02D-P66-FR_12-07_Chlorsulfuron_Soil_Residues_In_Seasonal_Floodplain-T4.5

CHLORSULFURON SOIL RESIDUES IN A SEASONAL FLOODPLAIN

ERP 02D-P66 PROJECT REPORT

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Keywords: *Lepidium latifolium, Telar®, chlorsulfuron, bioassay*

1.0 Task 4.5 Introduction

This report summarizes the findings of Task 4.5: Report on effect of *Lepidium latifolium* presence and chlorsulfuron application on soil properties of ERP-02D-P66.

1.1. Objective

The Lepidium Control Experiment (LCE) sought to understand the residual effects of chlorsulfuron herbicide treatment on future plant communities through a bioassay of soils collected on an experimental seasonal floodplain in Sacramento County, California. Land managers frequently use herbicides to control invasive weeds in natural areas. Although most commonly used herbicides have extremely low toxicities to humans and wildlife, there are concerns that herbicides may affect non-target plant species, with concomitant biomagnification. Herbicides with a long half-life are of particular concern, as they can accumulate in soils and effect vegetation for years to come.

1.2. Background

The Cosumnes River Preserve, in partnership with the UC Davis Information Center for the Environment, used both herbicides and tarping as part of a comprehensive adaptive weed management experiment to determine the most efficacious methods for controlling the highly invasive perennial pepperweed (*Lepidium latifolium*) at the Preserve. The experiment is testing the use of glyphosate (Rodeo™) and chlorsulfuron (Telar™) herbicides tested using two methods of application. Herbicide application methods were either (1) mowing followed by broadcast spraying on target plants, or (2) stem cut and direct application of herbicide.

Of the two herbicides used in the experiment, one – glyphosate – has undergone extensive testing with regards to soil and water contamination. Aquatic formulations of glyphosate-based herbicides degrade rapidly and are not considered a concern for soil or groundwater contamination (DuPont 2003). Chlorsulfuron, is highly recommended for the control of perennial pepperweed in terrestrial environments (see Task 4.1 Report), but may not be applied in aquatic environments. The bioassay study described here evaluated concerns regarding the potential for chlorsulfuron application to negatively affect treated floodplain soils at the Preserve and other similar environments. Our results provide needed information to managers on the likely effects of chlorsulfuron use in floodplain restoration areas.

Chlorsulfuron is a non-selective sulfonylurea herbicide which has high toxicity to dicotyledons and micro-organisms at very low doses. It functions by disrupting a single plant enzyme, acetolactate synthase, responsible for amino acid synthesis, and has extremely low toxicity to animals. Researchers have found mixed results of chlorsulfuron's effects on non-target vegetation, ranging from no detrimental effect on surrounding vegetation (Young, Palmquist et al. 1998; Renz 2002) to detrimental effects 2 years post-treatment (Guo and Sun 2002). These long-term effects are generally for crop species, which tend to be much more sensitive than wildland species (DiTomaso, personal communication). Several plants, including some common weeds, have acquired herbicide resistance to chlorsulfuron (Shaner and Lym 1991).

The breakdown of chlorsulfuron in soil is affected primarily by hydrolysis and microbial activity, and is aided by high soil temperatures, low pH levels and soil water content (DuPont 2003). The half-life on chlorsulfuron averages 4-6 weeks, and decreases with decreasing soil pH (Walker, Cotterill et al. 1989; PMEP 2003). Soils within our study sites at the Cosumnes River Preserve soils range 4.7-8.4 in pH based on soil tests conducted in this study (see Task 4.4 Report). Based on observed pH values and published information regarding chlorsulfuron breakdown (Guo and Sun 2002; PMEP 2003), we sought to understand the longevity of chlorsulfuron in the top 20 cm of floodplain soil.

