Title

Chemical and Mechanical Control of *Cytisus scoparius* Across the Life Cycle. Technical report submitted to Joint Base Lewis-McChord.

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Chemical and Mechanical Control of *Cytisus scoparius* Across the Life Cycle

Final Report,
Forest Regeneration under Scotch Broom Control, Phase I
Submitted to Joint Base Lewis-McChord
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Introduction and Findings to Date

Control of the invasive weed *Cytisus scoparius* (Scotch broom) is one of the primary land management challenges at Joint Base Lewis-McChord (JBLM). Management of this plant is the focus of hundreds of personnel hours per year at JBLM, and the conversion of both prairie and working forest land to *Cytisus* scrub results in both the loss of land available for military training as well as a loss of native habitat for plants and animals. Tree plantations have failed repeatedly in areas that had once supported Douglas fir forest, with *Cytisus* invasion replacing forest in what appears to be a permanent state change of the ecosystem (Figure 1). The economic impact of *Cytisus* outside of JBLM is also dramatic: *Cytisus* is responsible for an estimated $100 million in lost sales and personal income in Oregon alone (Oregon Department of Agriculture, 2000).

In fall 2007, a large-scale collaboration was formed among the JBLM Forestry Department, the University of California Santa Cruz (UCSC) and Northern Arizona University (NAU) to study the effectiveness of different land management approaches to *Cytisus* control in the context of forest regeneration. Planning for *Cytisus* control has become an unavoidable part of JBLM silviculture, and science-based decision-making is the key to efficient and effective forest management.

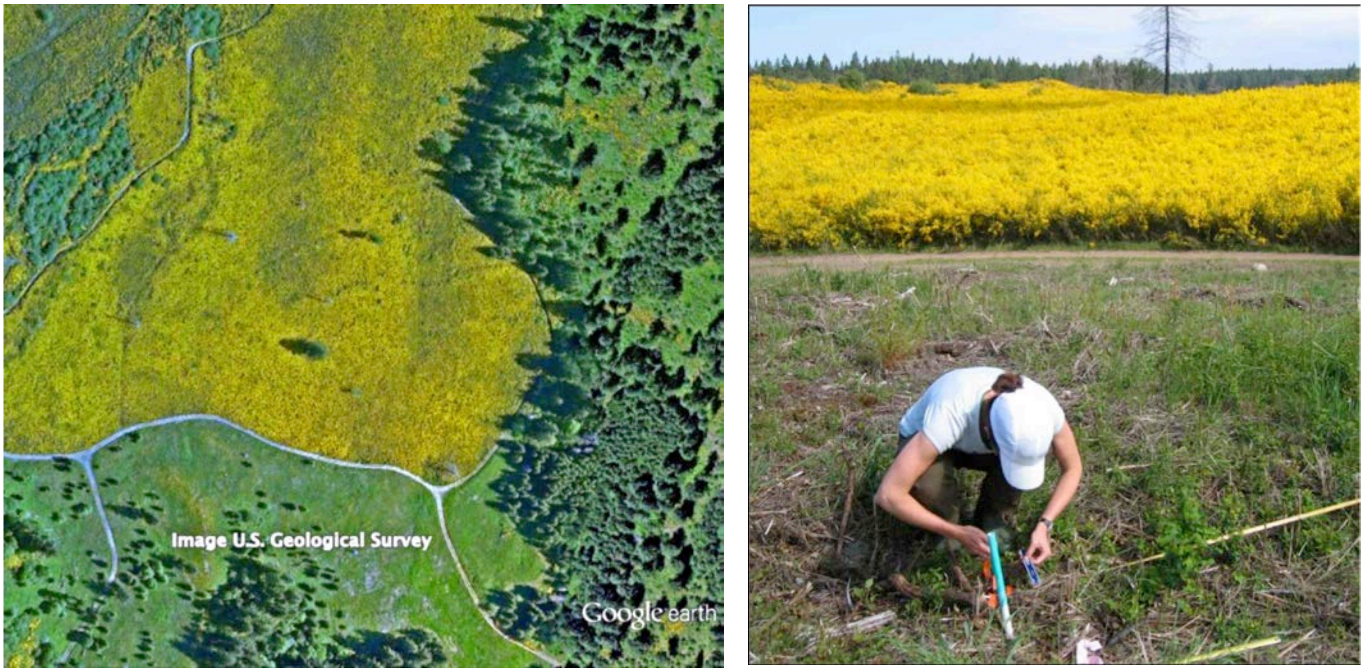

Figure 1. *Cytisus* invasion at Rumble Hill, May 2009, showing extent and impact of invasion. Area in the foreground had *Cytisus* removed by large machinery in fall 2007.

Our primary objectives were to examine approaches to *Cytisus* control specifically relevant to forestry application. In particular, JBLM forestry is constrained to strategies that avoid the use of fire and do not damage young tree seedlings. On the other hand, unlike other applications, in the forestry context non-target effects on native vegetation are not a focal point; silvicultural practices generally include the total removal of understory vegetation before planting. The studies presented here have two main foci: timing of control relative to the *Cytisus* life cycle, and chemical vs. manual control. The original design of the main field study considered the response of planted Douglas-fir seedlings equally with the response of *Cytisus*. However, massive Douglas-fir mortality in the early years of the study required a shift away from the tree responses, although subsequent experiments have addressed the interactions between *Cytisus* and Douglas-fir in both greenhouse and field. These experiments are ongoing and will not be discussed further in this report.

Our secondary objectives included understanding the mechanisms underlying *Cytisus*-Douglas-fir interactions. The discovery of massive tree mortality even in the absence of direct competition from the *Cytisus* led us to questions about how *Cytisus* invasion may alter the soil environment in ways that affect tree health even after *Cytisus* removal. Because *Cytisus* is a nitrogen-fixing plant, the interactions include a combination of positive effects through nitrogen fertilization and negative effects through inhibitory secondary chemistry. We continue to explore these mechanisms in field, lab and greenhouse experiments, however a discussion of these experiments is not included in this report.

Finally, our work has also focused on strategies of planting Douglas-fir that might mitigate the negative effects of *Cytisus* invasion in large clear-cuts. We have tested the relative success of planting seedlings along forest edges (edge effect experiment), and we have tested whether leaving land fallow for a period of time after removing *Cytisus* and before planting trees allows for system recovery (legacy effects experiment).

* * *

***Results from previous reports*.** Many insights that have been gained from our experiments both in the field and in the greenhouse. Following is a partial list of our conclusions from previous reports (Parker and Haubensak 2008, Parker et al. 2012), where data, statistics, and detailed methods are provided:

1. Douglas-fir seedlings planted into failed plantations died in the first year, **well before competition from *Cytisus* could cause mortality**.
2. Sites show **extreme variation in how many *Cytisus* seedlings germinate** after *Cytisus* removal. Germination patterns strongly affect how quickly sites are reinvaded and the physical structure of the

vegetation, which in turn strongly affects the type of mechanical control that is most effective.

3. Sites show extreme variation in the probability that *Cytisus* will resprout after cutting. Although the site with the smallest *Cytisus* plants (Rumble) had the highest resprout rate, within any given site resprouting was not higher for smaller plants. In addition, **resprout rate was not strongly predicted by how high off the ground operators cut the plants**.
4. Pre-treatment of sites to exhaust the *Cytisus* seed bank using **mechanical soil disturbance ("scarifying") was shown to have important disadvantages** compared to mechanical or herbicide removal of *Cytisus* regrowth later. Not the least of these was the significant wear and tear on brushcutting equipment caused by the need to intensively disturb the soil.
5. Double-scarification, in which the ground was disturbed to flush seedlings and then re-disturbed to kill seedlings, did not result in lower *Cytisus* cover than single scarification. **This approach should not be used**, unless the objective is to induce maximal germination followed by some other control method.
6. Seed germination drops off in control plots over time due to a **combination of interference from growing *Cytisus* overstory cover and lack of soil disturbance.**
7. Despite assumptions that *Cytisus,* a nitrogen-fixer, should promote Douglas-fir growth by fertilizing the soil, our initial greenhouse experiment showed that **Douglas-fir seedlings grew poorly in *Cytisus*-invaded soil**. This was the first study to demonstrate that *Cytisus* has a "legacy effect"—an impact on the soil that remains even after removal. This study was published in the journal Plant Ecology (Grove et al. 2012).
8. *Cytisus* litter may have a negative effect on plant growth through **allelopathy**, a theory supported by the positive response of Douglas-fir to adding activated carbon in combination with *Cytisus* mulch.
9. **Ectomycorrhizae associated with Douglas-fir were depressed** in *Cytisus*-invaded soils, which could partly explain why seedlings grew poorly.

This document reports on the final results from studies conducted by Ingrid Parker (Professor, UC Santa Cruz), Karen Haubensak (Research Professor, Northern Arizona University), and Sara Grove (PhD student, UC Santa Cruz), with help from many UCSC and NAU undergraduates and graduate students. Much of the work presented concerns a large, randomized experiment implemented at five clear-cut sites. This experiment was established in the fall of 2007, and here we report on data collected September 2012, after five years of growth following initial *Cytisus* removal.

