AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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Thomas N. Kaye

Prairies were once the dominant vegetation type in Oregon's Willamette Valley. Land use conversion, fire suppression, succession, and invasive species have reduced Willamette Valley prairies to less than 1% of their historical area. The remnant prairies that persist today are small in size and are highly fragmented. Marginal strips of habitat along roadsides and agricultural fields play an important role as refugia for native species and provide important resources for wildlife. These seemingly insignificant habitat units may also play an important role in facilitating gene flow between disjunct populations of prairie plants, thus reducing the potential for the negative effects of inbreeding depression. Presently, much of the land area in the Willamette Valley is dedicated to commercial agricultural which is heavily reliant on herbicides for weed control and field preparation. Since herbicide applications are imprecise and prone to drift, there is potential to impact the native plants surrounding these agricultural fields. Current EPA methods for assessing the ecological effects of herbicides may not be robust enough to account for potential impacts on native plants since the suggested test species are ten annual agricultural crops. To address the need for improved phytotoxicity testing protocols, we incorporated non-crop plant species into the EPA vegetative vigor test methodology for use in determining effects of low concentrations of chemical herbicides on Willamette Valley terrestrial plants. A separate experiment was conducted in order to determine how herbicides might be used to restore Butterfly Meadows, a degraded Willamette Valley prairie. The specific objective of this study were to: 1.) determine which herbicide treatments were most effective at reducing dominance of an invasive species, *Brachypodium*

sylvaticum, 2.) determine if native species declined following herbicide treatments, and 3.) describe the compositional changes in the plant communities over a four-year period.

The EPA vegetative vigor test study showed that there was a wide variety of responses among 17 species (14 native and 3 introduced) to each herbicide tested (glyphosate, tribenuron, and fluazifop). For glyphosate, *Potentilla gracilis* was the most sensitive species based on an EC_{25} value of 0.012 x f.a.r. for dry weight; while *Bromus carinatus*, *Clarkia amoena*, *Gilia capitata*, and *Lupinus albicaulis* were tolerant to glyphosate as indicated by no effect on dry weight. Seven Willamette Valley forb species were sensitive to tribenuron based on EC_{25} values ranging from 0.001 to 0.012 x f.a.r.; *Clarkia amoena, Collinsia grandiflora, Leucanthemum vulgare, Potentilla gracilis, Prunella vulgaris, Ranunculus occidentalis and Sanquisorba occidentalis*. Six grass species and *Eriophyllum lanatum* were resistant to tribenuron showing no reduction in dry weight. Fluazifop primarily affected grass species as expected due to the grass-specific activity for this herbicide. Two native grasses, *Elymus trachycaulus* and *Danthonia californica* were the most sensitive to fluazifop, based on low EC_{25} values of 0.002 to 0.010 x f.a.r. A native fescue grass, *Festuca roemeri*, and nearly all the forb species were resistant to fluazifop, showing no response at any herbicide rate applied. The results from this research will be useful as background information for evaluating potential modifications in the EPA's Vegetative Vigor Test to assess the risk of herbicides to non-target plants.

Seven different herbicide combinations were effective at reducing the cover of *Brachypodium sylvaticum* in test plots at Butterfly Meadows one year after treatment. The reduction of *B. sylvaticum* was short lived however, since the cover of this grass species was not different from control plots during the second growing season. Native plant species were not negatively impacted by the herbicide treatments, as shown by MRPP analysis. Successional trajectories illustrate that control plots that were dominated by *B. sylvaticum* remained relatively unchanged over the course of four years. Some treatments exhibited a sharp decline in dominance by graminoids after the first year, a recovery the second year, but never returned exactly to their pretreatment community composition after the third year. The reduction of the

dominant species after the first growing season was associated with colonization by a number of introduced species into the newly created open habitat. Over the same period there was no overall increase in native species cover, suggesting that the native species at this site may be recruitment limited. Future restoration activities at this site should include multiple years of *B. sylvaticum* control, with special attention to the seed bank and tolerant individuals. Seed additions of native species may help fill empty niches and afford resistance to invasion by introduced species.

Performance of Willamette Valley Native Plants Following Herbicide Exposure

by
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CONTRIBUTION OF AUTHORS

The first chapter of this thesis, *Incorporation of native plants into EPA phytotoxicity tests for herbicide registration*, represents the collaborative work performed by a number of people. David Olszyk and E. Henry Lee helped with experimental design and performed much of the statistical analysis. David Olszyk also contributed to the results and discussion sections, while Thomas Pfleeger helped with the introduction. Milton Plocher and George King assisted in writing the section on herbicide selection, formulation, rates, and application.

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Chapter 1 : Introduction

Prior to settlement of Oregon's Willamette Valley in the mid 1800s, prairies and oak savanna dominated the landscape (Habeck 1961). These two habitat types are characterized by a diverse assemblage of perennial grasses and forbs which are adapted to a Mediterranean-like climate with wet winters and summer drought. The historical disturbance regime that maintained these open habitats was frequent fire events initiated by native peoples (Johannessen 1971). The Cascade Mountains played an important role in isolating the Willamette Valley from the extensive prairie systems located in the central part of the country. This geographic isolation has lead to the evolution of a unique flora and a coevolving fauna in the Willamette Valley and Puget Trough ecoregions.

With the arrival of settlers in the 1800s came land conversion, introduced species, and fire suppression, all of which had negative consequences for native ecosystems. In 1998 the Oregon Department of Fish and Wildlife conducted a study on the vegetation of the Willamette Valley. They estimated that over 500,000 acres were dedicated to perennial grass seed production, 103,000 acres were in row crops, 94,000 acres were planted to annual grasses, and 114,000 acres were developed as urban areas (ODFW 1998). Another study, conducted by the Oregon Natural Heritage Information Center, found that 456,119 hectares of wetland and bottomland habitat and 732,432 hectares of upland habitat have been lost since 1850. The remaining upland habitat is composed of small remnant prairies that are geographically isolated. These endangered habitats harbor twenty-two species of rare plants and animals that are dependent on upland communities in the Willamette Valley (Titus 1996).

The nearly wholesale conversion of land into agriculture and urban areas in the Willamette Valley leaves less than 1% of the historical area once occupied by upland prairie (ODFW 1998). This prairie habitat is threatened by introduced species, woody plant encroachment, habitat fragmentation, and a plethora of human related threats such as off-road vehicles, grazing, and herbicide drift. The listing of a number of upland prairie dependent species under the federal Endangered Species Act has

prompted recent scientific study into the restoration and management of this rare habitat to facilitate the recovery of these species (U.S. Fish and Wildlife Service 2000; Schultz and Crone 1998).

Herbicides can play two seemingly opposing roles in plant conservation. Herbicides are used by restorationists to reduce non-native species abundance and favor native species (Annen 2005; Ewing 2002; Travenicek et al. 2005; Erickson et al. 2006), while intentional and accidental applications of herbicide threaten native plant communities along roadsides and adjacent to agricultural fields (Kleijn and Snoeijing 1997; Marrs et al 1992). Although management plans may involve the use of herbicides, our understanding of how Willamette Valley plant communities respond to these treatments often comes from anecdotal observations, other biomes, or agricultural settings - not from controlled experiments in natural areas. Additionally, current Environmental Protection Agency (EPA) phytotoxicity tests for herbicide registration do not include native plant species, despite the fact that current models indicate that plants growing adjacent to agricultural fields and public roads are potentially being exposed to low levels of herbicide drift (Gilbert and Bell 1988; Kleijn and Snoeijing 1997; Marrs et al. 1992; de Snoo 1998). With native plant communities declining nearly worldwide there is clearly a need to understand how to best restore these endangered systems as well as protect them from obvious threats.

Second only to outright habitat destruction, invasive species pose a substantial threat to most federally listed endangered plant species (Wilcove 1998; Pimentel et al. 2000). Compliance with the Endangered Species Act has stimulated a number of public land managers to actively address habitat degradation resulting from invasive species encroachment. Herbicide use for habitat manipulation has been limited because there is uncertainty as to how different species will respond to a particular management practice. Without active management, however, many of these rare species will continue to decline as they become displaced by introduced species. Careful evaluation of the control techniques available to land managers will lead them to make informed decisions regarding weed control in sensitive habitats. Land managers will likely require control methods other than herbicides since the history of the site, the species present, and varying environmental conditions make it doubtful

that any single tool will be appropriate for all sites (Sheley and Krueger-Mangold 2003; Hobbs and Humphries 1994).

One fundamental tool for ecological risk assessment is phytotoxicity testing. These tests allow the EPA to assess the potential for adverse ecological impacts associated with the use of a particular pesticide. For herbicides, the required EPA tests for plants are the Pesticide Assessment Guidelines Subdivision J, which have been refined as the Series 850, Ecological Effects Tests, especially the 850.4150 Tier I Vegetative Vigor Test (US EPA 1996a) and the 850.4250 Tier II Vegetative Vigor Test (US EPA 1996b). The vegetative vigor test routinely uses ten crop species to evaluate the effect of a toxin on plant growth. The ten crops species include tomato, cucumber, lettuce, soybean, cabbage, carrot, oat, perennial ryegrass, corn, and onion. These ten species are then the surrogates for all crops and native plants in the United States as well as in other countries where U.S. standards are accepted. The narrow range of growth form and taxonomic diversity of the ten required test species raises questions as to whether non-crop plant species will be adequately protected (Boutin et al. 2004; Boutin et al. 1995; but also see McKelvery et al. 2002). Currently there is no provision to include the wide diversity of native plants in non-target plant risk assessments. Therefore, an improved vegetative vigor test protocol is needed to determine the effects of herbicide exposure on non-target native plants (Touart and Maciorowski 1997).

The purpose of this thesis is twofold: to document the efficacy of several herbicide treatments in controlling an introduced species (*Brachypodium sylvaticum*) in a degraded upland prairie, and to develop guidelines that incorporate native plants into EPA phytotoxicity tests for herbicide registration.

Chapter 2 : Incorporation of native plants into EPA phytotoxicity tests for herbicide registration

INTRODUCTION

Phytotoxicity tests provide a powerful tool to evaluate the relative toxicity of a certain chemical on an individual plant species at a specific stage of development. Current EPA phytotoxicity test guidelines recommend the use of ten agricultural crops when registering new pesticides (US EPA 1996a, b, c). These guidelines restrict the scope of inference due to the limited number of test species and the experimental design. Crop plants have been highly bred for rapid growth and uniformity, while non-crop plants have more diverse gene pools which may result in unpredictable responses to phytotoxicity tests (but see Clark et al. 2004). For example, studies conducted on non-crop species *Cirsium arvense* and *Convolvulus arvensis* showed that different genotypes of the same species were differentially affected by 2,4-D (Hodgsen 1970; Whitworth and Muzik 1967). With North America's estimated 17,000 native species (Kartesz 1994) the use of ten agricultural species to predict potential harm to non-target species may greatly underestimate the risk that these species actually face due to their differences in genetic diversity, life history, morphology and physiology (Boutin et al. 2004).

In 1996 it was estimated that 70 million acres worldwide (an area roughly the size of Texas) were treated with just one herbicide, glyphosate (Woodburn 2000). During ground or aerial applications of herbicides, 100% of the applied chemical does not actually reach the targeted plants or the ground (Pimentel and Levitan 1986; Nellessen and Fletcher 1990). This may be due to a number of causes such as environmental conditions, spray equipment mechanics, or herbicide volatility. Herbicide drift has been documented to occur at distances of 1600 m downwind and may reach 0.1 x the applied rate, depending on topography and weather conditions (Chester and Ward 1984; Teske et al. 2002; Parkin and Merritt 1988). Herbicide drift can have negative effects on non-target plant growth (Kjaer et al. 2006a) and reproduction (Koger 2005; Kjaer et al. 2006b), although these effects are contingent on herbicide, species, and environmental conditions (Wall 1995). Herbicide drift is distinguished from herbicide misapplication, in which case herbicides are mistakenly

applied directly to non-target vegetation, resulting in herbicide concentrations and impacts that are much higher than drift events.

European and Canadian scientists have documented the effects of sub-lethal concentrations of herbicides on native plants, though there are few experimental results from the United States (but see Pfleeger and Zobel 1995). In Europe, observational data suggested that plant assemblages at field margins changed in species frequency and distribution due to differential susceptibility to herbicides (Kleijn and Snoeijing 1997). In Canada, Jobin et al. (1997) found lower species diversity in the herbaceous layers of hedgerows and woodland edges of cultivated fields with a history of herbicide use as compared with those near fields without herbicide use. In controlled experiments with plant communities, Marrs et al. (1991) demonstrated that variable species responses to herbicide exposure altered the composition of species within a community.

The EPA regulates the registration and use of herbicides under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). The current EPA required tests under FIFRA for plant phytotoxicity effects are the Pesticide Assessment Guidelines Subdivision J tests, which have been refined as the Series 850, Ecological Effects Test Guidelines. Guideline 850.4000 (US EPA 1996a) provides general information on conducting plant tests with pesticides and industrial chemicals. A key test to assess effects to non-target plants is the Vegetative Vigor Test (850.4150) (US EPA 1996b). The test is commonly used in a "Tier" sequence, i.e., for "Tier I" a single concentration of a pesticide is required to determine the general phytotoxicity of a chemical. When a chemical is known to have phytotoxic effects multiple concentrations of the pesticide are required to establish dose-response functions (the "Tier II" test). The Tier II dose-plant response data are then used to establish pesticide benchmarks for effective concentrations (ECs) which produce specific levels of effects. For example, for sub-lethal effects of pesticides on plants, the EC_{25} may be defined as the effective herbicide that reduces shoot dry weigh by 25% compared to the control (Bruce and Versteeg 1992). These EC values are a useful means to

compare the relative susceptibility of plant species to the same pesticide, or the relative effects of different herbicides to the same species.