2.0 Task 4.5 Methods

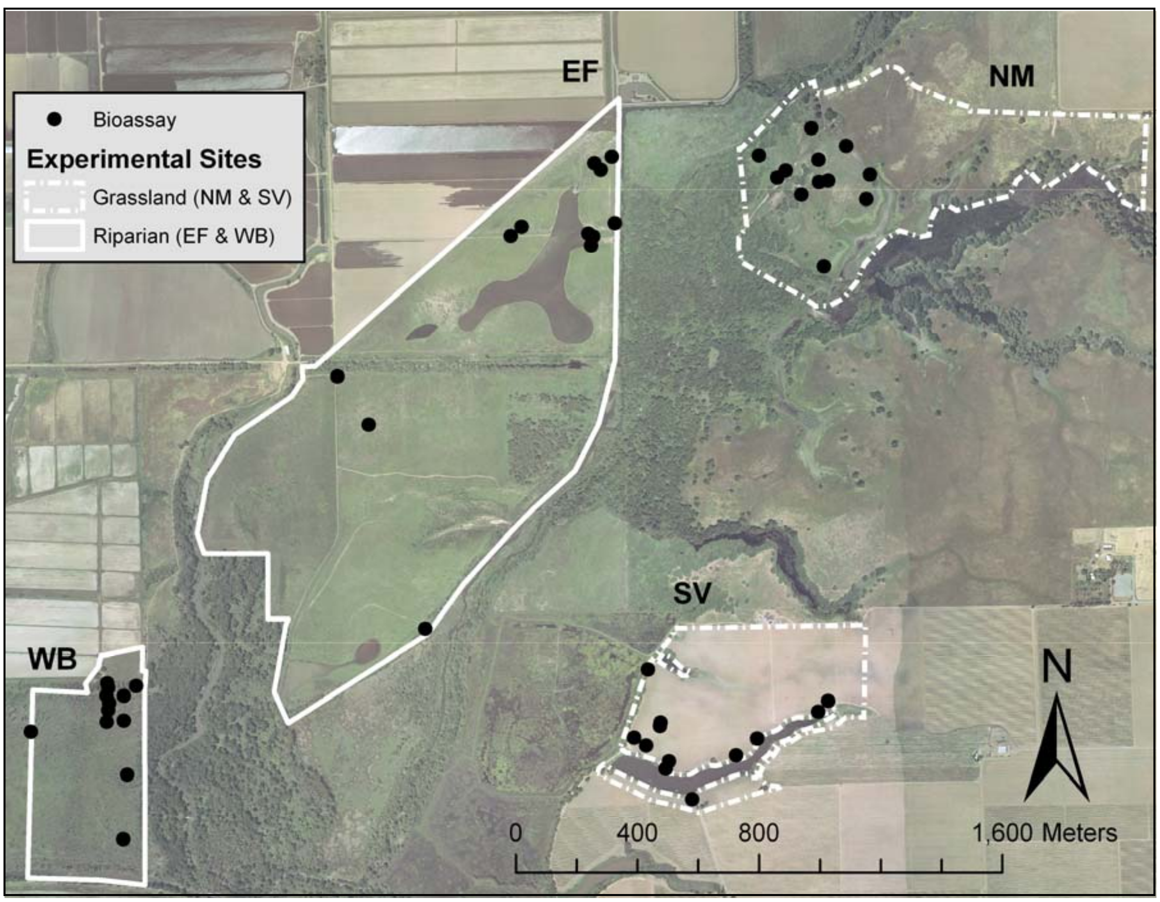

Figure 1. Collection sites and points used to conduct a soil bioassay at the Cosumnes River Preserve. These points were selected based on treatment method and site type.

The methods used in this study consist of a bioassay using soil collected prior and post herbicide application across four representative sites at the Preserve.

Soil samples were collected with a hand auger at four Preserve sites where herbicide trials took place; samples were obtained before herbicide treatment and at regular intervals following chlorsulfuron herbicide treatment (Figure 1). Samples were also taken from treatment control plots on all sample dates. We collected soils at depths of 0-10 and 10-25 cm at all plots, with three cores per plot, which were bagged in the field. Soil collection plots were established 3 m × 3 m areas used in other project tasks. In mow-broadcast treatment plots, soil sample location was chosen randomly from within the plot. In cut-stem treatment plots, samples were collected < 30 cm from a cut stem, where previously cut stems were flagged to allow for subsequent soil collection.

Soil was dried at 40° C and ground at the UC Davis Division of Agriculture and Natural Resources (DANR) soils laboratory using standard lab procedures[1]. Drying and grinding of soils resulted in effective mixing and allowed standardization of soil weight for the subsequent bioassay experiment. We labeled 7.62 cm (3") diameter plastic petri dishes and filled each with 200 g of DANR prepared soil. We prepared three replicate petri dishes per core-depth sample.

Using these prepared soil growth media, we initiated a bioassay inside a standard UC Davis greenhouse. We followed the maize root petri dish bioassay protocol published by Eleftherohorinos (1987). We used white, SE type Silverado variety untreated hybrid sweet corn (*Zea mays*), isolated from SHZ sweet corn and field corn (2318 seeds per pound) purchased from Harris Moran Seed Company, with a reported germination rate of 95% as tested in December 2004. Corn seeds for bioassay were pre-soaked in water for 6 hours, and then germinated in trays lined with paper towels in a dark growth chamber for 3 days using published temperature settings (Eleftherohorinos 1987). Following initial germination, 3 seeds with root radicals >1 cm long were added to each petri dish. Initial root length was recorded for each petri dish.

Exactly 40 ml of water was added to each petri dish. Petri dishes were covered, and then added to the growth chamber in stacks of three. The arrangement of petri dish samples within the chamber was fully randomized, and the order of petri dishes in the stack of three replicates per sample was recorded to test for possible effects of stacking on root growth. After 5 days, petri dishes were removed from the growth chamber, and root length was measured and recorded using a digital caliber.