Key research questions for this report are consistent with previous years' questions, but updated with 2012 data:

I. **The temporal and spatial dynamics of *Cytisus* invasion:**
   a) How does *Cytisus* germination vary among sites and across time after initial site preparation?
   b) How does the cover of *Cytisus* increase over time in untreated plots, and how does it vary among sites?
   c) How does height of *Cytisus* increase over time, and does this change vary across sites?

II. **Response to treatments targeting early life stages of *Cytisus***:
   a) How did one vs. two soil scarification treatments affect the cover of *Cytisus*
   b) Was the response to herbicide comparable to scarification?
   c) Does waiting longer for the seedbank to flush increase the effectiveness of scarification?

III. **Response to treatments targeting larger *Cytisus* plants**
   a) How did the seasonal timing of herbicide treatment affect *Cytisus* cover?
   b) What is the relative effectiveness of chemical control vs. manual control on older plants?
   c) Did plant age / size affect herbicide spray effectiveness or the amount of *Cytisus* cover in 2012?

IV. **Edge effects on tree seedling establishment, and soil amendment as a restoration technique**
   a) Is tree establishment consistently higher near forest edges?
   b) Are mycorrhizal fungi more abundant near forest edges?
   c) Can soils transplanted from nearby forests effectively restore EMF communities and ameliorate negative effects on Douglas fir?

Experimental Overview and Methods

In 2008 we implemented two field experiments at each of four sites (Nisqually Plantation, Rumble Hill, TankTable, and Johnson Marsh Plantation) with a fifth site (Beal Hill) containing only one of the field experiments. Although the two field 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). Tables 1 and 2 provide an updated list of the treatments implemented in each of the 6 treatments (with 4 blocks per site).

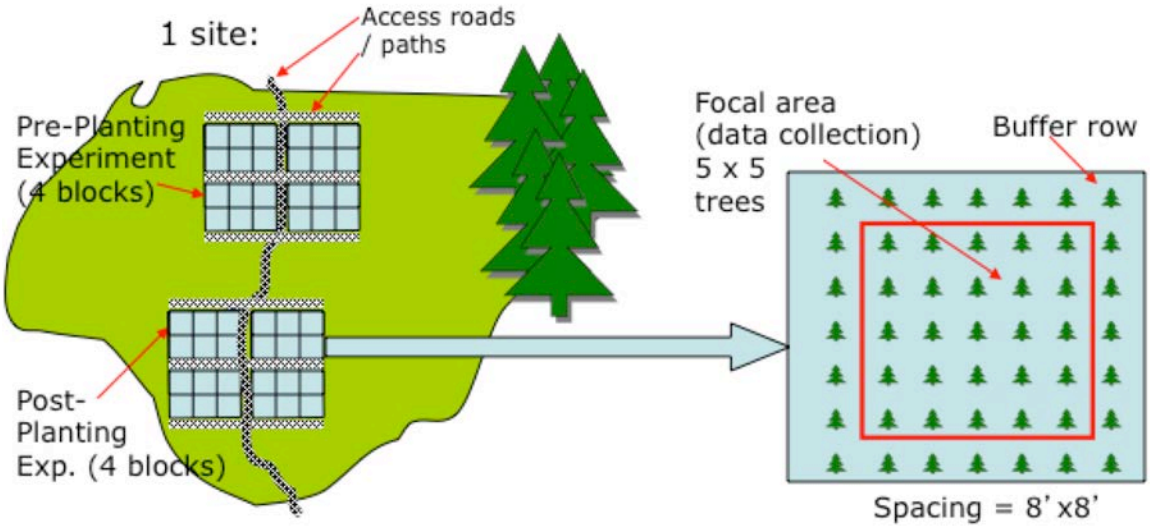

Figure 1. Generalized experimental design for PRE and POST experiments. Each site contains four blocks per experiment, with six 56' x 56' plots per block and 49 trees planted per plot.

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 were re-planted in Spring 2009. Three additional treatments (C, D, and E) were intended to be planted for the first time in Spring 2009 but planting did not occur. See text for further details.

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

Table 2. Treatments: POST-Planting Experiment. For the post-planting experiment, we have the following treatments. All treatments began with adult broom removal in fall/winter 2007 and were planted with DF seedlings in March 2008 (=Year 0). Because of mortality, all post-planting blocks were 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

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. These survival data were reported in Parker and Haubensak (2010).

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 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.

I. Temporal and spatial dynamics of *Cytisus* invasion

We measured broom germination and cover in the plots within the sites described 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 linear distance that it covered on the transect, 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, May 2010, September 2011, and September 2012. *Cytisus* percent cover was measured in May 2009, May 2010, September 2011 and September 2012.

1A) How does *Cytisus* germination vary among sites and across time after initial site preparation?

We examined broom germination in the control plots (with no additional treatment after initial broom removal) to understand spatial variation and dynamics of the seedbank. We found that germination spiked in 2009 but with a great deal of variability within sites, underscoring the patchiness inherent in this process (Fig. 2). By 2010 however, germination at all sites declined to very few new seedlings. This pattern persisted through 2012.

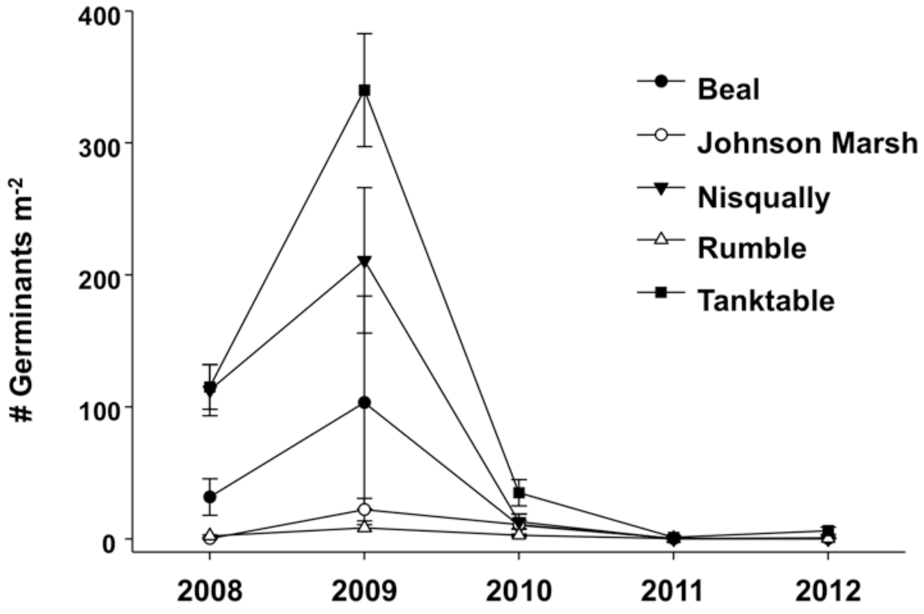

Figure 2: Germination (number of germinants m^{-2}) in control plots across all sites in May 2008-2012. Symbols represent means +/- 1 SE. Where standard error bars are not visible, they are smaller than the symbols.

1B) How does the cover of *Cytisus* increase over time in untreated plots, and how does it vary among sites?

The broom in all five sites was completely removed in fall 2007/winter 2008 in preparation for these experiments. We documented the recovery of broom in untreated (control) plots as percent cover increased over time in the absence of further removal treatments. Broom cover increased in all sites between 2008 and 2012, with the most rapid increase occurring between year 2 and year 3 (Fig. 3; Also see Appendix II). Interestingly, Tanktable (which had the highest initial germination rates) had the highest broom cover after one year of growth, but by year two Nisqually had overtaken it and remains the densest site. Johnson Marsh has shown a much slower invasion than the other sites, and even after four years the broom cover is only ~20%. The other sites are nearing or have surpassed 100% cover. Nisqually has the highest percent cover at 150%, which is effectively a closed canopy of broom.

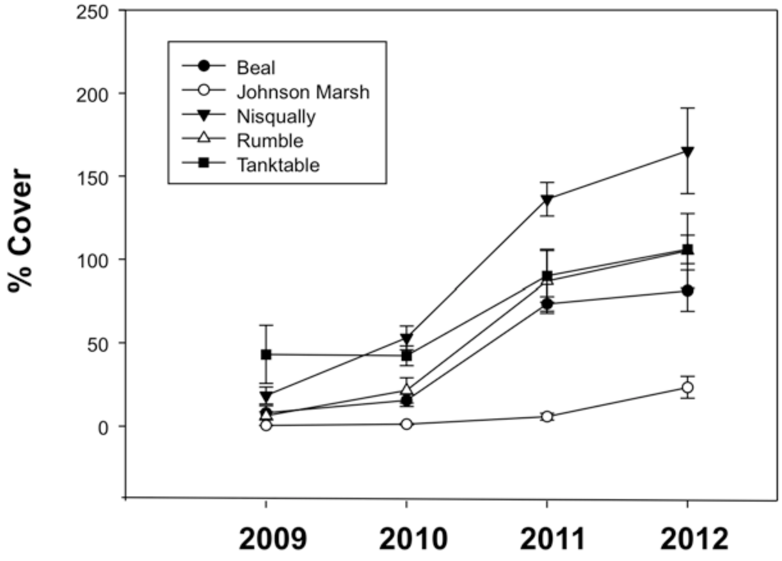

Figure 3: Percent cover of broom in untreated (control) plots in 2009-2012. Symbols represent means +/- 1 SE. Where standard error bars are not visible, they are smaller than the symbols.