Despite the need for research to improve non-target plant testing protocols, there has been little research in this area in the United States. Especially important is the need to evaluate non-crop (i.e., native) species for these protocols. Not only do non-crop plants serve as primary producers and therefore act as the base of most food webs, they also play a crucial role in creating the physical structure that provides critical wildlife habitat (Boutin et al. 2003; Marshall et al. 2006). Most research concerning plant responses to herbicides has been conducted primarily using crops such as peas (Al-Khatib and Tamhane 1999), lentils (Wall 1995), cherries (Bahtti et al. 2006), cotton (Patterson et al. 1990), and others.

To address the need for improvements in the plant testing protocols, we conducted research on the effects of three herbicides on vegetative growth of 17 species of container-grown plants from the Willamette Valley, Oregon. The research presented in this paper is modeled after the Tier II Vegetative Vigor Test and uses EC_{25} data to document the relative sensitivities of the Willamette Valley native plants to these herbicides. Specifically, studies were designed to answer two research questions: 1.) Can native plants be easily grown for standard EPA phytotoxicity tests? and 2.) can results from these phytotoxicity tests be used to measure the relative sensitivity of native plants to herbicides?

METHODS

Plant selection

Seventeen herbaceous plant species, representing eight families, were selected for study. Vegetation data collected from Butterfly Meadows (Clark et al. 2004), an upland prairie outside of Corvallis, Oregon, as well as knowledge of the Oregon flora was used to determine which species to include in this study. Species selection was also narrowed by seed availability and ease of cultivation in standard greenhouse conditions.

In an effort to include species with a wide range of growth forms and life history, ten perennial species and seven annual species were chosen. Since grasses contribute a large proportion of the biomass in prairie communities six grass species were chosen. The remaining eleven species were herbaceous forbs. Three introduced species were included since this accurately reflects the species composition in current upland prairies. A separate experiment on community-level responses to low-dose herbicide applications is currently underway, so introduced species were included in order to support that research.

Native plant material is becoming increasingly more available for purchase. Seeds for the native species used in this experiment were purchased from Heritage Seedlings (4194 71st Ave SE, Salem, OR 97301). The seeds were bulk collected from many remnant prairies throughout the Willamette Valley, planted as row crops, allowed to cross pollinate, and the resulting seeds were harvested and sold to buyers (Lynda Boyer, personal communication). Seeds for the three introduced species were locally collected from meadows and roadsides in Corvallis, Oregon in September, 2004. An ecoregion approach was used to define which species were native to the Willamette Valley and the USDA PLANTS database was used as the reference for taxonomic nomenclature (USDA, NRCS 2006).

Plant culture

Native plants of the temperate zone often have specific conditions that must be met to induce germination. Consequently, there is no single factor that will induce

germination for all species. In order to answer our first research question, detailed germination trials were conducted in order to determine the specific requirements for each of the 17 test species (see Table 2-1).

Those species which required stratification were started in 50 cell plastic germination trays (53 cm x 27 cm, with each cell measuring 5 cm x 5 cm x 6 cm deep) containing commercial potting soil for seedlings (OBC#1 soil without fertilizer). The OBC#1 mix contains peat moss, fine perlite, washed sand and wetting agent and its suggested use includes seed propagation. Seeds were started in the seedling trays and placed in a dark 8°C walk-in cooler for stratification. Following cold stratification of between one to six weeks, the seedling trays were placed on a mist bench in a greenhouse for five to seven days to allow seedlings to emerge and establish. Individual seedlings were then transplanted into 10 cm diameter plastic pots (10 cm diameter x 9.8 cm deep, volume of 350 ml from base to line 1.2 cm below top) and placed on a greenhouse bench. An artificial potting mix (OBC #3, containing mulch, peat moss, pumice, vermiculite, perlite, and wetting agent) to which was added slow release fertilizer (Osmocote® 14 x 14 x 14 3 to 4 month slow release) provided at a rate of 1.05 g per pot was used. Species that did not require stratification were planted directly into the 10 cm pots and placed on the mist bench until seedling emergence. *Danthonia californica* was planted in a sandy loam soil because this species exhibited nutrient deficiency symptoms when grown in the OBC#3 mix.

Greenhouses were maintained under the same environmental conditions as those recommended for the current EPA vegetative vigor test (Series 850, Ecological Effects Tests, US EPA 1996a). Air temperature was set at 25/20 °C day/night with supplemental HID lighting set for 16 hours to increase the average daily light intensity during winter months.

After the pots were transferred to greenhouse benches they were placed on large sheet trays for a week so they could be bottom watered. Once the seedlings were established, daily overhead watering was initiated. Care was given not to get water or soil on the leaves as some species such as *Prunella vulgaris* var. *lanceolata* exhibited marginal necrosis after leaves came in contact with wet soil.

Following emergence, plants were allowed to grow on the greenhouse bench for approximately twenty-one days prior to herbicide treatment. This was longer than the established EPA vegetative vigor tests which suggest seedlings be treated 14 days after emergence. Fourteen days appeared to be too short since most native species only developed true leaves one week after emergence. Since the plants were so small the scales would not be able to accurately detect differences in dried biomass. It was then decided to extend the growing period to three weeks prior to treatment. Following herbicide treatment, plants were returned to the greenhouse and allowed to grow for an additional 14 days. After this period all above-ground biomass was harvested and dried for 3 days at 60°C prior to determining dry weight.

Herbicide selection, formulations, rates, and application

Three herbicides were used in this experiment: glyphosate, tribenuron, and fluazifop. Herbicides were selected because they were widely used in the Willamette Valley study area, pose a potential risk due to high activity at low concentrations (Brown 1996), or have not been studied extensively.

Glyphosate was selected since it is the most commonly used herbicide in Benton County, Oregon, as well as worldwide (Woodburn 2000). Herbicide usage was determined by using the National Center for Agricultural Policy (1998) statewide herbicide usage data adjusted for crops grown in a county, as described by Pfleeger et al. (2006). Glyphosate, a foliar applied amino acid synthesis inhibitor, is a non-selective systemic herbicide used to control most pest plants. This herbicide is often used in agricultural settings prior to planting to ensure a weed-free seedbed, or in non-crop land where total vegetation control is desired (Vencill 2002; Tu et al. 2001). Glyphosate was applied in the commercial form of Roundup Original® (liquid) at a field application rate of 2.3 L/ha or 835 g/ha.

Tribenuron is an acetolactate synthase inhibitor (ALS), belonging to the Sulfonylurea class of herbicides, which are extremely active at very low use rates and pose a risk to non-target plants (Fletcher et al. 1993). Tribenuron is a broad-leaf specific herbicide which is labeled for use on *Triticum aestivum* (wheat), *Hordeum vulgare* (barley), and fallow fields. When applied at the proper stage of development,

grasses tend to be unaffected. Tribenuron, an ALS inhibitor, was applied in the form of Express®XP (solid) at a field application rate of 11.7 grams/ha.

Fluazifop is a lipid synthesis inhibitor herbicide used to control perennial and annual grasses, while leaving forbs unharmed. Grass-specific herbicides are of interest to restorationists due to their potential use in habitat management (Hitchmough et al. 1994). Since some of the most widespread weeds in Willamette Valley natural areas are members of the Poaceae (e.g. *Holcus lanatus*, *Festuca arundinacea*, *Phalaris arundinacea*, etc.) a grass specific herbicide could potentially remove invasive grasses while posing little threat to broadleaf plants (Clark et al. 2004). Fluazifop was applied in the commercial form of Fusilade®DX (liquid) at a field application rate of 0.88 L/ha or 210 g/ha.

Herbicide concentrations were selected at levels to reflect drift conditions, i.e., below typical field application rates (f.a.r.). Four herbicide rates were used for tribenuron (0.1, 0.01, 0.002, and 0.001 x f.a.r.). For fluazifop and glyphosate a 1.0 x f.a.r was included. Since fluazifop is labeled as a grass specific herbicide broadleaf plants should be resistant at the labeled rate. The 1.0 x f.a.r. for glyphosate served as an indicator of proper herbicide formulation. For all experiments the non-ionic surfactant Preference™ was applied at 0.5% v/v in the spray solution. A "no-spray" control as well as a "carrier control" were included for each experiment. We compared the "no-spray" controls with the "carrier controls" to determine if the surfactant had any affect on plant growth separate from the effects of the herbicide itself.

Herbicide rates were based on previous research that has shown that off-site deposition of herbicides may reach 0.1 x the applied rate at distances of 1600 m downwind, depending on topography and weather conditions (Chester and Ward, 1984). Other researchers have shown that the range of herbicide spray drift that is likely to occur off-site is between 0.1 and 0.01 x the applied rate (Al-Khatib and Peterson, 1999; Bailey and Kapusta, 1993; Snipes et al., 1991, 1992). The two lowest concentrations (0.002 and 0.001 x f.a.r.) were included to determine if extremely low concentrations, representing potential long-distance drift from point of application, could impact plant performance. The experiments therefore included concentrations

of herbicides that bracketed levels of known activity and levels of no suspected activity. Modeling non-linear responses to the herbicides was also made easier by the fact that the levels of 0.1, 0.01 and 0.001 represent a log10 sequential dilution in herbicide concentrations.

Herbicide solutions were applied in a RIC spray chamber (Model RC-5000-100EP Overhead Trolley Sprayer for Reproducible Agrochemical Research, Mandel Scientific Company, Ltd, Guelph, Ontario, Canada). The sprayer was equipped with an even flat fan spray nozzle (TeeJet TP8002E-VS, Spraying Systems Co., Wheaton, Illinois, USA). The nozzle was positioned 46 cm above mid-canopy and traversed the chamber at 3.0 km per hour. Spray was dispersed at 40 psi giving a delivery rate of 234 liters per hectare. The sprayer was calibrated to determine the volume of herbicide delivered per surface area of chamber (ml/cm2) gravimetrically. Sprayer calibrations were adjusted for the effects of the surfactant on herbicide delivery. Plants were treated with a single pass of the track sprayer with a concentration of herbicide solution prepared to deliver the target rate in g/ha. After treatment, plants were removed from the sprayer and allowed to dry until the leaves were dry. After drying, the plants were moved back into the greenhouse.

The surfactant Preference® had little effect on the plants from the glyphosate, tribenuron, and fluazifop experiments. Across all comparisons among 17 species, 3 herbicides, and 2 experiments there were only four instances that shoot dry weight of the carrier control plants differed significantly from the no-spray plants. Thus we concluded that treatment effects were generally due to the active ingredients and not the surfactant.

Experimental design

For each experiment there were six single pot replicates for each treatment level resulting in a total of 42 pots for the tribenuron experiment and 48 pots for the glyphosate and fluazifop experiments (which included the 1.0 x f.a.r. treatment). Approximately 10% extra plants were grown for each species to provide extra plants so unusually small, large or unhealthy individuals could be discarded.

Plant height was measured one day prior to herbicide treatment. If plant height was uniform (coefficient of variation < 20%) then all of the plants were randomly assigned to treatment level groups. If the coefficient of variation was greater than 20%, stratified random sampling was used for assignment to treatments. Pre-treatment stratification by size was useful when plants were not uniform in size and eliminated the need to include plant height as a covariate to account for effects of plant size.

All experiments were conducted twice for each herbicide and each species. Due to differences in germination dates and growth rates among species, growing space limitations in the greenhouse, and herbicide treatment capability of the track sprayer, all experiments could not be conducted on the same day. Experiments were repeated as close as possible in time to reduce the effect of differences in growing conditions between experiments.

Statistical Analysis

Analysis of variance (ANOVA) was performed for each species to test for herbicide treatment effects, replicate experiment effects, and treatment x experiment interactions (alpha = 0.05). Shoot dry weight was the response variable for all analyses. In some cases, a natural log transformation was used to stabilize the variances prior to ANOVA and regression analysis. The data for non-sprayed and carrier control plants were analyzed separately to determine the significance of any carrier effects.

The no observed effect concentration (NOEC) in shoot dry weight was calculated for treated versus control plants using Dunnett's one-tailed Test. The NOEC represents the lowest concentration that did not differ significantly from control plants (alpha = 0.05). If there was a significant herbicide effect but not a significant herbicide treatment by experiment interaction, the NOEC value was determined across both experiments for that species and herbicide. If there was a significant herbicide rate by experiment interaction, separate NOEC values were determined for each experiment. PROC GLM in SAS® for PC (Version 9.1) was used for the ANOVA analysis.

The EC_{25} was calculated for each species using nonlinear regression based on the probit model of Bruce and Versteeg (1992). When there was no hebicide rate by experiment interaction, the data for the two replicate experiments were combined to fit a common probit model. Otherwise a separate probit models was fit for each experiment. If there was no herbicide rate by experiment interaction, but a significant experiment effect, the probit model was calculated with separate y-intercepts for each experiment. We calculated 51 EC_{25s} so there is about a 2.5% chance that a type II error occured.

