ECOLOGICAL STUDIES OF WILLOW (*SALIX CAROLINIANA*): MONTHLY STATUS REPORT #6



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Ecological Studies of Willow (*Salix caroliniana*): Monthly Status Report #6 Covering the time period from June 1-31, 2009

This status report summarizes progress made on the Ecological Studies of Willow project through June 30, 2009, with reference to the tasks and timeline outlined in the Scope of Work and presented in Table 1 below.

Table 1. Timeline of tasks to be accomplished in Year 1 and later. Tasks initiated and underway in this reporting month are highlighted in blue, completed tasks in red.

YEAR 1		
Quarter	Months	Tasks accomplished
1st	Oct – Dec,	Initiate and complete Task 1 (Finalize research plan)
	2008	
2nd	Jan – Mar,	Initiate Task 2.1 (Germination & early survival and growth
	2009	experiments)
		Initiate Task 2.4 (<i>Life history</i>)
3rd	Apr – Jun,	Continue Task 2.1 (Germination experiment)
	2009	Initiate Task 2.2 (Willow transplantation)
		Initiate Task 2.3 (Fire response)
		Continue Task 2.4 (<i>Life history</i>)
4 _{th}	Jul – Sep,	Continue Task 2.4 (<i>Life history</i>)
	2009	Complete Tasks 2.1 & 2.2 (Germination experiment & Willow
		transplantation)
		Complete Task 3.1 (Data analysis and final report, Year1)

YEAR 2

Quarter	Months	Tasks accomplished
1^{st}	Oct – Dec,	Continue Task 2.3 (Fire response)
	2009	Continue Task 2.4 (<i>Life history</i>)
2^{nd}	Jan – Mar,	Continue Task 2.3 (Fire response)
	2010	Continue Task 2.4 (<i>Life history</i>)
		Initiate Task 2.5 (Spatial analysis of willow distribution)
$3^{\rm rd}$	Apr – Jun,	Initiate Task 2.2 (2nd iteration, Willow transplantation)
	2010	Continue Task 2.3 (Fire response)
		Continue Task 2.4 (<i>Life history</i>)
		Continue Task 2.5 (Spatial analysis of willow distribution)
4^{th}	Jul – Sep,	Complete Task 2.2 (2nd iteration, Willow transplantation)
	2010	Continue Task 2.3 (Fire response)
		Continue Task 2.4 (<i>Life history</i>)
		Continue Task 2.5 (Spatial analysis of willow distribution)
		Complete Task 3.2 (Data analysis and final report, Year2)

Progress on Task 1 – Finalizing the Research Plan

The UCF team revised and submitted the final research plan for approval.

Progress on Task 2.1 – Germination and Early Survival and Growth Experiments

The UCF team completed data entry for the two germination experiments that concluded in late April. Preliminary results are summarized below.

Greenhouse experiments on willow seedlings and cuttings continued during this month. We performed the third iteration of treatments in the cross-over design, as described in previous reports, and re-randomized positions of plants within the greenhouse twice.

Analysis of germination of Willow (Experiment 1)

This experiment evaluated six treatments in a gradient of moisture from continuously flooded pot soil to sandy soil watered every 8 days. We used the statistical program R for all the analysis.

We tested two survival analysis models to assess germination in Experiment 1.

- 1. Exponential: This is a one-parameter distribution in which the hazard function is independent of age. It describes a Type II survivorship curve.
- 2. Weilbull: In this model the risk of "death (= germination)" changes with time.

The Weibull model was a better representation of the germination schedule than the Exponential, supporting our observation that most germination occurred rapidly, during the first 5 d in the pots (Table 1, Figure 1, notice the scale far from 1.0).

Table 1. Results of survival analysis of the germination Experiment 1 comparing treatments simulating a water gradient.

Water	-2*Likelihood	-2*Likelihood	p between	Scale	p among
Treatment	Exponential	Weilbull	models		treatments
					Weilbull
Overall	5708.9	5599.4	< 0.0001	0.698	0.00045
Overall *	5339.9	5315.3	< 0.0001	0.668	0.087

* If extreme replicate in capillarity treatment is excluded.

