# TECHNICAL NOTE

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# EFFECTS OF LONG-TERM REFRIGERATED STORAGE ON HARDWOOD WILLOW CUTTINGS

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**This technical note describes performance of dormant hardwood cutting of two native willow species under varying lengths of storage in dark refrigerated conditions. It also compares survival and growth with decreased moisture loss through the use of polyethylene bags. Non-bagged cuttings stored under refrigerated conditions retained good vigor for at least four months. Increasing humidity and maintaining cutting moisture levels by bagging the cuttings in polyethylene extended vigor with no loss of survival for up to 6 months. Bagging the cuttings also appears to stimulate root and shoot growth.**

**Effects of long-term refrigerated storage and bagging in polyethylene on hardwood willow cuttings**

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Planting healthy, vigorous cuttings is essential to successful establishment of riparian willow plantings for streambank erosion practices. Vigor of cuttings during the initial planting phase is critical to establishment and long-term survival of the cutting. Cuttings are often harvested dormant in late winter prior to the scheduled planting, but schedules frequently become preempted by unforeseen circumstances. Installation is then forced to wait until the completion of groundwork, construction, etc, or until conditions become favorable, or when funding for the planting is approved. Willow cuttings are commonly kept in long-term storage for weeks to months after their scheduled installation date, which raises the question, “how long can cuttings be kept in storage and still retain enough vigor to be successfully used in riparian restoration projects?”

Cutting survival under storage conditions is dependent on water loss and the prevention of infection (Behrens, 1988). Unfavorable conditions can 1) kill cuttings, or 2) reduce rooting potential (Behrens, 1988). Best storage conditions for dormant cuttings are those that cause no water stress and prevent the spread of fungal pathogens. The best means to achieve this is to lower the temperature and increase humidity (Behrens, 1988; Davis and Potter, 1985). If possible, relative humidity should be kept near 100% (Behrens, 1988; Scianna et al., 2005). An optimum temperature for cutting storage is approximately -4° C (24° F) (Behrens, 1988; Cram and Lindquist, 1982).

The majority of cutting storage research involves small diameter cuttings used for nursery stock production. Meeting ideal storage conditions is more feasible with small nursery cuttings than for larger cuttings (poles or posts) intended for riparian bioengineering projects. Many riparian restoration projects are limited in resources and cutting storage locations may include basements, root cellars, walk-in refrigerators or in plastic bags stored outside during winter months.

Storage of dormant, leafless hardwood cuttings during winter months is not problematic and is a well-established practice without any major problems (Behrens, 1988). Cuttings should maintain vigor in freezing conditions outside fairly well if protected from disease, wind, sunshine, or insects. Heeling-in cuttings as well as fall dormant planting have also proven successful as means of storage and establishment (Cram and Lindquist, 1982; Tilley and Hoag, 2009).

However, long term storage effects on hardwood cuttings for restoration in less than ideal conditions are less well understood. Problems arise when storage becomes prolonged and conditions need to be maintained artificially. This study conducted by the Aberdeen Plant Materials Center addresses long term refrigerated storage on larger diameter cuttings of two willow species commonly used for riparian restoration projects in the Great Basin Region. It also compares the effects of storage in polyethylene versus cuttings stored in open conditions.

**Materials and Methods**

The test occurred over two seasons. During the first season we tested cuttings stored exposed (not bagged in polyethylene, hereafter referred to as the non-poly treatment) in a dark walk-in cooler. During the second season the cuttings were kept in large black polyethylene bags (hereafter poly treatment) tied shut to retain moisture. Dormant hardwood cuttings for the non-poly treatment were harvested on November 29-30, 2011. Cuttings used in the poly treatment were collected on November 26-28, 2012. Yellow willow (*Salix lutea*) was collected from a native stand at Quaking Aspen Spring at an elevation of 1,585 m (5200 ft) (42.2309642, -112.7885628). Coyote willow (*S. exigua*) was collected from a native stand on the Curlew National Grassland on Hwy 37, approximately 1 mi south of Twin Springs in Rock Creek at 1,554 m (5100 ft) (42.2423708, -112.7495098). All cuttings were trimmed to a length of 60 cm (24 in) with a basal diameter of 1.9 to 2.5 cm (0.75 to 1.0 in). Temperatures in the cooler ranged between from 1 to 2° C (34 to 39° F) with 77 to 82% relative humidity. Relative humidity inside the polyethylene bags was measured at 99%. The cuttings were stored for 60, 120, 180 and 240 days and a 0 day control treatment.

