation of the stem, we did not separate bark from cambium and investigated this complex together.

Key words: propagation, cloning

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Introduction

Previous experimentation by Kefeli *et al.* (2007) demonstrated the carbon polymer formation in leaves, wood, and bark of willow cuttings. The carbon polymers in leaves are mainly starch. In wood, the carbon polymers are lignin and cellulose. Carbon polymers found in bark are cellulose, lignin, and pectin. These secondary substances are formed from the primary products of photosynthesis such as glucose and amino acids (Kefeli *et al.,* 2007). These stored carbon polymers accumulate in the bark-cambium complex to be mobilized for building new materials. Low molecular phenolics could be excreted from roots and accumulated in leaves (Kefeli *et al.,* 2000). These phenolics may play the role of natural inhibitors and botanical herbicides (Kefeli *et al.,* 2003). In addition, after abscission, the leaves play a role in producing carbon soil substrate with nutrients that contribute to the regeneration of soil for future productivity. The present study is a continuation of research at the Center of Restoration of Biological Cycles: The Jennings Environmental Education Center, Slippery Rock, PA, in which mining site restoration is the focus for integrative studies on the restoration of water and soil cycles as well as shelter construction. Past projects include water restoration using filtration through both calcium carbonate and composted materials. Other investigations involve the relationships of bark, wood, and leaves; growth rhythms in willow plants; use of fabricated soils; protection of chestnuts by root exudates of willows; and building material fabrication from local plant sources. In this paper we are investigating the mechanism of rooting in pussy willows *(Salix discolor).* The pussy willow is an adaptable, rapid growing plant that is easily cloned. It has potential for soil restoration and fiber production for use in building materials.

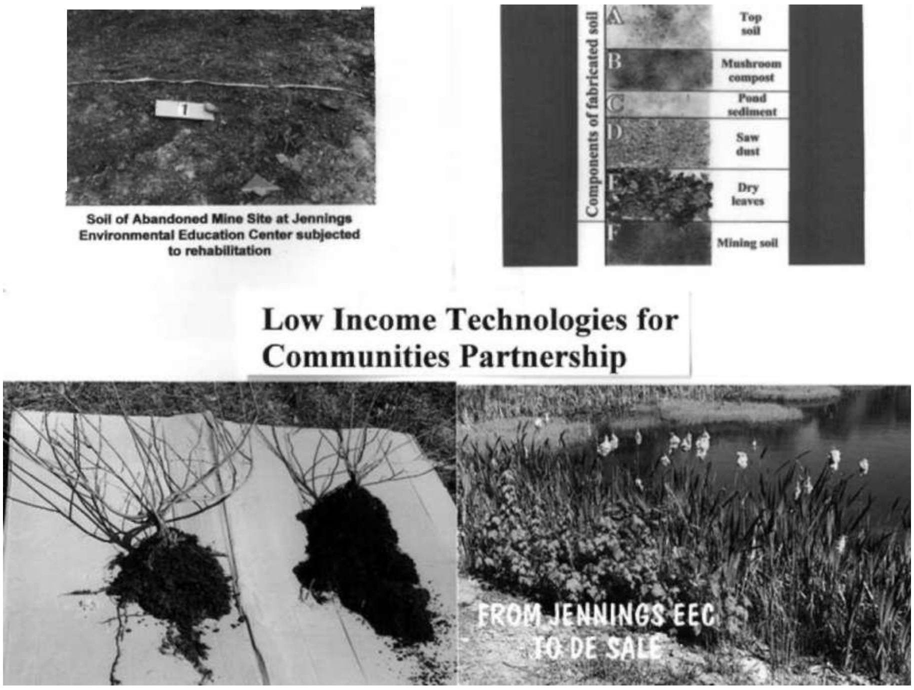


Figure 1. Illustration of regeneration process for mining soils at Jennings and De Sale using pussy willow, red willow, and poplar clones. Mother willow plants grown on fabricated soils at Jennings Environmental Education Center provide cuttings for clones. The plants are used to restore mine damaged soils at Jennings and De Sale.

Previous experimentation on red willow

Previous experimentation by B. Rihn investigated the relationship of bark and wood formation and stem construction in red willow shoots. Leaves were counted, weighed, and registered from five-year-old mother plants grown in fabricated soil at Jennings mining soil restoration site, one-year-old cuttings from the mother plants, and transplants at the DeSale Plot. The upper part of each shoot produced more leaves and had a higher weight (Kefeli *et al.,* 2007). The upper leaves are considered the centers of primary products of photosynthesis (Kefeli, 1992). On the other hand, the lower portion of the cuttings had the higher production of wood and bark. A rhythmic pattern was noted in the process of growth of red willow cuttings. First, photosynthesis in leaves produces primary products. Second, these primary products are used for stem elongation. Third, roots play a part in stem growth. Fourth, carbon polymers are formed while wood, cambium, and bark is differentiated (Kefeli *et al.,* 2007) (Figure 1-2).