The PROC NLIN procedure in SAS® was used fit the probit model to the data. The probit model was parameterized so that the EC_{25} was one of the model parameters. The probit model has the form:

$$Y = \quad Ro \ \Phi [(\log(EC_{25}) - \log (C)) / \sigma + 0.6745]$$

where C=herbicide rate, Ro is the predicted response at zero herbicide rate, σ is the shape parameter and Φ [ ] is the cumulative distribution for a standard normal distribution. The model parameters to be estimated were EC_{25}, σ, and Ro. The probit model is a monotonically decreasing function with a maximum at C=0 and a horizontal asymptote of zero. The probit model was fit to the data using weighted nonlinear regression with weights equal to the reciprocal of the predicted response (Bruce and Versteeg 1992). In several cases where heterogeneity of variance was severe, a natural log transformation was applied to the response variable and unweighted nonlinear regression was used to fit the probit model.

RESULTS

Plant propagation

Four native species, *Bromus carinatus*, *Clarkia amoena*, *Elymus trachycaulus*, and *Gilia capitata*, and the three introduced species, *Cynosurus echinatus, Leucanthemum vulgare*, and *Festuca arundinacea* germinated readily in the greenhouse in three to seven days without any special treatment (Table 2-1). One of the most common requirements for germination was for a seed to experience an extended cold and moist period. Cold stratification naturally occurs over the winter season, but can be simulated by placing seeds on a moist paper towel in a cold chamber. *Madia elegans* required one week of cold stratification, while *Sanguisorba occidentalis, Collinsia grandiflora, Festuca roemeri, Prunella vulgaris* var. *lanceolata,* and *Ranunculus occidentalis* required two weeks. Two perennial forb species, *Eriophyllum lanatum* and *Potentilla gracilis* and the perennial grass *Danthonia californica* required six weeks of cold stratification. *Danthonia californica* was the only species planted in a native mineral soil since it exhibited chlorosis and general poor growth in the potting medium.

Many hard seeded species will not absorb water unless their seed coat is scarified. One species, *Lupinus albicaulis,* required a scarification process in order to imbibe. This process occurs naturally by wind and soil abrasion, by freezing, or by passing through the digestive system of an animal. In this experiment scarification was achieved by rubbing seeds against a hard surface with sandpaper for about ten seconds.

All of the plants developed true leaves after one week. *Eriophyllum lanatum, Leucanthemum vulgare, Lupinus albicaulis,* and *Potentilla gracilis* were the slowest growing species. These plants required an additional seven days of growth to attain sizes similar to that of the other 14 species.

Glyphosate Responses

NOEC Values

The NOEC values for many of the species were either 0.1 or 0.01 x f.a.r., reflecting the general non-specific phytotoxicity of glyphosate (Table 2-2). The more sensitive species, *Festuca roemeri, Madia elegans, Potentilla gracilis, Prunella vulgaris* and *Sanguisorba occidentalis* had NOEC values of 0.01 x f.a.r. More glyphosate tolerant species, *Bromus carinatus, Clarkia amoena, Gilia capitata* and *Lupinus albicaulis* had no effects from glyphosate at the rate of 0.1 x f.a.r.

EC25 Values

The EC_{25} values and their standard errors for all of the species following the glyphosate treatments are listed in Table 2-3. *Ranunculus occidentalis* and *Elymus trachycaulus* had a significant rate by experiment interaction, so separate EC_{25} values are provided for these two species. Except for the four tolerant species noted in the NOEC section, the remaining EC_{25} values represent pooled data across experiments since there were no additional significant rate by experiment interactions. There were no apparent differences in glyphosate susceptibility among the annual or perennial grasses, annual or perennial forbs, and introduced species though this difference was not tested statistically since EC_{25} values are singly calculated values for a mean response (Figure 2-1).

Glyphosate had no effect, and, hence, there were no EC_{25} values for four species: *Bromus carinatus, Clarkia amoena*, *Gilia capita* and *Lupinus albicaulis*. Based on the data for dry weight, *Potentilla gracilis* was the most sensitive species to glyphosate with an EC_{25} of 0.012 x f.a.r. *Elymus trachycaulus, Festuca arundinacea, Festuca roemeri, Madia elegans, Prunella vulgaris* and *Ranunculus occidentalis* were all sensitive to glyphosate and had EC_{25} values ranging between 0.028 and 0.056 x f.a.r. *Collinsia grandiflora*, *Cynosurus echinatus*, *Danthonia californica, Eriophyllum lanatum, Leucanthemum vulgare* and *Sanguisorba occidentalis* showed intermediate responses to glyphosate with EC_{25} values from 0.096 to 0.156 x f.a.r.

Tribenuron Responses

NOEC Values

Four Willamette Valley forb species were highly sensitive to tribenuron based on reductions in shoot dry weight, with NOEC values of 0.001 or 0.002 x f.a.r.

(*Clarkia amoena, Prunella vulgaris, Ranunculus occidentalis* and *Sanquisorba occidentalis*) (Table 2-2). Five other forb species were moderately sensitive to tribenuron with NOEC values of 0.01 to 0.1 x f.a.r. (*Collinsia grandiflora, Gilia capitata, Leucanthemum vulgare, Lupinus albicaulis, Madia elegans* and *Potentilla gracilis*). One forb species (*Eriophyllum lanatum*) and six grass species (*Bromus carinatus, Cynosurus echinatus, Danthonia californica, Elymus trachycaulus, Festuca arundinacea* and *Festuca roemeri)* were resistant to tribenuron showing no reduction in dry weight even at the field application rate.

EC_{25} Values

Based on EC_{25} values for dry weight, there were six species that were considered to be the most sensitive to tribenuron ($EC_{25} \leq 0.008$ x f.a.r.) (Table 2-4, Figure 2-2): *Clarkia amoena, Collinsia grandiflora, Potentilla gracilis, Prunella vulgaris, Ranunculus occidentalis and Sanquisorba occidentalis*). These species were all native forbs, three of which were annuals. Three species of forbs were also affected, but were less sensitive, by tribenuron with EC_{25} values ranging between 0.034 and 0.091 x f.a.r., respectively, *Madia elegans, Gilia capitata*, and *Lupinus albicaulis.* The introduced forb *Leucanthemum vulgare* was sensitive to tribenuron with an EC_{25} of ≤ 0.022 x f.a.r.). Seven species showed no reduction in dry weight with the tribenuron treatment: all six of the grass species and one forb, *Eriophyllum lanatum.* Figure 2-2 shows that there was no great difference in tribenuron sensitivity between the annual and perennial forbs.

Fluazifop Responses

NOEC Values

As expected, the grass-specific herbicide fluazifop primarily impacted the growth of grasses. However, there were differences in fluazifop sensitivity among these species. The most sensitive species, *Cynosurus echinatus* and *Elymus trachycaulus*, had NOEC values of 0.001 to 0.01 (Table 2-2). Three other grasses, *Bromus carinatus, Danthonia californica* and *Festuca arundinacea* and one forb species, *Sanguisorba occidentalis*, were less sensitive to fluazifop, with NOEC values

of 0.01 to 0.1 x f.a.r. One grass species, *Festuca roemeri*, and the rest of the forb species were not affected by fluazifop even at the highest rate of 1.0 x f.a.r.

EC_{25} Values

The native grasses, *Elymus trachycaulus*, *Danthonia californica*, and *Bromus carinatus*, were by far the most fluazifop sensitive grass species, with low EC_{25} values for reductions in shoot dry weight of $\leq$ 0.044 x f.a.r. (Table 2-5, Figure 2-3). The introduced grass *Cynosurus echinatus* was of medium sensitivity, with a low EC_{25} value for reduced dry weight in experiment 2, but a higher EC_{25} value in experiment 1. The introduced grass *Festuca arundinacea* had a relatively high EC_{25} of 0.231 x f.a.r. Interestingly, the native annual forb *Sanguisorba occidentalis* was sensitive to fluazifop and had an EC_{25} value of 0.140 x f.a.r., while the native grass *Festuca roemeri* was tolerant at the highest rate (1.0 x f.a.r.).

DISCUSSION

This study indicates that native, non-crop grass and forb species can effectively be obtained and grown for phytotoxicity testing purposes. Through careful evaluation of stratification requirements and germination times it was possible to predictably grow uniform populations for phytotoxicity testing. Resources such as the Native Plants Propagation Protocol Database (http://www.nativeplantnetwork.org) have also improved the ability to grow plants that were once considered challenging. Thus, our study reinforces the view of Boutin et al. (2004) that non-crop plant species can be easily grown and maintained in a greenhouse for toxicity testing for risk assessment purposes.

As expected, due to the targeted efficacy of commercial herbicides for different types of plants, the 17 plant species used in this study differed widely in susceptibility to different herbicides as indicated by shoot dry weight (Tables 2-3, 2-4, 2-5; Figures 2-1, 2-2, 2-3). The greatest variability in the EC_{25} values was with tribenuron where there was a 45-fold difference in susceptibility between the sensitive species *Prunella vulgaris*, and the most resistant species that showed some reduction in dry weight, *Lupinus albicaulis* (Table 2-4, Figure 2-2). There was a greater than 10-fold difference in the EC_{25} for shoot dry weight in response to glyphosate between the most sensitive species, *Potentilla gracilis*, and the most resistant species which showed some response, *Cynosurus echinatus* and *Sanguisorba occidentalis* (Table 2-3, Figure 2-1). There was nearly a 100-fold difference in the EC_{25} for shoot dry weight in response to fluazifop between the sensitive species *Danthonia californica* and the more resistant species that showed some response *Festuca arundinacea* (Table 2-5, Figure 2-3). This variability of up to several orders of magnitude in response to the different herbicides used in our study was similar to that reported earlier by McKelvey et al. (2002) and Boutin et al. (2004).

In addition to wide variation among Willamette Valley species to the three herbicides, there was also variation among species within families. The17 species represented 8 plant families, but were primarily represented by the Poaceae and Asteraceae families. Each of these families had species that were both relatively tolerant and sensitive to glyphosate based on the EC_{25} data for shoot dry weight (Table

2-3). In the Poaceae, *Festuca roemeri* was resistant to fluazifop while *Elymus trachycaulus* was a highly sensitive species. Similarly, while nearly all of the forb species were sensitive to tribenuron, *Eriophyllum lanatum* was tolerant while *Leucanthemum vulgare* was sensitive despite the fact that these two species are both members of the Asteraceae. This may be due to the fact that the foliage of *Eriophyllum lanatum* is covered with hairs, which may impede the uptake of herbicide by the foliage (Colquhoun 2001). Our results were similar to those of Boutin et al. (2004) who found variability in response to glyphosate and a sulfonylurea, metsulfuron methyl, among species within the same family. For example, they reported that the Asteraceae species *Centaurea cyanus* was more resistant (higher EC_{50}) whereas other Asteraceae species such as *Inula helenium* were more sensitive to metsulfuron.

The results indicated that each non-crop species was resistant (no effects) and sensitive ($EC_{25} < 0.10$ x f.a.r.) to at least one of the herbicides based on dry weight data. This suggested that, separate from the relative adverse effects of herbicide drift to native plants, there is the potential to use herbicide dose / plant response data to favor the growth of species of interest while inhibiting the growth of undesirable species (usually introduced and/or invasive species) which are sensitive to specific herbicides. For example, three introduced species may be controlled by glyphosate (*Festuca arundinacea*), fluazifop (*Cynosurus echinatus)* and tribenuron (*Leucanthemum vulgare*). Particularly interesting is the relative resistance to fluazifop of a native fescue (*Festuca roemeri*) which showed no negative responses, while the introduced fescue (*Festuca arundinacea*) showed reduced dry weight between 0.1 and 1.0 x f.a.r. (Figure 2-3). This difference in sensitivity may be due to the fine, small diameter leaves of *Festuca roemeri* which may provide less surface area for herbicide interception compared to *Festuca arundinacea*; or there may be a physiological or biochemical basis for the different resistance to fluazifop between these grasses. To my knowledge there are no studies that conclusively report the source of resistance exhibited by *Festuca roemeri*. Other species that are resistant to fluazifop have a type of acetyl-coA carboxylase (the enzyme that catalyzes the first committed step in fatty

acid synthesis) that is not inhibited by fluazifop, metabolize the herbicide, or move it into a vacuole away from the site of action (Vencill 2002).

In this controlled greenhouse experiment, the high selectivity of some herbicides (i.e. grass-specific and forb-specific herbicides) was found to stress plant species differentially. In the field this differential susceptibility could result in shifts in dominance in a forb dominated plant community to a grass dominated community or vice versa. These shifts in plant assemblages can result in changes in frequency and even local extinction of desired species (Tilman 1988). In our study all six grasses were tolerant to the herbicide tribenuron while all of the forbs that were tested were injured well below the 10% estimated rate of drift that is proposed in the literature (Chester and Ward 1984; Teske et al. 2002; Parkin and Merritt 1988). A hypothetical drift event of tribenuron onto a sward of plants similar to the ones used in this study could conceivably shift the balance of the community away from forbs and towards grasses. This outcome becomes much more likely when one considers the fact that many agricultural fields are sprayed with herbicides multiple times a year (Mark Mellbye, personal communication).