Four replications of 6 cuttings each were placed in 9.5 l (10 qt) galvanized buckets in a growth chamber kept at 22-23° C (71 to 74° F). The design was a randomized complete block with 4 buckets acting as 4 replications. The growth chamber was equipped with six, 34 watt white fluorescent bulbs, 3 on the door and 3 on the back panel with a 24 hr light period. Photosynthetically active radiation (PAR), measured using a Decagon AccuPAR LP-80 ceptometer®, measured 45 $\mu mols/m^2s$. Relative humidity in the growth chamber was measured at 58%. The buckets were watered daily to maintain water depth at 23 cm (9 in). No soil medium was used in the experiment to facilitate root measurement.

Cuttings were evaluated after 32 days of growth chamber conditions for percent survival (visible active growth, root initials, or healthy green stem tissues) and for average shoot and root biomass. Biomass was measured after air drying for 14 days at 22° C (72° F).

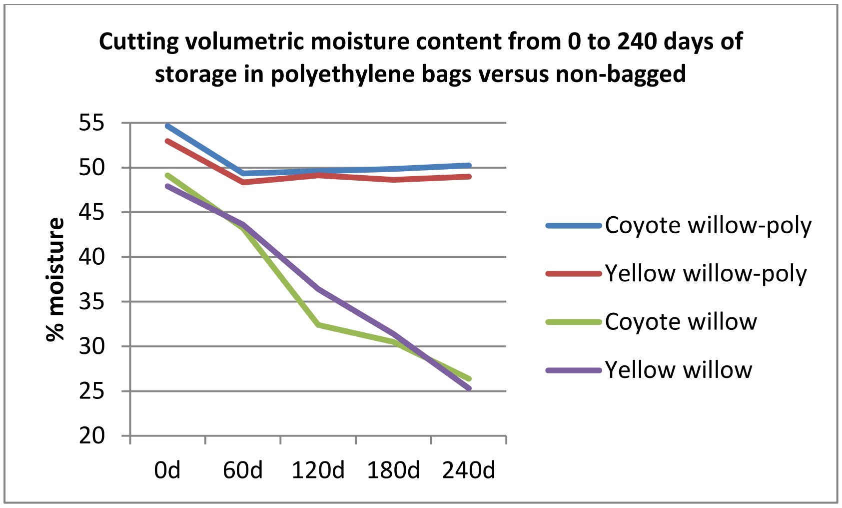
A second set of cuttings for each treatment was used to measure cutting volumetric moisture content. Four replications of one cutting per rep were used for each storage treatment. Fresh weights were measured after storage treatment conclusion, and dry weights were recorded after oven drying at 60° C (140 °F) for 10 days.

Comparisons of cutting survival and growth measurements were made using a one-way ANOVA. Significance was assessed at $\alpha$=0.05. Means were differentiated using a Least Significant Difference (LSD) test. Statistical analysis was conducted using Statistix 8 Analytical Software.

## Results

*Cutting volumetric moisture content*

The 2011 harvested non-poly cuttings began the storage treatment with between 45 and 50% moisture content. The poly treatment cuttings began with slightly higher moisture ranging between 50 and 55%. Cutting moisture decreased steadily in the non-poly cuttings at a rate of approximately 0.1% per day. After 240 days water content ranged from 25 to 30% (figure 1). The cuttings stored in polyethylene lost a small amount of moisture between 0 and 60 days, then leveled off at approximately 50% for the remainder of the trial.




*Cutting survival*

Fresh cuttings of both species planted with no storage had 100% survival (table 1). Excellent survival was achieved with fresh cuttings and from cuttings bagged in polyethylene up to 180 days of storage but then declined significantly at the 240 day evaluation. Cuttings not bagged in polyethylene had excellent survival with up to 120 days of storage. At 180 days and beyond coyote and yellow willow survival decreased significantly for cuttings in the non-poly treatment.

Table 1. Cutting Survival

| | Coyote willow | Yellow willow |
|---|---|---|
| 0d | 100 a[1] | 100 a |
| 0d poly | 100 a | 100 a |
| 120d poly | 100 a | 100 a |
| 180d poly | 96 a-b | 100 a |
| 60d | 92 a-b | 92 a-b |
| 60d poly | 88 a-b | 100 a |
| 120d | 79 a-b | 96 a |
| 240d poly | 75 b | 77 b-c |
| 180d | 17 c | 58 c |
| 240d | 8 c | 29 d |

[1] Means followed by the same letter are not significantly different at P≤0.05 using LSD (least significant difference) test.

