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Effect of Storage Conditions on the Survival and Growth of Willow Cuttings

Final Report

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**Effect of Storage Conditions on the
Survival and Growth of Willow Cuttings**

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Executive Summary

The establishment phase of willow biomass crops is critical to their economic and biological success. Survival rates in the field of less than 80% are considered to be unsuccessful at planting densities around 15,000 plants ha⁻¹. Planting healthy cuttings that quickly develop roots and shoots, combined with good site preparation and management, are essential to establishing successful and profitable willow biomass crops.

Planting stock for willow biomass crops consists of dormant, unrooted cuttings from one-year-old shoots harvested during the previous winter. Planting stock should be stored slightly below freezing and packaged to minimize desiccation. Cuttings should be removed from storage two to four days before planting and kept moist and cool.

Several factors have raised concerns about the feasibility of maintaining a two to four day period between removal of cuttings from cold storage and planting. The planting window for the Northeastern and Midwestern United States is limited to a four to six week period from late April to early June when field access is unpredictable due to snow melt and wet spring weather. The supply and distribution of planting stock and specialized planting equipment are additional complicating factors. Therefore, studies were implemented to address the following questions.

1. How long can cuttings be left out of cold storage and still maintain vigorous sprouting and rooting ability, and rapid growth rates necessary for successful establishment?
2. Once cuttings have been removed from the original freezer (maintained at -4°C) and thawed, does returning them to cold storage affect their sprouting and rooting ability and early growth? Three types of large-scale cold storage are practical, the original freezer at -4°C, a nursery cooler at +2°C, or a freezer at -20°C that is used primarily for food storage.

Four willow clones (S301, SA2, SH3 and SV1) from the SUNY – ESF collection were used in the study. Cutting length and diameter were standardized at 25.4 cm and 0.9 - 1.2 cm respectively for all experiments. Cuttings were produced late December 1996 and stored in a walk-in freezer at -4°C.

To address the first question, three replicates of ten cuttings of each clone were randomly selected and removed from the -4°C freezer on five different days in April 1996 (Experiment 1). They were stored at ambient temperatures in a shaded portion of a greenhouse for 2, 9, 12, 16 and 23 days before being planted in 23 cm tall pots in a greenhouse. Prior to planting, the proportion of cuttings with roots that had broken through the bark and buds that had opened was recorded for each replicate of ten cuttings. After three weeks in the greenhouse the plants were harvested. The proportion of cuttings with shoots and roots was recorded and oven dry shoot biomass was measured.

As a follow up to the first study, four replicates of ten cuttings of each clone were randomly selected and removed from the -4°C freezer and stored in a barn for 2, 12 and 23 days before planting in a field trial at Tully, NY (Experiment 2). Survival in the field was assessed on a regular basis during the first growing season. The plants were harvested at the end of the growing season to determine first-year above ground biomass production.

To address the second question, nine replicates of ten cuttings of each clone were randomly selected, removed from the freezer and stored at ambient temperatures in a greenhouse for 2, 5, and 9 days (Experiment 3). Three replicates of each clone were then returned to one of three treatments - a cooler at +2°C, a freezer at -4°C freezer, or a chest freezer at -20°C - for seven days. These cuttings were then considered to have been out of the original -4°C freezer for 9, 12, and 16 days respectively. The cuttings were then planted in 23 cm tall pots in a greenhouse. Prior to planting the

proportion of cuttings with roots that had broken through the bark and buds that had opened was recorded for each replicate of ten cuttings. After three weeks in the greenhouse the plants were harvested. The proportion of cuttings with shoots and roots was recorded and oven dry shoot biomass was measured.

Results from the first and second experiment indicated that leaving cuttings out of the -4°C freezer for up to 12 days did not have a significant impact on survival or biomass production. In the greenhouse experiment, cuttings left out for 23 days had similar or greater survival and biomass production than the cuttings planted after only two days out of the -4°C freezer. However, in the field trial, cuttings of three of the clones (S301, SA2, and SV1) left out for 23 days had lower survival and per tree biomass production than cuttings taken out of the -4°C freezer two days prior to planting.

In Experiment 3, returning cuttings to any of the three cold storage treatments ($+2^{\circ}\text{C}$, -4°C or -20°C) after being left out for 5 to 9 days slowed shoot development of clones SV1 and S301, and virtually stopped shoot development of SA2. Returning cuttings to cold storage had no effect on the shoot development of clone SH3 because it was slow regardless of the treatment.

The proportion of cuttings with roots at harvest was reduced in Experiment 3 if the cuttings had been left out of the freezer for 5 or 9 days and then returned to the -20°C freezer. Returning cuttings to either a $+2^{\circ}\text{C}$ cooler or a -4°C freezer after any length of time did not have any effect on the proportion of cuttings with roots at the time of harvest. Returning cuttings to a -20°C freezer reduced shoot biomass compared to the control or to cuttings returned to $+2^{\circ}\text{C}$ and -4°C cold storage for all clones.

These experiments suggest that cuttings can be left out of the -4°C freezer for longer than the commonly recommended period of two to four days before being planted and still achieve survival and first year production rates necessary for a successful plantation. However, the response varied among the four different clones in this experiment and may not be applicable to all other willow clones. Once cuttings have been removed from the -4°C cold storage they should not be returned to a -20°C freezer, which is a typical temperature for many food storage freezers that are readily available. Returning cuttings to a $+2^{\circ}\text{C}$ or -4°C cold storage may slow shoot and root development of the cuttings. This is an important consideration for large-scale plantings, where pre-emergent herbicides are applied immediately after planting. Many clones of willow have been shown to be sensitive to some of these herbicides once they have sprouted, so delaying sprout development would reduce herbicide damage.

Several conditions present in this experiment contribute to the reported results and need to be considered when applying them to large-scale operations. After cuttings were removed from the -4°C freezer, they were kept in cool, shady conditions, which moderated the rate of development. Secondly, cuttings were stored in sealed containers that prevented desiccation. Following these practices for handling cuttings will increase the rate of survival in the field when planting is delayed.

1.0 Introduction

Regional energy shortages, dependence on foreign supplies, and international agreements to reduce the level of CO₂ emissions have prompted the study and deployment of short-rotation woody crops (SRWC) in several countries. Since energy generated from SRWC is CO₂ neutral (Mann and Spath 1997, Hall et al. 1991), the use of this renewable and sustainable energy source has the potential to reduce global fossil fuel emissions of CO₂ by up to 20% (Dixon et al. 1994). These systems are unique because they have the potential to simultaneously produce renewable energy as well as other environmental and rural development benefits when they are established and managed strategically across the landscape. For example, SRWC can be planted to treat tertiary wastewater, control the movement of nutrients and agricultural chemicals into surface and groundwater, or remediate contaminated sites while producing biomass. Models indicate that the deployment of willow biomass systems across the landscape in upstate New York could create up to 75 jobs for each 4,000 ha established and harvested for energy production (Proakis et al. 1999).

Research on willow biomass crops has been conducted in Sweden since the 1970s. Yields of willow biomass crops in Sweden have exceeded 20 odt ha⁻¹ yr⁻¹ under experimental conditions (Christersson et al. 1993). Commercial yields of 10-12 odt ha⁻¹ yr⁻¹ are anticipated, which is the equivalent of 4 -5 m³ of oil, but have been lower than expected due to poor crop management and the use of undeveloped planting stock (Larsson et al. 1998). Experimental willow biomass crops in New York State have produced up to 27 odt ha⁻¹ yr⁻¹ (Adegbidi et al, in press). Similar commercial yields, 10 – 12 odt ha⁻¹ yr⁻¹, are anticipated (Abrahamson et al. 1988) with the current suite of clones available and with properly applied management. The first commercial harvests of willow biomass crops in the United States are scheduled to occur in western New York State in the winter of 2001/02.

The desired characteristics for species used in SRWC systems include ease of vegetative propagation, rapid growth, high biomass production per hectare, and ability to coppice prolifically and repeatedly. Several species and clones in the willow and poplar family (Salicaceae) have proven themselves for SRWC systems. In addition, willows (*Salix* spp.) have large genetic variability and a short time period to sexual maturity, making them relatively easy to work with in genetic improvement programs.

The establishment phase of willow biomass crops is critical to both their economic and biological success. Establishment costs account for 26% of total production costs for willow (White et. al. 1995). Supplemental planting in stands with marginal survival rates is costly and can be ineffective since interplanted cuttings are easily out competed by adjacent established plants. Cutting survival rates in the field of less than 80% are usually considered to be unsuccessful (Bergkvist et al. 1995). Willow biomass crops are susceptible to drought and competition from weeds during establishment and can be damaged by frost heaving during the first winter, especially on soils with high silt content. The use of high quality planting stock and the application of good management practices is essential in establishing a successful and profitable willow biomass crop.

Planting stock for willow biomass crops consists of dormant, unrooted cuttings harvested from one-year-old shoots between December and March. Cuttings should be stored in plastic bags to minimize desiccation and stored at -4°C (Cram and Lindquist 1982, Siren et al., 1987). Current recommendations are that cuttings should be removed from cold storage 2 - 4 days before planting. They should remain in their sealed containers and kept moist and cool until they are planted (Bergkvist et al. 1995, Danfors et al. 1998, Sennerby-Forsse 1986). It is anticipated that planting

success drops off rapidly if roots have penetrated the bark prior to planting (Siren et al., 1987), especially if mechanical planters are used (Cram 1969). Preformed root primordia in willow can become active in as little as 24 hours when the cuttings are soaked (Fjell 1985) and roots can begin to emerge in as few as three days (Siren et al. 1987).

The planting window for willow biomass crops in the Northeastern and Midwestern United States is limited to a four to six week period (late April to early June), when weather and soil conditions make field access unpredictable. The supply of planting stock to various sites across large distances and the restricted availability of specialized planting equipment have raised concerns about the feasibility of maintaining a two to four day period between removal of cuttings from cold storage and planting. Studies were designed to address these concerns. The first study examined how long cuttings can be left out of cold storage and still retain the vigor necessary for successful establishment. The second study addressed the question of whether returning cuttings to some type of cold storage, after they have been removed from the original -4°C and thawed, enhances or decreases their viability. Three types of large-scale cold storage are practically possible. The original freezer at maintained at -4°C, a nursery cooler at +2°C, or a freezer used for food storage at approximately -20°C.