Boutin and Jobin (1998) demonstrated that herbicides can contribute to shifts in plant communities adjacent to intensively cropped fields from native species toward more weedy species. These areas can then act as sources for seed dispersal and can further promote the spread of weed species. Furthermore, crops are currently being genetically engineered to be resistant to certain herbicides, which could result in more widespread use of those chemicals and subsequently shift the dominance of field margin vegetation towards tolerant non-native species (Maxwell and Weed 2001; Blackburn and Boutin 2003). In Oregon, creeping bentgrass (*Agrostis stolonifera*) was engineered to be resistant to glyphosate. It was later reported that long distance pollen-mediated gene flow between genetically modified creeping bentgrass and wild plants occurred 3.6 km beyond the "controlled" field sites (Reichman 2006; Watrud et al. 2004). This is a striking example of how the species composition of a field boundary could easily shift from that of a diverse plant assemblage to one dominated by a single tolerant (or resistant) species following a repeated disturbance such as herbicide drift. Couple the area and frequency of herbicide applications worldwide

with the apparent susceptibility of boundary vegetation to shifts in plant community composition and the consequences for ecological systems appear substantial.

The simplification of a plant community could have indirect negative consequences for wildlife that may depend on habitat diversity or require specific resources. For example, Schultz and Dlugosch (1999) suggested that population vigor of the endangered Fender's blue butterfly (*Icaricia icarioides fenderi*) was strongly associated with resource availability (hostplants and nectar sources) in Willamette Valley prairies. The authors noted that habitats with higher nectar plant diversity sustained larger butterfly populations for longer periods of time enabling the butterflies to lay more eggs.

Semi-natural habitat types play a crucial role in maintaining biological diversity in an otherwise uniform and biologically depauperate landscape. For example, field boundaries with higher habitat resources (diverse plant assemblages) sustain larger populations of bees and orthoptera (Marshall et al. 2006). Duelli and Obrist (2003) quantified the dependence of invertebrates on agricultural borders and found that 63% of the invertebrates captured were dependent on the semi-natural habitats adjacent to farmland.

In conclusion, the results from this study should help improve EPA's Series 850.4150 Vegetative Vigor Test to assess the risk of chemical herbicides to non-target plants. The great variability in EC_{25} values for the 17 species tested shows how genetically diverse native plants have a broad range in susceptibility to different herbicides. The differential sensitivity of non-crop plants to herbicides has interesting ecological implications because it suggests that species composition in plant communities may shift depending on the herbicides being applied and the species present. These changes in species composition could have profound impacts on the wildlife whose life histories are intricately intertwined with native plants. Future research should focus on field studies to document the relationships that exist between Willamette Valley native plant communities and wildlife and how we might mitigate the potential impacts that herbicides have on these ecological systems.

RECOMENDATIONS

Based on the results from these experiments with a range of Willamette Valley non-crop plants the following recommendations for improvements in the vegetative vigor test protocol are provided.

1. Selection. Select herbaceous species (grasses and forbs) based on regional importance. Use a spatial analysis, GIS based system as described in Pfleeger et al. (2006) to obtain specific areas of interest. Have an initial extended list of species with at least twice the number of species needed for testing since germination and seedling survival may necessitate the exclusion of some species.

2. Culture. Native plants generally are slower growing than crop plants and have a more compact form. Therefore it is recommended that small containers (e.g., 10.2 cm diameter pots) with one plant per pot be used. Use of a commercial potting mix is recommended since mineral soils can loose soil structure and pose issues with respect to watering. It is recommended that stratification requirements be tested as some species may require a cold-moist period to induce germination even though the seed supplier may not recommend it.

Even though attempts were made to grow the native plants as uniformly as feasible in a greenhouse, the resulting growth was variable from plant to plant. Thus, extra plants should be grown so that outliers, in terms of size, can be discarded when selecting a uniform population of plants for an experiment. For this study at least 10% more pots per species than required for a particular experiment were grown to allow us to remove potential outliers. Many seeds per pot could be started to provide an even larger number of plants for selection and to ensure a more uniform population. Additionally, since many species exhibited a moderate amount of variation in plant size prior to treatment, a larger number of replicates than 6 could be used per experiment in order to better understand levels of natural variation.

In greenhouse studies such as these, it is important to minimize environmental variability between replicate experiments by conducting them close together during the same season. For the Willamette Valley study there were few experiment by

herbicide rate interactions since the experiments were conducted close together in time.

3. Herbicide Treatments. Although a field application rate was included for some experiments, in a number of cases the highest rate applied was 0.1 x the f.a.r. Since some of the calculated EC_{25} values were above the 0.1 x f.a.r, it would improve the accuracy of the estimate if a higher rate was included, thus avoiding extrapolation beyond one's data.

4. Response Parameters. Shoot dry weight was the most reliable quantitative measure of plant vegetative growth and response to herbicides treatments. Height can be easily measured, but because many native plants initially form a rosette, height measurements may not be informative.

5. Reproduction Tests. Separate from the vegetative vigor tests, changes in fitness following low-dose herbicide exposure could be tested with a plant reproduction test. Measuring the impact of low-dose herbicides on plant reproduction may be more informative than biomass sampling since the long-term viability of plant populations depends on sucessful reproduction and establishment of new individuals.

Table 2-1. Willamette Valley species and their propagation requirements.[1]

Species	Code	Common Name	Family	Type	Stratification (5 C)	Days Mist Bench / Warm	Days of growth in seed tray	Days of growth in 10 cm pots
Forbs								
Clarkia amoena	CLAAMO	Farewell-to-spring	Onagraceae	Native	No	3	7	14
Collinsia grandiflora	COLGRA	Blue-eyed Mary	Scrophulariaceae	Native	2 weeks	5 to 7	7	14
Eriophyllum lanatum	ERILAN	Wooly sunflower	Asteraceae	Native	6 weeks	3 to 5	7 to 14	14
Gilia capitata	GILCAP	Globe gilia	Polemoniaceae	Native	No	3 to 5	7	14
Leucanthemum vulgare	LEUVUL	Oxeye daisy	Asteraceae	Introduced	No	5 to 7	7 to 14	14
Lupinus albicaulis	LUPALB	Sicklekeel lupine	Fabaceae	Native	2 weeks	3	7 to 14	14
Madia elegans	MADELE	Common madia	Asteraceae	Native	1 week	1 to 3	7	14
Potentilla gracilis	POTGRA	Slender cinquefoil	Rosaceae	Native	6 weeks	3 to 5	7 to 14	14
Prunella vulgaris	PRUVUL	Self-heal	Lamiaceae	Native	2, 4 weeks [3]	5 to 7	7	14
Ranunculus occidentalis	RANOCC	Western buttercup	Ranunculaceae	Native	2, 4 weeks [3]	5 to 7	7	14
Sanguisorba occidentalis	SANOCC	Western burnet	Rosaceae	Native	2 weeks	3 to 5	7	14
Grasses								
Bromus carinatus	BROCAR	California brome	Poaceae	Native	No	3 to 5	7	14
Cynosurus echinatus	CYNECH	Bristly dogstail grass	Poaceae	Introduced	No	5	7	14
Danthonia californica [2]	DANCAL	California oatgrass	Poaceae	Native	6 weeks	7 to 14	7	14
Elymus trachycaulus	ELYTRA	Slender wheatgrass	Poaceae	Native	No	3 to 5	7	14
Festuca arundinacea	FESARU	Tall fescue	Poaceae	Introduced	No	3 to 5	7	14
Festuca roemeri	FESROE	Roemer's fescue	Poaceae	Native	2 weeks	5 to 7	7	14

[1] Generally plants were allowed to develop as seedlings on the mist bench in seedling trays with OBC soil #1 for 7 days. Plants were then transplanted into 10 cm pots with OBC soil # 3 with Osmocote® fertilizer added. They were allowed to grow for two weeks prior to herbicide application.

[2] Native soil was used because potting soil resulted in poor growth.

[3] Some germination occurs after 2 weeks, but best germination occurs after 4 weeks.

Table 2-2 The lowest concentration of an herbicide below that which produced a statistically significant reduction in growth (No Observed Effect Concentration or NOEC), for 17 Willamette Valley prairie plant species. Values are x field application rate of 834 g ha^{-1} active ingredient for glyphosate, 8.8 g ha^{-1} for tribenuron, and 210 g ha^{-1} for fluazifop. [1]

Species [1]	Glyphosate	Tribenuron	Fluazifop
Forbs			
Clarkia amoena	ns	0.001	ns
Collinsia grandiflora	0.1	0.01,0.1	ns
Eriophyllum lanatum	0.1	ns	ns
Gilia capitata	ns	0.01	ns
Leucanthemum vulgare ‡	0.1	0.002,0.01	ns
Lupinus albicaulis	ns	0.01	ns
Madia elegans	0.01	0.01	ns
Potentilla gracilis	0.01	0.01	ns
Prunella vulgaris	0.01	0.001,0.002	ns
Ranunculus occidentalis	0.01,0.1	0.002	ns
Sanguisorba occidentalis	0.01	0.002	0.1
Grasses			
Bromus carinatus	ns	ns	0.01, 0.1
Cynosurus echinatus ‡	0.1	ns	0.001,0.002
Danthonia californica	0.1	ns	0.01
Elymus trachycaulus	0.01,0.1	ns	0.002,0.01
Festuca arundinacea ‡	0.1	ns	0.1
Festuca roemeri	0.01	ns	ns

[1] Introduced species indicated by a "‡".

Table 2-3. EC_{25} values for Willamette Valley prairie plant species treated with glyphosate. The EC_{25} is the effective herbicide concentration reducing biomass of treated plants by 25% compared to controls. Values are x field application rate of 834 g ha^{-1} active ingredient. [1]

Species [2]	Experiment 1		Experiment 2		Average/Combined	
	EC_{25}	SE EC_{25}	EC_{25}	SE EC_{25}	EC_{25}	SE EC_{25}
Forbs						
Clarkia amoena	ns across experiments				ns	
Collinsia grandiflora	significant across experiments				0.096	0.091
Eriophyllum lanatum	significant across experiments				0.1	0.109
Gilia capitata	ns across experiments				ns	
Leucanthemum vulgare ‡	significant across experiments				0.1	0.064
Lupinus albicaulis	ns across experiments				ns	
Madia elegans	significant across experiments				0.031	0.012
Potentilla gracilis	significant across experiments				0.012	0.027
Prunella vulgaris	significant across experiments				0.056	0.024
Ranunculus occidentalis	0.035	0.035	0.028	0.02	0.031	
Sanguisorba occidentalis	significant across experiments				0.156	0.09
Grasses						
Bromus carinatus	ns across experiments				ns	
Cynosurus echinatus ‡	significant across experiments				0.154	0.098
Danthonia californica	significant across experiments				0.117	0.061
Elymus trachycaulus	0.036	0.031	0.029	0.041	0.032	
Festuca arundinacea ‡	significant across experiments				0.043	0.041
Festuca roemeri	significant across experiments				0.046	0.028

[1] When there is a significant herbicide rate x experiment interaction based on the analysis of variance, separate EC_{25} values for each experiment are given, and an average EC_{25} value for the two experiments is calculated. When there is no significant interaction, the EC_{25} value for the combined data is given in the average column for a parameter. A "ns" indicates no significant rate effect at $p<0.05$.

[2] Introduced species indicated by a "‡".

[3] PROC NLIN failed to stabilize. There was a significant rate effect, but NOEC was ns.

[4] Converged for EC_{25} value, but SE was infinity.

Table 2-4. EC_{25} values for Willamette Valley prairie plant species treated with tribenuron. The EC_{25} is the effective herbicide concentration reducing biomass of treated plants by 25% compared to controls. Values are x field application rate of 8.8 g ha^{-1} active ingredient. [1]

Species [2]	Experiment 1		Experiment 2		Average/Combined	
	EC_{25}	SE EC_{25}	EC_{25}	SE EC_{25}	EC_{25}	SE EC_{25}
Forbs						
Clarkia amoena	0.002	0.003	0.001	0.001	0.002	
Collinsia grandiflora	0.003	0.007	0.011	0.008	0.007	
Eriophyllum lanatum	ns across experiments				ns	
Gilia capitata	significant across experiments				0.041	0.038
Leucanthemum vulgare [4] ‡	0.022	0.019	0.002	0.005	0.012	
Lupinus albicaulis	significant across experiments				0.091	0.002
Madia elegans	significant across experiments				0.034	0.053S
Potentilla gracilis	significant across experiments				0.007	0.005
Prunella vulgaris	0.001	0.001	0.002	0.002	0.001	
Ranunculus occidentalis	significant across experiments				0.002	0.002
Sanguisorba occidentalis	significant across experiments				0.008	0.003
Grasses						
Bromus carinatus	ns across experiments				ns	
Cynosurus echinatus ‡	ns across experiments				ns	
Danthonia californica [3]	ns across experiments				ns	
Elymus trachycaulus	ns across experiments				ns	
Festuca arundinacea ‡	ns across experiments				ns	
Festuca roemeri	ns across experiments				ns	

[1] The EC_{25} is the effective herbicide concentration affecting the relevant biotic parameter by 25% (Bruce and Versteeg 1992). When there is a significant herbicide rate x experiment interaction based on the analysis of variance, separate EC_{25} values for each experiment are given, and an average EC_{25} value for the two experiments is calculated. When there is no significant interaction, the EC_{25} value for the combined data is given in the average column for a parameter. A "ns" indicates no significant rate effect at p=0.05.

[2] Introduced species indicated by a ‡".

[3] Four experiments.

[4]Three experiments. The data here for exp. 2 (in 1 column) and 3 (in 2 column), group 1 ns.

