Title

Forest Regeneration under Scotch Broom Control, Phase I Progress. Technical Report submitted to Joint Base Lewis-McChord and The Nature Conservancy.

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Publication Date

2011-06-01

Forest Regeneration Under Scotch Broom Control

Phase I

Progress Report June 2011

Submitted to Joint Base Lewis-McChord and The Nature Conservancy

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Awarded to the University of California, Santa Cruz

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Introduction

The pest plant Scotch broom (*Cytisus scoparius*) is hindering effective reforestation at Fort Lewis, resulting in both a loss of land available for military training as well as a loss of native forest habitat for native plants and animals. Sustainable forestry has been practiced on the 40,000 acres (16,000 ha) of commercial forest lands on Joint Base Lewis-McChord for over a hundred years. When Scotch broom invaded the base over the later decades of the 20th century, it created new challenges for reforestation efforts. Large areas of forest have been essentially taken over by Scotch broom after trees were harvested. Tried-and-true approaches to site preparation (removal of all competing standing vegetation, planting T1 plugs at 8-foot spacing) have not resulted in successful reforestation; in fact, plantations have shown repeated failures, resulting in serious financial losses to the Base as well as having a negative effect on the use of these areas for training.

The primary objective of our original Phase I project was to examine the effectiveness of different approaches to Scotch broom control in the forestry context. This included the response of broom plants themselves to the control treatments, as well as the survival and growth response of Douglas fir seedlings planted into these different treatments. Massive mortality of the Douglas fir transplants (see below) has required a shift of focus away from the response of trees in the Phase I experiments, toward an exclusive focus on controlling the broom, with the objective that these results will be useful in any future Department of Forestry activities requiring broom control. In addition, the information we have gathered on the extent and timing of Douglas fir mortality has led us into an extensive inquiry into how the invasion of Scotch broom may alter the soil environment in ways that affect tree health.

This document reports on the results so far obtained from a series of studies conducted by Ingrid Parker (Professor, UC Santa Cruz) and Karen Haubensak (Adjunct Professor of Forestry, Northern Arizona University), along with Sara Grove (PhD student, UC Santa Cruz), and a number of UCSC undergraduates.
Key research questions for this phase of the project are:

1) Response of Douglas Fir:

    a) How did Douglas fir seedling mortality vary across sites and between November replanting and March replanting?

    b) Did treating plots multiple times before planting trees have a significant effect on the survival of Douglas fir trees?

c) Did the distance between a Douglas fir seedling and the nearest adult tree affect the probability of mortality?

d) Was mortality lower for trees in plots close to existing forest edges?

2) Response of Scotch broom:

a) In the absence of broom control treatments, how does germination vary across time and sites? How did germination from the seedbank respond to different broom control treatments?

b) How does the cover of Scotch broom increase over time, and how does that vary across sites? Comparing sites, how does the resprout rate, initial conditions of seed bank, and/or initial vegetation response appear to influence broom cover?

c) How did the total cover of broom in the plots over time respond to different broom control treatments?

d) How did the total cover of broom in the plots respond to herbicide treatments at different times of the year?

3) Scotch broom effects on soil (Greenhouse experiment)

a) Does soil from broom-invaded areas have a positive or negative effect on Douglas fir seedling growth, relative to soil from nearby uninvaded forest sites?

b) Does adding broom mulch to soil have a positive or negative effect on Douglas fir seedling growth?

c) What is the role of mycorrhizae in these plant-soil interactions?

Part I: Response of Douglas Fir Seedlings

Table 1. Treatments: PRE-Planting Experiment. For the pre-planting experiment, the following treatments were planned. Two of these (A and B) were planted in Spring 2008 and will need to be re-planted in Spring 2009. Three additional treatments (C, D, and E) will be planted for the first time in Spring 2009.

I.D.	Treatment
A	Control: Initial cut and mulch (Fall '07) only
B	Initial cut + Spring '08 soil scarification/seedling removal before planting
C	Initial cut + Spring '09 herbicide before planting
D	Initial cut + Spring '09 scarification/broom removal before planting
E	Initial cut + stimulation of seedbank in fall '08 + scarification/broom removal in Spring '09 before planting
F	Initial cut + seedling removal in Spring '09 + Spring '10 before planting

Experimental Overview and Methods

For details of the design, layout, maps of the sites, and methods of the Scotch broom control experiments, the reader is referred to earlier reports from Parker and Haubensak. Briefly, there are two field experiments, at each of four sites (Nisqually Plantation, Rumble Hill, TankTable, and Johnson Marsh Plantation). Although the two experiments were originally designed to be analyzed separately, inferences can (cautiously) be drawn from comparing them because the experiments were in contiguous areas and analyses have not shown significant differences between control plots in the two experiments. The "PRE" experiment was originally designed to study scarification treatments that would reduce the broom seed bank before planting trees. Only one-third of the plots were planted in the first year (Spring 2008). The "POST" experiment was originally designed to study control methods implemented after all Douglas fir seedlings were planted, and trees were planted into all plots at the same time (Spring 2008). A fifth site, Beal Hill, was included only in the POST experiment. Tables 1 and 2 provide an updated list of the treatments implemented in each of the 6 treatments (with 4 blocks per site).

Survival of 3,800 focal trees (all focal trees in POST blocks and focal trees in the subset of planted PRE blocks) was censused in May 2008, September 2008, May 2009, and September 2009.

Mortality of the Douglas fir seedlings after the first dry season (summer 2008) led to a re-planting of all sites in the second year. All dead trees were replaced in the POST experiment in November 2008. All focal trees (dead and alive) were

Table 2. Treatments: POST-Planting Experiment. For the post-planting experiment, we have the following treatments. All treatments start with adult broom control in fall/winter 2007 and were planted March 2008 (=Year 0). Because of mortality, all post-planting blocks will be partially replanted November 2008.

I.D.	Treatment
A	Control: Initial control and planting (Year 0)
B	Herbicide in September 2009 (Year 2).
C	Herbicide in May 2009 (Year 2)
D	Herbicide in September 2010 (Year 3)
E	Manual Cut in September 2010 (Year 3)
F	Herbicide in September 2011

replaced in the PRE experiment in March 2009. Live focal trees were transplanted to the border areas or else collected for assessment of mycorrhizal colonization.

We designed two small studies designed to test whether tree seedlings were more successful when planted near forest edges and established trees, than when they were planted in sites isolated from living Douglas fir trees. At the end of the planting season in March 2008, 425 trees were divided roughly equally between a north-facing edge and a south-facing edge at the Johnson Marsh plantation site. At each edge, a rectangular area was delimited roughly 10m x 100m, with the long side perpendicular to the forest edge. Trees were planted haphazardly with 8' spacing, not in a set grid. In the following year, we used a Trimble Geo-XH GPS with sub-meter accuracy to map the location of every tree, noted whether the tree was alive or dead, and also mapped the location of every nearby mature Douglas fir tree. We revisited each live tree in May 2009 and in September 2009 and recorded mortality.

Second, in the 2009 planting, we added a study to compare tree performance in the experimental plots (PRE and POST) with tree performance when planted on the edges of these large failed plantations. At the four sites with PRE experiment blocks (not Beal), we added one "edge plot" along a north-facing forest edge. Each edge plot had 4 rows of 15 trees (60 total) at 8-foot spacing. Background vegetation was not removed before trees were planted.