2.0 Materials and Methods

2.1 Plant Material

Four willow clones (S301, SA2, SH3 and SV1) (Table 1) from the SUNY–ESF collection were used in the experiments. Twenty-five centimeter long cuttings of each clone were produced from one-year-old coppice stems between December 12 and 21, 1995. All cuttings were made from material harvested from fertilized and irrigated plots in Tully and Lafayette, NY. Four bundles of 25 cuttings each were double-bagged in 4 ml plastic bags and stored in a walk-in freezer at -4°C.

Table 1. Parentage of willow clones.	
Clone	Parentage and Origin
S301	Salix interior 62 x S. eriocephala 276
SA2	S. alba var. sanguinea. Novi Sad, Yugoslavia
SH3	S. purpurea. Munden, West Germany
SV1	S. dasyclados. Brantford, Ontario, Canada.

In each of the experiments described below, all the cuttings were handled similarly before each storage period or planting experiment. On March 12, 1996, cuttings were removed from the -4°C storage and sorted for uniformity in an adjacent, unheated barn. Cutting length and diameter were standardized at 25.4 cm and 0.9 - 1.2 cm respectively. Bundles of 10 cuttings were placed in a 4 ml plastic bag and sealed. Ten of these bundles were then placed into a second 4 ml plastic bag and returned to the -4°C storage within an hour of removal.

Ten cuttings of each clone were left in the -4°C storage until two days prior to planting. Fresh weight (± 0.01 g) and number of buds was recorded for each cutting. Cuttings were dried at 65°C to a constant weight and dry weight was measured (± 0.01 g). Moisture content expressed on a wet weight

basis was calculated.

2.2 Experiment 1: Number of Days Between Removal from -4°C Storage and Planting

Three bundles of ten cuttings each of were randomly selected for each clone and removed from -4°C storage and stored in their plastic bags in a shaded portion of a greenhouse for 23, 16, 12, 9 or 2 days prior to planting on April 5. Minimum and maximum daily ($n = 23$) greenhouse temperatures (mean \pm standard error) were $5.4 \pm 0.8^\circ\text{C}$ and $15.6 \pm 1.0^\circ\text{C}$, respectively. The experiment employed a completely randomized design with a one way classification treatment structure with three replications. A total of 600 cuttings were used.

On April 4, 1996 the cuttings from all storage periods were soaked in 18 - 20 cm of tap water for 18 to 26 hours. Each bundle of 10 cuttings was then planted, 22 - 23 cm deep in a 23 cm diameter pot filled with Vit Hume (Hyponex Corp., Marysville, OH) topsoil. Pots were randomly distributed on two center benches in a greenhouse. At the time of planting the number of cuttings in each bundle that had broken bud or had roots penetrating the bark was recorded. Pots were watered everyone to two days as needed to maintain high moisture conditions. Relative humidity and minimum and maximum temperatures (mean \pm standard error) in the greenhouse over the next three weeks were $57.6 \pm 3.9\%$, $14.3 \pm 0.4^\circ\text{C}$, and $26.6 \pm 0.7^\circ\text{C}$, respectively. After three weeks, the proportion of cuttings with shoots and roots was recorded. New above ground biomass (stems and leaves) for all ten cuttings was harvested, dried at 65°C to a constant weight, and weight recorded (± 0.01 g).

2.3 Experiment 2: Field Test of Number of Days Between Removal from -4°C Storage and Planting

Four bundles of 25 cuttings of each clone were randomly selected and removed from -4°C storage and stored in their plastic bags in a barn for 23, 12, or 2 days prior to planting at the SUNY-ESF Genetics Research Station in Tully, NY ($42^\circ 47' 30''$ N, $76^\circ 07' 30''$ W). The experiment used 1,200 cuttings in a completely randomized block design with a one way classification treatment structure with four replications.

The soil at the site is a Glossoboric Hapludalf of the Palmyra series (Hutton 1997) developed in a gravelly sandy outwash parent material. It has a loam texture with a gravel content varying from 25% in the Ap horizon to 60% in the IIC horizon. Rooting depth is 40 to 100 cm; available water capacity is moderate to high and pH ranges from medium acid to neutral in the surface horizon. The site had been plowed, disked and capped with oxyflouofen (2.2 kg ai/ha) in the fall of 1994 and was fallow in 1995. Immediately prior to planting the site was cross-disked. On June 13, 1996 the proportion of cuttings in each bundle with shoots and roots was recorded. Cuttings from all storage periods were soaked in 18-20 cm of tap water for 20 to 29 hours. Each bundle of 25 cuttings was then hand planted with a dibble at 61 cm x 61 cm spacing in 25 tree plots. One to two centimeters of the cutting remained above the soil surface. Simazine (2.2 kg ai/ha) was applied two days after planting. Survival of the inner nine plants was assessed on July 16, August 5, and October 18. Glyphosate was spot sprayed to control weeds on July 17. The survival assessment on August 5th showed that some herbicide damage had occurred, so the July assessment was used as an indicator of treatment effects on survival. The inner nine trees were harvested on December 18, dried at 65°C to a constant weight, and weight recorded on a per tree basis (± 0.01 g).

2.4 Experiment 3: Cold Storage Treatments

Nine bundles of each clone were randomly selected from the -4°C storage, and stored in their plastic bags, under the same greenhouse conditions described above, for 2, 5, and 9 days. Three bundles of each clone were then placed in one of three cold storage treatments ($+2^{\circ}\text{C}$, -4°C , and -20°C) for seven days. These cuttings were then considered to be out of the initial storage conditions for 9, 12, and 16 days respectively. The 9, 12 and 16 days treatments from experiment 1 above were used as controls. Then on April 4 all the cuttings were removed from storage, soaked, planted, and measured as described above. A total of 1,080 cuttings were used in a completely randomized design in a 3×4 factorial treatment structure with three replications.

2.5 Experiment 4: Screening Cuttings in Water versus Soil

Three bundles of clone SV1 were removed from the -4°C storage on April 2 (two days prior to planting Experiments 1 and 2) and placed in 18 cm of tap water. Cuttings remained in the tap water for the length of experiments 1 and 2 and were measured as described above. In addition, roots were harvested, dried at 65°C to a constant weight and dry weight was measured ($\pm 0.01\text{g}$).

2.6 Data Analysis

For cuttings that were not subjected to any of the above treatments, comparisons in moisture content and number of buds per cutting between clones were made using a one-way analysis of variance (ANOVA). Means were differentiated using Fisher's Protected Least Significant Difference (FPLSD). For experiments 1 and 2, comparisons among treatments for all variables for each clone were measured with a one-way ANOVA. Trends were analyzed by testing orthogonal contrasts for linear, quadratic and cubic response curves for experiment 1 and linear and quadratic response curves in experiment 2 (Stehman and Meredith 1995).

Comparisons among treatments by clone in experiment 3 were made by two-way ANOVA. When significant differences were found ($\alpha = 0.15$), interactions were analyzed by testing the following simple effect contrasts:

1. Control minus the average of the three freezer treatments for the 9, 12, and 16 days out treatments.
2. The average of the $+2^{\circ}\text{C}$ and -4°C treatments minus -20°C storage treatment for the 9, 12 and 16 days out treatments.
3. The 9 days treatment minus the average of 12 and 16 days out treatment for the control, $+2^{\circ}\text{C}$, -4°C , and -20°C treatments.

Clones with non-significant interactions ($\alpha = 0.15$) were analyzed by testing the following main effect contrasts:

- Control minus all the freezer treatments across the number of days out.
- The average of $+2^{\circ}\text{C}$ and -4°C treatments minus -20°C storage treatment across the number of days out.
- Linear and quadratic contrasts for the effect of the number of days out across all cold storage treatments.

Differences between SV1 cuttings grown in soil (from experiment 1) versus water (from experiment 4) were analyzed with t-tests. All statistical analysis was done with SAS 6.09 (SAS 1996).

3.0 Results

3.1 Clonal differences in cutting characteristics

The number of buds per cutting ($p < 0.001$) and moisture content ($p < 0.001$) varied significantly by clone (Table 2). The number of buds was similar on clones SH3 and SV1, both of which were significantly greater than the other two clones. The moisture content of SH3 (49.8%) and SV1 (50.9%) was significantly less than S301 (54.2%) and SA2 (55.8%).

Table 2. Mean and standard error (SE) of number of buds and moisture content of willow clones when removed from -4°C storage.				
Clone	Number of Buds per Cutting		Moisture Content (%)	
	Mean	SE	Mean	SE
SH3	12.40 a	0.16	49.75 a	0.43
SV1	11.20 a	0.07	50.91 a	0.09
S301	6.70 b	0.16	54.21 b	0.09
SA2	7.00 b	0.07	55.49 b	0.20

Means within the same column followed by the same letter are not statistically different (Fisher's protected LSD $\alpha = 0.05$)

3.2 Experiment 1: Number of Days Between Removal from -4°C Storage and Planting

The proportion of cuttings with buds that had broken at planting varied significantly by the number of days left out of the -4°C storage, for three of the four clones ($\alpha = 0.05$, Table 3, Figure 1). The exception was SA2 ($p = 0.074$). Each of the four clones showed a significant positive linear trend ($\alpha = 0.05$). Two of the clones, SH3 and S301 had significant quadratic trends ($\alpha = 0.05$) and two clones, SV1 and S301 had significant cubic trends ($\alpha = 0.05$).