1C) How does height of *Cytisus* increase over time, and does this vary across sites?

We began collecting plant height data in 2011; over the following year the average height of broom plants in untreated plots increased substantially (Fig. 4). Nisqually had the tallest plants, with the average height in 2012 over 2.5 m (~8.5 ft), while plants at Johnson Marsh were relatively small at less than 1 m (~3 ft).

The difference in plant height between 2011 and 2012 gives us an indication of plant growth rates. Interestingly, the absolute amount of height increase was remarkably similar among sites, which means that the sites with smaller plants grew more as a proportion of their size. These relative growth rates varied substantially among sites, with plant heights at Johnson Marsh and Tanktable increasing by ~40% and those at Nisqually and Rumble increasing by ~20%.

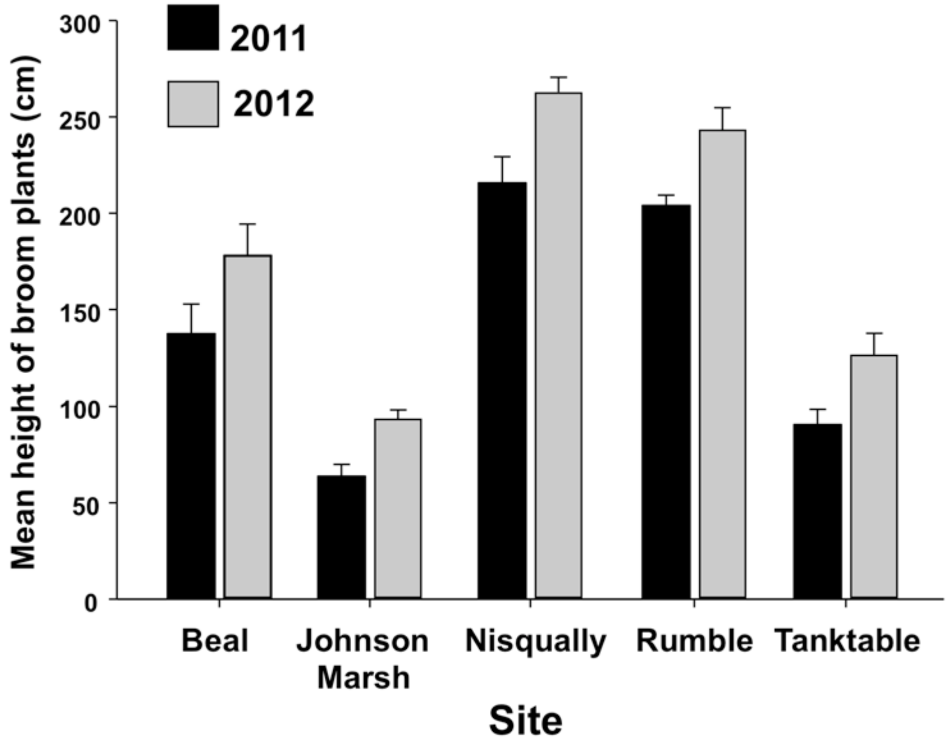

Figure 4: Height of broom (cm) in untreated (control) plots in 2011 and 2012. Bars represent means +/- 1 SE.

II. Response to Treatments Targeting Early Life Stages of *Cytisus*

Since 2010 we have examined the question of whether chemical treatment is preferable to mechanical (or vice versa), and whether there is any benefit to treating plots twice (once in each of two years) versus once. This year we have added the question of whether it is better to wait for one or two years following initial adult broom removal from a site; we examined this additional question in the context of both single-year versus two-year treatments. 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).

2A) How did the cover of broom over time respond to one vs. two soil scarification treatments?

2B) Was the response to herbicide comparable to scarification?

In 2010 we found that there was a clear benefit to any removal treatment (except at Johnson Marsh where there was hardly any *Cytisus*); all treated plots had significantly lower broom cover than control plots (Appendix I, Fig. A). Measured one year after treatment, the herbicide treatment was trending to be more effective than scarification, although this difference was not significantly different with a Tukey's post-hoc test.

By 2011 plants had grown up substantially, and the treatment effect was not significant at either Johnson Mash or Tanktable (Appendix I, Fig. B). At Nisqually and Rumble, however, treated plots overall maintained lower cover relative to the control plots, with herbicide plots having lowest cover (but not significantly lower than 2x scarification).

In 2012, we continued to observe a significant effect of the one-time treatment of small seedlings that was dependent on site (site x treatment interaction term, $F_{9,27} = 5.144$, $p = 0.0002$). Johnson Marsh and Tanktable continued to show no differences among treatments, as in 2011 (Fig. 5). At Nisqually, herbicide and twice scarified plots had similar, lower percent cover compared to the control plots, while the single scarified plots were no longer different than control plots. At Rumble, on the other hand, only herbicide plots had lower percent cover than control plots.

Only at Nisqually was it a significantly better strategy to scarify soils two years in a row rather than scarifying once. This result was seen both in 2011 and 2012.

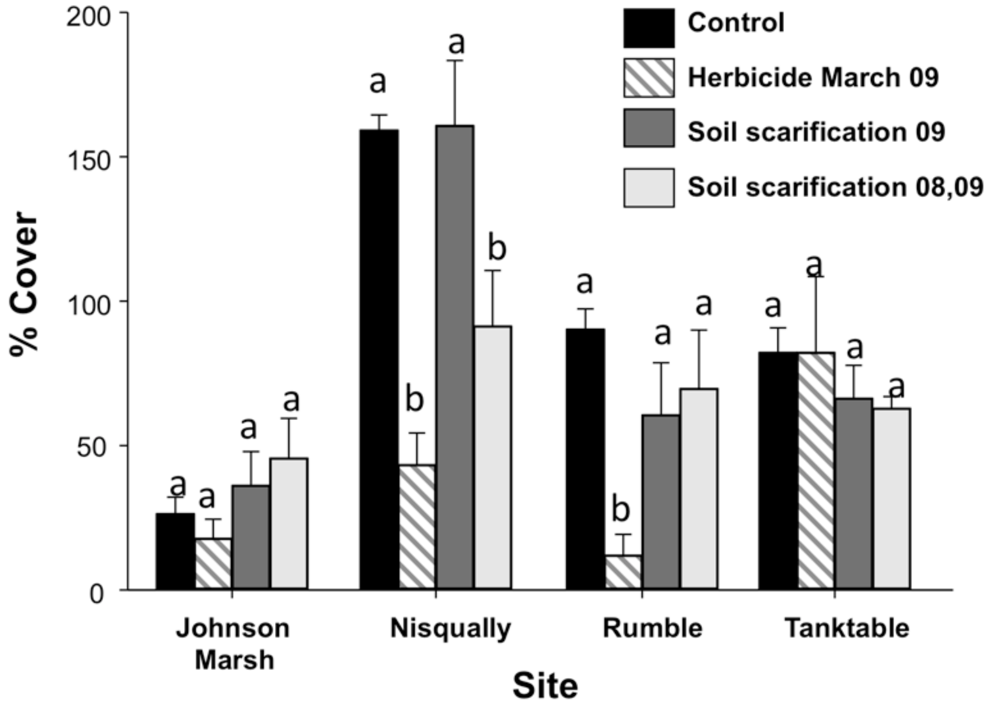

Figure 5: Percent cover of broom in 2012 in control, herbicide (2009), single soil scarification (2009), and double soil scarification (2008 and 2009) treatments. Length of time since last treatment is equal across treatments. Bars are means + 1 SE.

C) Does waiting longer for the seedbank to flush increase the effectiveness of scarification?

For this report we also compared plots that differed in the amount of time seeds were allowed to germinate but otherwise experienced similar treatments (once scarified or twice scarified). First, we compared percent cover for plots treated only one time with soil scarification, but that differed in time after initial adult broom removal. That is, after broom was removed from the site in fall 2007/winter 2008, a set of plots was scarified that March (2008) and another set was scarified one year later in March 2009. We found that by 2012, 4.5 and 3.5 years following treatment, respectively, there was a significant and surprisingly consistent effect of scarification (treatment effect $F_{2,24} = 11.59$, $p = 0.0003$). **Scarifying a few months after initial broom removal resulted in significantly higher cover of broom compared to control plots** (Fig. 6). The plots that were scarified in 2009, the following year after initial broom