Results

1A) How did Douglas fir seedling mortality vary across sites and between November replanting March replanting?

Mortality of Douglas fir seedlings planted in 2009 was as catastrophic as in 2008 (for 2008 data, refer to October 2008 Report). By September 2009, more than 90% of the trees had died, and that was true across the five sites (Figure 1).

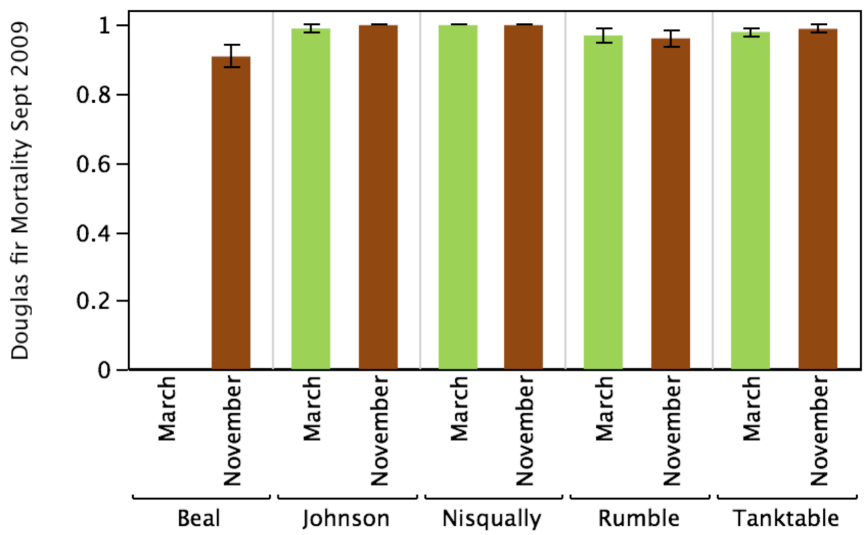

Figure 1. Mortality measured in September 2009 of Douglas fir seedlings replanted in November 2008 (in the POST experiment) and replanted in March 2009 (in the PRE experiments) across the same five sites. Beal only has POST plots, and therefore only experienced replanting in November.

The trees that were replanted in November 2008 during the height of the rainy season did not have either lower or higher mortality than trees replanted in March 2009, which was the more typical planting schedule for Joint Base Lewis-McChord (two-way ANOVA using four sites, Effect of Month: $F_{1,24}$=0.098, P=0.76). The effect of site was marginally significant ($F_{3,24}$=2.94, P=0.053), but there was no significant interaction between site and planting date ($F_{3,24}$=0.24, P=0.87).

We are forced to conclude that replanting in November instead of March did not produce any dramatic improvement in the ability of Douglas fir seedlings to establish and survive through the summer drought. It is possible that with different summer conditions, there might have been a difference between November and March planting; however, the extreme mortality we saw with little variance across plots equalized any small differences there might have been.

1B) Did treating plots multiple times before planting trees have a significant effect on the survival of Douglas fir trees?

In the PRE experiment, we were able to compare tree seedling performance across a range of treatments focused on removing competing vegetation, including the seedlings and resprouts of Scotch broom (Table 1). We used mixed-model ANOVA with block as a random effect nested within site, and with site and treatment as fixed effects, and the response variables were 1) mortality in May 2009, 2) tree height in May 2009, and 3) mortality in September 2009.

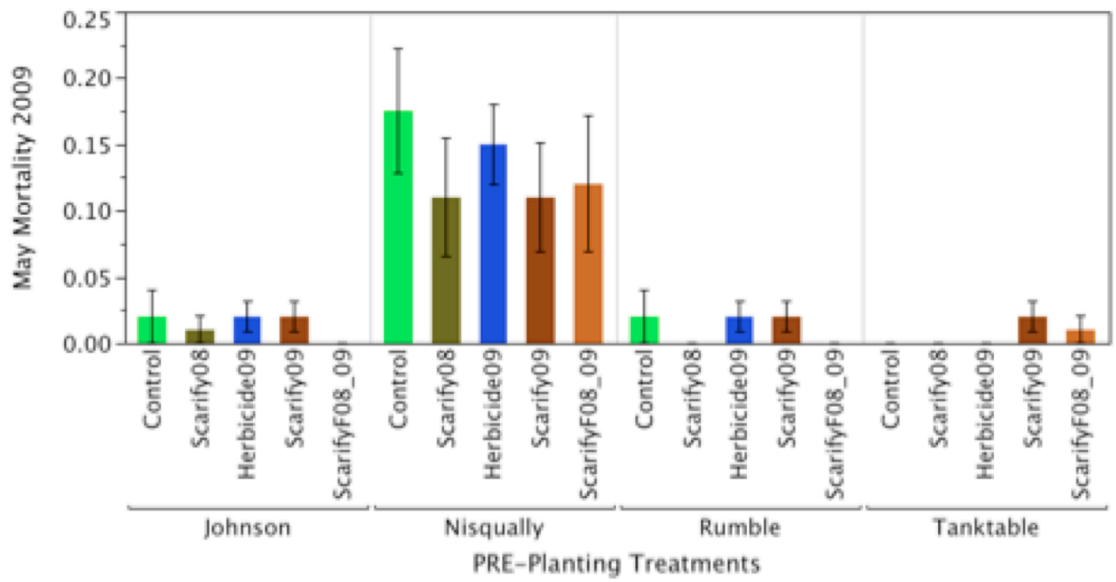

Figure 2. Mortality of Douglas fir tree seedlings measured in May 2009 in the PRE experiment. Trees were planted in March 2009. Treatments are as in Table 1.

We found a highly significant difference among sites in mortality between March and May (Figure 2; $F_{3,12}$=47.59, P<0.0001), but no difference among the treatments ($F_{4,60}$=0.76, P=0.55). For tree height in May, there was no significant effect of either site ($F_{3,12}$=1.87, P=0.19) or treatment ($F_{4,60}$=1.84, P=0.13).

By September 2009, mortality was so high that the significant difference among sites seen in May had disappeared (Figure 3; $F_{3,12}$=2.25, P=0.14). Treatment was also not significant for September mortality ($F_{4,60}$=1.23, P=0.31).

Overall, we could find no significant differences among the soil scarification and herbicide treatments for early tree establishment, in the face of intense summer mortality. This is in contrast to the results from the 2008 planting, when the mortality of trees was slightly higher in control plots

than in plots scarified a second time before planting (See Parker and Haubensak 2008 Report, Figure 11).