2.1. Data Methods

Root growth was calculated as final root length minus initial root length (cm). Values were normalized through $\log_e$ transformation and analyzed in JMP IN 5.1 using

[1] http://groups.ucanr.org/danranlab/Methods_of_Analyses/Soil.htm

standard statistical techniques, such as *t*-tests and analysis of variance (ANOVA). All responses are *LN(Root Growth cm)* unless otherwise noted.

3.0 Task 4.5 Results

A total of 1599 observations were made in the bioassay trial (Table 1). There were 600 treatment controls, which exhibited no detectable linear productivity trend across time at either soil depth (p = 0.98 for 0 - 10 cm; p = 0.07 for 10 - 25 cm; Appendix 4.5.1). However, we did observe differences within experimental controls among replicate position in the controlled environment. We subsequently excluded all bottom tray petri dish measurements because they were significantly affected by treatment position (p < 0.001). Therefore, we only report top and middle tray responses hereafter, which were not significantly different (p = 0.18). We observed no detectable differences among pre-treatment observations, modeled as a treatment by soil depth factorial (p = 0.17).

LCE Site	Treatment	Observations
SV	CS-HC-CH	120
SV	LELA-CO	150
SV	MB-CH	168
NM	CS-HC-CH	129
NM	LELA-CO	162
NM	MB-CH	156
WB	CS-HC-CH	96
WB	LELA-CO	144
WB	MB-CH	114
EF	CS-HC-CH	96
EF	LELA-CO	144
EF	MB-CH	120

Table 1. Bioassay Petri Dish Observations by Site and Treatment.

To test the effect of herbicide application method by residual soil effects, we used two methods. One, we contrasted pretrial observations with day of treatment observations by treatment method and soil depth. We hypothesized that pre-treatment observation would have a higher growth rate as it was unaffected by the herbicide treatment; our observations concluded that the mow-broadcast treatment had a significant negative effect on growth rates in the bioassay trial at 0.95 confidence interval at both soil depths (Table 2), whereas the cut-stem treatment had a negative, but insignificant, effect on growth rates regardless of soil depth (Table 2; Appendix 4.5.1). Further, there was a significant difference between soil depths post treatment with mow-broadcast deeper soil sample observations (10 - 25 cm) having higher growth (Diff = 1.34 cm; *t*-Ratio = -2.72; DF = 46; p = 0.005; Appendix 4.5.1). The cut-stem treatment was not significantly different across soil depths post-treatment (p = 0.34). Two, we regressed treatment response across number of months since treatment. Both treatments showed positive trends post treatment (Appendix 4.5.1); in other words, the observed growth values were greater as time progressed. The cut-stem treatment slope, however, was only marginally significantly different than zero (p = 0.1) when all depths were pooled, and insignificant when separated (p = 0.16 for 0 - 10 cm; p = 0.34 for 10 - 25 cm). The mow-broadcast treatment did show significant positive growth over time pooled (p = 0.02), and for the upper soil layer (p = 0.05 for 0 - 10 cm; p = 0.12 for 10 - 25 cm).

Treatment × Soil Depth	Mean Difference (Post - Pre cm)	*t*-Ratio	DF	Prob < *t*
CS 0 - 10 cm	1.41	-1.34	36	0.09
CS 10 - 25 cm	1.42	-1.21	34	0.12
MB 0 - 10 cm	3.63	-9.07	52	0.00
MB 10 - 25 cm	3.23	-8.62	52	0.00

Table 2. Results of *t*-tests by treatment method (CS = cut-stem; MB = mow-broadcast) and soil depth.

Lastly, we examined soil property influence on latent soil residency for the mow-broadcast treatment in the 0 - 10 cm layer, as it was the only treatment with observable effects in the bioassay trial longer than one month. We regressed root growth against time and several covariates. Of the prominent soil factors, pH played a significant role in the observed latency of chlorsulfuron in the soil. Although day-of-treatment growth was negatively associated with pH [*Ln(RtGrowth) = 5.7 - 0.8 * pH*], the slope was not significantly different than zero (p = 0.17). However, after one week, the slope becomes significantly negative (p = 0.04) [*Ln(RtGrowth) = 4.0 - 0.4 * pH*] and explained 7% of the observed variance. After two weeks, negative relationships between chlorsulfuron affected growth and soil pH disappeared. We used residuals from growth regressed against time to examine potential independent soil factors that might exacerbate observed residue latency (Appendix 4.5.1). We partitioned sites as either upland (North Moyer Slough, Silverado) or floodplain-riparian (Experimental Floodplain, West Bottoms) to block geomorphic position as it related to soil chemistry. We observed that upland sites exhibited no additional residue effects with changes in soil chemistry; however, floodplain riparian sites did exhibit significant add-on effects by soil component. Namely, the growth rates adjusted for time since herbicide application were higher for sites with more percent sand (Residuals *Ln(RtGrowth)* = -0.67 + 0.02 * Sand; R^2_{adj} = 0.16; p = 0.001) and lower for sites with more percent silt (Residuals *Ln(RtGrowth)* = 2.14 - 0.05 * Silt; R^2_{adj} = 0.19; p = 0.0005; Appendix 4.5.1).