Materials and methods

Eighteen centimeter cuttings of pussy willow were isolated from the mother plants at Jennings Environmental Education Center. Cuttings were then prepared for rooting in water, at 68° F. Sink water was used. The pH registered 6.33. Water temperature was maintained at 60° F (16° C). A week later, four groups were established. Two control groups had no surgery. One group had the bark-cambium layer girdled an inch above the base of the cutting. The forth group had a narrow strip of bark removed from the base of the cutting to two inches up the stem. After 7 days, the first primordia were registered. After another 7 and 14 days, the number of roots were registered on each cutting, above and below girdling and on the strips. Length of roots was also measured. On the fifth week, the number of shoots was registered and the average length of roots and shoots were recorded. The plants received no natural light, and only occasional artificial light. No nutrients were added, but fresh sink water was exchanged each week.

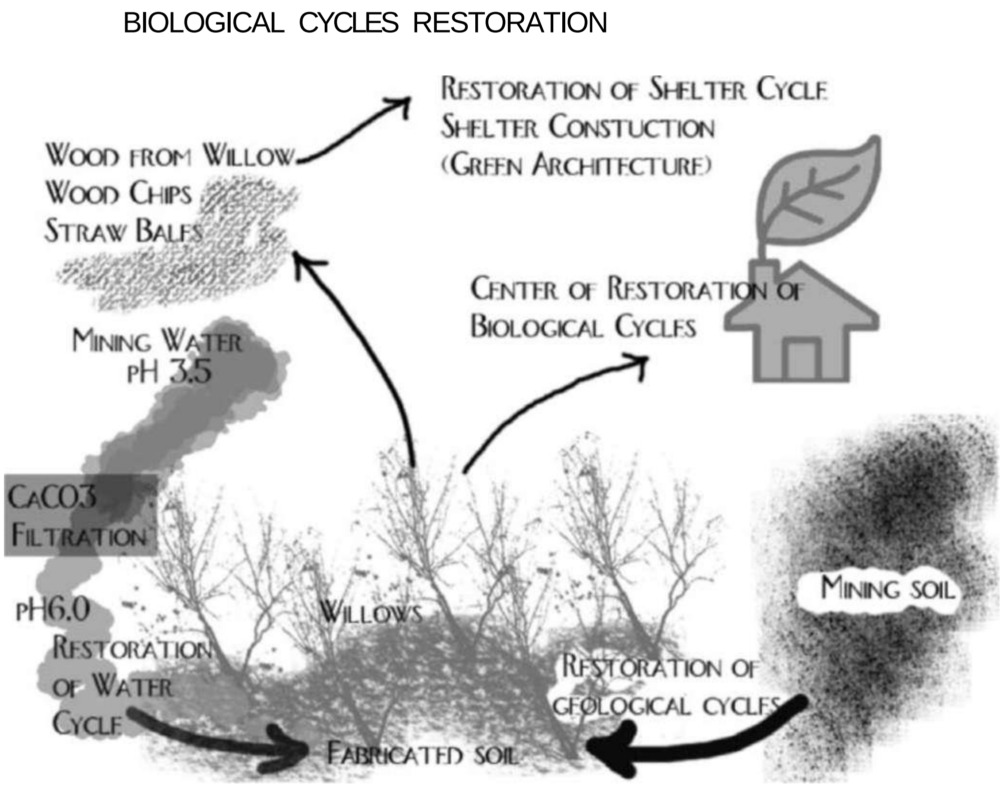


Figure 2. Integration of cycles restoration at Jennings Environmental Center.