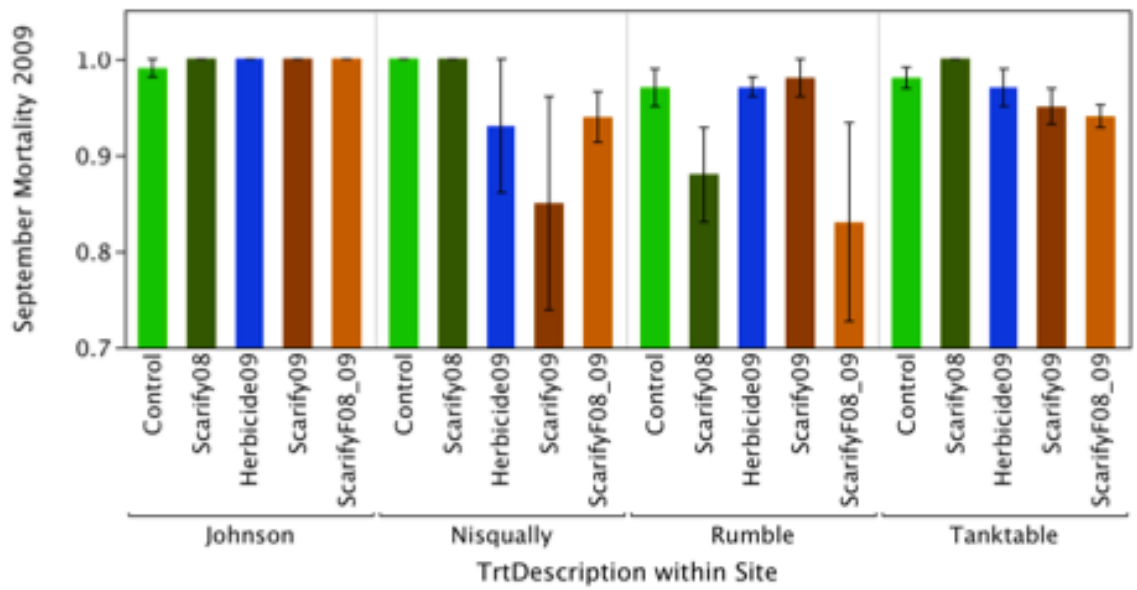

Figure 3. Mortality of Douglas fir tree seedlings measured in September 2009 in the PRE experiment. Trees were planted in March 2009. Treatments are as in Table 1.

1C) Did the distance between a Douglas fir seedling and the nearest adult tree affect the probability of mortality?

For the trees planted across a broad swath perpendicular to the forest edge in 2008, we used logistic regression with survival (May and September) as the response variable and distance to the nearest tree as the explanatory variable. We found a significant effect of distance to adult tree on mortality through the first year (Figure 4; May 2009 survival, DF=421, effect of distance: χ^2 =14.5, P<0.0001), with no significant difference between north-and south-facing edges (χ^2 =2.52, P=0.11). There continued to be a significant effect of distance on

Table 3. Numbers of trees surviving at Johnson Marsh Plantation in experiment to test the effect of distance to nearest adult tree on survival.

Edge	Total number of trees	Number Alive in May 2009	Number Alive in September 2009
North-facing	204	9	3
South-facing	220	4	4

mortality through the second summer (September 2009 survival, effect of distance: χ^2 =6.60, P<0.0102), with no significant difference between north-and south-facing edges (χ^2 =0.10, P=0.75). By the end of the experiment, the number of surviving trees was so low that effects of distance were hard to test because of small sample size (Table 3).

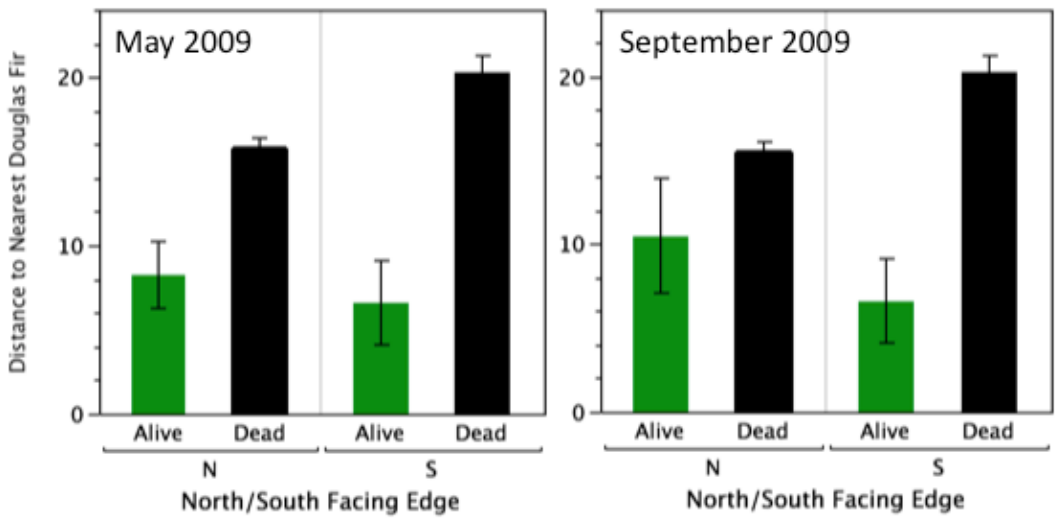

Figure 4. Distance to the nearest established Douglas fir tree for tree seedlings that died compared to those that did not die; trees were planted in March 2008 and data were collected in May 2009 (left panel) and September 2009 (right panel).

1D) Was mortality lower for trees in plots close to existing forest edges?

At the end of May 2009, after two months of growth in the field, trees in the small plots placed along North-facing edges showed lower tree mortality than plots in the center of the plantations (Figure 5). Using a very conservative statistical approach to avoid pseudoreplication, we still find a marginally significant effect of edge vs. center with the four sites as replicates (single-tailed, paired t = 2.04, P = 0.067).

However, four months later, all sites had experienced such high rates of mortality that there was not enough variation in mortality to see any meaningful differences (Figure 5). The difference between edge and center plots in September was not significant (single-tailed, paired t = 1.00, P = 0.19). However, at Rumble Hill, a surprisingly high proportion of trees were still alive in edge plots in September 2009. When this plot was revisited in May 2010, we found that only an additional 12% of the trees alive in September had died over

that eight-month period. As has been noted before, the first dry season appeared to be a critical time for the Douglas fir seedlings in this study.

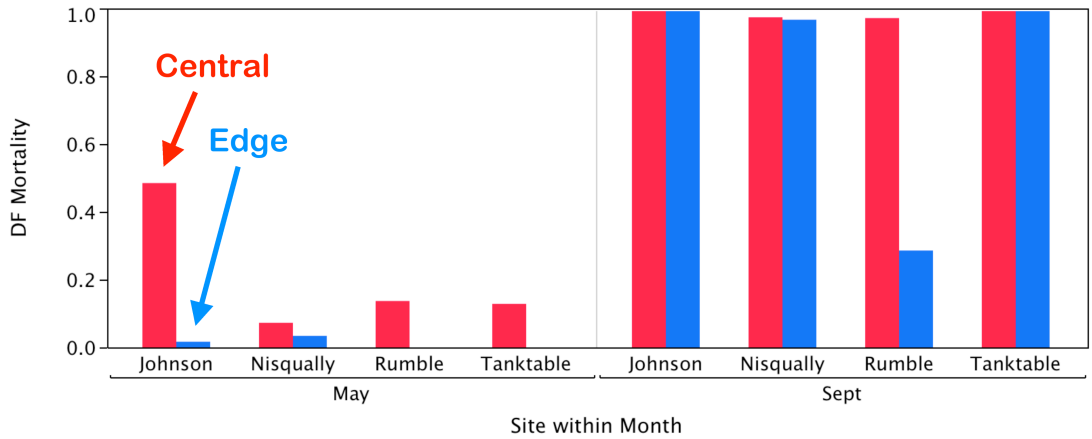

Figure 5. Mortality of Douglas fir seedlings planted in March 2009, showing the proportion of dead + nearly dead trees for plots at the center of the plantation site (red bars), and plots along the North-facing edge of remaining forest adjacent to the plantation site (blue bars). Edge trees were more successful than center trees at surviving through the first few months. By September, catastrophic mortality masked the edge effect at three of the four sites.