4.0 Task 4.5 Discussion

We observed no significant trend among treatment controls across time in regards to changes in productivity, either positive or negative, suggesting that each site was environmentally stable during the period of soil collection, as the bioassay did not exhibit a response from potential changes in soil conditions, such as nutrients leached during wet periods. This observation of environmental stability is important, because aside from initial interactivity with pH, chlorsulfuron efficacy in the soil diminished readily over the seven-month period for which the bioassay trials were conducted. Soil residual effects were observed, however, at sites with high silt fraction even after time since treatment was factored out. In other words, floodplain-riparian areas with high silt content are more likely to be adversely affected by chlorsulfuron applications regardless of time since herbicide application, and sites that are better presumably better drained with high sand content will be less adversely affected.

Additional anecdotal evidence from the field suggests that some plots positioned in floodplain-riparian areas were adversely affected by mow-broadcast chlorsulfuron treatments after 2 years of treatment (Figure 2). While we observed no difference in seed bank germinants two years after the initial treatments, some lingering effects in the field were evident (see Subtask 4.6 Report). Some of these effects might be explained by the lack of moisture and subsequent increase in bare soil in all plots in 2007. For example, the bioassay trials were subjected to a uniform growing environment with ample water availability, whereas field conditions are subjected to high variability in environmental conditions (e.g., water, shade, disturbance, etc.).

Figure 2. Plots treated by Telar® at the Experimental Floodplain and West Bottoms (left to right) after 2 years of mow broadcast treatment.

When selecting perennial pepperweed treatment methods on Preserve lands, testing for silt content and pH may be two simple and inexpensive ways to make decisions about which chemicals or which method would be most beneficial to an individual site. By combining these results with the results of our soil chemical and physical analysis, vegetation surveys and treatment success in our adaptive management plan we will have a clear picture of how each site differs. For example, in order to identify the impacts of chlorsulfuron in test plots, we sprayed the entire 3 m by 3m plot area. Based on our results from the bioassay and vegetation sampling, which documented initial and potential long term impacts of chlorsulfuron on plant growth, restricting application to include only the target plant is suggested. Management guidelines at the preserve should stress the detrimental side effects of excessive herbicide application, especially in the case of Telar®. Other herbicides, including Rodeo®, are safer and very effective at controlling perennial pepperweed as well as other common riparian weeds. Based in part on these findings, the Cosumnes River Preserve has set management guidelines that restrict the use of chlorsulfuron to greater than 1,200 feet from any water body. This restriction will help protect sensitive riparian areas and its vegetation.

5.0 Task 4.5 Conclusion

Our experiment was intended to identify the legacy effect of chlorsulfuron on non-target vegetation through a bioassay of soils collected on an experimental seasonal floodplain in Sacramento County, California. We collected soil from experimental plots treated with herbicide in one of two methods: broadcast application or cut-stem swab. We found little evidence for legacy effects in the cut-stem swab application; however, we did document significant negative effects in the first two weeks following broadcast application up to 25 cm in soil depth. This observation of negative residual effects was exacerbated by elevated pH in soils. Although negative residual effects of broadcast chlorsulfuron application were detected over a seven month period, the effects diminished over time in a linear fashion. This trend, however, showed significant continued soil residuals for floodplain-riparian sites with high silt fraction, but not high sand fraction sites that are presumably well drained. We recommend curtailing use of broadcast chlorsulfuron application in floodplain-riparian sites with either high pH or high silt fraction.