*Root development*

Root biomass of coyote willow was significantly greater in treatments with cuttings stored in polyethylene bags compared to non-bagged cuttings and the 0d control treatment (table 2). Root production of coyote willow cuttings stored in poly did not differ significantly between storage durations. Likewise, root weight of coyote willow cuttings not stored in poly did not differ significantly from one another.

Differences in root growth were detected between storage durations among yellow willow cuttings. Greatest root biomass was found with 180d poly treatment which differed significantly from the 240 and 60d poly treatments but not the 120d poly treatment. Root biomass among the non-poly treatments of yellow willow cuttings did not differ significantly from one another regardless of length of storage.

Table 2. Root biomass

| | Coyote willow | Yellow willow |
|---|---|---|
| | ---------------(g)--------------- | |
| 240d poly | 0.76 a[1] | 0.48 b-c |
| 120d poly | 0.67 a | 0.57 a-b |
| 60d poly | 0.67 a | 0.36 c-d |
| 180d poly | 0.58 a | 0.72 a |
| 0d | 0.06 b | 0.11 e-f |
| 120d | 0.04 b | 0.07 f |
| 180d | 0.02 b | 0.04 f |
| 60d | 0.01 b | 0.28 d-e |
| 0d poly | 0.00 b | 0.11 e-f |
| 240d | 0.00 b | 0.00 f |

[1] Means followed by the same letter are not significantly different at P≤0.05 using LSD (least significant difference) test.

*Shoot development*

Poly treatments all produced significantly more shoot growth in coyote willow than the non-poly treatments (table 3). Coyote willow stored in polyethylene also produced more shoot biomass than fresh cuttings of the 0d control treatment. Storage for 180d in poly had two times more shoot production than the next highest rated treatment.

Yellow willow cuttings showed significant difference in shoot production between the 0d poly and 0d non-poly treatment. This may be a reflection of different moisture levels at start of trial. Poly treatments produced significantly more growth of yellow willow shoots than all non-poly treatments but not the 0d control of the 2012 harvested cuttings. 120d poly had significantly greater shoot production than all other treatments, two times more shoot biomass than the next highest rated treatment.

Table 3. Shoot biomass

| | Coyote willow | Yellow willow |
|---|---|---|
| | ---------------(g)--------------- | |
| 180d poly | 13.76 a[1] | 6.60 b |
| 120d poly | 6.63 b | 12.64 a |
| 60d poly | 4.95 b-c | 6.93 b |
| 240d poly | 3.83 c | 8.06 b |
| 60d | 0.45 d | 2.64 c |
| 0d | 0.43 d | 2.86 c |
| 120d | 0.40 d | 1.31 c |
| 180d | 0.04 d | 0.46 c |
| 0d poly | 0.00 d | 6.22 b |
| 240d | 0.00 d | 0.00 c |

[1] Means followed by the same letter are not significantly different at $P \leq 0.05$ using LSD (least significant difference) test.

## Discussion

Although cuttings for the two storage treatments (poly and non-poly) were harvested in different years, fresh cuttings from each treatment (0d poly and 0d non-poly) did not differ significantly from one another in the characters measured.

Mold and fungus development was visible in the polyethylene bagged cuttings at 60 days of storage and beyond. Black spots and a fuzzy white fungus were observed on cut tips and pruned branches. The presence of fungus however did not seem to inhibit survival or growth under shorter lengths of storage when compared to fresh cuttings. Only after 240 days of poly treatment did we observe a significant decrease in survival. We also observed fungus on the non-poly cuttings, but not to the degree of that on the cuttings stored under high relative humidity in the polyethylene.

Our results indicate that non-bagged cuttings stored under refrigerated conditions will retain good vigor for at least four months. Increasing humidity and maintaining cutting moisture levels by bagging the cuttings in polyethylene extends vigor with no loss of survival for up to 6 months. Bagging the cuttings also appears to stimulate root and shoot growth. 120 to 180 days of storage in polyethylene resulted in significantly greater shoot production than all other treatments including freshly harvested cuttings. This information may be useful for stream bank restoration or bioengineering where more root and shoot production is useful for bank protection.

## References

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