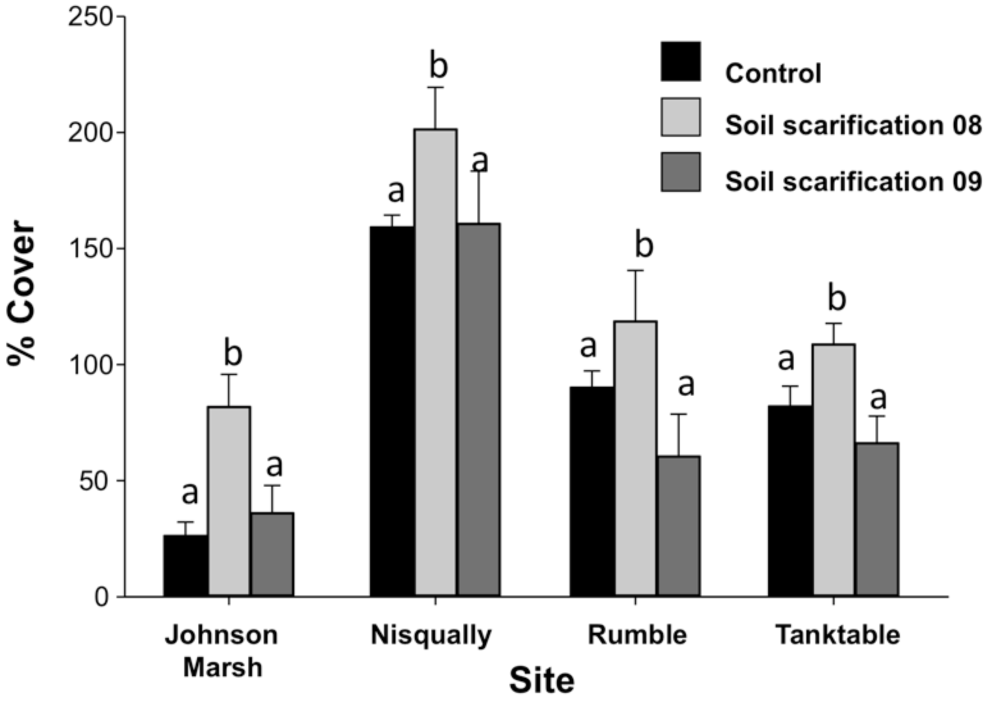

Figure 6: Percent cover of broom in 2012 in single soil scarification treatments several months (2008) and about 15 months (2009) following initial adult broom removal. Bars are means + 1 SE.

removal, did not differ significantly from control plots in 2012 (Fig. 6). Sites also differed from one another (site effect $F_{3,24} = 39.50$, $p = 0.0001$).

Next we compared percent cover in plots that had been scarified two years in a row, but as above differed in the amount of time that had elapsed since the initial adult broom removal from the sites. We compared plots that had been scarified in 2008 and 2009 to plots that were scarified in 2009 and 2010. Across all four sites, the two scarification treatments were not different from one another (Fig. 7). For three of the four sites they were not even different from the control plots (significant site x treatment interaction term $F_{6,24} = 2.73$, $p = 0.03$).

Taken together, our studies of treatments targeting the early life stages of *Cytisus* show very limited success. **Control of *Cytisus* was not achieved by using large equipment to stir up the seed bank and eliminate seedlings**. Multiple scarifications, designed to stimulate and then exhaust the seed bank, did not result in lower broom cover several years later. **In fact, the one consistent effect of scarification was to increase, not decrease, broom density when implemented in the spring of the first year. In contrast, herbicide control of one-year seedlings was effective in some sites,**

even though the seedlings were very difficult to see at the time of application.

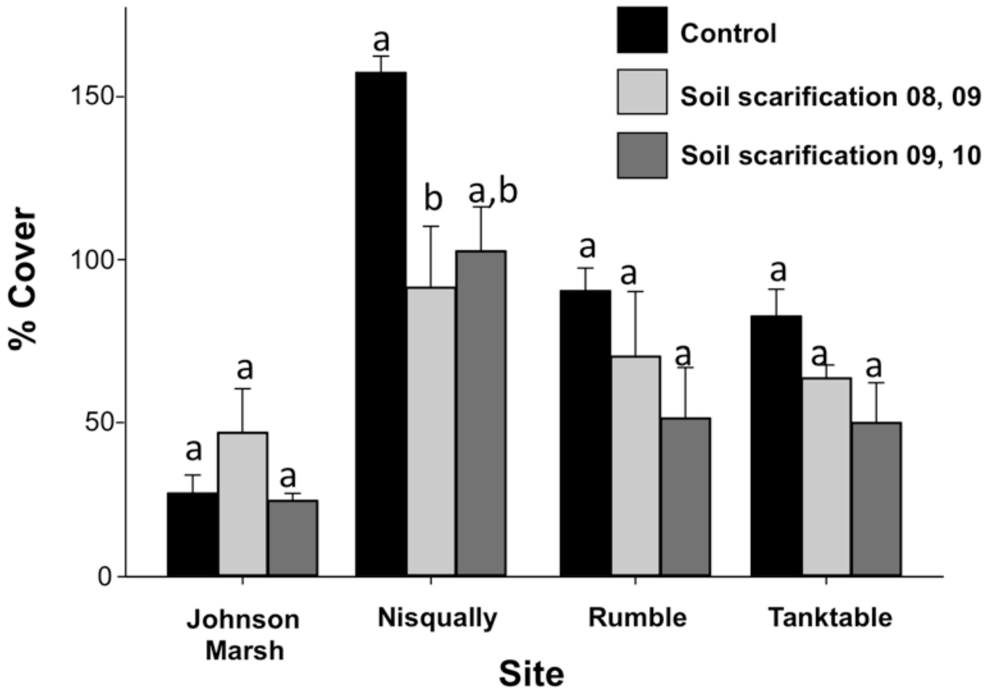

Figure 7: Percent cover of broom in 2012 in double soil scarification treatments occurring in 2008 and 2009, versus 2009 and 2010. Bars are means +/- 1 SE.

III. Response to Treatments Targeting larger *Cytisus* Plants

3A) How did the seasonal timing of herbicide treatment affect *Cytisus* cover?

We incorporated the question of seasonality into the herbicide component of our experiment because of a lack of consensus among practitioners about the best time of year to spray *Cytisus*. We included a spring (March) spray, an early summer (May) spray, and an early fall (September) spray. All treatments took place in 2009 when broom plants were in their second year of growth. 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. Some citizen groups advocate for controlling broom in May while it is flowering. Others argue that the plants are most vulnerable at the end of the dry season. Some companies refuse to spray broom plants during the rainy season, maintaining that it doesn't work.

In 2010, we found no significant difference in broom cover among the three seasons (Appendix I, Fig. C). Spraying herbicide on broom in spring, summer, or fall all had dramatic but similar effects on broom cover. Thus we concluded that overall, herbicide was extremely effective in reducing broom cover, and the time of year for herbicide application was relatively unimportant.

In 2011, the same pattern continued in which herbicide spray was very effective in reducing percent cover irrespective of time of year applied (Appendix I, Fig. D). At Johnson Marsh, however, the effect of herbicide spray at any time of year was completely undetectable.

Interestingly, the effects of herbicide at all sites except Johnson Marsh persisted into 2012, three years after initial treatment. As in previous years, we observed different treatment effects across sites (significant site x treatment interaction term $F_{11,40} = 5.32$, $p = 0.001$). This pattern was driven by the lack of treatment effects at Johnson Marsh, where the sprayed plots had the same percent cover as the control plots, whereas **spraying at all other sites effectively reduced broom cover irrespective of time of year that spraying occurred** (Fig. 8c). The only exception was at TankTable, where the effect of the March spray diverged from the May and September treatments. Three years after treatment, the March spray had the same amount of broom cover as untreated plots at Tanktable (Fig. 8).

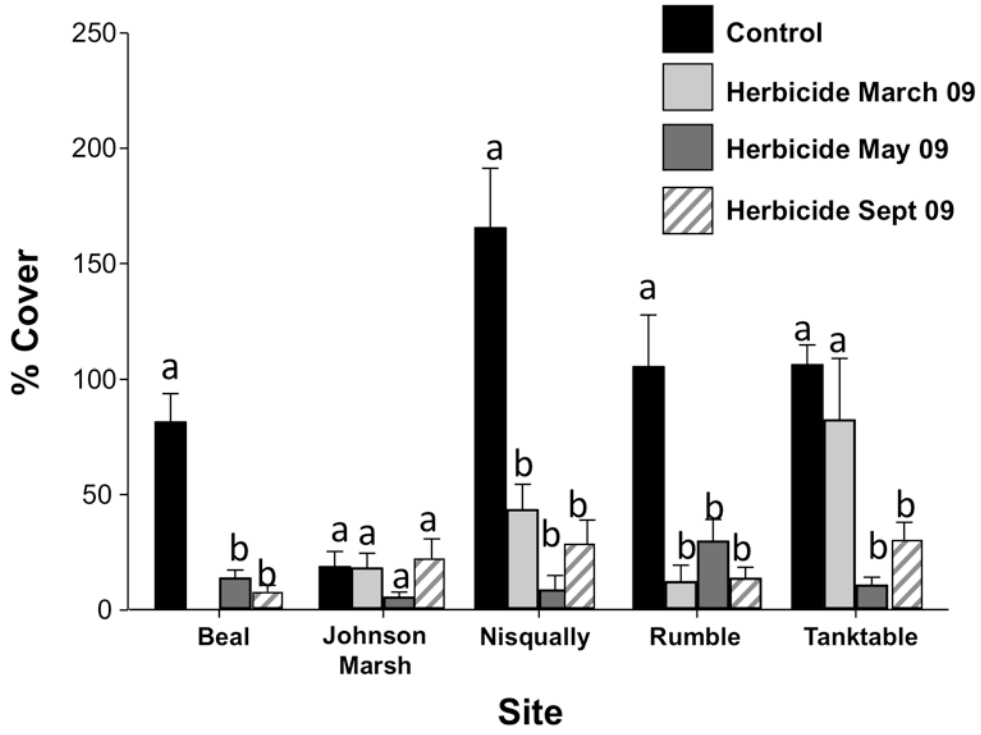

Figure 8: Percent cover of broom in 2012 in control versus herbicide spray in March, May, and September 2009. Bars are means + 1 SE.