Table 3. Percentage of cuttings for four willow clones showing shoot development at planting after being left out of -4°C storage for 2-23 days.								
Days out of storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	0.0	0.0	13.3	8.8	10.0	10.0	6.7	6.6
9	0.0	0.0	43.3	8.8	6.7	6.6	20.0	10.0
12	40.0	23.1	53.3	12.0	36.7	3.4	90.0	10.0
16	66.7	33.4	100	30.0	30.0	15.3	100	0.0
23	76.7	23.4	96.7	86.7	86.7	13.4	100	0.0

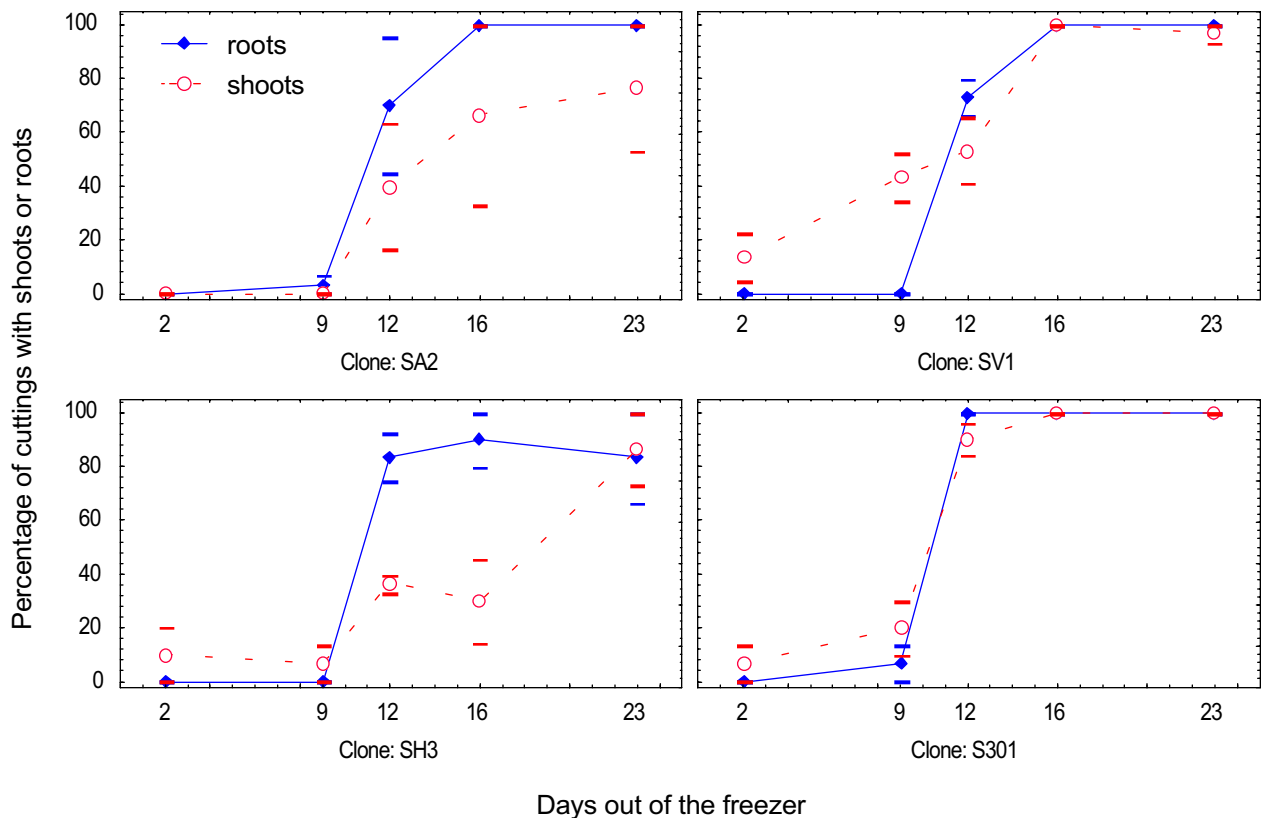


Figure 1. Percentage of cuttings with shoots and roots at planting for four willow clones after being left out of -4°C storage for 2, 9, 12, 16, and 23 days. Bars represent ± 1 standard error.

The proportion of cuttings with visible roots at the time of planting varied significantly by the number of days left out of -4°C storage for all four clones ($\alpha = 0.05$, Table 4, Figure 1). At 9 days out, less than 7% of the cuttings of all the clones had roots. At 16 and 23 days out, over 80% of the cuttings all clones had developed roots. All four clones had significant positive linear and cubic trends ($\alpha = 0.05$). Three of the four clones, the exception being SA2 ($p = 0.36$), had a significant quadratic trend.

Three weeks after planting $\geq 90\%$ of the cuttings had produced shoots, except for SV1 at 16 days out, where the proportion was 83% (Table 5). Oven dry biomass of the harvested shoots was not significantly different ($\alpha = 0.05$) between the number of days left out of the freezer for three of the four clones, the exception being S301 ($p = 0.0005$) (Table 6, Figure 2). Three of the four clones had a significant positive linear trend ($\alpha = 0.05$) between biomass and days left out. The exception was SH3 ($p = 0.27$). None of the clones had a significant quadratic or cubic trend.

Table 4. Percentage of cuttings for four willow clones showing root development for 2-23 days out of -4°C storage before planting.

Days out -4°C of storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	3.3	3.6	0.0	0.0	0.0	0.0	6.7	6.6
12	70.0	25.2	73.3	6.6	83.3	8.8	100	0.0
16	100	0.0	100	0.0	90.0	10.0	100	0.0
23	100	0.0	100	0.0	83.3	16.7	100	0.0

Table 5. Percentage of cuttings with shoots at harvest for 2-23 days out of -4°C storage before planting for four different willow clones.

Days out -4°C Storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	100	0.0	100	0.0	90.0	10	100	0.0
9	100	0.0	100	0.0	96.7	3.3	100	0.0
12	96.7	3.3	93.3	6.6	100	0.0	100	0.0
16	100	0.0	83.3	12.0	100	0.0	100	0.0
23	100	0.0	96.7	3.3	100	0.0	100	0.0

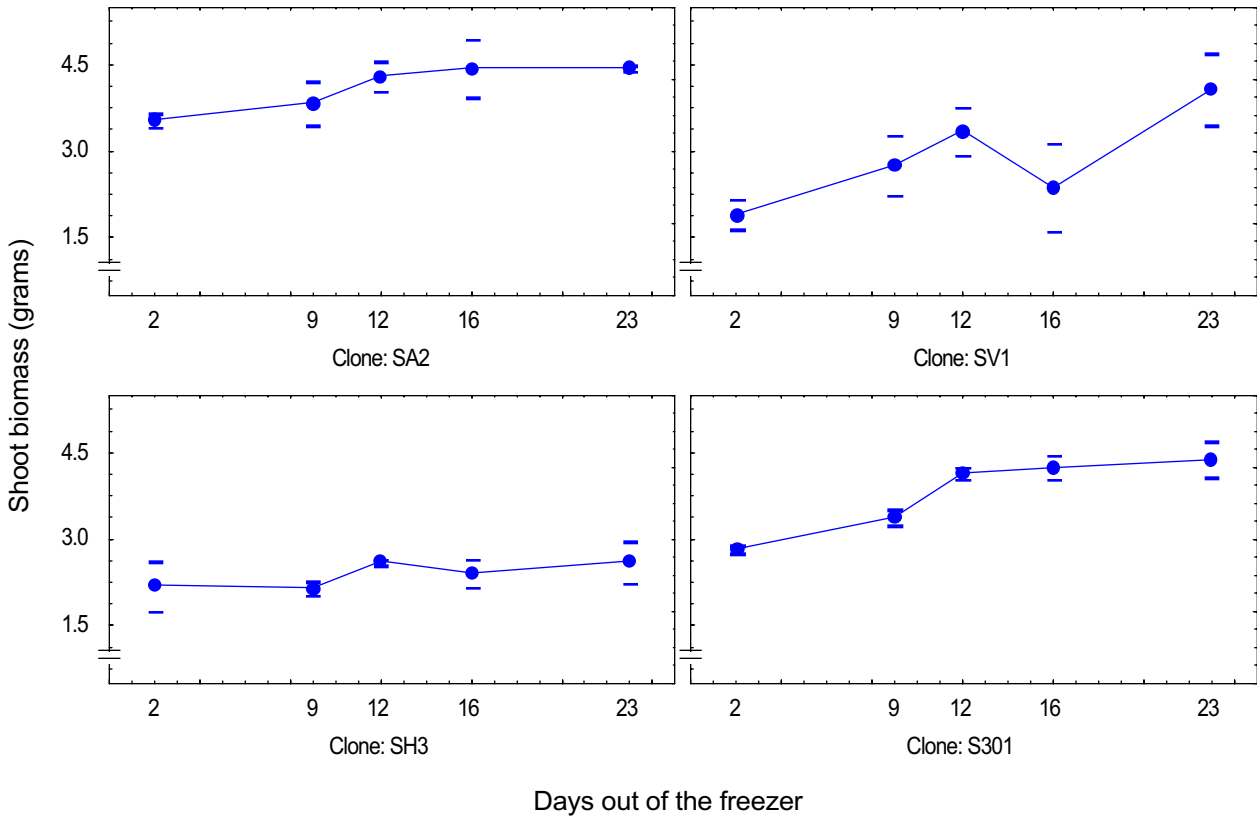


Figure 2. Oven dry shoot biomass (g) at harvest for four willow clones after being left out of -4°C storage for 2, 9, 12, 16, and 23 days before planting. Bars represent ± 1 standard error.

Table 6. Oven dry shoot biomass (g) at harvest for 2-23 days out of -4°C freezer for four different willow clones.

Days out -4°C Storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	3.54	0.11	1.89	0.26	2.19	0.45	2.82	0.58
9	3.85	0.39	2.75	0.53	2.14	0.13	3.38	0.13
12	4.30	0.26	3.36	0.43	2.61	0.06	4.05	0.12
16	4.43	0.51	2.37	0.78	2.41	0.23	4.24	0.20
23	4.44	0.06	4.08	0.63	2.61	0.37	4.39	0.31

Three weeks after planting $\geq 90\%$ of the cuttings of all of the clones had developed roots (Figure 3 and Table 7). Differences in the proportion of cuttings with roots between the days out of the -4°C storage were not significant ($\alpha = 0.05$) for any of the clones. Only SH3 had a significant linear trend ($p = 0.03$).



Figure 3. Typical root development on clone S301 after three weeks of growth. Cuttings were left out of the -4°C storage for 2 (pot 118), 9 (pot 30) and 23 (pot 25) days before being planted.

Table 7. Percentage of cuttings for four willow clones with roots at harvest for 2-23 days out of -4°C storage.