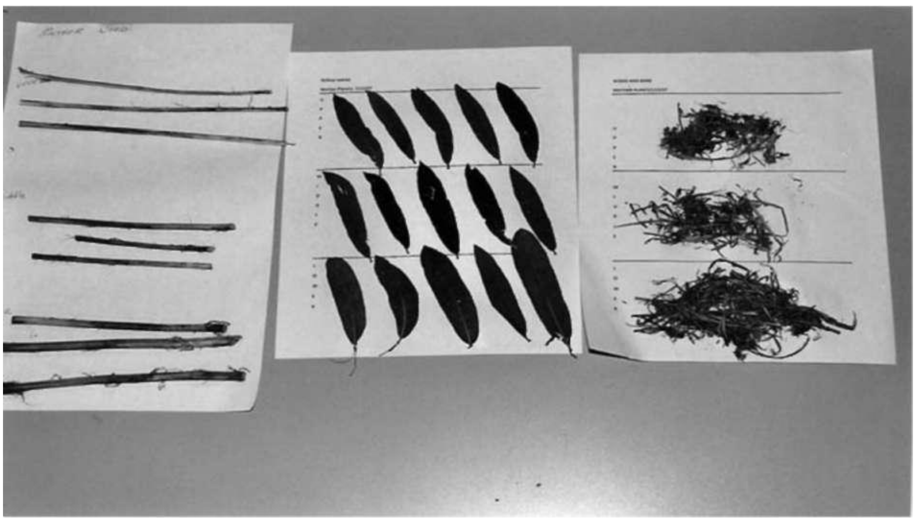


Figure 3. H is pointed out that the connection between monomer production in leaves to polymer production in leaves, wood, and bark.

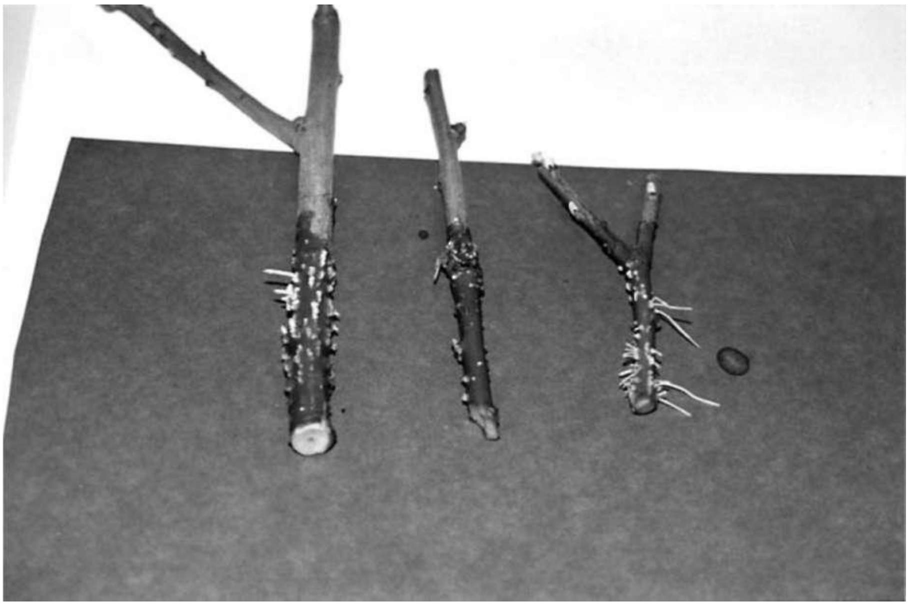


Figure 4. Process of root initiation on willow cuttings. Stages of primordia and root development

Figure 5. Willow cuttings taken 2/5/08. Control, girdles, strips.

Table 1. 02/19/08 Week Three of Pussy Willow Cuttings: Observations of Primordia and Roots.

	I. Control 1	II. Girdles	III. Strips	IV. Control 2
Primordia	24	7.5	12	29
Roots	0.4	0	0.09	0
Primordia below girdle		0.2		

Table 2. Week four observations of primordia and roots of pussy willow cuttings.

	I. Control 1	II. Girdles	III. Strips	IV. Control 2
Primordia	22	12	16	30
Roots	15	7	17	23
Primordia below girdle		3		

Table 3. Week five observations of primordia, roots, and shoots.

	I. Control 1	II. Girdles	III. Strips	IV. Control 2
Primordia	18	8	13	23
Roots	32	22	27	34
Shoots	6	5	6	6
Avg. longest Root (cm)	6	6	6	8
Avg. longest Shoot (cm)	8	6	5	8