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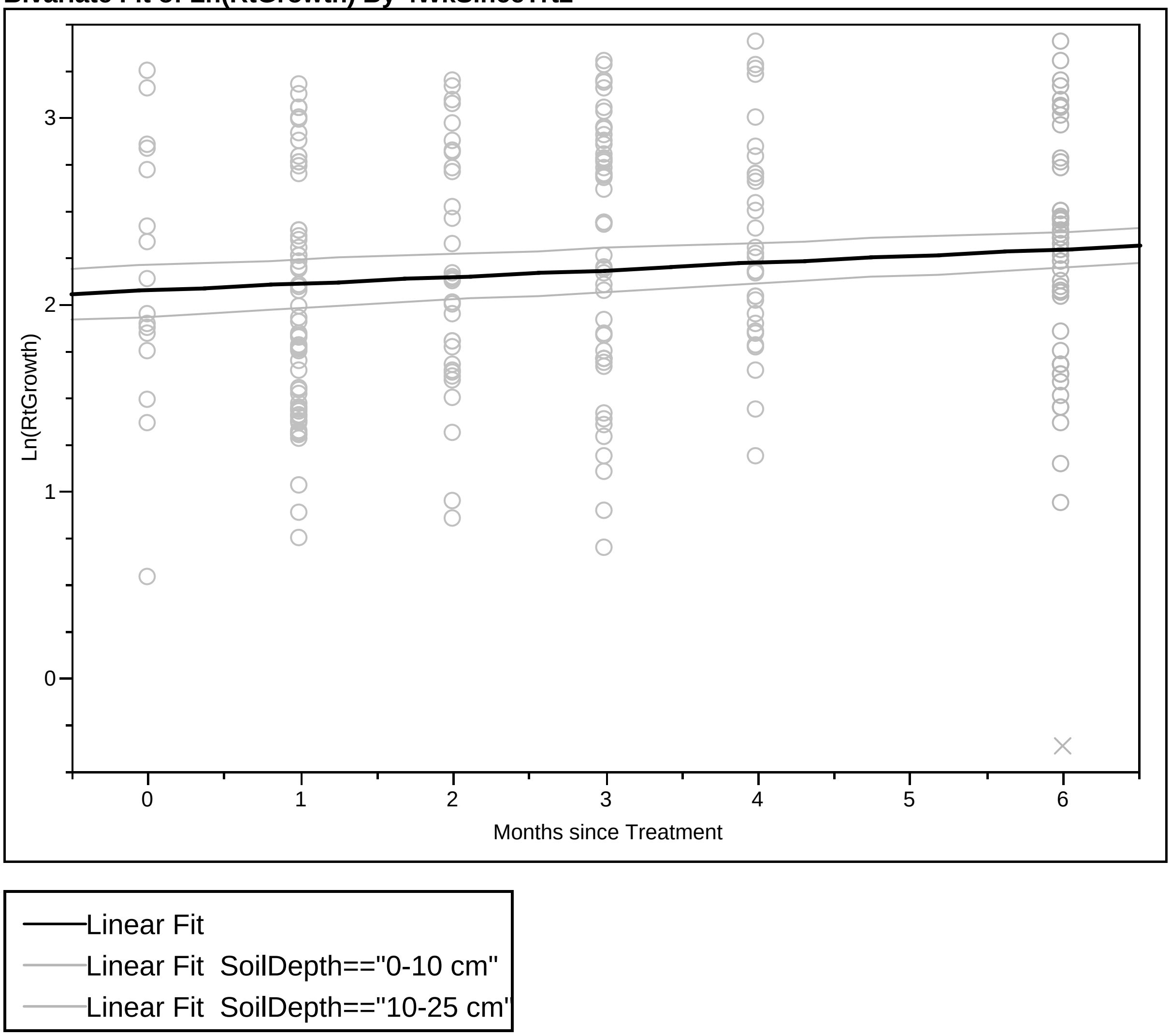
7.0 APPENDIX 4.5.1

Trt=CS-HC-CH

Bivariate Fit of Ln(RtGrowth) By 4WkSinceTrt2



Linear Fit

Ln(RtGrowth) = 2.0764835 + 0.0368368 4WkSinceTrt2

Summary of Fit

RSquare	0.01144
RSquare Adj	0.007215
Root Mean Square Error	0.663137
Mean of Response	2.183248
Observations (or Sum Wgts)	236

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.19081	1.19081	2.7079
Error	234	102.90177	0.43975	Prob > F
C. Total	235	104.09258		0.1012

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.0764835	0.077928	26.65	<.0001
4WkSinceTrt2	0.0368368	0.022385	1.65	0.1012

Linear Fit SoilDepth=="0-10 cm"

Ln(RtGrowth) = 1.9401246 + 0.0436475 4WkSinceTrt2

Summary of Fit

RSquare	0.017034
RSquare Adj	0.00856
Root Mean Square Error	0.644856
Mean of Response	2.066629
Observations (or Sum Wgts)	118

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.835923	0.835923	2.0102
Error	116	48.237349	0.415839	Prob > F
C. Total	117	49.073272		0.1589

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.9401246	0.107168	18.10	<.0001
4WkSinceTrt2	0.0436475	0.030785	1.42	0.1589

Linear Fit SoilDepth=="10-25 cm"

Ln(RtGrowth) = 2.2128424 + 0.0300261 4WkSinceTrt2

Summary of Fit

RSquare	0.007635
RSquare Adj	-0.00092
Root Mean Square Error	0.665751
Mean of Response	2.299867
Observations (or Sum Wgts)	118