3B) What is the relative effectiveness of chemical control versus manual control on older plants?

In September 2010 we implemented treatments designed to compare mechanical and chemical control methods on plants that had experienced three growing seasons (i.e., plants were 2.5 years old). We applied control methods that, unlike the scarification approaches used earlier, targeted individual stems: mechanical control was done with hand-held brushcutters, and herbicide control was done with backpack sprayers targeting individual plants. We evaluated the efficacy of the different treatments in two ways: first, we flagged seven individual plants in each plot and followed their fates for a year. Second, we quantified the response of broom cover at the plot level in 2011 and again in 2012.

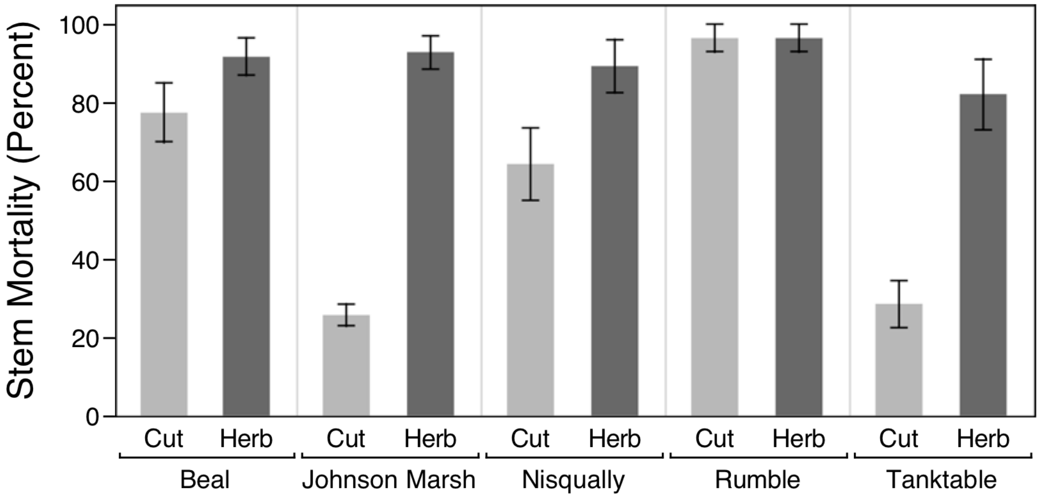

Figure 9: Percent mortality (measured in 2011) of marked 2.5-yr broom plants brushcut ("Cut") or treated with herbicide ("Herb") in September 2010. Bars are means +/- 1 SE.

The kill rate of individual plants in herbicide plots was high (>80%) across all sites (Fig. 9). In contrast, the kill rate of cut plants varied substantially from a high of close to 100% to a low of about 25%. Overall, **herbicide led to significantly higher mortality than cutting** (Logistic regression, Chi-square = 49.7, P< 0.0001, N= 260, treatment nested within site).

In September 2011, both cut and spray treatments were equally effective at reducing cover relative to untreated controls at all sites, with the exception of Johnson Marsh where neither treatment reduced cover. At that site, however, cover was extremely low (~10%).

At two years post-treatment (September 2012), the herbicide plots had significantly lower broom cover than controls at all sites but Johnson Marsh (Fig. 10). The cutting treatment was sometimes equally as effective as herbicide (Nisqually, Rumble), and sometimes intermediate between herbicide and control plots (Beal, Tanktable). Johnson Marsh was still less than 10% cover across all treatments, and there was a significant site x treatment interaction ($F_{8,28} = 4.09$, p = 0.003).

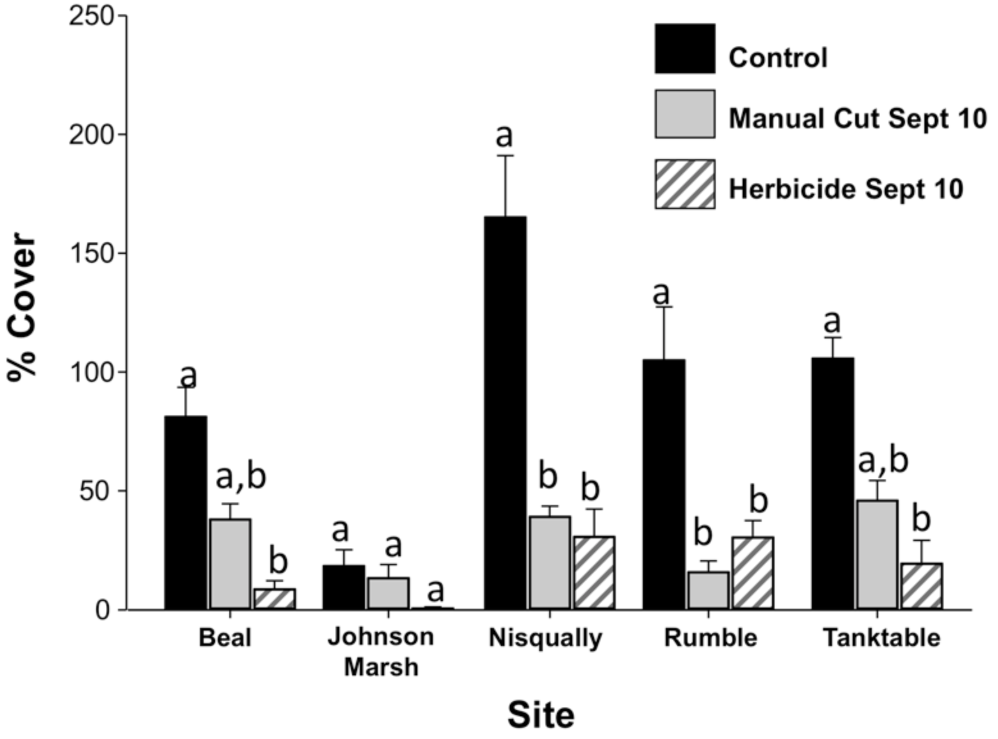

Figure 10: Percent cover of broom in 2012 in control plots versus 2010 herbicide spray and 2010 manual cut. Bars are means + 1 SE.

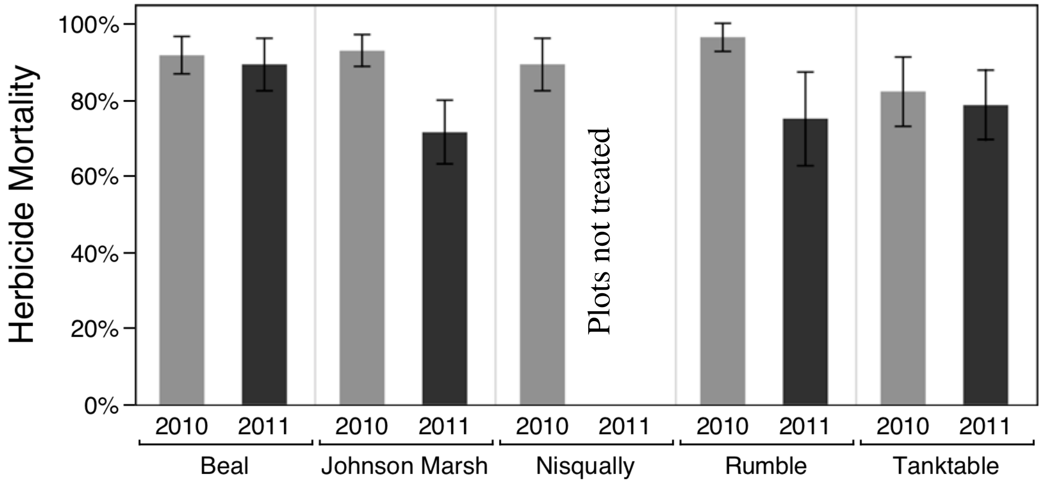

Figure 11: Percent mortality of marked individual broom plants treated with herbicide after 2.5 years of growth (September 2010) and after 3.5 years of growth (September 2011). Bars are means +/- 1 SE.

3C) Did plant age / size affect herbicide spray effectiveness or the

amount of *Cytisus* cover in 2012?

Following the mortality of marked individuals, we found that **3.5-yr-old plants sprayed in 2011 showed slightly lower kill rates than 2.5-yr-old plants** sprayed in 2010 (Fig. 11). Unfortunately, herbicide treatments were not implemented in 2011 at the Nisqually site, and so statistical analyses are based on only four sites, but even so year is significant (F = 4.97, DF = 1, 27, P= 0.034).

Interestingly, comparing plants within a given year**, the initial height of a plant did not predict whether herbicide spray would kill that plant**, either in 2010 (Logistic regression N = 137, X^2 = 0.007, P = 0.93), or in 2011 (Logistic regression N = 112, X^2 = 0.005, P = 0.94; site included in the model).