Table 2-5. EC_{25} values and their standard errors (SE) for Willamette Valley native plants treated with fluazifop. The EC_{25} is the effective herbicide concentration reducing biomass of treated plants by 25% compared to controls. Values are x field application rate of 210 g ha^{-1} active ingredient.[1]

Species [2]	Experiment 1		Experiment 2		Average/Combined	
	EC_{25}	SE EC_{25}	EC_{25}	SE EC_{25}	EC_{25}	SE EC_{25}
Forbs						
Clarkia amoena	ns across experiments				ns	
Collinsia grandiflora	ns across experiments				ns	
Eriophyllum lanatum	ns across experiments				ns	
Gilia capitata	ns across experiments				ns	
Leucanthemum vulgare ‡	ns across experiments				ns	
Lupinus albicaulis	ns across experiments				ns	
Madia elegans	ns across experiments				ns	
Potentilla gracilis	ns across experiments				ns	
Prunella vulgaris	ns across experiments				ns	
Ranunculus occidentalis	ns across experiments				ns	
Sanguisorba occidentalis	significant across groups				0.14	0.222
Grasses						
Bromus carinatus	0.073	0.039	0.015	0.008	0.044	
Cynosurus echinatus ‡	0.118	0.037	0.048	0.018	0.083	
Danthonia californica	significant across experiments				0.01	0.009
Elymus trachycaulus	0.003	0.003	0.002	0.002	0.003	
Festuca arundinacea ‡	0.153	0.065	0.309	0.026	0.231	
Festuca roemeri	ns across experiments				ns	

[1] When there is a significant herbicide rate x experiment interaction based on the analysis of variance, separate EC_{25} values for each experiment are given, and an average EC_{25} value for the two experiments is calculated. When there is no significant interaction, the EC_{25} value for the combined data is given in the average column for a parameter. A "ns" indicates no significant rate effect at p=0.05. An "ns" indicates no significant rate effect at p=0.05.

[2] Introduced species indicated by a "‡".

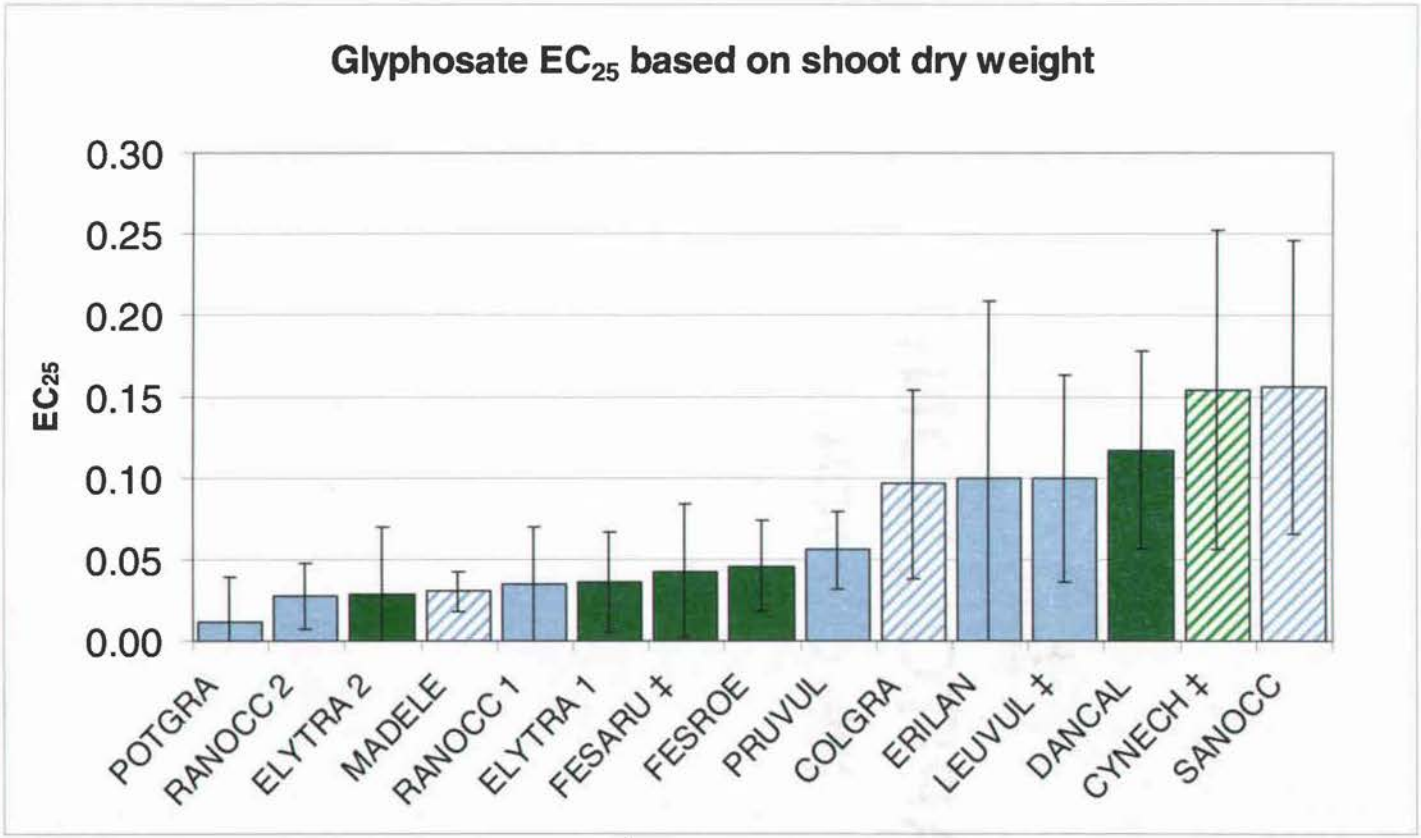

Figure 2-1. Calculated EC_{25} values (x f.a.r. of 834 g ha^{-1} active ingredient) based on shoot dry weight data for Willamette Valley prairie species treated with glyphosate. The EC_{25} is the effective herbicide concentration reducing biomass of treated plants by 25% compared to controls. Solid blue bars are perennial forb species, hatched blue bars are annual forb species. Solid green bars are perennial grass species, the hatched green bar is an annual grass species. The numbers/symbols following the species name indicates whether data originate from the first experiment (1), the second experiment (2) or combined if no symbol (no significant rate*experiment interaction), or introduced species (‡). Error bars are ± one standard error. For species abbreviations see Table 2-1.

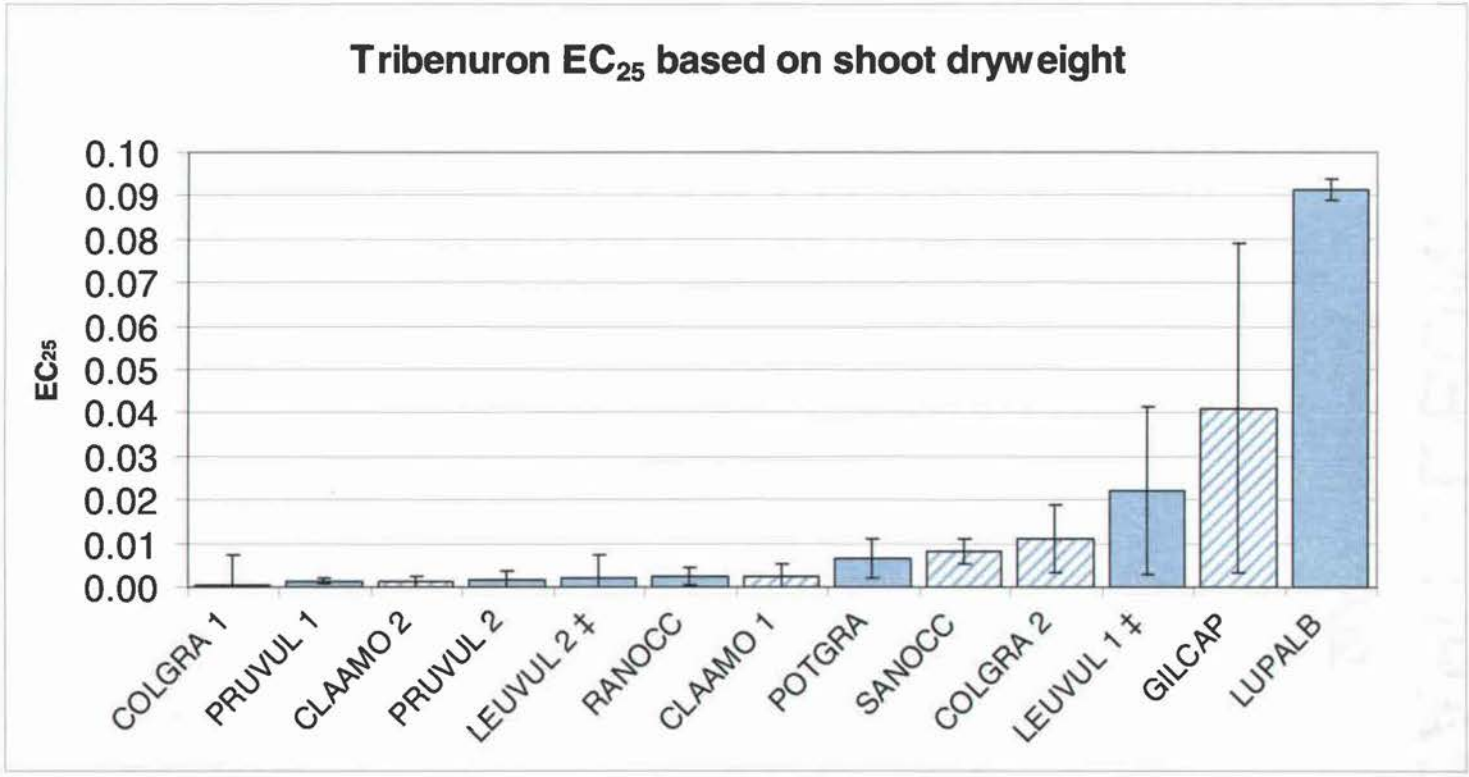

Figure 2-2. Calculated EC_{25} (x f.a.r. of 8.8 g ha^{-1} active ingredient) for Willamette Valley prairie species based on shoot dry weight for tribenuron. Solid blue bars are perennial forb species and hatched blue bars are annual forb species. The EC_{25} is the effective herbicide concentration reducing biomass of treated plants by 25% compared to controls. All seven species of grasses were not significantly affected by the highest rate of tribenuron (10% field application rate) and are not included in the graph. The numbers/symbols following the species name indicates whether data originate from the first experiment (1), the second experiment (2) or combined if no symbol (no significant rate*experiment interaction), or introduced species (‡). Error bars are ± one standard error. For species abbreviations see Table 2-1.

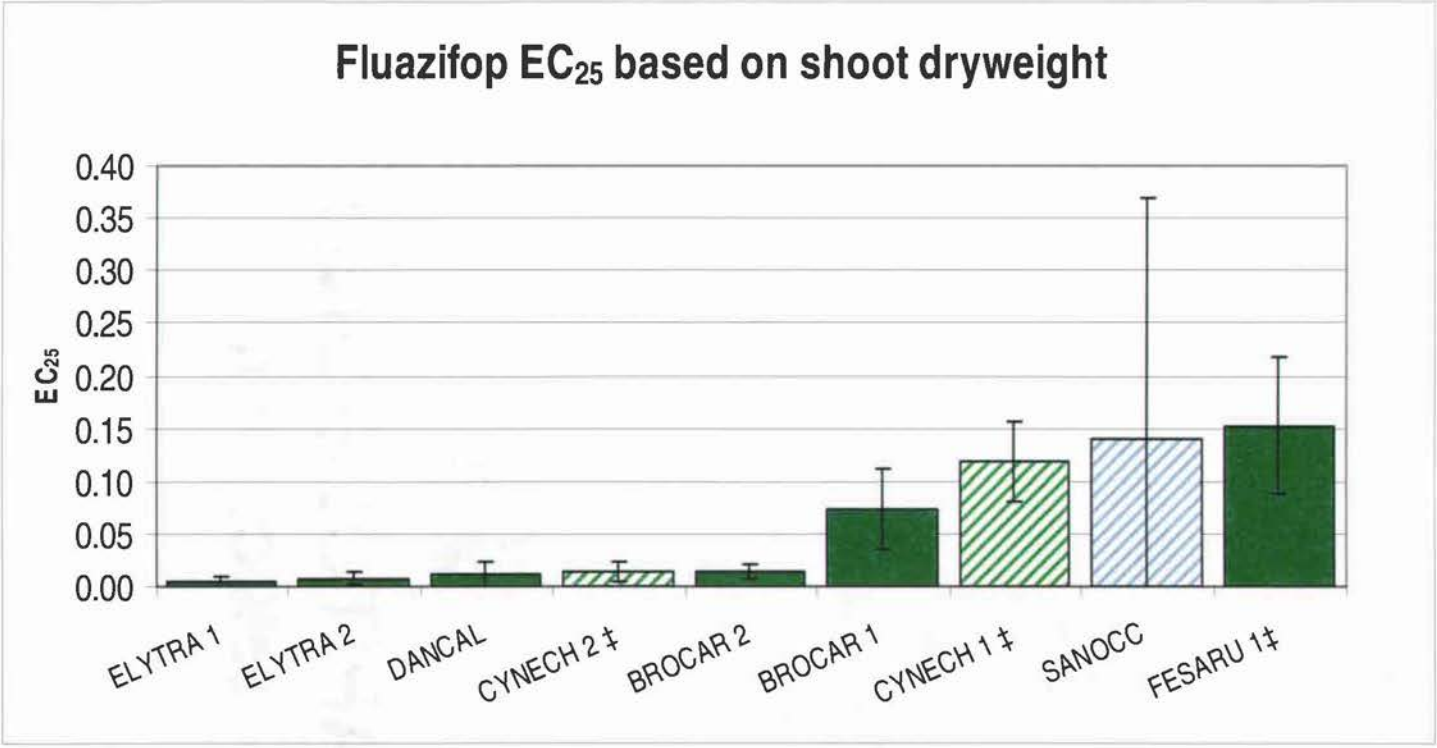

Figure 2-3. Calculated EC25 (x f.a.r. of 210 g ha-1 active ingredient) for Willamette Valley species based on shoot dry weight for fluazifop The EC25 is the effective herbicide concentration reducing biomass of treated plants by 25% compared to controls. Solid green bars are perennial grass species, hatched green bars indicate annual grass species, and the hatched blue bar indicates an annual native forb. The numbers/symbols following the species name indicates whether data originate from the first experiment (1), the second experiment (2) or combined if no symbol (no significant rate*experiment interaction), or introduced species (‡). Shoot dry weight of eleven species of forbs were not significantly affected by the field rate of fluazifop and are not included in the graph. Error bars are ± 1 standard error. For species abbreviations see Table 2-1.