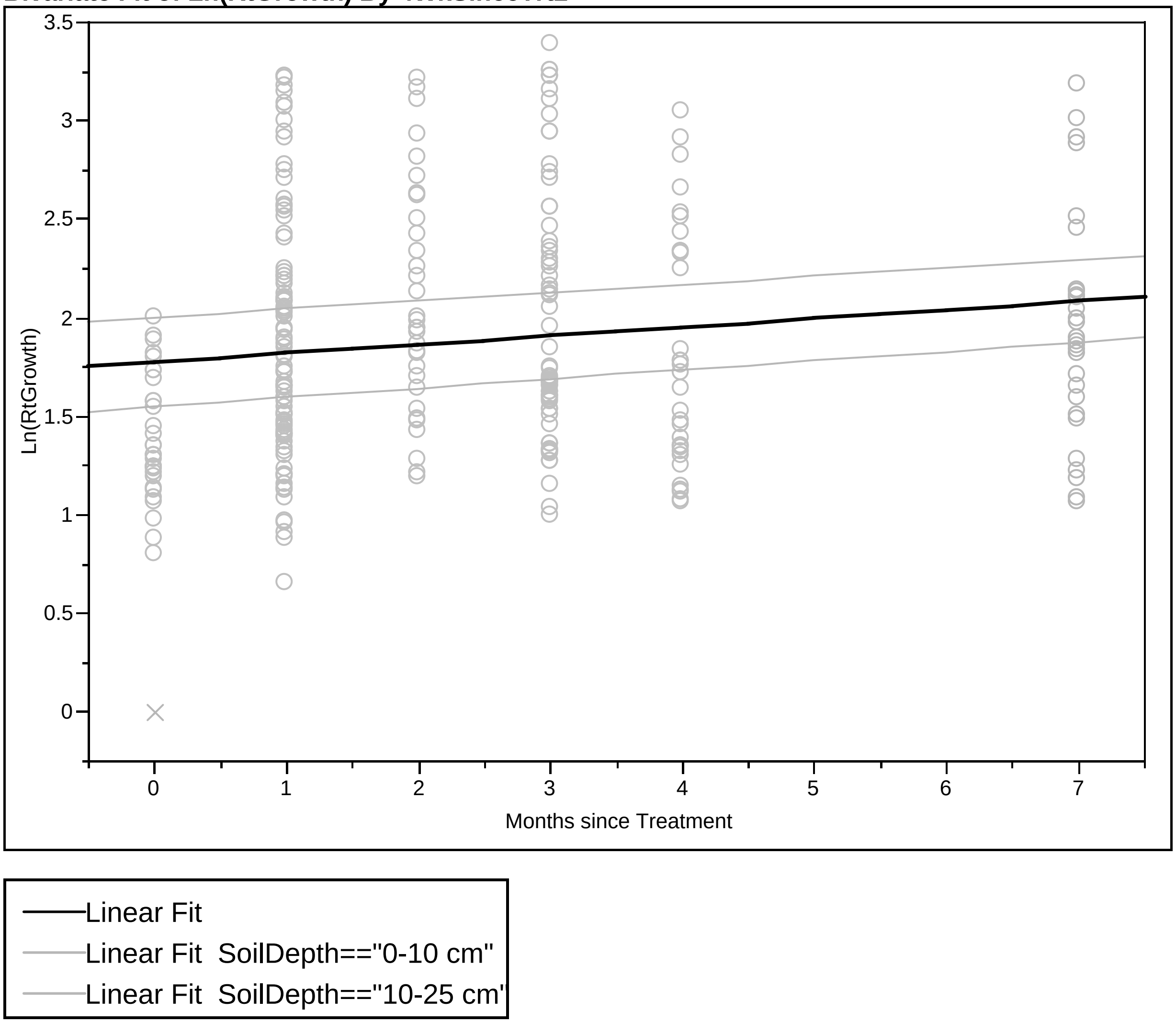
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.395589	0.395589	0.8925
Error	116	51.414101	0.443225	Prob > F
C. Total	117	51.809690		0.3468

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.2128424	0.110641	20.00	<.0001
4WkSinceTrt2	0.0300261	0.031783	0.94	0.3468

Trt=MB-CH
Bivariate Fit of Ln(RtGrowth) By 4WkSinceTrt2



Linear Fit
Ln(RtGrowth) = 1.7765564 + 0.0441948 4WkSinceTrt2

Summary of Fit

RSquare	0.01904
RSquare Adj	0.015511
Root Mean Square Error	0.621532
Mean of Response	1.882624
Observations (or Sum Wgts)	280

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	2.08443	2.08443	5.3959
Error	278	107.39205	0.38630	Prob > F
C. Total	279	109.47648		0.0209

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.7765564	0.058861	30.18	<.0001

Term	Estimate	Std Error	t Ratio	Prob>|t|
4WkSinceTrt2	0.0441948	0.019026	2.32	0.0209

Linear Fit SoilDepth=="0-10 cm"

Ln(RtGrowth) = 1.5494605 + 0.0467079 4WkSinceTrt2

Summary of Fit

RSquare	0.027355
RSquare Adj	0.020306
Root Mean Square Error	0.547673
Mean of Response	1.66156
Observations (or Sum Wgts)	140

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.164118	1.16412	3.8811
Error	138	41.392560	0.29995	Prob > F
C. Total	139	42.556678		0.0508

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.5494605	0.07335	21.12	<.0001
4WkSinceTrt2	0.0467079	0.023709	1.97	0.0508

Linear Fit SoilDepth=="10-25 cm"

Ln(RtGrowth) = 2.0036522 + 0.0416816 4WkSinceTrt2

Summary of Fit

RSquare	0.017414
RSquare Adj	0.010294
Root Mean Square Error	0.615673
Mean of Response	2.103688
Observations (or Sum Wgts)	140