While we cannot conclude from the Edge Effects study that these edge plots were a great success in 2009, the survival of over 66% of the trees in the plot at Rumble is suggestive that edge planting may be a way to start rehabilitating large failed plantations, where large-scale reforestation efforts have failed.

Part II: Response of Scotch broom

Experimental Overview and Methods

We measured broom germination and cover in the plots within the sites described in Part I (above). In order to assess germination, we collected data on broom seedling density along a 24m x 0.1m-wide belt transect across the entire hypotenuse of the plot. To assess broom cover, we used the line-intercept method, recording every individual and the distance on the transect that it covered, along a 24 m transect tape laid across the plot perpendicular to the one for germination. This method was chosen after a pilot study found it to be the most accurate and efficient method across a wide range of broom densities.

Seedlings were counted in May 2008 (on a subset of plots), May 2009, and May 2010. Broom percent cover was measured in May 2009 and May 2010.

2A) What were background germination rates across sites and years? How did germination from the seedbank respond to different broom control treatments?

We were interested in quantifying germination across sites, as broom is notorious for the buildup of a large, untreatable seedbank. We found that in the control plots (i.e., in the absence of broom control treatments aboveground), germination declined predictably across sites, from 2008 following initial site preparation, to 2010 (Figure 6). However, there was a great deal of variation among sites. For example, at Rumble and Johnson, there was virtually no germination from the seedbank. Tank Table, in contrast, had abundant germination (~ 300 seedlings m^{-2}) in 2008 and 2009, decreasing to nearly zero by 2010.

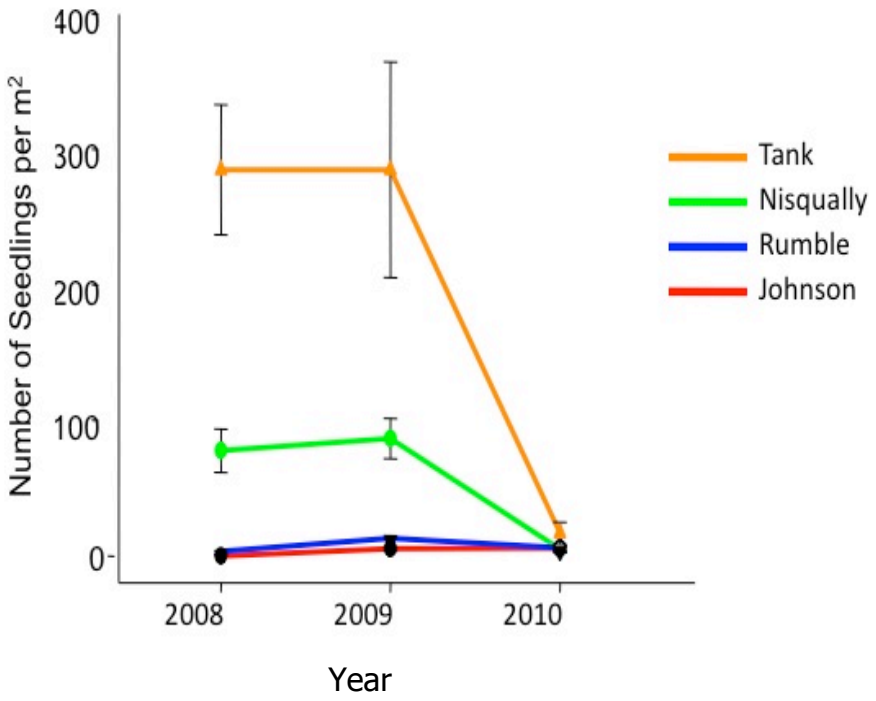

Figure 6: Germination (number of seedlings per m^2) in control plots across four sites in May 2008-2010.

We hypothesized that the lack of germination in 2010 resulted from one of two causes: 1) increased shading or other interference from the overstory, due

primarily to broom returning in these plots; and/or 2) lack of soil disturbance. We were able to examine the relative importance of these two causes by looking at the germination response to the particular broom control treatments that were designed to remove the overstory and to disturb the soil. The herbicide treatment caused removal of the overstory without soil disturbance; the soil scarification treatment removed the overstory while simultaneously causing soil disturbance. We compared plots that received either herbicide or scarification in spring 09, and measured for cover in 2010.

We found that there was a strong effect of treatment on broom germination (Figure 7; Two-way factorial ANOVA, $F_{11,47} = 5.28$, $p<0.0001$). There was a significant effect of site ($F_{3,36} = 7.74$, $p=0.0004$) and no significant interaction between site and treatment ($F_{6,47} = 1.73$, $p=0.14$). Using planned contrasts, we found that herbicide caused a significant increase in germination compared to the control ($F_{1,36} = 4.39$; $p=0.043$), and scarification also caused a significant increase compared to the control ($F_{1,36} = 23.48$; $p=0.00002$). In addition, scarification resulted in significantly more germination than herbicide ($F_{1,36} = 7.57$, $p=0.0092$).

From these results we conclude that seed germination drops off in control plots over time because of interference from growing broom overstory cover, and that, in addition, lack of soil disturbance suppresses germination further.

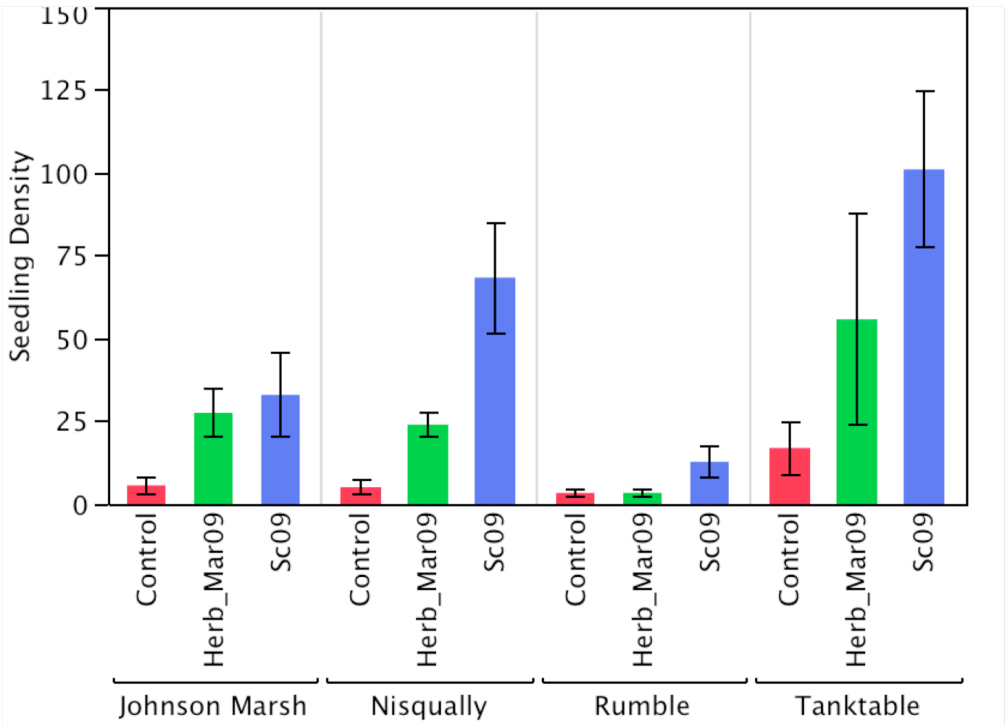

Figure 7: Germination (number of seedlings per m^2) in control (red bars), herbicide (green bars) and scarification (blue bars) treated in 2009 and measured in 2010, across sites. Bars are means +/- 1 SE.