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Chapter 3 : Herbicides as a restoration tool

INTRODUCTION

Introduced species pose a major threat to the ecological integrity of natural areas worldwide (Pimentel et al. 2000; Vitousek et al. 1997). Introduced species can displace native species through competitive interactions and cause substantial reductions in biodiversity (Cronk and Fuller 1995). Of particular concern is when introduced species contribute to the decline of rare or endangered species. Restorationists therefore employ a number of tools such as mowing, tilling, burning, solarization, carbon additions, and competitive plantings in order to improve conditions for native species by reducing the dominance of introduced species (Pavlik et al. 1993; Alpert and Maron 2000; Wilson et al. 2004; Wilson and Clark 2001; for a review specific to the Willamette Valley see Fitzpatrick 2004). Herbicides are an additional tool that can provide effective control of introduced species (Marrs 1984; Grilz and Romo 1995; Hurst and John 1999a; Rice et al. 1997), though much of the work thus far has been anecdotal or site specific.

Native prairies, which once dominated the landscape of the Willamette Valley, are considered among the rarest of Oregon's ecosystems and are in critical need of conservation (Titus et al. 1996). One of the largest remaining parcels of native upland prairie, Butterfly Meadows (Benton County), is being invaded by false-brome (*Brachypodium sylvaticum* (Huds.) Beauv), an invasive perennial grass that is capable of completely dominating understory and open habitats to the exclusion of most other native species (Kaye 2001). At some sites it competes directly with rare and endangered species, such as the federally threatened Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [C.P. Smith] L. Phillips), host plant for the endangered Fender's blue butterfly (*Icaricia icariodes fenderi* [Lycaenidae]) (Schultz et al. 2003; Wilson et al. 2003).

Widespread herbicide use for weed control in commercial agriculture began in the 1940's following the invention of 2,4-D and MCPA. It was not until recent decades, however, that herbicides were specifically used to remove weeds from natural areas with the intent of promoting native plant diversity (Marrs 1984; Marrs

1985; Rice et al. 1997; Merriam 1999; Travenicek 2005; Grilz and Romo 1995). One of the main concerns with herbicide use in natural areas is that native species and non-native species are often intermixed in complex patterns, making it difficult to avoid injury to desired species (Erickson et al. 2006). This is especially important when restoration occurs in areas containing endangered species, where non-target injury is unacceptable. Additionally, herbicides have been reported to have both direct and indirect effects on non-target organisms in natural communities (Freemark and Boutin 1995; Jobin et al. 1997; Kleijn and Snoeijing1997; Marrs et al. 1991), and have been detected in remote areas far from the site of application (Donald et al. 2001). Even though some herbicides such as glyphosate have been reported to be relatively environmentally benign due to low animal toxicity and persistence (Carlisle and Trevors 1988; Tu et al. 2001), reports still emerge to the contrary regarding impacts on plants (Cornish and Burgin 2005), amphibians (Relyea et al. 2005), and fish (Mitchell et al. 1987).

Managing degraded Willamette Valley prairie habitats for a particular species composition will require restorationists to actively manipulate plant communities since they are presently on a trajectory towards dominance by both introduced and woody species (Schultz and Crone 1998; Schultz and Dlugosch 1999; Wilson et al. 2003). The goal of this study was to test chemical herbicides as a means to direct a degraded plant community to a desired composition. The specific objectives were to 1.) determine which treatments were most effective at reducing *Brachypodium sylvaticum* dominance, 2.) determine if native species declined following herbicide treatments, and 3.) describe the compositional changes in the plant communities over a four year period. Treatments included the use of a broad-spectrum herbicide, grass-specific herbicide, and pre-emergent herbicides in various combinations as well as varying concentrations and different dates of application, as well as one mowing treatment, for a total of 13 treatments and a no-spray control.

METHODS

Site description

Butterfly Meadows is one of only four high quality native upland prairie sites remaining in the Willamette Valley (Wilson 1996). This experiment was established in a portion of the meadow (named the Quarry site) that is heavily invaded by the non-native grass *Brachypodium sylvaticum*. The quarry site is a southwest facing meadow (aspect 230 degrees) with a slope ranging from 0 to 5 % and an elevation of 316 meters. The NAD27 UTM coordinates for the site are 0472451 easting and 4939752 northing.

Brachypodium sylvaticum *description*

False-brome is a caespitose perennial grass that regenerates primarily by seed (Grime et al. 1988), while individual clumps coalesce and form dense monocultures. These monocultures exclude native species and have resulted in declines in local plant diversity where it is well established (Kaye 2001). European biologists report that the seeds of false-brome are short-lived in the seed bank (Grime et al. 1988); whether that holds true in western Oregon is yet to be determined (Tom Kaye personal communication). A related species, *Brachypodium pinnatum*, has been the focus of control efforts in European grasslands due to its negative impacts on local plant diversity (Hurst and John 1999a; Hurst and John 1999b).

Experimental design

In August 2003 84 2 m x 1.5 m treatment plots were established at the quarry site of Butterfly Meadows. Each plot contained at least 10% cover of *Brachypodium sylvaticum* and was randomly assigned one of 14 herbicide treatments including a no-spray control, yielding six replicates per treatment (Table 6). The percentage cover of each vascular plant species within a 1 m x 0.5 m sub-sample was estimated to the nearest 1%. Percentage cover readings were recorded during August 2003 (pre-treatment), May 2004 (first growing season), May 2005 (second growing season), and June 2006 (third growing season).

Herbicide selection

Herbicide selection was based on a 2002 pilot study which was conducted in a different part of Butterfly Meadows. This study identified a number of different herbicides that were effective at controlling *Brachypodium sylvaticum* while minimizing harm to native species (Clark et al. 2004). Based on the findings from the pilot study, the broad spectrum herbicide glyphosate (Accord™ concentrate) and the grass specific herbicide fluazifop (Fusilade DX™) were selected. Tank mixtures including each of the above herbicides with one of two pre-emergent herbicides, Pendimethalin (Pendulum 3.3 EC ™) and Oryzalin (Surflan A.S.™), were also used. In all, 13 experimental treatments with different herbicide combinations, concentrations, and dates of application were applied, as well as a no-spray control (Table 3-1).

Herbicide applications

The herbicide treatments were sprayed with a gas operated backpack sprayer equipped with a six nozzle spray boom. Herbicides were applied at a rate of ten gallons per acre with water as the carrier in all treatments. Herbicides were applied on August 1, 2003 and September 29, 2003. Concentrations for the thirteen herbicide treatments can be found in Table 3-1. The goal was to avoid damaging the native species by timing the applications such that most native prairie species had senesced while *B. sylvaticum* was still actively growing.

Statistical analysis

Analysis of variance was used to determine which treatment was most effective at reducing *B. sylvaticum* dominance over time. Two separate analyses were performed. The first analysis measured how effective the herbicides were at killing *B. sylvaticum* by comparing the total percentage cover of *B. sylvaticum* in the treatment plots to the control plots. In the second analysis the proportion of the total vegetative cover contributed by *B. sylvaticum* was used as the response variable. A comparison of the mean proportion of *B. sylvaticum* for each treatment was made to the mean proportion of *B. sylvaticum* for the control for each individual year using Dunnett's

test. This analysis is more useful for determining the relative dominance of a single species with respect to the community. Four years of treatment means for species richness and diversity (Shannon's index) were also compared to the control plots using Dunnett's test. All univariate analyses were performed using PROC GLM in SAS version 9.1 (SAS Institute).

Multivariate statistics were used to answer the remaining two research questions. Prior to performing any of the analysis percent cover data were merged into a single matrix of 336 sample units (84 plots x 4 years) by 53 species (total number of species observed in all plots over 4 years). The values for species abundance ranged over three orders of magnitude, so a square root transformation was performed in order to reduce this large distribution and improve the power of the analyses. A square root transformation was chosen since it applies an intermediate level of compression to higher values, thus retaining some of the original weight of dominant species over minor species

Rare species occurring in less than 5% of the sample units were deleted from the matrix to reduce overall noise and emphasize patterns of the abundant species (McCune and Grace 2002). A separate response variable matrix contained all of the categorical treatment combinations as well as thirteen quantitative variables: total plant cover, total species richness, Shannon's diversity index, percentage of bare ground, and proportion of cover attributed to introduced species, native species, annual species, perennial species, graminoids, forbs, vines, ferns, and *B. sylvaticum*. All multivariate statistical analyses were performed with PC-ORD statistical software, version 5.0 (McCune and Mefford 2006).

Multi-response permutation procedure (MRPP) was used to determine if native species declined following herbicide treatments. MRPP is a nonparametric test of the hypothesis of no difference between two or more a priori groups and can employ Sørensen's distance measure (Mielke and Berry 2001). MRPP is a useful tool for analyzing community data since it does not rely on distributional assumptions (Zimmerman et al. 1985). A priori groups were defined as the 14 treatment types. MRPP was first used 14 times to test the hypothesis of no significant difference in native species composition between pre-treatment and one-year post-treatment plots.

MRPP was also used to test for differences in total species composition between pre-treatment and three-years post-treatment for each of the 14 treatments.

Any treatments with significant differences in native species composition (as determined by MRPP) were subsequently analyzed with indicator species analysis (ISA) to determine which individual species played an important role in altering species composition. ISA quantifies how individual species were distributed among treatment groups by assigning each species an indicator value (IV) ranging from 0 to 100% (Dufrêne and Legendre 1997). An indicator value is based on both a species abundance and frequency in a particular group, whereby a perfect indicator (100% IV) occurs exclusively in one particular group and is always present in that specific group. A Monte Carlo test with 1000 randomizations was used to test the hypothesis that an indicator value for a given species was no larger than expected by chance.

In order to graphically display how species composition changed over time, non-metric multidimensional scaling (NMS) was used to ordinate the sample units in species space. NMS is an ordination technique that minimizes noise while emphasizing the strongest structure in the dataset, enabling it to extract structure from datasets with non-linear relationships between variables (McCune and Grace 2002). Initially NMS was performed using PC-ORD's autopilot menu, set on slow and thorough, while using Sørensen's distance measure. A 2-D solution was chosen and the analysis was re-run requesting Sørensen's distance measure, two axes, 100 iterations with the real data, no step down in dimensionality, and no randomized runs. The final stress of the model was 16.8. The ordinations were rotated ten degrees in order to load the strongest explanatory variable on axis one, as suggested by McCune and Grace (2002). Joint plots from the second matrix were used as overlays in the ordinations to clarify the relationship between the ordination scores and related variables such as species diversity, proportional cover of introduced species, bare ground, etc. An r^2 of 0.4 was used as a cutoff for including a particular vector. Successional vectors were also used to connect plots that had been repeatedly measured four times.

RESULTS

Brachypodium sylvaticum response

Prior to treatment, there was no difference in mean *B. sylvaticum* cover between the treatment plots and the control plots ($p = 0.52$). One year after herbicide applications were applied, mean cover of *B. sylvaticum* in the control plots was signifcantly different from treatment plots ($p < 0.001$). Plots that were mowed in August and treated with glyphosate in September declined from 26.7% (± 4.9 SE) to 2.7% (± 0.5) *B. sylvaticum*. Plots that were treated with a medium rate of Fusilade and Pendulum in September declined from 23.7% (± 4.7) to 9.5% (± 2.9). Plots treated with Fusilade at a medium rate and Surflan in September dropped from 34.3% (± 3.6) to 10.2% (± 4.1). Those plots that were treated with glyphosate and Pendulum in September dropped from 37.0% (± 11.1) to 10.0% (± 4.4). *B. sylvaticum* cover also declined from 40.0% (± 10.6) to 1.9% (± 0.6) in the plots that were treated with glyphosate and Surflan in September. The two remaining treatments, glyphosate applied in September with Surflan and glyphosate applied alone in August, were associated with a decline of *B. sylvaticum* from 23.5% (± 3.1) to 2.3% (± 1.2) and 30.2% (± 6.7) to 4.8% (± 1.5) respectively. The declines in *B. sylvaticum* cover were short-lived, however, since there were no significant differences in mean *B. sylvaticum* cover between the treated plots and the controls in 2005 and 2006 (Table 3-2).