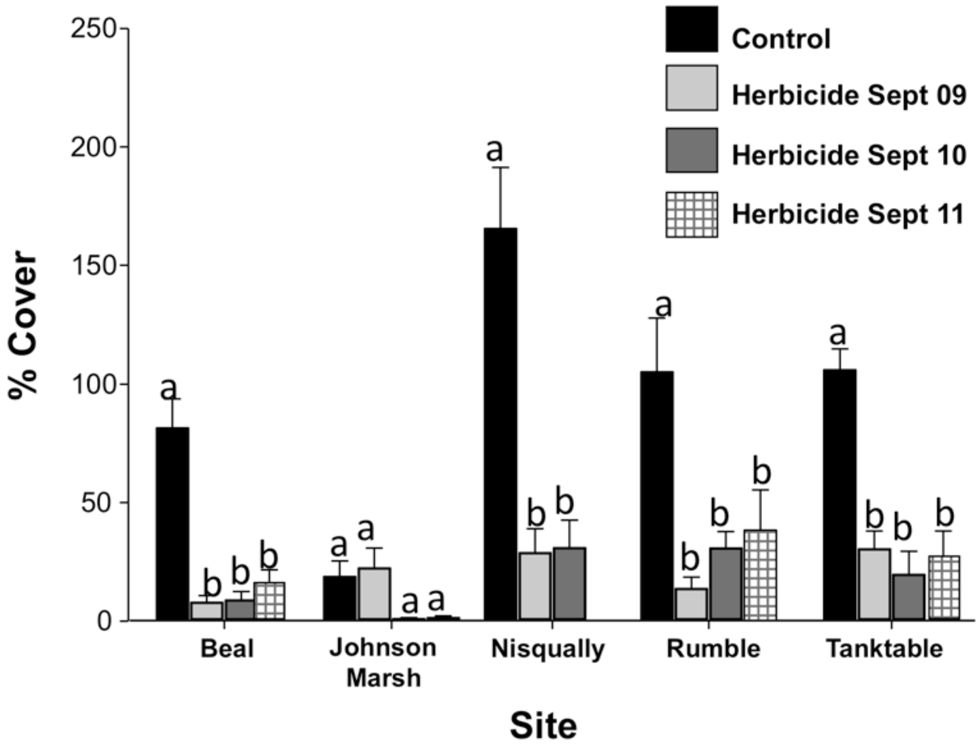

Figure 12: Percent cover of broom in 2012 in control plots versus herbicide spray in 2009, 2010, and 2011. Herbicide sprays were not completed in Nisqually in 2011. Bars are means +/- 1 SE.

In addition, we compared 2012 cover for plots that were sprayed in 2009, 2010, and 2011 (sprayed as 1.5, 2.5, and 3.5 year old plants, respectively). The plots sprayed in 2009 would have had smaller plants to be treated, but then would have had longer for new broom plants to germinate and grow. Conversely, the 2011 spray plots had much larger plants to kill but then less time for new plants to grow in them. These two factors seem to have cancelled each other out. We found that all sprayed plots had significantly less broom cover compared to untreated plots, irrespective of plant size/age (Fig. 12). There was a significant site x treatment interaction term ($F_{11,40} = 4.13$, $p = 0.004$) which was likely due to a lack of treatment effects at Johnson Marsh.

IV. Edge effects on tree seedling establishment, and soil amendment as a restoration technique

Our early studies suggested that Douglas-fir seedlings planted near adult trees or forest edges established with much higher success than more isolated seedlings, and also that seedlings near established trees had higher colonization by ectomycorrhizal fungi (EMF) (Parker and Haubensak 2008, Parker and Haubensak 2010, Makagon 2010). Some fungal species form mycorrhizal networks that can pass important resources from established mature trees to conspecific seedlings. In collaboration with JBLM Forestry staff, we designed a large-scale, well-replicated experiment **to test whether tree establishment at JBLM is consistently higher near forest edges, and also whether mycorrhizal fungi play a role in this edge effect.**

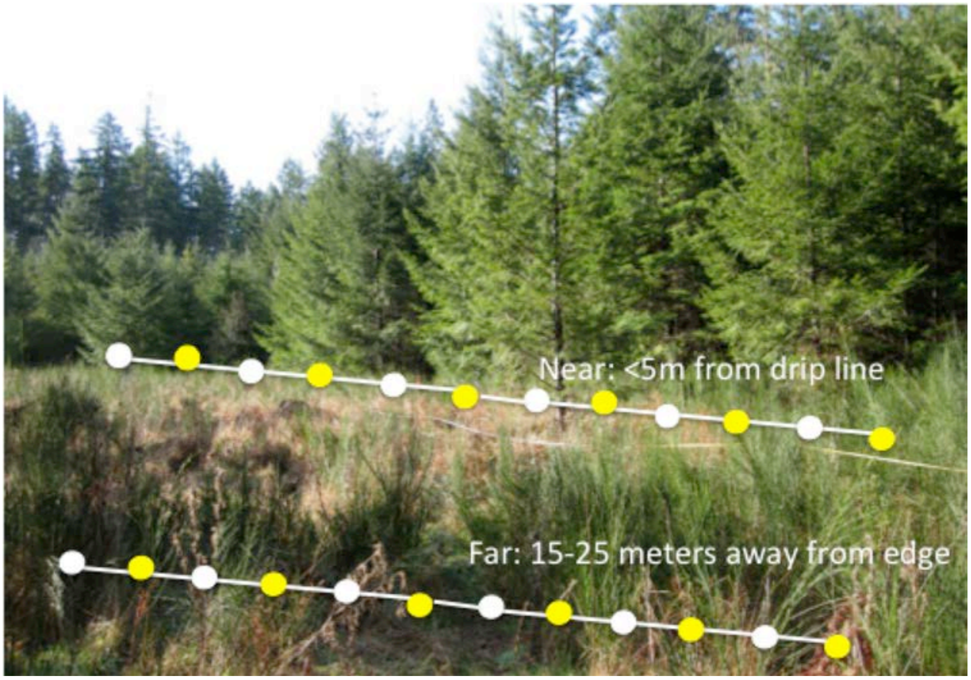

Figure 13. Experimental design for Edge Effects / Soil Transplant Experiment. Pairs of transects are near to or far from forest edges. Dots represent planting points for Douglas fir seedlings; yellow and white represent treatments, alternating soils from the forest with soils from the broom-invaded clear-cut.

In March of 2011, we installed transects at 5 sites (Beal, Johnson Marsh, Tank Table, Nisqually and Rumble Hill). We cleared *Cytisus* along the transects. To test **the effect of forest edges**, we established transects in pairs, with one transect along the forest edge and the other 15-25 meters into the *Cytisus*-invaded clear-cuts (Table 4, Figure 13).

Table 4: Experimental design and sample sizes for the Edge Effects / Soil Transplant experiment.

Site	Total Transects	Transect Pairs	Seedling Number
Johnson Marsh	14	7	172
Nisqually	16	8	175
Tank Table	14	7	245
Beal Hill	12	6	186
Rumble Hill	12	6	180
Total	68	34	958

To determine **if soils transplanted from nearby forests can effectively restore EMF communities** and improve the survival of Douglas-fir seedlings in *Cytisus*-invaded clear-cuts, we implemented a soil transplant treatment. We planted the Douglas-fir seedlings into 3 liters of soil collected from either the invaded clear-cut (control) or a nearby uninvaded forest (treatment). Seedlings were planted every 3m along each transect, with soil types alternating.

On May 6-12, 2011, we measured the initial heights and diameters of 958 seedlings. Seedling size and mortality were again measured on September 12-16, 2011 and November 7-12, 2012. On the final date, we collected, dried and weighed the aboveground portion of all surviving seedlings to obtain aboveground dry biomass. To test for the effects of forest edge and soil type on Douglas-fir seedling survival, we used a two way logistic regression model. We used two-way ANOVA to analyze Douglas-fir seedling growth and biomass.

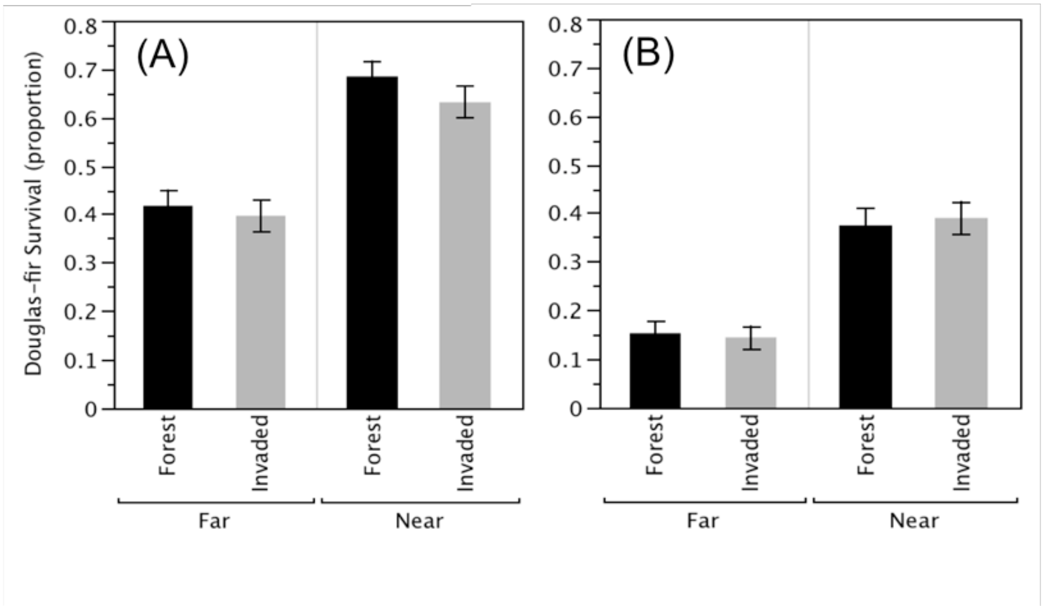

Figure 14. Survival of transplanted Douglas-fir seedlings measured in September 2011 (A) and September 2012 (B). Seedlings were planted either near forest edges ("Near") or out in the *Cytisus*-invaded clearcut ("Far"), with either *Cytisus*-invaded soil ("Invaded") or forest soil ("Forest") added at the time of transplant. Error bars are ± 1 SE with sites as replicates.