2B) How does Scotch broom cover increase over time, and did that increase vary across sites? Comparing sites, how does the resprout rate and initial conditions of seed bank appear to influence these broom responses?

Here we examined broom cover in untreated (control) plots only, to determine the extent to which broom cover is increasing in the absence of broom removal treatments. We found that broom generally and predictably increased in cover between 2009 and 2010 (Figure 8). Some sites increased very little (e.g., Johnson Prairie, from an average of around 2% in 2009 to close to 5% in 2010), while others increased over three-fold (e.g., Nisqually, from ~15% in 2009 to over 50% cover in 2010). Broom cover at Tank Table, on the other hand, had already reached more than 40% mean broom cover when we began assessing total cover in May 2009, and it did not change between years.

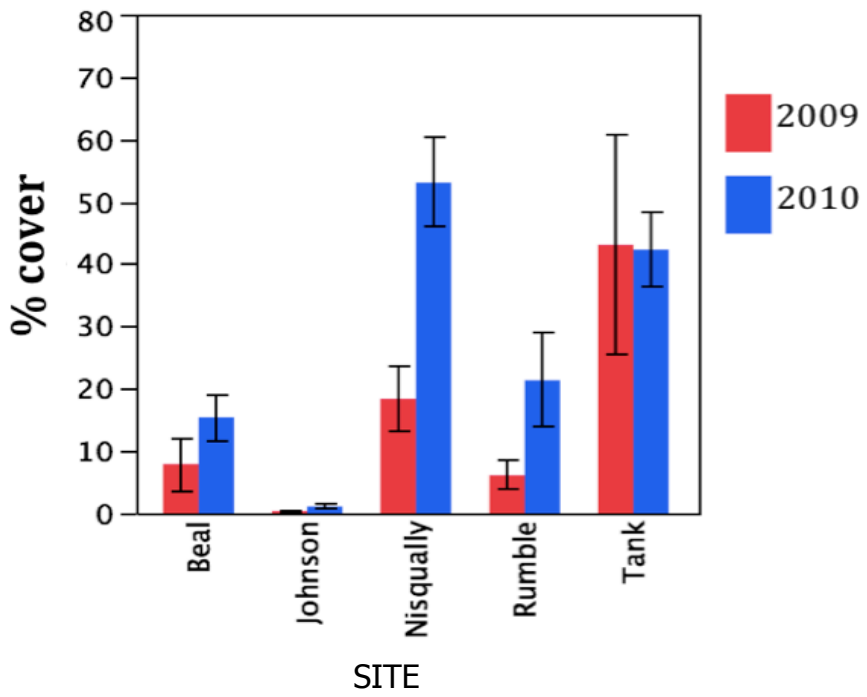

Figure 8: Percent cover of broom in untreated (control) plots in 2009 (red bars) and 2010 (blue bars) across all sites. Bars are means +/- 1 SE.

Qualitatively comparing the different sites, we find that **variation in germination rates seemed to be more predictive of broom cover two to three years after site prep than variation in resprouting.** Rumble was the only site that had high levels (nearly 40%) of resprouting, approximately four

times as high as any other site, but it was one of the slower sites to build up substantial broom cover. The initial seedling densities in 2008 were ranked as Tanktable > Nisqually > Beal > Rumble > Johnson Marsh; this is also the ranking of the sites for overall percent cover of broom in 2009. Tanktable in particular experienced germination of thick "blankets" of broom seedlings after the initial adult Scotch broom removal in fall 2007 (see Figure 6). Although initial germination seemed to drive percent cover of broom in 2009, already by 2010, other factors seemed to be influencing the rankings. Nisqually overtook Tanktable as having the most broom, and Rumble surpassed Beal. This may reflect individual growth rates being higher at these sites.

2C) How did the total cover of broom in the plots over time respond to different soil scarification treatments? Was this response comparable to herbicide?

Here we were interested in asking whether chemical treatment was preferable to mechanical (or vice versa), and whether there was any benefit to treating plots twice (once each of two years) versus once. For these questions we compared plots sprayed with Garlon herbicide in spring 09 to those scarified at the same time (chemical versus mechanical); we secondly compared plots that had been scarified once in spring 09 to those that had been scarified both in fall 08 and spring 09 (1x versus 2x scarification).

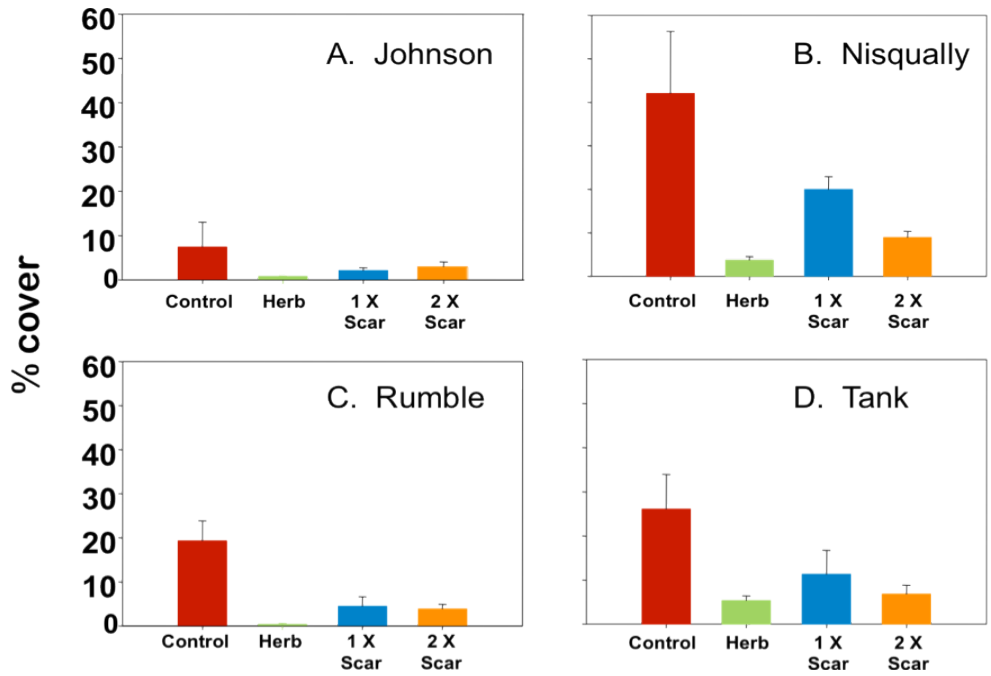

Figure 9: Percent cover of broom in control (red bars), single application of herbicide (green bars), single application of scarification (blue bars), and double application of scarification (orange bars), all treated in 2009 and measured in 2010, across sites. Bars are means +/- 1 SE.