Days out -4°C Storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	100	0.0	100	0.0	90.0	5.8	100	0.0
9	100	0.0	100	0.0	96.7	3.4	100	0.0
12	96.7	3.4	96.7	3.4	100	0.0	93.3	6.6
16	100	0.0	100	0.0	100	0.0	100	0.0
23	100	0.0	100	0.0	100	0.0	100	0.0

3.3 Experiment 2: Field Test of Number of Days Between Removal from -4°C Storage and Planting

The proportion of cuttings that had broken bud was significantly different for all clones among the days out of the -4°C storage treatments. Bud break was particularly slow on three of the four clones – SH3, SV1, and S301. After being out of cold storage for 23 days less than 40% of the cuttings of these clones had broken bud (Table 8, Figure 4). After 23 days out 100% of the cuttings of SA2 had broken bud. All clones had significant linear and quadratic relationships, except SV1, which only had a significant positive linear relationship.

Table 8. Percentage of cuttings for four willow clones with shoots at time of planting for 2-23 days out of -4°C storage.

Days out -4°C Storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0	0.0	13.0	6.6	0.0	0.0	37.0	11.4
23	100	0.0	17.0	2.5	7.0	1.9	20.0	4.9

The proportion of cuttings with roots at the time of planting varied significantly for all clones among the day out of the -4°C treatments. After being out of the -4°C storage for 12 days, roots were developing on greater than 80% of the cuttings of S301 and SV1, but less than 20% of the cuttings of SA2 and SH3 (Table 9). After 23 days out, roots were developing on greater than 94% of the cuttings of all clones. All clones had significant linear and quadratic relationships.

Only clone SH3 had significant differences in survival among the days out treatments in July ($p = 0.0018$) (Figure 5). Both SH3 ($p = 0.005$) and SA2 ($p = 0.0368$) had significant negative linear trends. There were no significant differences in survival for the other two clones ($p = 0.2603$ for SV1, $p = 0.1881$ for S301).

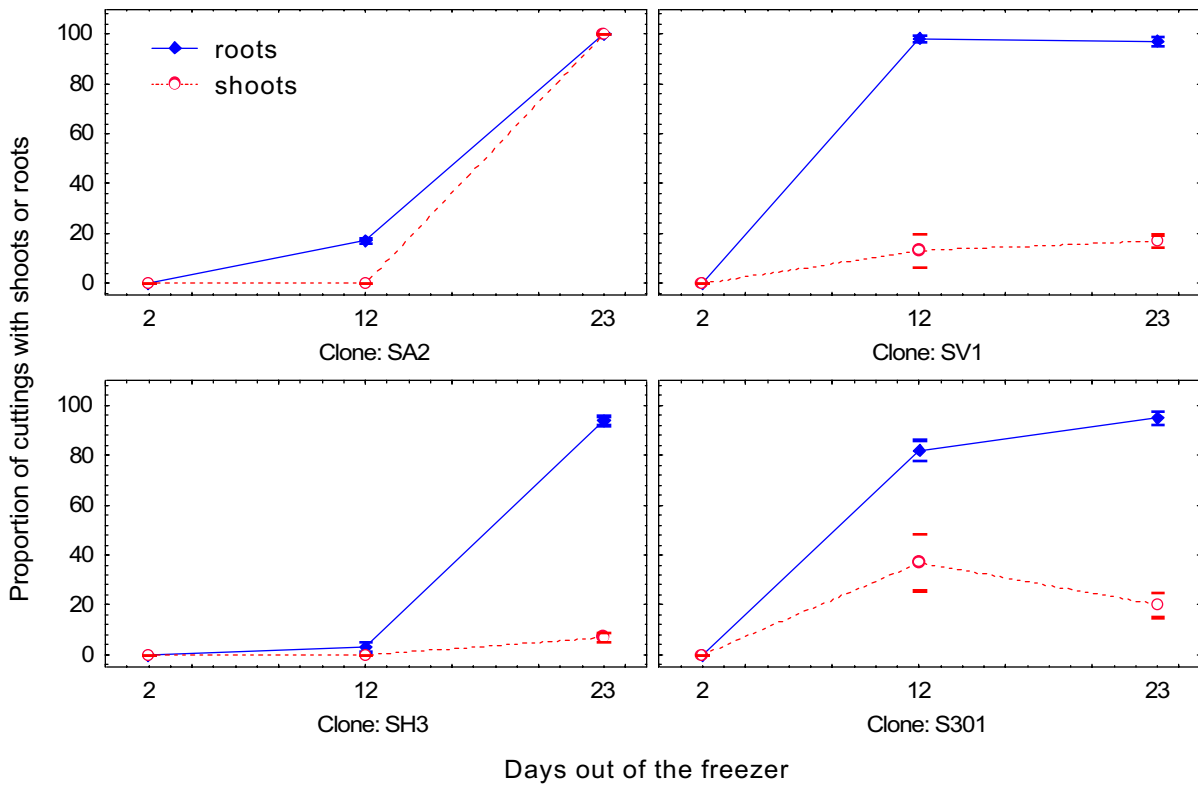


Figure 4. Percentage of cuttings with shoots and roots at planting for four willow clones after being left out of -4°C storage for 2, 9, 12, 16, and 23 days. Bars represent ± 1 standard error.

Table 9. Percentage of cuttings for four willow clones with roots at time of planting for 2-23 days out of -4°C storage.

Days out -4°C Storage Before Planting	Clone							
	SA2		SV1		SH3		S301	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	17	1.0	98.0	1.2	3.0	1.9	82.0	4.2
23	100	0.0	97.0	1.9	94.0	2.0	95.0	2.5

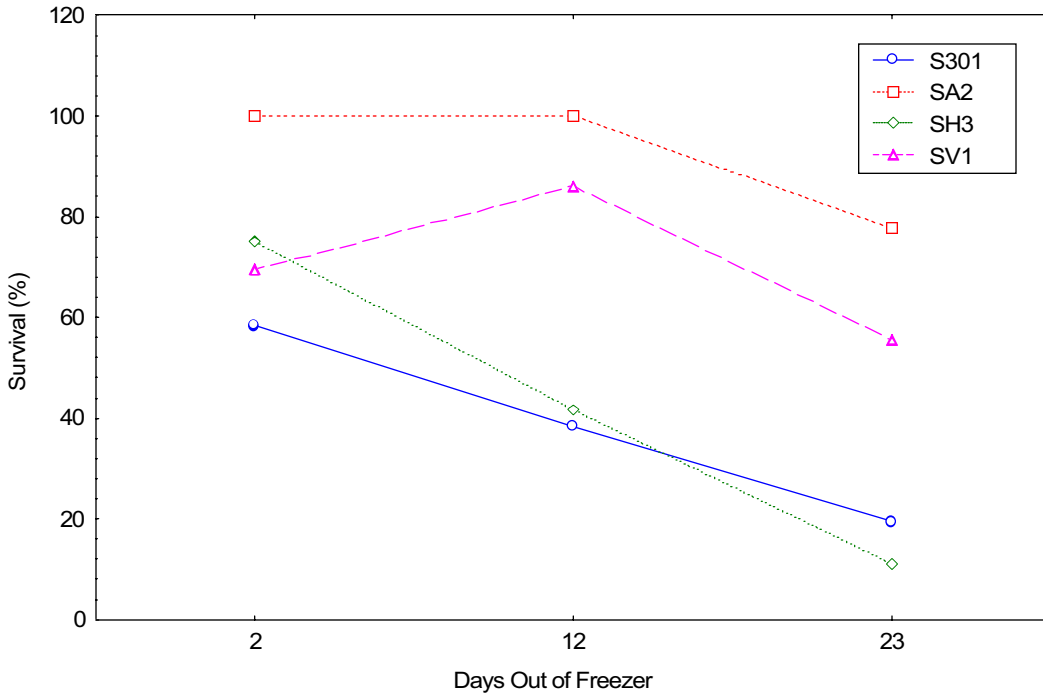


Figure 5. Survival for four willow clones after being left out of the freezer for 2, 12, 23 days before planting in a field trial in Tully, NY.

There were no significant differences for biomass production on a per tree basis among the days out of the -4°C storage. None of the clones had significant linear or quadratic trends (Figure 6). However, the trend for three of the four clones – SV1, SA2, and S301 – was for a decrease in per tree biomass production at the 23 days out treatment.

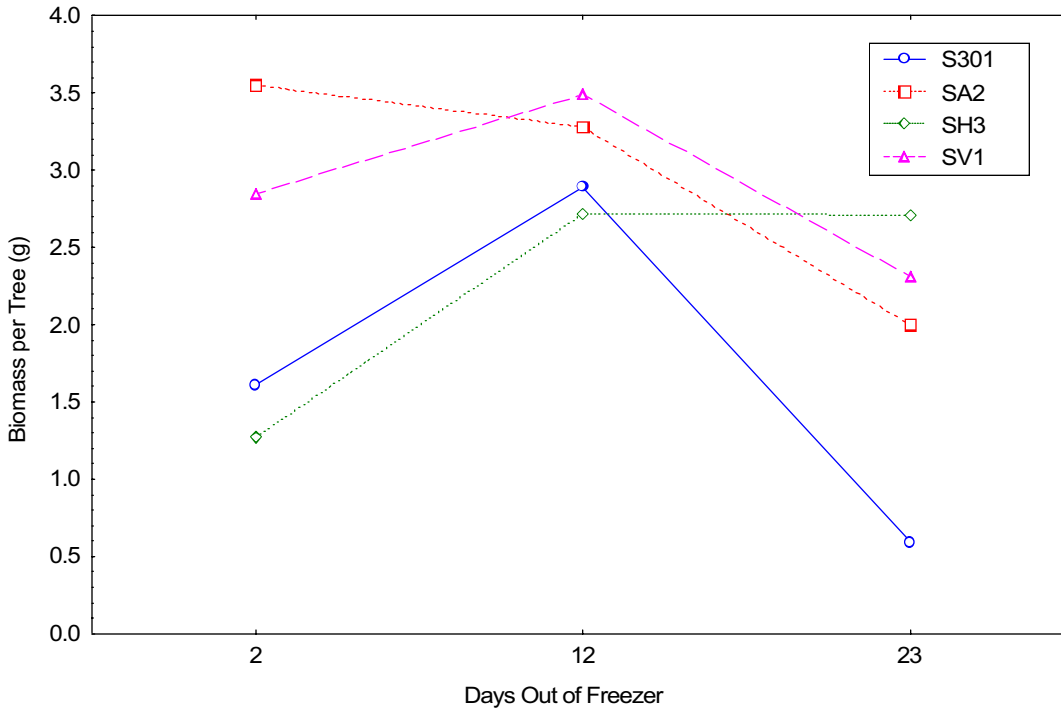


Figure 6. Individual tree biomass for four willow clones after being left out of -4°C storage for 2, 12, 23 days before being planted in a field trial in Tully, NY.