In all cases except one in 2005, *B. sylvaticum* contributed equally over all four years to the proportion of total vegetative cover in the treatment plots when compared to the control plots. The exception occurred in the plots that were treated with glyphosate and Surflan in September. *B. sylvaticum* in these plots contributed 54% less to the cover of total vegetation than it did in the control plots (95% confidence interval -100% to -6%).

Community response

A total of 53 vascular plant species were recorded in the 84 sample plots over the course of the four growing seasons. Species richness ranged from 3.8 (± 0.61) to

8.8 (±1.3) species per plot per treatment prior to herbicide applications, with a mean alpha diversity of 5.6 for all 84 plots (Table 3-2). Values for Shannon's diversity index ranged from 0.66 (± 0.2) to 1.38 (± 0.2) prior to treatment. Species richness and diversity did not differ significantly between treatment and control plots over the three years following herbicide applications (native and introduced species combined).

One year after herbicide application native species abundance of the four treatments listed below increased significantly when compared to their pretreatment condition. Plots that were mowed in August and treated with glyphosate in September changed significantly in native species composition ($p = 0.012$) (Table 3-4). There were no strong indicator species associated with these plots, however, suggesting that existing species increased in cover, while establishment of new species was not significant. Native species composition in plots treated with glyphosate and Pendulum in September also changed significantly ($p = 0.029$), and indicator species analysis suggested that the native annual forb *Clarkia amoena* was weakly associated with the post-treatment plots ($p = 0.062$) (Table 3-5). Plots that were treated with glyphosate in August and September both differed in native species composition following treatment ($p = 0.005$ and $p = 0.008$ respectively). The native species *Vicia americana* and *Bromus carinatus* increased in cover following the August glyphosate treatment and were found to have very high indicator values ($p = 0.002$ and $p = 0.016$) (Table 3-5). The native forbs *Lotus purshianus* and *Clarkia amoena* both increased in cover following the September application of glyphosate ($p = 0.015$ and $p = 0.08$). Also of interest were treatments that did not differ in native species composition following herbicide treatment, since this indicates that the treatments did not negatively impact the native component of the plant community. Included in this category were the two treatments that were most successful at maintaining low *B. sylvaticum* cover. Those treatments included the August application of Fusilade at a medium rate with Surflan and glyphosate applied in September with Surflan ($p = 0.65$ and $p = 0.73$ respectively).

NMS was used to ordinate sample units in species space and to display the successional patterns that developed over four years (Figure 3-1). The ordination accounted for 80% of the variance in the data, with 55% loaded on axis one and 25%

loaded on axis two. Axis one represented a strong gradient in life forms. Plots falling on the left side of the ordination were highly correlated with annual vegetation (r^2 = 0.657), while plots falling on the right side of the ordination were dominated by perennials (r^2 = 0.657). Axis two represented a strong gradient in dominance by graminoids (r^2 = 0.780), much of which was associated with *B. sylvaticum* (r^2 = 0.525).

Four treatments and the control are shown in Figure 3-1. The ordination was reduced to include only four treatments and the control over four years since the original ordination contained 56 plots (14 treatments x 4 years) and was not visually interpretable. The treatments in the ordination include a September application of Fusilade at a low rate with Pendulum, a September application of Fusilade at a medium rate with Surflan, a September application of glyphosate and Surflan, an August application of just glyphosate, and a no-spray control.

The September application of Fusilade applied at a low rate with Pendulum changed the least in species composition (Figure 3-1). The minor shifts in the community were similar to the control plots. Although there was a shift in importance of graminoids in the second growing season after treatment, this effect disappeared during the next growing season. The September application of Fusilade at a medium rate with Surflan was associated with a community level decline in graminoids and an increased abundance of annuals one year after treatment. Change in the community over the next two years was subtle, but the proportion of graminoids began to rise gradually. The September application of glyphosate and Surflan was associated with a sharp decline in the proportion of graminoid cover after the first year of treatment. Two years following treatment there was a gradual recovery of graminoid cover, but the major trend was toward community dominance by annuals. There was little change in this community during the third growing season. The application of just glyphosate in August was associated with a very strong shift from a perennial dominated community to an annual dominated community after the first year. During the second growing season the importance of graminoids increased, though there was a subsequent decrease the following year. Although the control plots exhibited annual variation in species composition, there was little net change over the four years.

DISCUSSION

Brachypodium sylvaticum response

One year after herbicide applications, six of the thirteen treatments were effective at significantly reducing the cover of *B. sylvaticum* to levels below that of controls. The treatments that contained the broad spectrum herbicide glyphosate were most effective at controlling the weed after one year. Treatments that included Fusilade had mixed results. Two of the fusilade treatments were effective at reducing the cover of *B. sylvaticum* after the first year, while six treatments failed to have an impact. The low application rates of some of the treatments may have been responsible for the poor performance of this herbicide, or the herbicide may have been applied during a period of low growth for *B. sylvaticum*. Fusilade moves within the phloem of grasses and inhibits the synthesis of lipids in areas of new growth. If the grass is under drought stress as was likely in August with low soil moisture and high light intensity, resources allocated towards new growth may be low, thus reducing the effectiveness of the herbicide. With the arrival of cooler temperatures and light rain in September (2.5 cm in September 2003 as measured at the nearby Hyslop Weather Station, Corvallis, Oregon), environmental conditions may have been more favorable for plant growth than they were in August. It is during this period of active transport of photosynthate that glyphosate and Fusilade are most effective (Colquhon 2001). The two effective Fusilade treatments were applied in September and included the preemergent herbicide Pendulum.

The treatment effects that resulted in reduction of *B. sylvaticum* were short lived. *B. sylvaticum* returned to pre-treatment levels in all of the plots by the second growing season. Although abundance of *B. sylvaticum* cover declined significantly in some plots after the first year, the proportion of cover this species contributed to the total cover was fairly constant over the four years (Table 3-3). This result could have been used earlier in the study to predict the eventual return to dominance of *B. sylvaticum*. The re-establishment and eventual dominance of introduced species following a single control measure may be a common occurrence in restoration projects (Sveinson and McLachlan 2003; Wilson et al. 2004; Travnicek et al. 2005).

For example, Hurst and John (1999a) applied glyphosate to an English meadow that was dominated by a closely related grass species, *Brachypodium pinnatum*. Dominance of *B. pinnatum* was initially reduced by the glyphosate applications, but the species quickly recolonized and increased in cover after one growing season. Treated plots never developed into the species rich community that grew adjacent to the *B. pinnatum* dominated meadow. Although our study did not include soil analyses, differences in a species' ability to exploit soil resources may be one factor that confers competitive advantage (Hurst and John 1999b) and should be investigated at Butterfly Meadows.

Community response

Although one of the main objectives of this study was to determine which herbicide treatments were most effective at reducing the cover of *B. sylvaticum*, a second objective was to consider what effects the treatments had on the entire plant community.

The combined approach of using MRPP and indicator species analysis enabled us to determine that overall native species composition was not significantly harmed by any of the treatments. In fact, the treatments that did differ in species composition following the treatments were associated with increases in the native species *Clarkia amoena*, *Bromus carinatus*, *Vicia americana*, and *Lotus purshianus*. Two introduced species, *Geranium dissectum* and *Poa pratensis*, were strongly associated with the pre-treatment plots, but declined markedly one year following treatments.

Although the herbicides were successful at removing the dominant introduced species (*B. sylvaticum*) for one year, the newly created habitat was colonized primarily by introduced species (Table 3-5). By the third growing season introduced species were strongly associated with six of the treatments. *Torilis arvensis*, a member of the Apiaceae, was the most common indicator species, while widespread weeds such as *Hypericum perforatum* and *Cirsium arvense* increased in some treatment plots. *Bromus carinatus* was the only native species to be associated with a treatment (glyphosate in August) in the third growing season. In a Willamette Valley restoration site Wilson et al. (2004) found that complete recovery by introduced species also occurred by the second growing season after various non-herbicide control techniques

were implemented (tilling, burning, and solarization). They suggested that a replete seed bank, plants that were tolerant to control measures, and dormant plants acted as sources for reestablishment of the introduced species. Further, since control measures had greatly reduced the cover of other plants, the surviving plants grew quickly in a competition-free and resource rich environment. Similarly, the seedbank in a degraded tallgras prairie greatly affected the species composition that developed following a glyphosate treatment (Sveinson and McLachlan 2003). Bareground that was not colonized by species from the seedbank allowed for establishment of wind dispersed introduced species (especially grasses) the following growing season.

Indicator species analysis from this study showed that changes in the vegetation following herbicide treatment were best explained by new species establishing in the herbicide treated plots. Since the study area has been dominated by *B. sylvaticum* and other introduced species for many years, the seedbank likely contained propagules from these species. Although native species were present, they did not proliferate after being released from intense competition by *B. sylvaticum.* Since much of the remaining Willamette Valley upland prairies are small in size and lack connectivity (Wilson 1996), it is very likely that many species are suffering from inbreeding depression due to reduced gene flow. A plant's ability to set viable seed, and hence its colonization and expansion capacity, has been shown to decline under conditions of isolation and habitat fragmenation (Soons and Heil 2002).

If the native species at Butterfly Meadows were not recruitment limited one would expect a large increase in cover of native species following removal of the dominant species. For example, Grilz and Rome (1995) used a wick application of glyphosate to control *Bromus inermis* in a native prairie in Saskatchewan. Although brome control was not complete after one application, the native species *Festuca altaica* ssp. *hallii*, was unharmed. Abundant recruitment from the seedbank supported the authors' hypothesis that recovery of native vegetation was likely to occur.

Case studies like the ones noted above have sparked many debates in the scientific community that seek to explain the mechanisms behind invasability. A recent article by MacDougal and Turkington (2005) frames the question nicely; "Are invasive species the drivers or passengers of change in degraded ecosystems?" Two

main camps have developed; those who argue that invasive species are competitively superior and therefore suppress native species, and those who suggest that introduced species are less susceptible to propagule dispersal, altered disturbance regimes, or stochastic events and are thus passengers by chance. Seed addition studies have commonly been used to show that plant species of native grasslands are strongly recruitment limited (Tilman 1997; Seabloom et al. 2003a; Seabloom et al. 2003b; Turnbull et al. 2000; Foster and Tilman 2003; Wilson, S.D. et al. 2004). On the other side of the argument, many restoration practitioners report that invasive species competitively exclude native species and must be repeatedly controlled in order for native species to re-establish (Annen et al. 2005; Randall 1996; Rice et al. 1997). Despite the appearance of two strong opinions regarding invasive species being drivers or passengers some authors suggest that ecological restoration will require an integrated approach that addresses both control of harmful invasives and augmentation of native species (Wilson, M.V. et al 2004; Hurst and John 1999a; MacDougal and Turkington 2005).

The results from Butterfly Meadows suggest that both competitive exclusion and low native species recruitment influenced the development of the vegetation following herbicide applications. Successional trajectories created using NMS provide a useful way for visualizing the complex patterns of community change that occurred over time (Figure 3-1). The treatments selected included the control, a treatment that failed to control *B. sylvaticum* (Fusilade applied at a low rate in September with Pendulum), the treatment with the greatest *B. sylvaticum* control after one year (glyphosate applied in August), and the two treatments with the greatest resistance to *B. sylvaticum* re-invasion during the second growing season (Fusilade applied at a medium rate in September with Surflan and glyphosate applied in September with Surflan).

Although the control and the failed Fusilade treatment were not static in species space, the observed shifts in community composition were minor compared to the other three treatments. For the control plots, the minor shifts may be attributed to the further invasion of false-brome into the site, the presence of highly transitory annual species, or variation among years in environmental conditions such as water

availability that affected plant growth. Since the Fusilade treatment was not effective at removing the dominant species, the pre-existing vegetation remained relatively unchanged over the sampling period. The treatment that included just glyphosate showed a drastic shift in community dominance from perennials to annuals after the first year. The second year was marked by an increase in the dominance of graminoids, possibly due to recruitment of *B. sylvaticum* from the seedbank. The proportion of graminoids then dropped slightly, possibly due to the fact that many newly established plants failed to persist through the winter. The ordination also depicts a concurrent recovery by the forbs during the decline of the graminoids. Both of the treatments that included the pre-emergent herbicide Surflan exhibited a decline in dominance by graminoids after the first year, a slight recovery the second year, neither fully returned to their pretreatment condition after the third year.

This study illustrates how herbicides can be used to quickly change the trajectory of a given community by reducing the cover of introduced species while creating favorable conditions for native species. Some herbicide treatments were very effective at controlling *B. sylvaticum* in Butterfly Meadows over a one year period. This particular meadow and other heavily infested sites will most likely require multiple herbicide treatments in order to reduce the initial cover of the weed, control plants that were tolerant to the first application, and control subsequent recruitment of the weed through the seedbank. The use of a preemergent herbicide may increase the efficacy of the treatments in the short-term by limiting seedling emergence after one year, though a single application of these herbicides still failed to afford long-term control of *B. sylvaticum*. Since seed production by *B. sylvaticum* at this site was observed to be prolific during all four years, addressing recolonization of this site by recruitment of seedlings is crucial. In areas where native plant cover is low, a seeding treatment should be considered due to the apparent lack of available native propagules. If preemergent herbicides are used one must be certain that these chemicals have degraded before attempting to seed an area. Finally, local conditions will dictate the selection of an appropriate weed management tool. Successful weed management strategies will combine multiple approaches for weed control over many years in order to shift the balance toward native dominated communities.