We found that seedlings planted close to forest edges had significantly higher survival through the first summer ($\chi^2 = 47.0$, P = 0.0001; Fig. 14), and the survival benefit of growing close to the edge was even more pronounced in year two ($\chi^2 = 63.5$, P = 0.0001; Fig. 14). Adding soil from Douglas-fir forest increased survival marginally significantly in the first year ($\chi^2 = 2.46$, P = 0.11, Fig. 14), but that effect was small and entirely disappeared by the second year (F = .079, P = 0.39, Fig. 14). There was no significant interaction between location and soil amendment at either time period ($P > 0.1$). Harvested biomass showed the same patterns as final survival (results not shown)

We collected the entire root system from a subset of surviving Douglas-fir seedlings at the time of the final harvest. Root samples were transported on ice to the University of California, Santa Cruz to be assessed for EMF colonization and fungal community composition. We quantified the proportion of roots colonized by EMF across treatments at Tank Table using a compound microscope. We used two-way ANOVA to test the effects of soil type (forest/invaded) and proximity to forest edge (near/far) on proportion colonization of EMF.

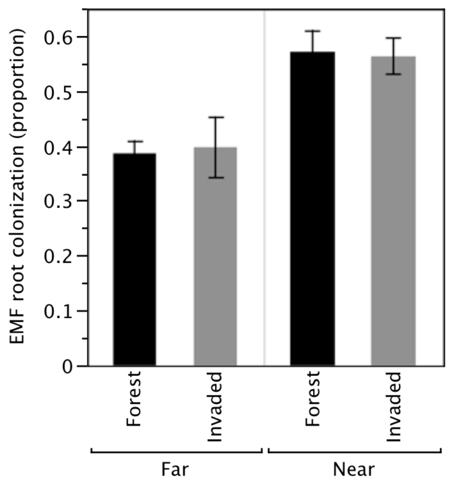

Figure 15. Proportion of roots colonized by ectomycorrhizal (EMF) fungi on Douglas-fir seedlings planted into areas invaded by Cytisus "near" and "far" from intact forest edges, with either *Cytisus*-invaded soil ("Invaded") or forest soil ("Forest") added at the time of transplant. Error bars are ± 1 SE with sites as replicates.

EMF colonization was 32% higher for seedlings near the forest edge than for those out in the clear-cut ($F_{1,52} = 18.6$, $P = 0.001$; Fig. 15). Mirroring the results for survival and biomass, there was no significant effect of transplanting forest soil on EMF two growing seasons later ($F_{1,52} = 0.001$, $P = 0.97$). There was no significant interaction between location and soil amendment ($F_{1,52} = 0.058$, $P = 0.81$).

The establishment of young Douglas-fir trees was strongly facilitated along forest edges. The effect of the edge was strong after one year and even stronger after two. Our results suggest that **sites that are otherwise inhospitable for young trees and in which plantations have failed may still show potential to regenerate slowly, starting at the edges**. These results also have implications for how the geometry of forest harvest methods influences reforestation success.

Less clear is what mechanism(s) mediate the negative effects of broom-invaded clear-cuts on Douglas-fir growth. Our original hypothesis, based on our earlier greenhouse results, was that broom "legacy effects" on the soil through allelopathy and/or suppression of EMF could be responsible for poor tree seedling performance in clear-cuts. Indeed, ectomycorrhizal fungi were clearly suppressed in the clear-cuts relative to forest edges, and molecular work funded by NSF will reveal whether particular species of EMF are missing in the clear-cuts. Amending tree plantings with forest soil and its associated fungi had a small positive effect on early seedling survival**, but the effect of soil addition was only marginally significant and short-lived, while the overall edge effect was large and amplified over time.** Since the soil amendment treatment was only implemented once and could involve only a moderate volume (3 liters of soil), perhaps it is not surprising that the effects of the treatment were small. Much remains unanswered about the details of the below-ground ecology of broom, Douglas-fir, and its fungal associates.

V. Conclusions and Management Recommendations

Many lessons have been learned from this five-year study, ranging from the mundane to the profound. Here we share what we consider to be our most practical messages based on our data and experience studying broom control at JBLM.

> When estimating broom cover, run a random transect and use line-intercept method. This is the most flexible and repeatable method across a broad range of broom stand ages and densities.

> When estimating seed germination in the field, use narrow belt transects. Seedlings are very patchy at the scale of meters and vary greatly between sites, but are surprisingly consistent at the scale of tens to hundreds of meters across a site.

> When broom is cut with machinery, the height at which the stumps are cut does not seem to matter for resprout potential.

> After initial broom removal, go into the sites the following summer to assess both the density of germinating seedlings and the frequency of resprouting stumps. These two pieces of information will predict how fast broom will cover the site (germination) and how fast new seeds will be produced (=1-2 years faster when plants come from resprouts). Manual cutting later may also be more effective when the plants are resprouts rather than new plants.

> Targeting the earliest life stages (seeds and seedlings) might appear to be the most efficient way to control broom populations mechanically, but it was not an effective method at JBLM. Multiple years of flushing the seedbank with soil scarification did not reduce broom cover three years later. In addition, the cost of the treatment in terms of labor and broken blades was excessive.

> Sites with high amounts of germination (e.g. Tanktable and Nisqually in our study) can jump to 50+ percent cover of broom within two years. These thick carpets of broom seedlings seem to compete heavily with other vegetation. Broom cover in sites with mostly resprouting stumps can catch up by year 3.

> Herbicide (Garlon 4) was the only treatment that showed any effect on small (1-year-old) seedlings, and the effect was measurable three years later even where spraying was done through thick grass. This technique appeared to be effective in part because it does not require the operator to see the broom seedlings. The use of dye ensures complete coverage.

> The kill rate with 2.5-year-old-plants was more consistently high for chemical than mechanical control. The best results for brush-cutting were as good as herbicide, but most sites showed poor results.

> It is not possible to predict death from herbicide by looking at the plants 6 weeks after spray. We found that % green tissue at 6 weeks did not predict whether the plants were dead 12 months later.

> Taller plants were NOT less susceptible to herbicide (within a year and within a site, on 2.5 or 3.5-year old plants).

> In this study, season was NOT a critical factor in herbicide effectiveness. Spraying in March, May, and September gave similar results. This is an important finding because managers are free to determine when plots are sprayed based on logistical priorities.

> The kill rate for herbicide was as high for 3.5-year-old plants as for 2.5-year plants in some sites, but had dropped from 90% to about 70% in two of the sites.

> The use of Garlon 4 on broom did not seem to harm Douglas fir trees in several different experiments.

> Herbicide treatment gave long-lasting results (Figures 16 & 17). While control plots at most sites were at 100-150% broom cover by 2012,

Figure 16. Herbicide plots at Tanktable in Sept. 2012, after three years of regrowth. Untreated area shown on the left for comparison (initial broom control in winter 2008).

Figure 17. Herbicide Plots at Beal in Jan. 2014, after four+ years of regrowth. Untreated area in the background for comparison.

plots sprayed in 2009 were still at 10-30% cover. In January 2014, herbicide plots were still easily distinguished (Figure 17).

> Trees planted near forest edges survive better, even in sites where plantations have failed multiple times. Among several possible explanations, mycorrhizal connections with established trees may be involved.

> Harvest and reforestation strategies should maximize the benefits of forest edges. This has implications for the geometry and optimal size of timber harvests.