We found after measurement in spring 2010 that there was a clear benefit to any removal treatment; all treated plots had significantly lower broom cover compared to control plots ($F_{15,63} = 5.42$, $p<0.0001$) (Figure 9).

Using planned contrasts to compare means, we found that scarifying once versus twice resulted in a similar outcome: broom cover was ~25-50% lower in scarified plots compared to control, and there was no difference between a single versus double scarification ($F_{1,48} = 1.23$; $p=0.27$). Again using planned contrasts, we found that herbicide was better than scarification at reducing broom cover ($F_{1,48} = 4.07$; $p=0.049$). Overall, however, it is clear that any treatment is preferable to doing nothing for reducing broom cover.

2D) How did the total cover of broom in the plots respond to herbicide treatments at different times of the year?

In developing the design of this experiment, we were struck by the variability in opinion among restoration experts about the best time of year to apply herbicide for broom control. Because of this, we incorporated the question of seasonality into the herbicide component of our experiment. We included a spring (March) spray, an early summer (May) spray, and an early fall (September) spray. The March spray took place during one dry day in the midst of a fairly consistent block of days of rain, and was part of the PRE experiment. The May spray was conducted during a dry period, as was the September spray; both were part of the POST experiment. We predicted that because broom is in different physiological states at these different periods, efficacy of spraying should depend not only on the weather conditions, but also on whether broom is actively growing, allocating resources to reproduction, etc.

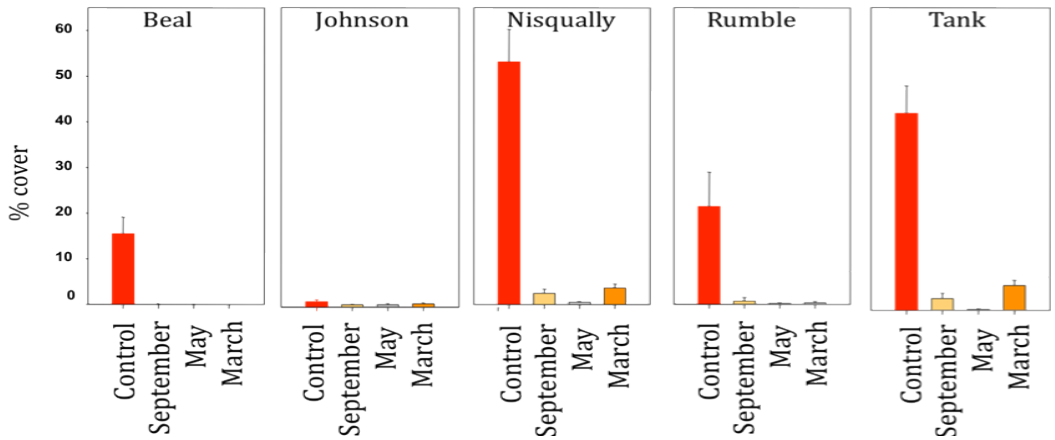

Figure 10: Percent cover of broom in control (red bars), September spray (yellow), May spray (gray) and March spray (orange) across sites. Plots were treated in 2009 and measured in May 2010. Bars are means +/- 1 SE.

We found that despite large differences in conditions among the three time points that we applied herbicide, there was no significant difference in broom cover among the three treatments (Figure 10). All appear to have dramatic effects on broom cover; in particular, Nisqually and Tank Table have 40-50% cover in control plots, and less than 10% cover in sprayed plots. **Overall, herbicide was extremely effective in reducing broom cover, and the time of year for herbicide application appears to be relatively unimportant.**

Part III Legacy Effects: Scotch broom effects on soil

Experimental Overview and Methods

In fall 2008 we began a greenhouse experiment to examine multiple potential effects of Scotch broom on soils. Scotch broom is a nitrogen-fixing plant and therefore may 'fertilize' soils with increased N availability. At the same time, however, broom produces N-rich defense compounds (alkaloids) that have been shown elsewhere to inhibit the growth and activity of some plants and microbes. Both these effects may not only directly affect DF growth, but also indirectly via mycorrhizal associations which may themselves be altered by changes in soil chemistry. With our greenhouse experiment, we first wanted to test whether Douglas fir grew better in soils collected from broom-invaded, harvested areas, or in uninvaded forest soils.

Because broom control in the field includes mulching of biomass into surface soils, we included a treatment of broom mulch addition. We also implemented treatments in the greenhouse to separate the effects of N and alkaloids by adding 1) sucrose to reverse the fertilization effect and 2) activated carbon to reverse the toxic effect of alkaloids. This fully-factorial experiment included 32 different treatments (2 sites x 2 soils (forest/broom) x 2 mulch treatments (+/-) x 2 activated carbon treatments x 2 sugar treatments).

Field soils were collected from paired, broom-dominated and uninvaded forest soils at two locations on Joint Base Lewis-McChord in September 2008. Soils from each site were homogenized and passed through a large-screen sieve to remove rocks and large root masses. At UC Santa Cruz, soils were mixed with broom mulch, activated carbon, and/or sucrose. These soils were distributed into 1L conetainers, with 15 samples per treatment. Into these conetainers, we planted Douglas-fir seedlings grown from southwestern Washington seed sources and purchased from Silvaseed in Roy, WA. The seedlings were left to grow outdoors in the natural light and temperature conditions for 19 month*s.*

Douglas fir growth

We measured initial height, timing of bud break, and survival and height in Jan 2009, April 2009, July 2009, and Jan 2010. In April 2010, we measured final tree heights then harvested the seedlings to measure aboveground biomass for all trees, and the belowground biomass of a subset of 232 trees. Biomass was dried at approximately 61°C for at least 4 days and then weighed.

Ectomycorrhizae

We assessed the diversity and colonization rates of ectomycorrhizal fungi on 120 trees across 8 of the 32 soil treatments: two sites x two soil types (broom/forest) for the control (unamended) soils, plus two forest soils + mulch, plus two forest soils + mulch + activated carbon.

The roots selected for mycorrhizal assessments were carefully cleaned in a series of water baths. We took great care not to remove any hyphae from the root tips. The cleaned and cut root tips were stored in a falcon tube of DI water and kept in a refrigerator at 3 degrees C and examined for EMF no more than seven days after the initial processing.

To measure EMF colonization rates, we placed the prepared root tips into gridded petri dishes. We assessed the presence or absence of ectomycorrhizal fungi for each of 56 randomly-selected root tips per tree seedling. Each mycorrhizal root tip was assigned a morphotype based on a number of morphological features. If the fungal mantle was not readily identified through the dissecting scope, had ambiguous morphology or was presumed to be absent of ectomycorrhizal fungi, we further examined cross sections of the root under a compound light microscope. Six-15, stained cross sections were observed until a clear presence or absence diagnosis could be made.

Additionally, we stored the first 36 observed roots in a 2X CTAB solution for species identification by DNA sequencing of the ITS region, This sequencing work is currently underway.