3.4 Experiment 3: Cold Storage Treatments

All four clones had significant freezer treatment by days out interactions ($\alpha = 0.15$) for the proportion of visible shoots at the time of planting. Returning cuttings to any of the cold storage treatments significantly ($\alpha = 0.05$) slowed shoot development of SV1 at 9, 12, and 16 days out and of SA2 and S301 at 12 and 16 days out (Table 10, Figure 7). Shoot development in clone SA2 was virtually stopped, with less than 6.6% of the cuttings having shoots at the time of planting after being returned to any of the three cold storage treatments. Returning cuttings of SH3 to any cold storage treatment had no effect on bud break. Bud break of the control treatment – not returning the cuttings to any cold storage – was slow with only 36.7% and 30.0% of the cuttings breaking bud after being out of cold storage for 12 and 16 days respectively. Returning the cuttings to a -20°C freezer had no significant effect ($\alpha = 0.05$) on bud break compared to the -4°C storage and $+2^{\circ}\text{C}$ cooler for all clones. The length of time the cuttings were left out (2 days versus 5 and 9 days) before being returned to cold storage had no significant impact on bud break in most cases. The exceptions were a reduction in the proportion of cuttings with shoots on S301 when it was returned to a cooler ($p < 0.001$) or -4°C freezer ($p = 0.014$) or returning SV1 to a cooler ($p = 0.0014$).

Table 10. Percentage of cuttings with shoots at planting for four willow clones (a. SA2, b. SV1, c. SH3, and d. S301) after being left out of the -4°C storage for 2, 5, or 9 days and then returned for one week (respectively 9, 12, and 16 days out of the freezer) to one of three cold storage treatments.

a) SA2		Days out of freezer			b) SV1		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	0.0	40.0	66.7	Control	Mean	43.3	53.3	100
	SE	0.0	23.1	33.3		SE	8.8	12.0	0.0
Cooler (+2°C)	Mean	0.0	0.0	3.3	Cooler (+2°C)	Mean	0.0	36.7	53.3
	SE	0.0	0.0	3.3		SE	0.0	8.8	3.3
Freezer (-4°C)	Mean	0.0	0.0	3.3	Freezer (-4°C)	Mean	26.7	30.0	33.3
	SE	0.0	0.0	3.3		SE	8.8	15.3	16.7
Freezer(-20°C)	Mean	0.0	6.7	0.0	Freezer (-20°C)	Mean	16.7	23.3	43.3
	SE	0.0	6.7	0.0		SE	8.8	14.5	6.7
c) SH3		Days of of freezer			d) S301		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	6.7	36.7	30.0	Control	Mean	20.0	90.0	100
	SE	6.7	3.3	15.3		SE	10.0	5.8	0.0
Cooler (+2°C)	Mean	30.0	6.7	30.0	Cooler (+2°C)	Mean	6.7	46.7	30.0
	SE	17.3	6.7	17.3		SE	6.7	23.3	43.3
Freezer (-4°C)	Mean	10.0	30.0	10.0	Freezer (-4°C)	Mean	3.3	23.3	43.3
	SE	10.0	0.0	13.3		SE	3.3	8.8	8.8
Freezer (-20°C)	Mean	0.0	3.3	36.7	Freezer (-20°C)	Mean	6.7	0.0	26.7
	SE	0.0	3.3	18.6		SE	3.3	0.0	4.5

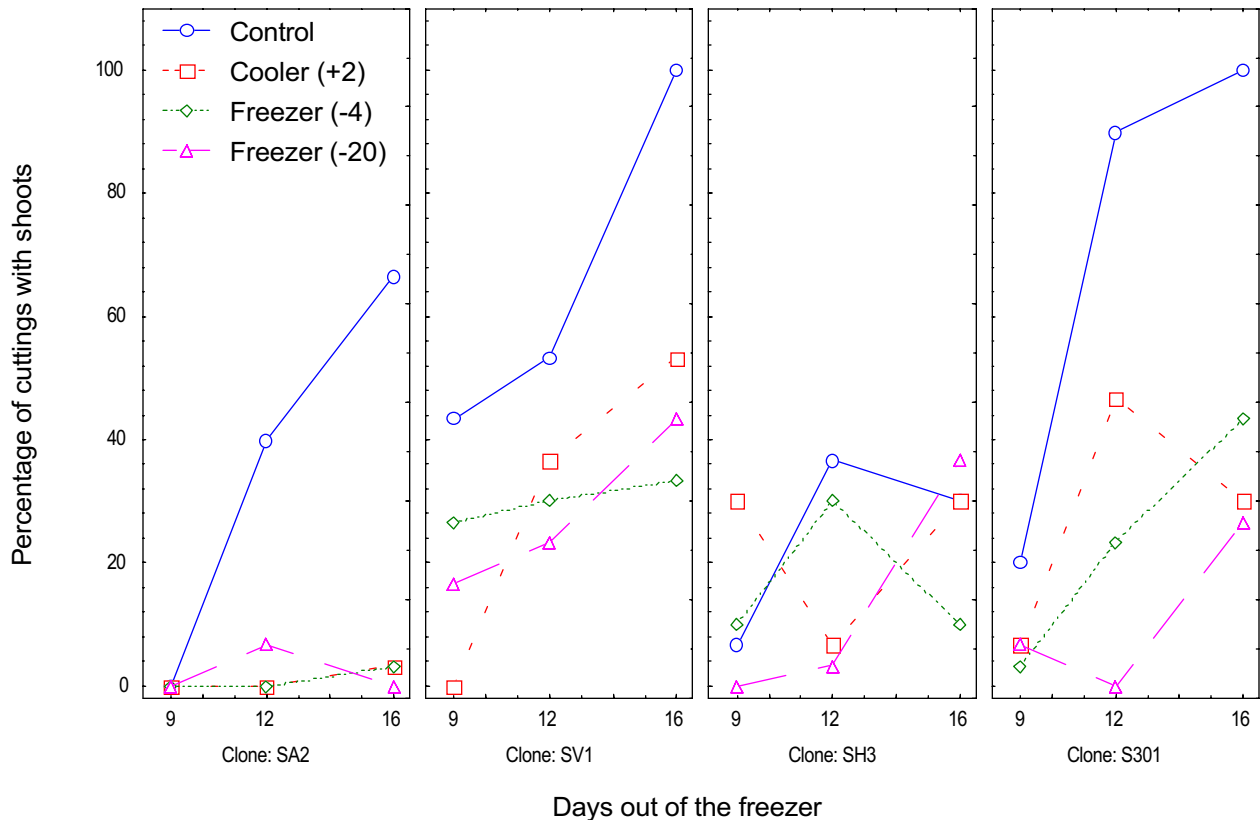


Figure 7. Percentage of cuttings with shoots at planting for four willow clones after being left out of the -4°C storage for 2, 5, or 9 days and then returned for one week to one of three cold storage treatments.

The freezer treatment by days out interaction was significant for all clones ($\alpha = 0.15$) for the proportion of cuttings with roots at the time of planting. Returning the cuttings to any of the cold storage treatments (cooler (2°C) or -4°C or a -20°C freezer) after 5 or 9 days (12 and 16 days out treatments) significantly ($\alpha = 0.05$) reduced the proportion of cuttings with roots for three of the four clones (SA2, SH3, SV1) (Figure 8, Table 11). The effect was only significant for S301 when it was returned to cold storage after 5 days. There was no significant difference for SA2, SH3, and SV1 cuttings that were returned to cold storage treatments after being out of cold storage for two versus five to nine days. In all cases the cold storage treatments dramatically curtailed root development. None of cuttings of SA2, SH3, and SV1 had roots after being returned to cold storage after 5 days, and the proportion of cuttings with roots was $\leq 40\%$ for any of the cold storage treatments after 9 days out. In contrast, greater than 95% of the cuttings of clone S301 had roots after being out of -4°C storage for 9 days and returned to $+2^{\circ}\text{C}$ and -4°C for one week. After 9 days out, 66.7% of S301 cuttings had roots after being returned to -20°C for one week.