Using all replicates, treatment capillarity was the only statistically significant different treatment from all the other treatments (Table 2)

Treatment	Value	Std Error	Z	Р
Daily	0.316	0.0904	3.49	0.0005
Every 3 days	0.270	0.0903	2.99	0.003
Every 5 days	0.260	0.0903	2.88	0.004
Flooded	0.302	0.0904	3.34	0.0008
Sand (8 days)	0.418	0.0906	4.61	< 0.0001



Figure 1. Germination schedule of Willow seeds among six different moisture treatments.

Most Willow germination occurred rapidly after the initial watering, reducing the effect of the moisture treatments. Water by capillarity was the best condition for germination. It was notable the some seeds germinated and survived in flooded soil.

Analysis of mortality of Willow seedlings (Experiment 1)

We analyzed survival of germinated seedlings among the above treatments after 30 days of initiating the experiment. We transformed data (percent mortality of germinated seedlings) using Arcsin (sqrt(p)) and analyzed it with ANOVA by treatment (Table 3).

Table 3. Analysis of variance of percent mortality (data arcsin transformed) among moisture treatments.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatments	5	5.1	1.02	8.3742	< 0.0001
Residuals	30	3.7	0.12		

We did posthoc Tukey HSD (Honestly Significant Difference) and found that the driest treatment (only sand with 8 d between watering) was the only one that differed significantly treatment from the others (Figure 2, Table 4).

Table 4. Tukey	HSD multi	ple comparis	sons among	treatments
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	diff	p adj
Comparisson		
every_5-capillarity	0.306	0.65
every_3-capillarity	0.361	0.49
daily-capillarity	0.438	0.28
Flooded-capillarity	0.526	0.13
sand-capillarity	1.237	0.00001
every_3-every_5	0.053	0.99
daily-every_5	0.130	0.99
Flooded-every_5	0.219	0.88
sand-every_5	0.930	0.001
daily-every_3	0.077	0.99
Flooded-every_3	0.165	0.96
sand-every_3	0.876	0.002
flooded-daily	0.088	0.99
sand-daily	0.799	0.005
sand-flooded	0.711	0.016



Figure 2. Plot of proportion mortality by treatment (\pm one standard variation). Bars with the same letter were not significantly different.

Analysis of germination of Willow (Experiment 2)

This experiment evaluated germination in six soil types, all watered by capillarity. Seeds came from four different sites. We tested the same two survival analysis models as in Experiment 1, Exponential and Weilbull. We had > 80 % germination overall. As before, the Weibull model better represented the germination schedule than the Exponential model, supporting our observation that most of the germination occurred rapidly (Table 5, Figures 3, 4 and 5, notice the scale far from 1.0).

Using the Weibull model we tested differences among soils and seed sources (Table 6).

Soil Treatment	-2*Likelihood Exponential	-2*Likelihood Weilbull	P among models	Scale	P among seed sources
	<u>r</u>				Weilbull
BC	664.0	656.9	0.007	0.834	0.000004
BC / D	673.6	667.5	0.014	0.848	0.007
BC / U	627.5	603.6	< 0.00001	0.690	< 0.00001
D	606.3	585.2	< 0.00001	0.736	0.00012
U	675.3	670.9	0.04	0.873	0.036
U / D	627.2	534.5	< 0.00001	0.439	< 0.00001
Overall	4005.7	3992.9	0.00035	0.913	0.095

Table 5. Results of survival analysis of the germination Experiment 2 comparing soils and seed sources.

Our data were not enough to demonstrate differences in germination among soil types (P =0.095, see Figure 4). Seeds from southern sources tended to have lower germination compared to the other sites (See Figure 5), except soil U/D were seeds from near Hw 60 had lower germination (see Figure 4). The model including the interaction between soil type and seed source had the best fit of all possible nested models of these variables (p=<0.0001 compared to the additive model of these variables).



Figure 3. Germination schedule of Willow seeds in different soils and from different seed sources.