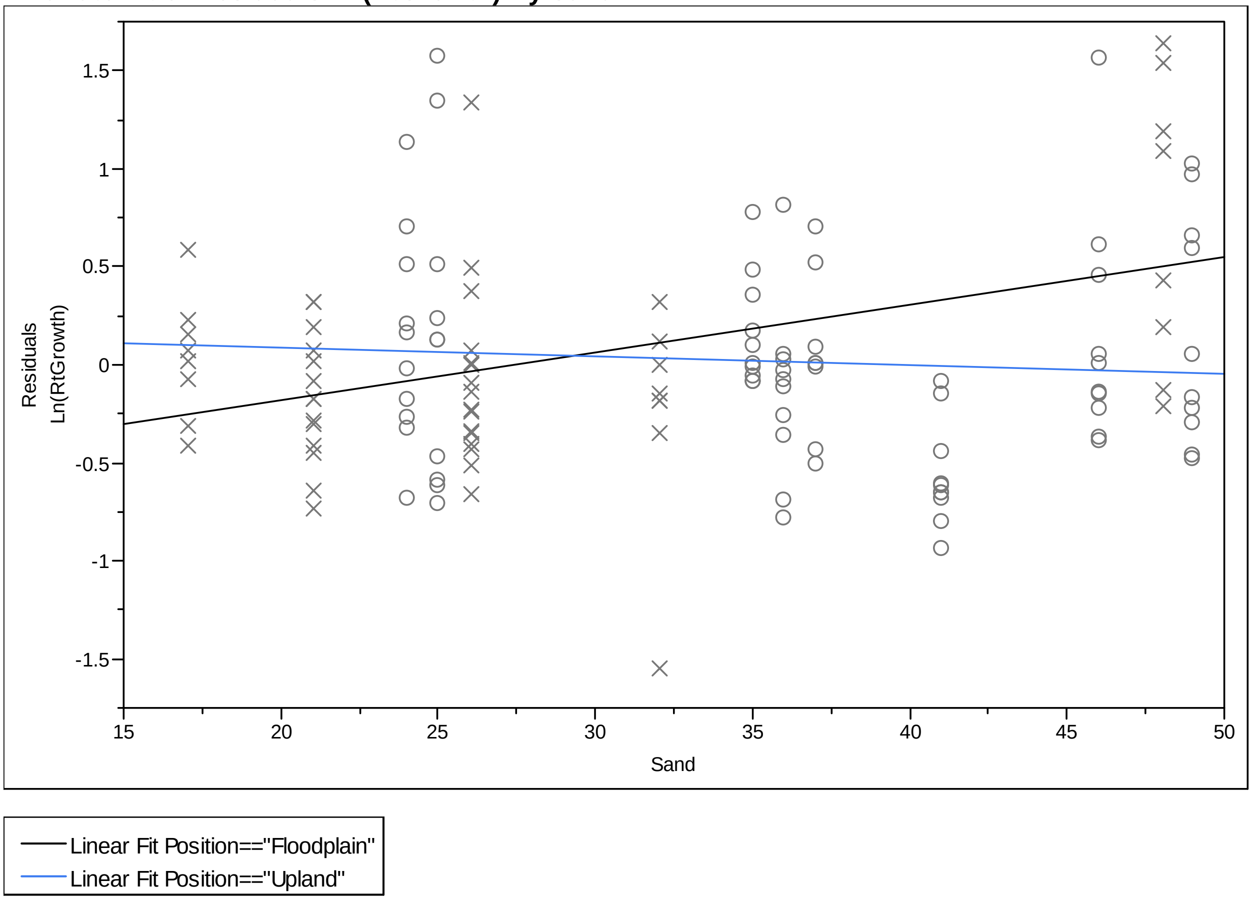
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.927052	0.927052	2.4457
Error	138	52.309328	0.379053	Prob > F
C. Total	139	53.236380		0.1201

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.0036522	0.082458	24.30	<.0001
4WkSinceTrt2	0.0416816	0.026653	1.56	0.1201

Bivariate Fit of Residuals Ln(RtGrowth) By Sand



Linear Fit Position=="Floodplain"

Residuals Ln(RtGrowth) = -0.669942 + 0.0244436*Sand

Summary of Fit

RSquare	0.179264
RSquare Adj	0.163481
Root Mean Square Error	0.514579
Mean of Response	0.002709
Observations (or Sum Wgts)	54

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3.007446	3.00745	11.3578
Error	52	13.769171	0.26479	**Prob > F**
C. Total	53	16.776618		0.0014

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.669942	0.21152	-3.17	0.0026
Sand	0.0244436	0.007253	3.37	0.0014

Linear Fit Position=="Upland"

Residuals Ln(RtGrowth) = 0.1843863 - 0.004672*Sand

Summary of Fit

RSquare	0.005144
RSquare Adj	-0.00795
Root Mean Square Error	0.556646
Mean of Response	0.01332
Observations (or Sum Wgts)	78