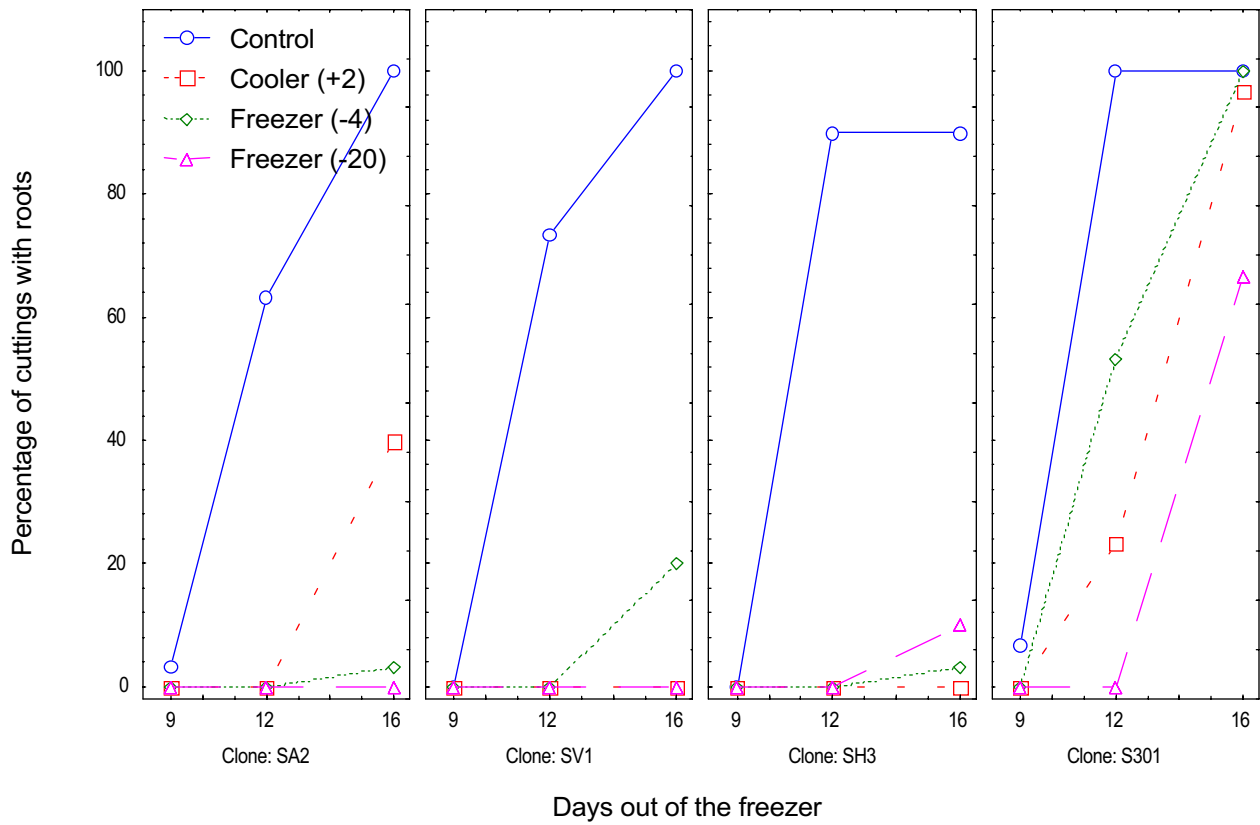


Figure 8. Percentage of cuttings with visible roots at planting for four willow clones after being left out of the -4°C storage for 2, 5, or 9 days and then returned for one week (respectively the 9, 12 and 16 days out of the freezer) to one of three cold storage treatments.

Table 11. Percentage of cuttings with roots at planting for four willow clones (a. SA2, b. SV1, c. SH3, and d. S301) after being left out of the -4°C freezer for 2, 5, or 9 days and then returned for one week (respectively 9, 12, and 16 days out of the freezer) to one of three cold storage treatments.

a) SA2		Days out of freezer			b) SV1		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	3.3	63.3	100	Control	Mean	0.0	73.3	100
	SE	3.3	31.8	0.0		SE	0.0	6.7	0.0
Cooler (+2°C)	Mean	0.0	0.0	40.0	Cooler (+2°C)	Mean	0.0	0.0	0.0
	SE	0.0	0.0	20.8		SE	0.0	0.0	0.0
Freezer (-4°C)	Mean	0.0	0.0	3.3	Freezer (-4°C)	Mean	0.0	0.0	20.0
	SE	0.0	0.0	3.3		SE	0.0	0.0	20.0
Freezer (-20°C)	Mean	0.0	0.0	0.0	Freezer (-20°C)	Mean	0.0	0.0	0.0
	SE	0.0	0.0	0.0		SE	0.0	0.0	0.0
c) SH3		Days of of freezer			d) S301		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	0.0	90.0	90.0	Control	Mean	0.0	100	100
	SE	0.0	15.3	10.0		SE	0.0	0.0	0.0
Cooler (+2°C)	Mean	0.0	0.0	0.0	Cooler (+2°C)	Mean	0.0	23.3	96.7
	SE	0.0	0.0	0.0		SE	0.0	23.3	33.3
Freezer (-4°C)	Mean	0.0	0.0	3.3	Freezer (-4°C)	Mean	0.0	53.3	100
	SE	0.0	0.0	3.3		SE	0.0	8.8	0.0
Freezer (-20°C)	Mean	0.0	0.0	10.0	Freezer (-20°C)	Mean	0.0	0.0	66.6
	SE	0.0	0.0	5.8		SE	0.0	0.0	33.3

Freezer treatment by days out interaction was significant for the proportion of SV1 and SA2 cuttings with roots at harvest ($\alpha = 0.15$). Leaving the cuttings out of the freezer for 5 or 9 days before returning them to the -20°C freezer resulted in a significant ($\alpha = 0.05$) reduction in the proportion of cuttings with roots for both clones (Figure 9 and 10, Table 12). Returning cuttings to either a $+2^{\circ}\text{C}$ cooler or -4°C storage did not have any effect on the proportion of cuttings with roots at the time of harvest. Clones SH3 and S301 did not have a significant treatment by day out interaction ($\alpha = 0.15$) for the proportion of cuttings with roots at the time of harvest. Both SH3 and S301 cuttings returned to -20°C freezer had a significantly lower proportion of cuttings with roots compared to cuttings returned to the other cold storage treatments across all three days out treatments ($p = 0.001$). Compared to the control, returning cuttings to either a $+2^{\circ}\text{C}$ or a -4°C storage did not have any effect on the proportion of cuttings with roots at the time of harvest.

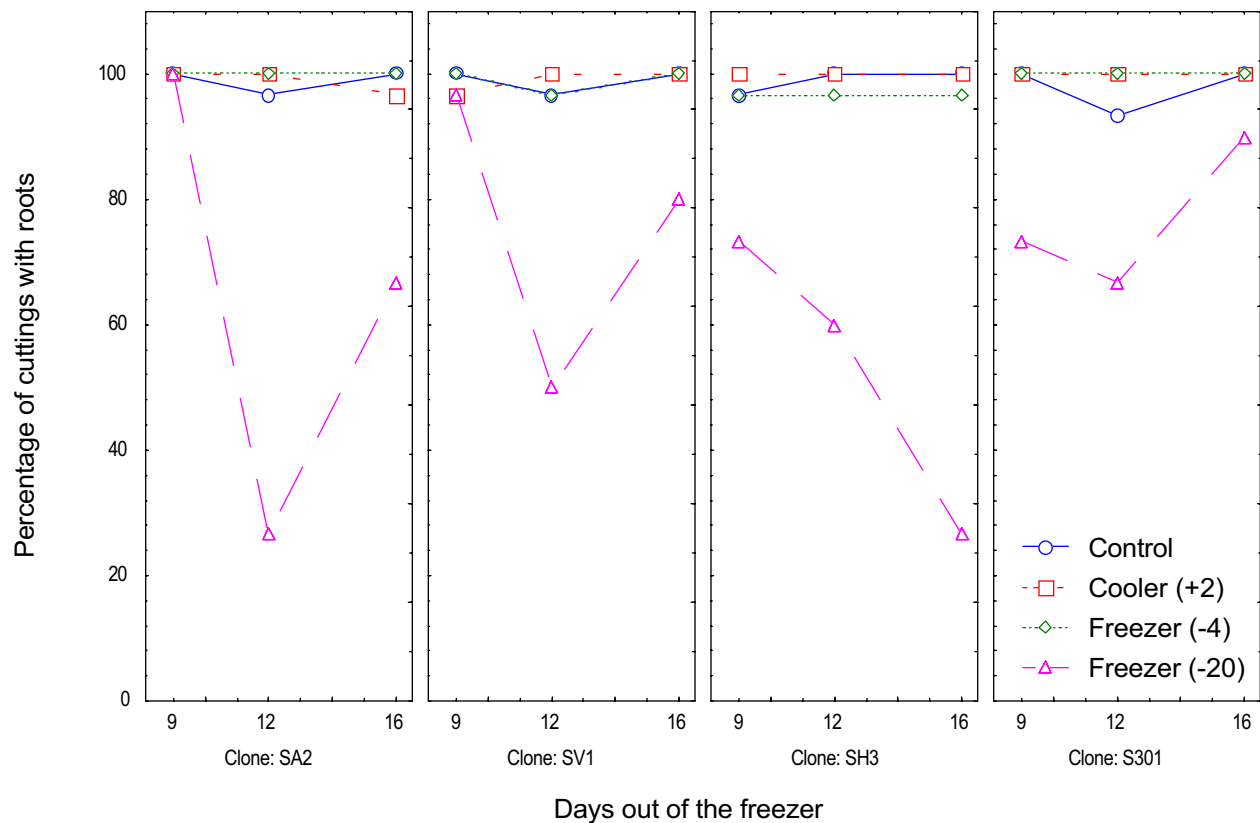


Figure 9. Percentage of cuttings with roots at harvest for four willow clones (a. SA2, b. SV1, c. SH3, d. S301) after being left out of -4°C storage for 2, 5, or 9 days and then returned for one week (respectively the 9, 12 and 16 days out of the freezer) to one of three cold storage treatments.



Figure 10. Typical root development on clone SA2 after three weeks of growth. Cuttings were left out of -4°C storage for 5 days and returned to a -20°C freezer for seven days (pot 19) and not returned to any freezer (i.e. control) (pot 23) before being planted.

Clones SA2, SV1, and SH3 had significant freezer treatment by days out interactions ($\alpha = 0.15$) for shoot biomass. Shoot biomass was significantly lower for cuttings returned to the -20°C freezer compared to the average of the $+2^{\circ}\text{C}$ and -4°C treatments for all three clones at each of three days out treatments ($\alpha = 0.05$) (Table 13, Figure 11). Returning cuttings to either a $+2^{\circ}\text{C}$ cooler or a -4°C freezer did not have any effect on the shoot biomass compared to the control for any of the clones. Clone S301 did not have a significant freezer treatment by days out interaction for shoot biomass ($p = 0.61$). Shoot biomass was significantly lower for the -20°C treatment compared to the other two cold storage treatments across the days out treatments ($p < 0.0001$). The number of days the cuttings were left out of the freezer before being returned to a cold storage treatment had no effect on shoot biomass.