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APPENDIX I: SELECTED FIGURES FROM 2010-2011

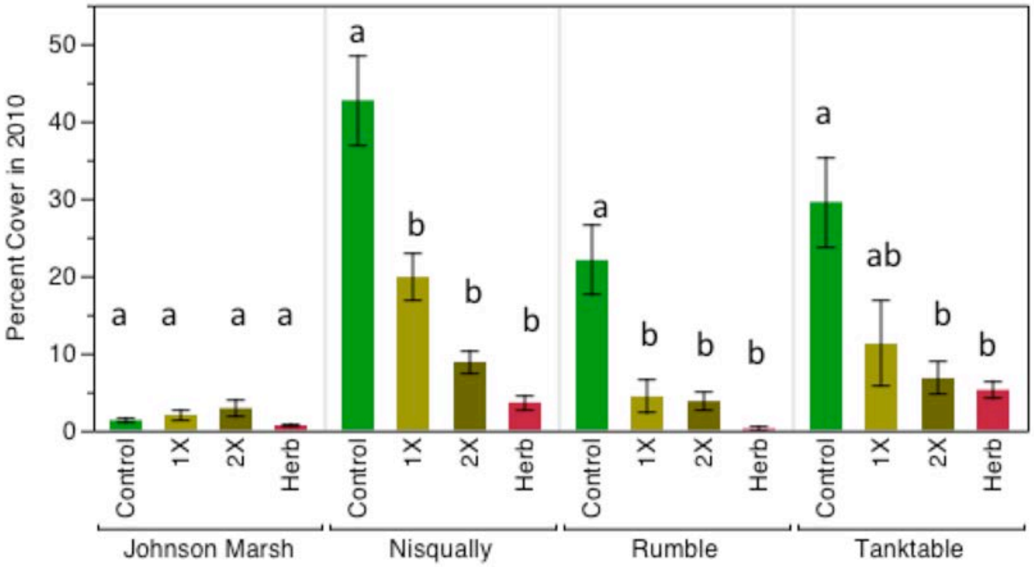

Figure A. Percent cover of broom in May 2010 in control (green bars), single application of scarification (gold bars), double application of scarification (olive bars), and single application of herbicide (red bars), all treated in 2009, across sites. Length of time since last treatment is equal across treatments. Bars are means +/- 1 SE.

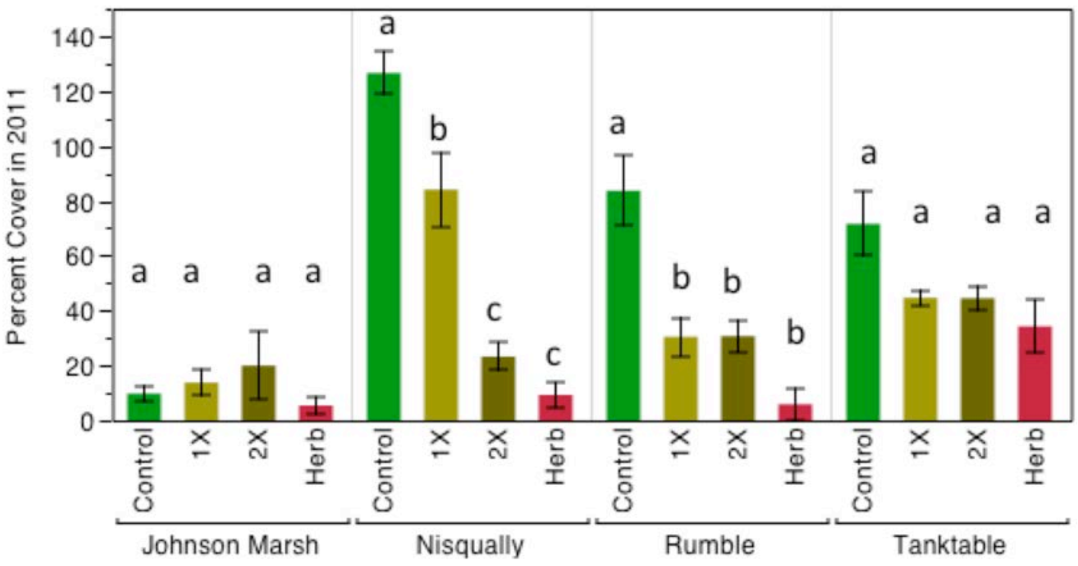

Figure B. Percent cover of broom in September 2011 in control (green bars), single application of scarification (gold bars), double application of scarification (olive bars), and single application of herbicide (red bars), all treated in 2009, across sites. Length of time since last treatment is equal across treatments. Bars are means +/- 1 SE.

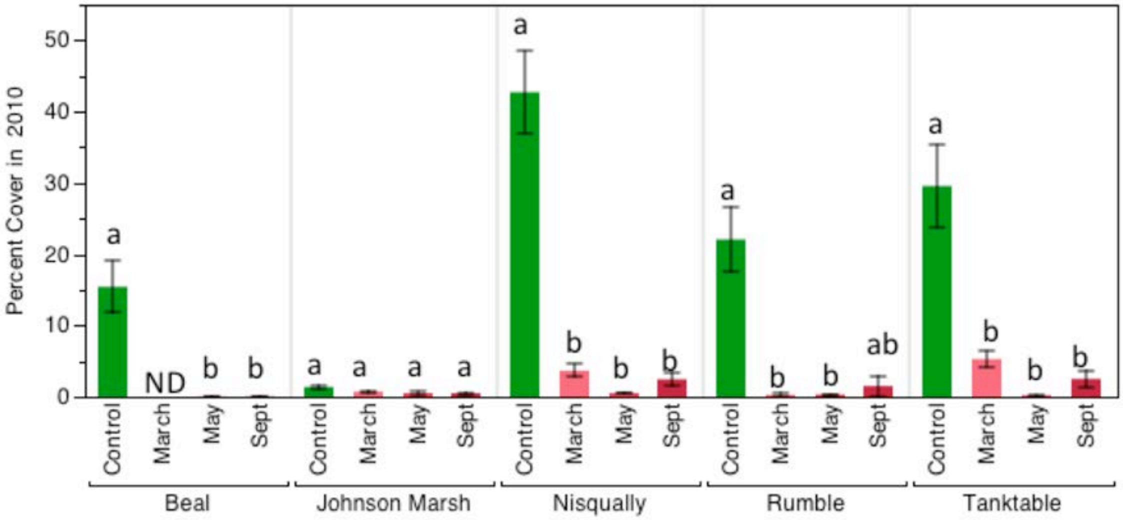

Figure C: Percent cover of broom measured in May 2010 in control plots (green bars), plots with March spray (light red bars), May spray (red bars) and September spray (dark red bars) across sites. Plots were treated in 2009. Bars are means +/- 1 SE. ND = not determined because plots were not sprayed in March 2009 at Beal.

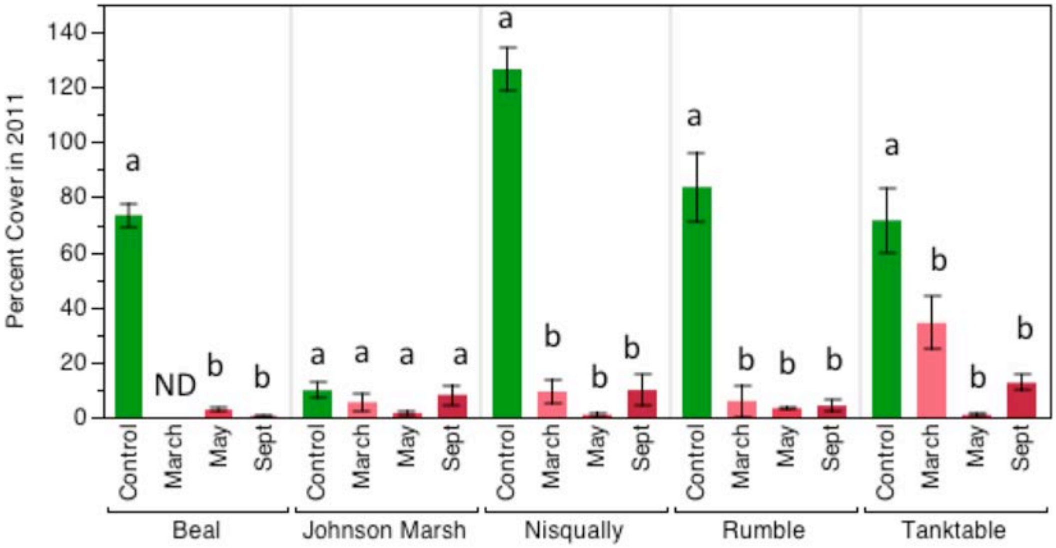

Figure D: Percent cover of broom measured in September 2011 in control plots (green bars), plots with March spray (light red bars), May spray (red bars) and September spray (dark red bars) across sites. Plots were treated in 2009. Bars are means +/- 1 SE. ND = not determined because plots were not sprayed in March 2009 at Beal.

APPENDIX II: PHOTO DOCUMENTATION

Temporal progression at Beal Hill site. Broom was cut initially winter 2008. Broom seedlings germinated through May 2008, then grew slowly for two years. Plant growth exploded in the third and fourth years.

March 2008, Initial treatment

May 2009, Into second growing season

May 2010, Into third growing season

Sept 2011, After fourth growing season (plants 3.5 yrs old)

Mechanical Control Early in the Life Cycle: Scarification

Mechanical treatment ("**scarification**") designed to kill one-year-old seedlings flushed from the seedbank in the previous year. Top panel: Equipment used for scarification, note finely ground soil surface (Tanktable March 2009). Bottom panel: scarification plot with herbicide plot behind it showing blue dye (Rumble Hill: March 2009). This herbicide treatment was fairly effective; the scarification treatment was not.

Mechanical Control vs. Chemical Control Later in the Life Cycle
Treatments comparing control of broom by chemical means (upper panel: Garlon 4 spray with back-pack sprayer) vs. mechanical means (middle panel: brush-cutting by hand). These treatments were done in September 2010, on 2.5-yr old plants (after 3rd growing season). Bottom panel shows the treatments side by side a few weeks after implementation, at Rumble Hill.