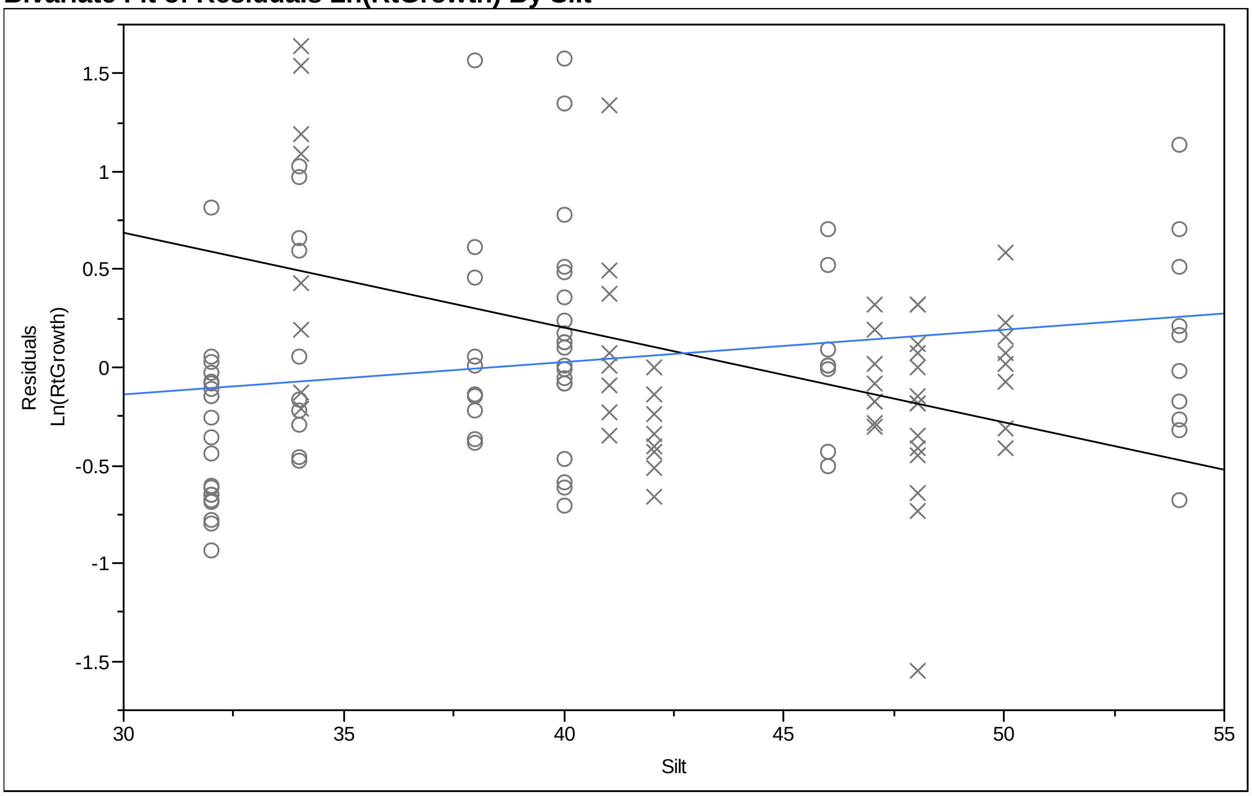
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.121763	0.121763	0.3930
Error	76	23.548925	0.309854	**Prob > F**
C. Total	77	23.670687		0.5326

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.1843863	0.280072	0.66	0.5123
Sand	-0.004672	0.007453	-0.63	0.5326

Bivariate Fit of Residuals Ln(RtGrowth) By Silt



Linear Fit Position=="Floodplain"
Linear Fit Position=="Upland"

Linear Fit Position=="Floodplain"

Residuals Ln(RtGrowth) = 2.1438208 - 0.0484983*Silt

Summary of Fit

RSquare	0.208452
RSquare Adj	0.19323
Root Mean Square Error	0.505346

Mean of Response	0.002709
Observations (or Sum Wgts)	54

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3.497119	3.49712	13.6941
Error	52	13.279498	0.25537	**Prob > F**
C. Total	53	16.776618		0.0005

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.1438208	0.582665	3.68	0.0006
Silt	-0.048498	0.013106	-3.70	0.0005

Linear Fit Position=="Upland"

Residuals Ln(RtGrowth) = -0.632157 + 0.0164104*Silt

Summary of Fit

RSquare	0.044295
RSquare Adj	0.03172
Root Mean Square Error	0.545583
Mean of Response	0.01332
Observations (or Sum Wgts)	78

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.048485	1.04849	3.5224
Error	76	22.622202	0.29766	**Prob > F**
C. Total	77	23.670687		0.0644

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.632157	0.349426	-1.81	0.0744
Silt	0.0164104	0.008744	1.88	0.0644