Table 12. Percentage of cuttings with roots at harvest for four willow clones (a. SA2, b. SV1, c. SH3, and d. S301) after being left out of -4°C storage for 2, 5, or 9 days and then returned for one week (respectively 9, 12, and 16 days out of the freezer) to one of three cold storage treatments.

a) SA2		Days out of freezer			b) SV1		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	100	96.7	100	Control	Mean	100	96.7	100
	SE	0.0	3.3	0.0		SE	0.0	3.3	0.0
Cooler (+2°C)	Mean	100	100	96.7	Cooler (+2°C)	Mean	96.7	100	100
	SE	0.0	0.0	3.3		SE	3.3	0.0	0.0
Freezer (-4°C)	Mean	100	100	100	Freezer (-4°C)	Mean	100	96.7	700
	SE	0.0	0.0	0.0		SE	0.0	3.3	0.0
Freezer (-20°C)	Mean	100	26.7	66.7	Freezer (-20°C)	Mean	96.7	50.	80.
	SE	0.0	30.6	57.7		SE	3.3	5.8	15.3
c) SH3		Days of of freezer			d) S301		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	96.7	100	100	Control	Mean	100	93.3	100
	SE	3.3	0.0	0.0		SE	0.0	6.7	0.0
Cooler (+2°C)	Mean	100	100	100	Cooler (+2°C)	Mean	100	100	100
	SE	0.0	0.0	0.0		SE	0.0	0.0	0.0
Freezer (-4°C)	Mean	96.7	96.7	96.7	Freezer (-4°C)	Mean	100	100	100
	SE	3.3	3.3	3.3		SE	0.0	0.0	0.0
Freezer (-20°C)	Mean	73.3	60.0	26.7	Freezer (-20°C)	Mean	73.3	66.7	90.0
	SE	14.5	30.6	13.3		SE	26.7	33.3	10.0

Table 13. Oven dry shoot biomass (g) at harvest for four willow clones (a. SA2, b. SV1, c. SH3, and d. S301) after being left out of -4°C storage for 2, 5, or 9 days and then returned for one week (respectively 9, 12, and 16 days out of the freezer) to one of three cold storage treatments.

a) SA2		Days out of freezer			b) SV1		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	3.85	4.30	4.44	Control	Mean	2.75	3.36	2.37
	SE	0.39	0.26	0.51		SE	0.53	0.43	0.78
Cooler (+2°C)	Mean	4.47	4.29	4.09	Cooler (+2°C)	Mean	2.96	2.37	3.79
	SE	0.08	0.24	0.45		SE	0.44	0.37	0.38
Freezer (-4°C)	Mean	3.45	3.56	4.46	Freezer (-4°C)	Mean	2.10	2.93	3.47
	SE	0.09	0.30	0.26		SE	0.17	0.05	0.50
Freezer (-20°C)	Mean	2.61	1.11	1.91	Freezer (-20°C)	Mean	1.51	0.33	1.21
	SE	0.07	0.47	0.67		SE	0.36	0.17	0.36
c) SH3		Days of of freezer			d) S301		Days out of freezer		
Cold Storage Treatment		9	12	16	Cold Storage Treatment		9	12	16
Control	Mean	2.15	2.61	2.41	Control	Mean	3.38	4.15	4.24
	SE	0.13	0.06	0.23		SE	0.13	0.12	0.20
Cooler (+2°C)	Mean	2.34	2.64	2.81	Cooler (+2°C)	Mean	3.58	3.38	3.53
	SE	0.26	0.09	0.13		SE	0.25	0.25	0.10
Freezer (-4°C)	Mean	2.11	2.50	2.45	Freezer (-4°C)	Mean	2.69	2.96	3.42
	SE	0.15	0.08	0.09		SE	0.06	0.11	0.37
Freezer (-20°C)	Mean	1.38	0.89	0.46	Freezer (-20°C)	Mean	1.78	1.44	1.77
	SE	0.11	0.36	0.20		SE	0.61	0.70	0.37

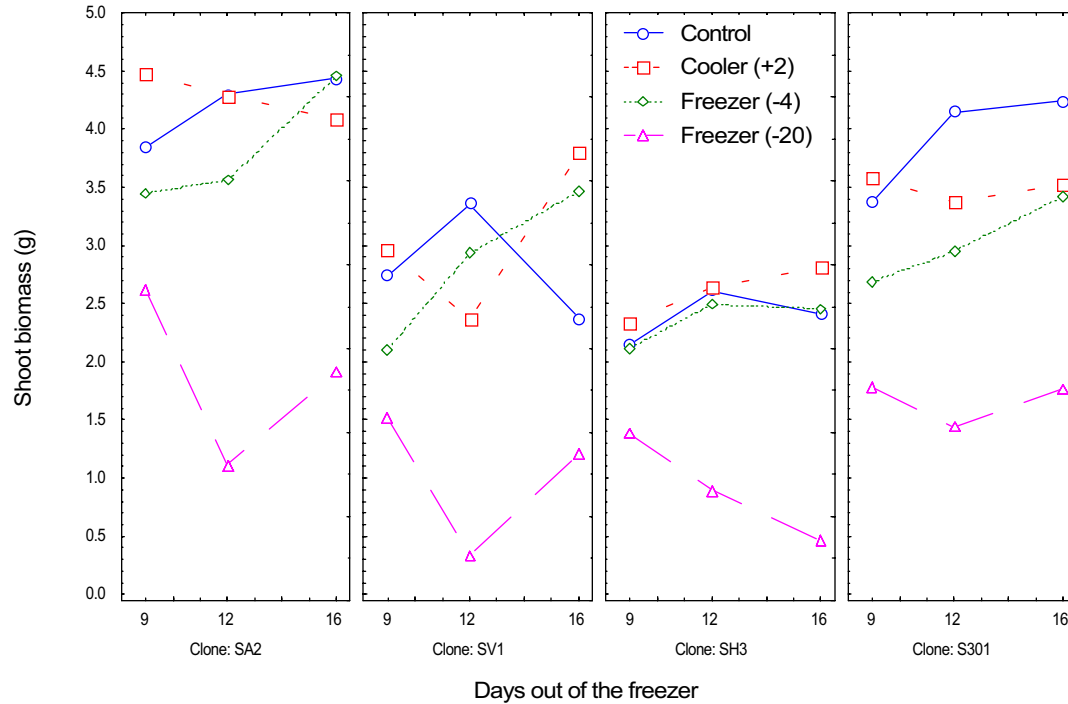


Figure 11. Oven dry shoot biomass (g) at harvest for four willow clones after being left out of -4°C storage for 2, 5, or 9 days and then returned for one week (respectively the 9, 12 and 16 days out of the freezer) to one of three cold storage treatments.

3.4 Experiment 4: Screening Cuttings in Water versus Soil

There were no significant differences between cuttings grown in water versus soil for the proportion of shoots and roots at planting and at harvest or in shoot biomass at harvest ($\alpha = 0.05$). Root biomass, however, was significantly greater ($p = 0.049$) for the cuttings grown in soil (Table 14) due to the more extensive branching of the roots grown in the soil.

Table 14. Percentage of cuttings with shoots and roots, and shoot and root biomass at planting and harvest for cuttings grown in water and soil.

	Soil		Water	
	Mean	SE	Mean	SE
Percentage of cuttings with shoots at planting	13.3	8.8	6.7	6.6
Percentage of cuttings with roots at planting	0.0	NA	0.0	NA
Percentage of cuttings with shoots at harvest	100	0.0	96.7	3.4
Percentage of cuttings with roots at harvest	100	0.0	93.3	6.6
Shoot biomass per 10 cuttings (g) at harvest	1.89	0.26	1.40	0.14
Root biomass per 10 cuttings (g) at harvest	0.21	0.26	0.039	0.001

4.0 Discussion

The moisture content of all clones is within the range (46.5 – 55.3%) recorded for freshly harvested one-year old material (Volk et al. 2000), indicating that the cuttings had not dried out during storage. Variation in moisture content of one-year-old willow between different clones and within a clone from year to year has been reported in other studies (Mosseler et al. 1988, Sennerby-Forsse 1985). Variation in bud arrangement accounted for differences in the number of buds per clone.

4.1 Experiment 1: Number of Days Between Removal from -4°C Storage and Planting

Under similar conditions, the onset of bud break and shoot development has been shown to vary between clones (Kenney et al. 1990, Sennerby-Forsse et al. 1993). About 10% of SV1, SH3, and S301 cuttings had signs of bud break after being left out of the freezer for only two days, while SA2 cuttings did not show any indication until after 12 days out. Despite the slower start, shoot biomass for SA2 was similar to both SV1 and S301 and greater than SH3 after three weeks of growth. The proportion of SH3 cuttings with shoots did not exceed 40% until they were left out for 23 days. While the storage location for cutting in experiments 1 and 3 was atypical, the temperatures recorded in the greenhouse were similar to outdoor air temperatures for central New York in late April and early May, when planting typically occurs.

After three weeks, survival was $\geq 90\%$ for all treatments and all clones, except SV1 at 16 days out, which was 83%. These results suggest that cuttings can be left out of the -4°C freezer for longer than the commonly recommended period of two to four days (Bergkvist et al. 1995, Siren et al. 1987) before planting and still achieve survival rates of $>80\%$ necessary for a successful plantation. This assumes that the cuttings removed from cold storage are protected from desiccation and excessively high temperatures, and receive adequate moisture during establishment. Initial root development from willow cuttings is typically slower than shoot development (Sennerby-Forsse et al. 1993). This was the case with three clones (SV1, SH3 and S301) in this study. Between 9 and 16 days out, all the clones increased from $<10\%$ of the cuttings showing root initiation to $\geq 90\%$. This slower than anticipated root development was probably due to the relatively cool storage conditions. There was no root initiation on three different poplar clones stored at 5°C for 10 days. Root density was similar when the cuttings were stored at 15°C or 25°C for 10 days (Bloomberg 1963). Optimal rooting temperatures are typically 18 - 25°C for cool season plants (Loach 1988). Fjell (1985) found that *S. viminialis* roots were beginning to emerge from the cutting after 6 days in tap water at temperatures of 18 - 20°C. Early root development is important for successful performance of willow biomass crops (Burgess et al. 1990). Cuttings with poor root development are more sensitive to drought conditions during the establishment period and to frost heaving during their first winter, especially on soils with a high silt content.