Figure 4. Germination schedule of Willow seeds among different seed sources



Figure 5. Germination schedule of Willow seeds among soil types

Appendix 1. Contrasts from the model including the interaction from seed sources and soil types

Call:

survreg(formula = Surv(death, status) ~ treatment * Site)

	Value	Std. Err	Z	p
(Intercept)	2.33	0.1394	16.714	0.000
treatmentBC_D	-0.0642	0.1955	-0.329	0.742
treatmentBC_U	0.0774	0.1955	0.396	0.692
treatmentD	-0.1824	0.1955	-0.933	0.351
treatmentU	-0.2134	0.1955	-1.091	0.275
treatmentU_D	-1.1081	0.1962	-5.646	0.000
SiteS_46	-0.8066	0.1955	-4.125	0.000
SiteS_50	-1.0268	0.1962	-5.233	0.000
SiteS_60	0.0507	0.1955	0.259	0.795
<pre>treatmentBC_D:SiteS_46</pre>	0.5807	0.2765	2.1	0.036
treatmentBC_U:SiteS_46	-0.4653	0.2767	-1.682	0.093
treatmentD:SiteS_46	0.1432	0.2765	0.518	0.604
treatmentU:SiteS_46	0.3429	0.2765	1.24	0.215
treatmentU_D:SiteS_46	0.9047	0.2765	3.272	0.001
<pre>treatmentBC_D:SiteS_50</pre>	0.1556	0.2765	0.563	0.574
treatmentBC_U:SiteS_50	-0.282	0.2765	-1.02	0.308
treatmentD:SiteS_50	0.206	0.2765	0.745	0.456
treatmentU:SiteS_50	1.188	0.2769	4.291	0.000
<pre>treatmentU_D:SiteS_50</pre>	1.0536	0.277	3.804	0.000
<pre>treatmentBC_D:SiteS_60</pre>	-0.2982	0.2765	-1.078	0.281
treatmentBC_U:SiteS_60	-0.3134	0.2765	-1.133	0.257
treatmentD:SiteS_60	-0.6739	0.2766	-2.436	0.015
treatmentU:SiteS_60	-0.4172	0.2765	-1.509	0.131
treatmentU_D:SiteS_60	1.5254	0.2767	5.513	0.000
Log(scale)	-0.2782	0.0263	-10.577	0.000

Scale= 0.757

Weibull distribution Loglik(model)= -1889.2 Loglik(intercept only)= -2001.1 Chisq= 223.85 on 23 degrees of freedom, p= 0 Number of Newton-Raphson Iterations: 5 n= 720

Analysis of mortality of Willow (Experiment 2)

We analyzed survival of germinated seedlings among the above soils and seed sources after 30 days of initiated the experiment. We transformed the data (percent mortality of germinated seedlings) using Arcsin (sqrt(p)) and analyzed it with ANOVA by treatment. There was no support for differences among soil types or seed sources in proportional seedling mortality.

Table 6. Analysis of variance of percent mortality (data arcsin transformed) among seed sources and soil types.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Soils	5	0.26	0.052	1.01	0.45
Seed Source	3	0.02	0.006	0.11	0.95
Residuals	15	0.777	0.052		





Figure 6. Proportion of seedling mortality vs. type of soil (\pm one standard variation).

Figure 7. Proportion of seedling mortality vs. type of seed source (\pm one standard variation).

Progress on Task 2.2 – Willow Transplantation

A. Competition Experiment – We monitored all blocks of the field experiment to evaluate responses of willow seedlings and cuttings to competition. Already strong differences in survival area apparent: only one cutting remained alive in the northern block and two in the central block, and all seedlings perished. In contrast, multiple cuttings and seedlings were alive and growing in the southernmost block.

B. Hydrology Experiment – We monitored the willow island experiment, which tests the ability of seedlings and cuttings to survive at different elevations in the marsh (Fig. 8). As in the southernmost block of the competition experiment, which is located nearby, survival of seedlings and cuttings was high. Almost all cuttings survived, even those atop the islands (Fig. 2). Most seedlings also survived, with the limited mortality higher on those at the lowest elevation (presumably due to erosion) and atop the islands, which were about halfway inundated in water. Due to the high water, for seedlings we only

assessed survival (presence/absence) because we could not see these small plants underwater. Cuttings were large enough for use to find and obtain all data, even when most of the plant was submerged. Several cuttings were fouled by bladderwort (*Utricularia* sp.).

Fig. 8. Willow island almost halfway submerged. Note the vigorous cuttings atop the island and emergent willows at the bottom.



Progress on Task 2.4 - Life History

Our attention during this period was focused on entering data from the germination experiments, monitoring the two field experiments and beginning the two greenhouse experiments. We expect to begin gathering life history data again next month, from sites near the field experiments.

Progress on Task 2.5 – Spatial Analysis of Willow Distribution.

We concentrated on other activities and therefore did not modify the spatial model. We are refining this model during July and August.