We used ANOVA to explore the effects of soil type, site, and treatments on Douglas fir growth. For the purposes of this report, we are focusing on final aboveground biomass at harvest. Seed lot was treated as a (random variable) blocking factor. The sugar treatment turned out to have no effect on the measured variables, so we removed the sugar effect from the model. This leaves a four-factorial ANOVA with Site, Soil Type, Mulch, and Activated Carbon as the four factors (Table 4).

Table 4. Four-factorial ANOVA for final aboveground biomass of Douglas fir seedlings at harvest. The degrees of freedom for all tests are 1, 425. Seed source is included in the model as a (random) blocking factor.

Effects	F Ratio	P-value
Site	1.15	0.28
Soil type	25.53	<0.0001
Mulch	5.57	0.019
Activated Carbon	0.31	0.58
Site * Soil type	0.36	0.55
Site * Mulch	14.25	0.0002
Soil type * Mulch	1.34	0.25
Site * Soil type * Mulch	0.38	0.54
Site * Act. Carbon	1.30	0.25
Soil type * Act. Carbon	7.85	0.005
Site * Soil type * Act. Carbon	0.38	0.54
Mulch * Act. Carbon	2.79	0.095
Site * Mulch * Act. Carbon	0.01	0.93
Soil type * Mulch * Act. Carbon	4.58	0.033
Site * Soil type * Mulch * Act. Carbon	0.04	0.84

3A) Does soil from broom-invaded areas have a positive or negative effect on Douglas fir seedling growth, relative to soil from nearby uninvaded forest sites?

The clearest and most consistent result from the greenhouse experiment was that Douglas fir seedlings grew more poorly in the two broom-invaded soils than in the two forest soils (Figure 11). The main effect of Soil type was highly significant, and there was no significant interaction between Site and Soil type (Table 4). **This provides support for the idea that the dominant effect of Scotch broom on the soil's ability to support Douglas fir is negative, not positive.** However, because these soils were collected from different areas, a number of features might differ between them. For example, the broom-invaded areas had been clearcut and the forested areas had not. We cannot say definitively that the presence of the broom per se caused the soil to be poor for Douglas fir growth. The pattern, however, is suggestive.

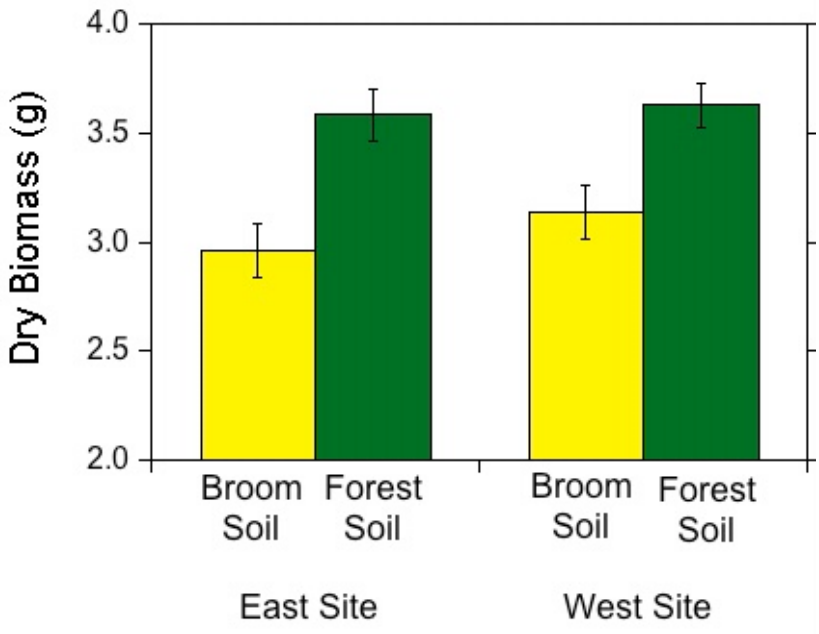

Figure 11. Final aboveground biomass of Douglas fir seedlings grown in either broom-invaded soils from harvested areas, or uninvaded forest soils. Bars are means +/- 1 SE.

3B) Does adding broom mulch to soil have a positive or negative effect on Douglas fir seedling growth?

In contrast to the above result, when broom mulch was added to soil, Douglas fir seedlings grew more, rather than less (Figure 12). The pattern was seen in both broom-invaded and forest soils, but only for soils from the West site, not the East site. This is reflected in the significant main effect of Mulch, the significant interaction between Mulch and Site, and the lack of significant interaction between Mulch and Soil type or three-way interaction between Mulch, Site, and Soil type (Table 4).

Our results from mulch addition suggest that there may also be a role for positive (fertilization) effects of the broom vegetation on this system. We are puzzled by the fact that this positive effect of mulch addition was so clear in soils from one site and not from the other site. We suspect the West site is more nitrogen limited than the East site, and we are investigating this hypothesis with professional soil analysis. These data have not been analyzed yet.

Another interesting aspect of these data is that, for forest soils, there is an interaction between mulch addition and activated carbon. That is, when mulch is added, there is an increase in Douglas fir growth, but only in the presence of

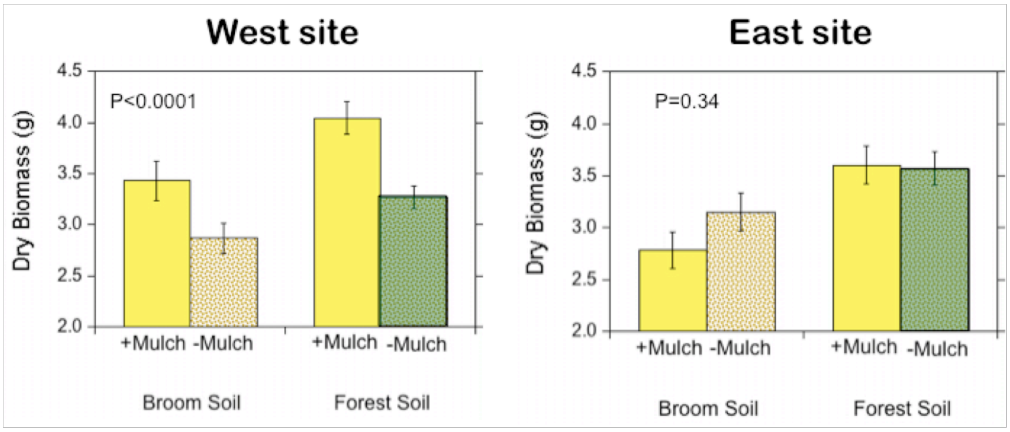

Figure 12. Final aboveground biomass of Douglas fir seedlings grown with or without mulch, in either broom-invaded soils from harvested areas, or uninvaded forest soils. Bars are means +/- 1 SE.

Activated Carbon (AC) (Figure 13). This can be seen with a planned contrast comparison of +Mulch/+AC vs. +Mulch/–AC using forest only soils, which is significant for both the West site ($F_{1,425}$ =5.40, P=0.02) and the East site ($F_{1,425}$ =6.91, P=0.009). These results suggest that there could be a positive fertilization effect of the mulch that is counteracted by a negative allelopathy effect.