Abrasive damage during planting primarily damaged longer roots on the cuttings, which would be similar to hand or machine planting in the field. Cuttings with longer roots also had numerous other emerging that could develop under favorable conditions, so damage probably had little impact on the ability of the cuttings to develop a root system and survive. Bloomberg (1963) reported that the survival of three poplar clones was only affected when all the initial roots were removed from

cuttings. Removal of 75% of the roots did not significantly effect root mass, shoot length, or shoot dry weight after nine months for three poplar clones. However, Siren et al. (1987) noted that survival of willow cuttings was affected when roots were damaged during planting. Mechanical planting of poplar cuttings was both more difficult and less successful once both shoots and roots had developed (Cram 1969). Due to the large proportion of cuttings with shoots and roots, the success of SA2, SH3 and S301 may be affected after being left out of cold storage for >9 days if planting is done mechanically. SV1 may be affected sooner than 9 days out, since 43.3% of the cuttings had signs of shoot development at that point.

The significant positive linear trend ($\alpha=0.05$) for shoot biomass for clones SA2, SV1 and S301 indicated that early shoot growth was enhanced by leaving the cuttings out of cold storage for up to 12 days. Since cutting length and diameter were standardized at 25.4 cm and 0.9 - 1.2 cm respectively, they should not have had an impact on the results, as has been show in other studies (Burgess et al. 1990, Rossi 1999). The majority of the biomass increase occurred over the 2 - 12 days out period, which is the same time period when most of the increase in the proportion of cuttings with roots and shoots occurred. Biomass at 16 and 23 days out was similar for these three clones. By 16 days out the proportion of cuttings with roots had reached its peak for all four clones. After 16 and 23 days out, cuttings had numerous roots, some up to 5mm in length. Damage to the roots may have resulted in the loss of any advantage these cuttings had over the 2 to 12 days out treatment. These early differences in growth were reflected over a longer period of time for S301 and SV1 in the field trial where both clones had their peak biomass production at 12 days out. SA2 had a decreasing, although not statistically significant, trend. Bloomberg (1963) found that poplar cuttings with the roots removed at planting had significantly lower shoot growth after one and three months compared to cuttings where the roots were not removed. The differences were no longer evident after nine months of growth. However, any early growth advantages could make a significant difference in the plants ability to compete with weeds and tolerate droughts early in the growing season under field conditions.

There was no significant difference in shoot biomass for SH3 ($p = 0.67$) across all the treatments. Although it was not quantified, SH3 had the most extensive root system of any of the clones when harvested. Biomass partitioning has been shown to vary among clones (Dickman and Pregitzer 1992). Root growth may have occurred at the expensive of shoot growth in SH3.

Several conditions present in this experiment contribute to the reported results and need to be considered when making recommendations for field applications. First, after cuttings were removed from the cooler, they were kept in cool, shady conditions, which moderated the rate of development. Secondly, cuttings were stored in double polyethylene bags, preventing desiccation, which is a major cause of cutting mortality (Sennerby-Forsse 1993). Finally, once they were planted, the cuttings had a consistent supply of water during the three week growing period.

4.2 Experiment 2: Field Test of Number of Days Between Removal from -4°C Storage and Planting

The proportion of cuttings with shoots at the time of planting was considerably less in this experiment compared to the greenhouse experiment, except for SA2 at 23 days out. When cuttings were stored in the greenhouse, 36 – 80% and 76 – 100% of the cuttings had broken bud after 12 and 23 days out respectively. For the field experiment 0 – 37% and 7 – 100% had broken bud after 12 and 23 days out respectively. Storage temperatures for the two experiments were quite

similar. For the field experiment cuttings were stored in boxes in a barn where light levels were very low. In contrast, for the greenhouse experiment the cuttings were stored in a high light environment in open plastic buckets. This storage environment was required in order to simulate outside temperatures fluctuations that occur outside during planting season in this region in late April and May. By the time the field experiment was conducted, maximum greenhouse temperatures would have been much higher than outside temperatures. Differences in light intensity have a strong effect on bud break in dormant hardwood cuttings. Storing cuttings in cool, dark environment where the chance for desiccation is minimized is the best way to minimize bud break.

At 12 days out of -4°C storage SH3 and SA2 respectively had 3 and 17% of their cuttings with roots, a much lower proportion than in the field experiment (83% for SH3 and 70% for SA2). The higher light intensity of the storage conditions in the greenhouse study may have contributed to this difference by retarding root development. However, by 23 days out the proportion of cuttings with roots was very similar for all clones between the greenhouse and field experiments.

Survival of cuttings in the control treatment – two days out before planting – was low in this trial for SH3, SV1, and S301 but similar to other field trials for SA2. First-year survival in field trials planted in between 1993 and 1998 for SV1 (11 trials), S301 (11 trials), SA2 (seven trials), and SH3 (five trials) respectively averaged $94.0 \pm 2.2\%$, $93.7 \pm 2.2\%$, $92.7 \pm 3.9\%$ and $82.5 \pm 8.1\%$ respectively (Tharakan et al. 2001). All these trials were planted by late May. The late planting date, June 16, of this trial may have resulted in higher air temperatures, which can promote bud break at the expense of root development and increase water loss (Loach 1998).

Differences in the survival patterns suggest that the clones respond differently to being left out of cold storage prior to planting. For SH3 the survival rate declines the longer the cuttings are kept out of cold storage. The sharp decline in survival coincides with a drop in biomass production as well. For SA2, leaving the cuttings out for up to 12 days had little effect on survival or biomass production. Survival and biomass production was highest when the cuttings were left out for 12 days prior to planting. The values for two days out or 23 days out were similar, suggesting that having this material out of cold storage for several days may enhance first year growth and production. Survival was so poor for S301 in the field across all treatments that effects could not be differentiated. The reasons for this poor survival and production were not clear. In other field trials S301 has had good survival and it was been one of the better biomass producers in the SUNY-ESF program (Tharakan et al. 2001).

4.3 Experiment 3: Cold Storage Treatments

Once cuttings have been removed from -4°C cold storage they should not be returned to a -20°C freezer, which is a typical temperature for many freezers that are used for storage and are readily available. Better survival and early growth would occur by leaving them in sealed containers in a shaded location for up to nine days. Ice crystals form in willow tissue at -2°C , thus a slightly lower storage temperature is recommended to avoid the reformation of ice crystals if temperatures fluctuate around -2°C . Other studies have found that leafless hardwood cuttings can be stored at -18°C if they are exposed to several days of freezing temperatures first and are thawed very slowly when removed from the freezer (Behrens 1988). Cuttings in this experiment were not exposed to these freezing temperatures before returning them to the -20°C condition.

Shoot development was slowed for clones SA2, SV1 and S301, and root development was slowed for SA2, SV1 and SH3, by returning them to either +2°C or -4°C cold storage after being out for 5 - 9 days. However, after three weeks of growth there were no differences in shoot biomass between the control and these two storage treatments, except for SV1 at 16 days out where stored cuttings produced 53% more biomass than the control. Returning cuttings to storage at +2°C or -4°C may extend the period of time cuttings can be held beyond 23 days after being removed from -4°C and thawed. Other wise, these storage treatments appear to have minimal impact on the early survival and growth of these four clones. If a pre-emergent herbicide cap is applied immediately prior to or immediately after planting, then slowing the rate of shoot and/or root development may reduce herbicide damage. This is an important consideration since many willow clones are sensitive to some of the more effective pre-emergent herbicides (Kopp et al. 1991). Therefore, slowing the rate of shoot development may be an important factor in reducing potential herbicide damage.

4.4 Experiment 4: Screening Cuttings in Water versus Soil

These results suggest that the viability of cuttings on shoot biomass production can be assessed effectively using either soil or water as the growth medium. If root biomass production is going to be assessed then the medium needs to be consistent and the soil medium will give a more realistic representation of how the cuttings will perform in the field.

5.0 Summary

Results from the first and second experiment indicated that leaving the cuttings out for up to 12 days did not have a significant impact on survival or biomass production. In the field trial (experiment 2), cuttings of three of the clones (S301, SA2, and SV1) left out for 23 days had lower survival and per tree biomass production. These results suggest that cuttings can be left out of the -4°C freezer for longer than the commonly recommended period of two to four days before being planted and still achieve survival and first year production rates necessary for a successful plantation, although this response may vary among different clones.

Returning cuttings of SV1, SA2, and S301 to any of the three cold storage treatments (2°C, -4°C or -20°C) after being left out for 5 to 9 days slowed shoot and root development as assessed at the time of planting. Since pre-emergent herbicides are applied to willow crops immediately after planting, and many clones of willow have been shown to be sensitive to these herbicides once they have sprouted, slowing the rate of shoot and root development may be an important factor in reducing potential herbicide damage.

At harvest the proportion of cuttings with roots was reduced if the cuttings had been left out of the freezer for 5 or 9 days and then returned to the -20°C freezer. Returning cuttings to either a +2°C cooler or a -4°C freezer after any length of time did not have any effect on the proportion of cuttings with roots at the time of harvest. Returning cuttings to a -20°C freezer reduced shoot biomass compared to the control or to cuttings returned to +2°C and -4°C cold storage for all clones. Once cuttings have been removed from the -4°C cold storage they should not be returned to -20°C storage, which is a typical temperature for many freezers that are readily available because it is commonly used for food storage.

These experiments suggest that cuttings can be left out of the -4°C freezer for longer than the commonly recommended period of two to four days before being planted and still achieve survival and first year production rates necessary for a successful plantation. However, the response varied among the four different clones in this experiment and may not be applicable to all other willow clones. Once cuttings have been removed from the -4°C cold storage they should not be returned to a -20°C freezer, which is a typical temperature for many freezers that are readily available because it is a common temperature used for food storage. Returning cuttings to a +2°C or -4°C cold storage may slow shoot and root development of the cuttings. This is an important consideration for large-scale plantings, where pre-emergent herbicides are applied immediately after planting. Many clones of willow have been shown to be sensitive to some of these herbicides once they have sprouted, so delaying sprout development would reduce herbicide damage.

Several conditions present in this experiment contribute to the reported results and need to be considered when applying them to large-scale operations. After cuttings were removed from the -4°C freezer, they were kept in cool, shady conditions, which moderated the rate of development. Secondly, cuttings were stored in sealed containers that prevented desiccation. Following these practices for handling cuttings will increase the rate of survival in the field when planting is delayed.

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