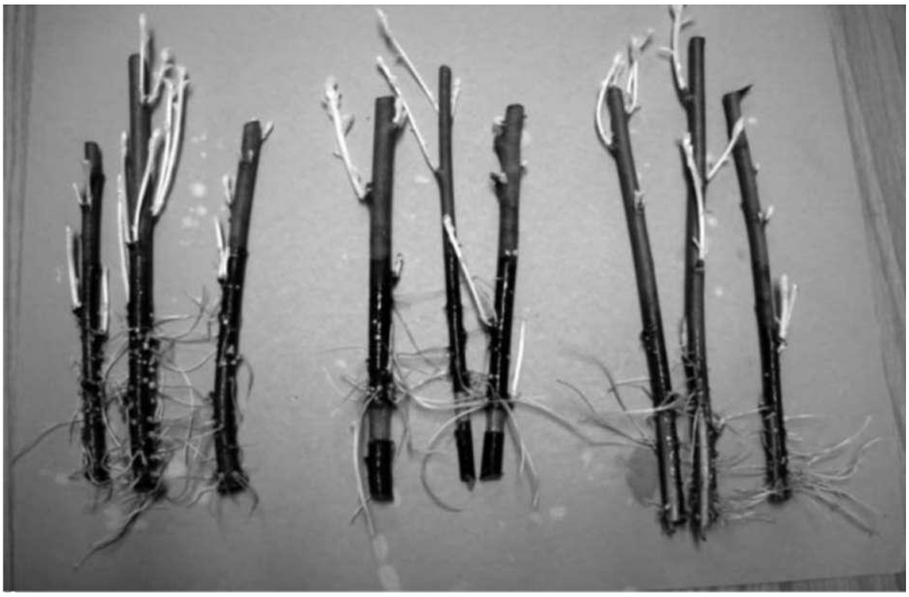
Table 4. Average of five weeks showing percentage of combined control groups.

	I. Control 1-2	II. Girdles	III. Strips
Primordia	24	9 (37 %)	14 (58 %)
Roots	17	14 (82 %)	15 (88 %)
Shoots	6	5 (83 %)	6 (100 %)
longest Root (cm)	7	6 (86 %)	6 (86 %)
longest Shoot (cm)	8	6 (75 %)	5 (63 %)

Results

General conception of this article is based on the activity of the willow shoot elements; leaves, wood and bark. In general, wood ia the system of water transportation (Figure 3 left), leaves as the center of photosynthesis and primary metabolite production (center) and bark and cambium (left) centers of root formation on the stem cuttings.

In the mechanism of rooting experiment, stages of root formation were observed. First, the primordial



Photograph 5. Week five of pussy willow cuttings.

tissue erupted through the bark. This tissue then developed into roots after 14 days of incubation in water. The roots went through the process of elongation, forming the new clones from the cuttings of the mother plants. No primordia or roots formed on the wood where the bark-cambium layer had been either stripped or girdled. Though a few primordia formed below the girdles, no roots developed from them. The cuttings with the girdles and strips showed reduced numbers of primordia and roots, as well as reduced root and shoot elongation. The pH measured before the water was replaced with new sink water, was lower than the pH from the sink water. On the third week, control group one had a pH of 5.74; cuttings with girdles measured 6.19; cuttings with strips measured 6.17; and control group two measured 5.23.

Stem willow cuttings could be transformed into individual plants via following stages- root primordial formation (after 2 weeks expose in water, temperature plus 22 C (Fig.4). Rooting process could be modified if we girdle the cuttings (no bark and cambium in the ring) or if we make longitudinal strips. Operation with bark (girdling and stripping) depressed root activity on cuttings after 3 weeks exposure in water (Table 1). Four weeks is a time of the partial recovery of cuttings after operation (Table 2-3). More advanced recovery we can observe after 5 weeks of surgery (Table 4). In general surgery on the cuttings (lower, water submerged part) leads to reduction of primordial formation which is connected with the cambium activity, length of shoots and roots are less sensitive (Table 4 and Figure 6)

Conclusion

Three centers of C-polymer formation were investigated on willow cuttings: leaves, wood and bark. After stem girdling, we observed higher amounts of primordia above the girdle. Wood was inert tissue for root initiation on willow cuttings. The bark-cambium complex was a center of root formation of willow cuttings. Wood and xylem are not regions for root formation. Though water could flow up the xylem, the breach in the phloem did not allow necessary materials for root development to flow down below the girdles. In addition, photosynthate necessary for rooting does not pass through the xylem. For formation of roots, there must be sufficient amount of flow from the upper part of the cutting.

The cuttings were taken in a dormant state and not exposed to natural sunlight. Nevertheless, materials were stored in the bark and cambium to establish initial primordia and root development as well as the formation of new shoots. Further investigation may reveal the limitations of root and shoot growth without sunlight and soil nutrients. The surgery on the cuttings slightly interfered with root and shoot development. Signals for root and shoot differentiation and for the mobilization and utilization of stored materials proceeded in the cuttings to produce clones of the mother plants. The rooted cuttings were potted in fabricated soil in gallon containers and placed outdoors for future planting on mine soil restoration sites. Though primordia will form and develop into roots, photosynthesis is necessary for further root growth and development.

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