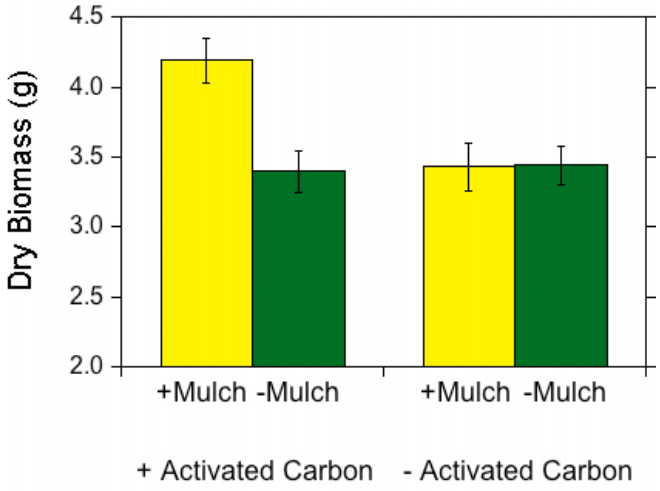

Figure 13. Final aboveground biomass of Douglas fir seedlings is higher when Scotch broom mulch is added, but only in the presence of Activated Carbon.

In contrast, the negative effects of broom soil compared to forest soil are not counteracted by the addition of Activated Carbon. This is consistent with the hypothesis that it is not direct chemical interference between the broom and the Douglas fir, but rather indirect effects through soil microbes, that may be mediating the reduction of growth in broom soil.

3C) What is the role of mycorrhizae in these plant-soil interactions?

Mycorrhizal colonization varied from 14% to 100% of the root tips on an individual plant. Congruent with the biomass data, we found that mycorrhizal colonization was higher in forest soils than in broom-invaded soils (Figure 14). Using just control soils (no mulch), we saw a significant effect of Soil type ($F_{1,55}$=7.66, P=0.0078) and a marginally significant effect of Site ($F_{1,55}$=3.00, P=0.089), but no significant interaction between Soil type and Site ($F_{1,55}$=0.74, P=0.39).

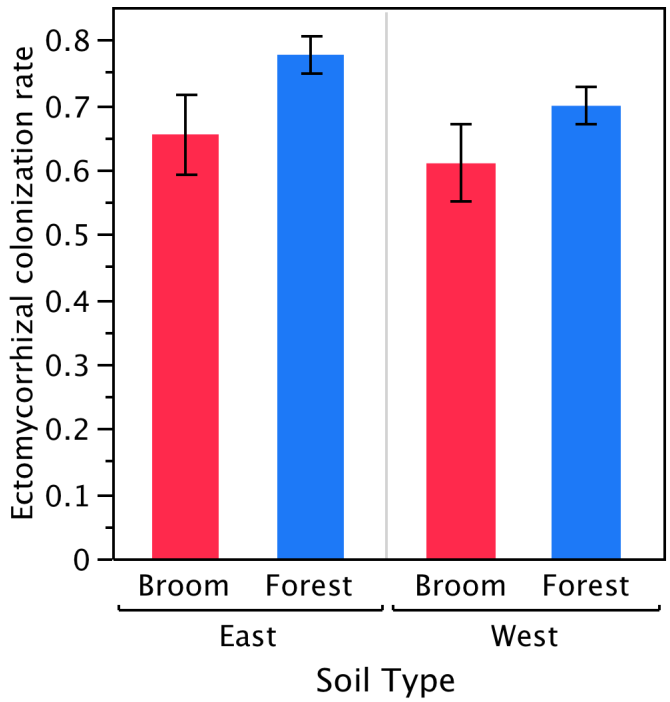

Figure 14. Ectomycorrhizal colonization of roots of Douglas fir seedlings grown in broom-invaded and forest soils for East and West sites. Bars are means +/- 1 SE.

When we used the forest soils alone to test for an effect of Mulch, we expected to see a reduced mycorrhizal colonization for the mulch addition treatment, and an amelioration of the effect of mulch when Activated Carbon was added.

However, although the trends were in the expected direction (Figure 15), we did not find any significant differences among these three treatments ($F_{2,78}$=1.09, P=0.34)

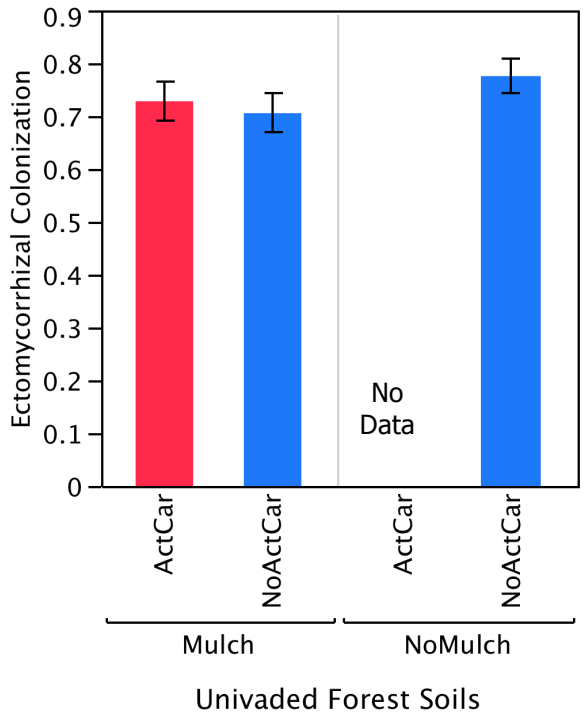

Figure 15. Ectomycorrhizal colonization of roots of Douglas fir seedlings grown in forest soils, to which Mulch was added (or not) and to which Activated Carbon was added (or not). Bars are means +/- 1 SE.

Given the rather small dataset, we feel it is difficult to draw strong conclusions from the mycorrhizal results except to say that mycorrhizae seem to be depressed in broom-invaded soils. In November-December 2010, PhD student Sara Grove and undergraduate Megan Bontrager set up two new greenhouse experiments designed to explore different aspects of the relationship between broom, mycorrhizae, and Douglas fir growth.

In addition, UCSC undergraduate Holly Makagon completed her senior thesis in December 2010, testing two aspects of mycorrhizal colonization in field-collected Douglas fir seedlings from the original 2008 planting. First, she used 8 trees per site to compare colonization rates among the four PRE sites (Tanktable, Nisqually, Rumble, and Johnson Marsh). She found no significant variation among sites, and most of the variance was partitioned among individual seedlings rather than among blocks or sites. Second, she used seedlings from

Nisqually Bridge Plantation to test whether seedlings collected from close to an established Douglas fir tree had higher mycorrhizal colonization than those collected from farther away from the same tree. She found that yes, indeed, mycorrhizal colonization was lower in the more isolated seedlings.

One criticism of this type of work is that the degree of overall mycorrhizal colonization does not capture potentially important variation in the presence of particular "key" mycorrhizal species. Colonization rate may mask variation in fungal composition that is very important to how the plant responds. Our work in progress on this system will address this problem in two ways: first, by gaining information on the identity of the fungi, building on morphotype work we have been doing at the microscope and adding molecular identification approaches. Second, one of our greenhouse experiments will directly test whether growth of Douglas fir may be mycorrhizae-limited. We are using a dose-response approach, growing Douglas fir seedlings with different dilutions of field soil 'inoculum' in the greenhouse. Through this work we hope to gain insights into why Douglas fir seedlings planted into sites long-invaded by Scotch broom experience extremely high mortality, even after the broom has been removed.