Table 3-1 Herbicide treatments, formulations, and date of application for plots containing *Brachypodium sylvaticum* adjacent to Butterfly Meadows. Each treatment was replicated 6 times. Glyphosate was applied as Accord concentrate™, fluazifop was applied as Fusilade DX™, Pendimethalin was applied as Pendulum 3.3 EC ™, and Oryzalin was applied as Surflan A.S.™. All herbicide applications included water as a surfactant, while Activator 90 was used as a sufactant for the glyphosate treatments and MSO (methylated seed oil) was used for the Fusilade treatments. The abbreviation a.i. means active ingredient and v/v is volume/volume. Fusilade was applied at three different concentrations: low, medium, and high. The August treatment was applied on August 1, 2003 and the September treatment was applied on September 29, 2003.

	Treatments	Rate of Application	Treatment dates	
			Aug.	Sept.
1	Fusilade (high)	0.375 lb a.i./acre and MSO (1% v/v)	X	
2	Fusilade (high)	0.375 lb a.i./acre and MSO (1% v/v)		X
3	Mow (Aug) & glyphosate (Sept)	2 lb a.i./acre + Activator 90 (0.5% v/v)	X	X
4	Fusilade (low) and Pendulum	0.094 lb a.i./acre and 3.96 lb a.i./acre and MSO (1% v/v)		X
5	Fusilade (med) and Pendulum	0.188 lb a.i./acre and 3.96 lb a.i./acre and MSO (1% v/v)	X	
6	Fusilade (med) and Pendulum	0.188 lb a.i./acre and 3.96 lb a.i./acre and MSO (1% v/v)		X
7	Fusilade (high) and Pendulum	0.375 lb a.i./acre and 3.96 lb a.i./acre and MSO (1% v/v)	X	
8	Fusilade (high) and Pendulum	0.375 lb a.i./acre and 3.96 lb a.i./acre and MSO (1% v/v)		X
9	Fusilade (med) and Surflan	0.188 lb a.i./acre and 6 lb a.i./acre and MSO (1% v/v)		X
10	Glyphosate and Pendulum	2 lb a.i./acre and 3.96 lb a.i./acre and Activator 90 (0.5% v/v)		X
11	Glyphosate and Surflan	2 lb a.i./acre and 6 lb a.i./acre and Activator 90 (0.5% v/v)		X
12	Glyphosate	2 lb a.i./acre and Activator 90 (0.5% v/v)	X	
13	Glyphosate	2 lb a.i./acre and Activator 90 (0.5% v/v)		X
14	No spray control			

Table 3-2. Mean *Brachypodium sylvaticum* cover, species richness, and Shannon's diversity index for four years. Numbers in parenthesis are standard errors. Dunnett's procedure was used to compare each treatment to the control. Treatments that are significantly different from the control are marked with *. Refer to Table 3-1 for a description of treatments.

***Brachypodium sylvaticum* cover**

treatment	2003		2004			2005		2006	
1	32.3	(6.4)	22.6	(7.2)		50.5	(16.1)	29.8	(10.0)
2	26.8	(6.5)	16.2	(8.1)		40.3	(11.3)	39.5	(5.4)
3	26.7	(4.9)	2.7	(0.5)	*	55.3	(10.1)	65.0	(6.7)
4	43.5	(3.9)	34.3	(9.8)		58.5	(13.2)	45.5	(10.3)
5	35.5	(4.2)	30.5	(6.3)		64.0	(11.9)	46.8	(7.4)
6	23.7	(4.7)	9.5	(2.9)	*	27.5	(6.6)	43.7	(6.2)
7	35.3	(5.7)	21.2	(6.2)		52.7	(7.2)	41.0	(7.1)
8	40.0	(7.9)	22.7	(5.8)		53.3	(10.1)	50.3	(6.7)
9	34.3	(3.6)	10.2	(4.1)	*	19.2	(8.4)	36.8	(7.1)
10	37.0	(11.1)	10.0	(4.4)	*	63.5	(10.0)	39.6	(9.4)
11	40.0	(10.6)	1.9	(0.6)	*	11.8	(7.0)	23.4	(5.3)
12	23.5	(3.1)	2.3	(1.2)	*	27.0	(15.3)	27.0	(10.6)
13	30.2	(6.7)	4.8	(1.5)	*	70.5	(9.9)	50.8	(6.0)
14	30.2	(5.0)	35.0	(9.3)		54.8	(14.0)	37.7	(6.8)

species richness

treatment	2003		2004		2005		2006	
1	4.33	(0.84)	8.33	(0.88)	8.00	(1.39)	8.17	(1.54)
2	7.00	(0.86)	8.83	(1.14)	7.33	(1.23)	9.83	(1.01)
3	3.83	(0.54)	7.17	(0.87)	6.83	(0.54)	8.00	(1.18)
4	5.33	(1.09)	6.33	(0.33)	6.50	(0.89)	6.83	(1.51)
5	4.50	(0.43)	7.67	(0.49)	6.00	(0.73)	7.83	(1.11)
6	8.83	(1.33)*	7.83	(0.79)	7.83	(0.75)	9.83	(1.11)
7	3.83	(0.61)	8.33	(0.84)	7.33	(0.21)	9.50	(0.72)
8	6.33	(1.15)	5.50	(0.34)	6.33	(0.88)	7.33	(1.31)
9	5.50	(0.89)	6.17	(1.05)	6.67	(0.84)	9.33	(1.82)
10	5.50	(0.85)	4.67	(0.61)	7.33	(1.05)	6.83	(0.83)
11	5.67	(1.05)	3.83	(0.54)	8.33	(0.99)	10.50	(0.76)
12	4.17	(0.48)	8.67	(0.92)	7.83	(0.48)	9.33	(1.54)
13	8.33	(1.31)	6.33	(0.71)	6.33	(1.12)	8.33	(1.02)
14	4.67	(0.99)	6.33	(1.15)	5.67	(0.88)	7.50	(0.99)

Shannon's diversity index

treatment	2003		2004		2005		2006	
1	0.55	(0.18)	1.18	(0.15)	0.90	(0.26)	1.16	(0.35)
2	0.94	(0.20)	1.11	(0.18)	1.13	(0.24)	1.21	(0.18)
3	0.70	(0.21)	1.18	(0.14)	0.85	(0.13)	0.84	(0.18)
4	0.66	(0.20)	0.83	(0.16)	0.78	(0.25)	0.85	(0.21)
5	0.72	(0.10)	1.44	(0.08)	0.67	(0.08)	1.07	(0.16)
6	1.38	(0.20)	1.48	(0.07)	1.32	(0.14)	1.32	(0.11)
7	0.60	(0.15)	1.49	(0.19)	1.05	(0.10)	1.11	(0.16)
8	0.74	(0.14)	1.05	(0.08)	0.84	(0.16)	0.98	(0.13)
9	0.77	(0.22)	0.95	(0.24)	1.05	(0.26)	1.18	(0.30)
10	0.83	(0.17)	1.02	(0.20)	0.70	(0.14)	0.90	(0.22)
11	0.87	(0.24)	0.88	(0.16)	1.29	(0.16)	1.44	(0.06)
12	0.77	(0.11)	1.24	(0.18)	1.00	(0.16)	1.26	(0.23)
13	1.19	(0.24)	0.82	(0.18)	0.69	(0.21)	0.86	(0.22)
14	0.71	(0.21)	1.16	(0.17)	0.69	(0.23)	1.07	(0.21)

Table 3-3. Difference in the relative cover of *Brachypodium sylvaticum* compared to control plots. Dunnett's procedure was used to make multiple comparisons. Treatments that are significantly different from the control are marked with *. The numbers in parenthesis are 95% confidence intervals. Refer to Table 1 for treatment descriptions.

trtmt	2003 / pretreatment		2004		2005			2006	
1	0.04	(-0.03 to 0.37)	-0.01	(-0.46 to 0.43)	-0.10	(-0.58 to 0.37)		-0.11	(-0.51 to 0.30)
2	-0.27	(-0.60 to 0.07)	-0.24	(-0.68 to 0.21)	-0.20	(-0.68 to 0.27)		-0.15	(-0.56 to 0.25)
3	-0.07	(-0.41 to 0.26)	-0.34	(-0.79 to 0.11)	-0.10	(-0.58 to 0.37)		0.05	(-0.36 to 0.46)
4	0.00	(-0.33 to 0.34)	0.13	(-0.32 to 0.57)	-0.02	(-0.50 to 0.45)		0.09	(-0.32 to 0.49)
5	-0.06	(-0.39 to 0.28)	-0.07	(-0.52 to 0.38)	0.01	(-0.47 to 0.48)		-0.02	(-0.43 to 0.39)
6	-0.24	(-0.58 to 0.09)	-0.23	(-0.68 to 0.21)	-0.37	(-0.85 to 0.11)		-0.14	(-0.54 to 0.27)
7	0.02	(-0.32 to 0.35)	-0.18	(-0.63 to 0.26)	-0.14	(-0.62 to 0.34)		0.01	(-0.40 to 0.41)
8	-0.02	(-0.36 to 0.31)	-0.03	(-0.48 to 0.41)	-0.04	(-0.52 to 0.43)		0.03	(-0.37 to 0.44)
9	-0.03	(-0.36 to 0.31)	-0.07	(-0.52 to 0.37)	-0.30	(-0.77 to 0.18)		-0.13	(-0.53 to 0.28)
10	-0.03	(-0.37 to 0.30)	0.08	(-0.36 to 0.53)	0.10	(-0.37 to 0.58)		0.07	(-0.34 to 0.48)
11	-0.20	(-0.53 to 0.13)	-0.20	(-0.64 to 0.25)	-0.54	(-1.01 to -0.06)	*	-0.37	(-0.78 to 0.04)
12	-0.13	(-0.46 to 0.21)	-0.38	(-0.83 to 0.07)	-0.41	(-0.89 to 0.07)		-0.27	(-0.68 to 0.13)
13	-0.16	(-0.50 to 0.17)	-0.28	(-0.72 to 0.17)	0.09	(-0.39 to 0.56)		0.10	(-0.31 to 0.51)

Table 3-4. Results of MRPP analysis testing differences in species composition. Only native species composition was considered for the first year comparisons, while total vegetation was considered for the analysis of the third year following treatment.

	pretreatment vs. year one			pretreatment vs. year three		
treatment	A	*p*		A	*p*	
fusilade high Aug	0.059	0.085		0.025	0.208	
fusilade high Sept	0.046	0.144		0.018	0.346	
mow (Aug)+glyphosate (Sept)	0.301	0.012	*	0.241	<0.001	*
fusilade low+pendulum high Sept	0.016	0.329		0.007	0.346	
fusilade med+pendulum high Aug	0.079	0.061		0.079	0.059	
fusilade med+pendulum high Sept	-0.005	0.485		0.147	0.007	*
fusilade high+pendulum high Aug	0.005	0.415		0.079	0.088	
fusilade high+pendulum high Sept	-0.045	0.813		-0.007	0.535	
fusilade med + surflan high Sept	-0.030	0.647		0.055	0.079	
glyphosate+pendulum high Sept	0.126	0.029	*	0.079	0.253	
glyphosate+surflan high Sept	-0.040	0.729		0.103	0.017	*
glyphosate Aug	0.153	0.005	*	0.215	0.003	*
glyphosate Sept	0.160	0.008	*	0.126	0.032	*
control	0.000	0.360		0.019	0.276	

Table 3-5. Indicator species associated with a positive response following herbicide treatments. The indicator value, IV, ranges from 0 to 100% and represents how strongly the species is associated with a group (treatment). Introduced species are marked with a *.

species	IV	*p*	year	treatment
*Geranium dissectum**	83.3	0.016	0	fusilade med + pendulum high Sept
*Poa pratensis**	83.3	0.017	0	glyphosate Sept
Clarkia amoena	66.7	0.062	1	glyphosate + pendulum high Sept
Bromus carinatus	83.3	0.016	1	glyphosate Aug
Vicia americana	98.9	0.002	1	glyphosate Aug
Clarkia amoena	64.6	0.084	1	glyphosate Sept
Lotus purshianus	83.3	0.015	1	glyphosate Sept
*Torilis arvensis**	100	0.003	3	fusilade high + pendulum high Aug
*Brachypodium sylvaticum**	64.9	0.028	3	fusilade med + pendulum high Sept
*Hypericum perforatum**	78.3	0.045	3	fusilade med + pendulum high Sept
*Sherardia arvensis**	83.3	0.016	3	fusilade med + pendulum high Sept
*Torilis arvensis**	83.3	0.015	3	fusilade med + pendulum high Aug
*Bromus sterilis**	83.3	0.015	3	glyphosate + surflan high Sept
*Cirsium arvense**	100	0.002	3	glyphosate + surflan high Sept
Bromus carinatus	83.3	0.015	3	glyphosate Aug
*Hypericum perforatum**	98.9	0.004	3	glyphosate Aug
*Torilis arvensis**	83.3	0.015	3	glyphosate Aug
*Brachypodium sylvaticum**	70.9	0.001	3	mow (Aug)+ glyphosate (Sept)
*Torilis arvensis**	83.3	0.013	3	mow (Aug)+ glyphosate (Sept)

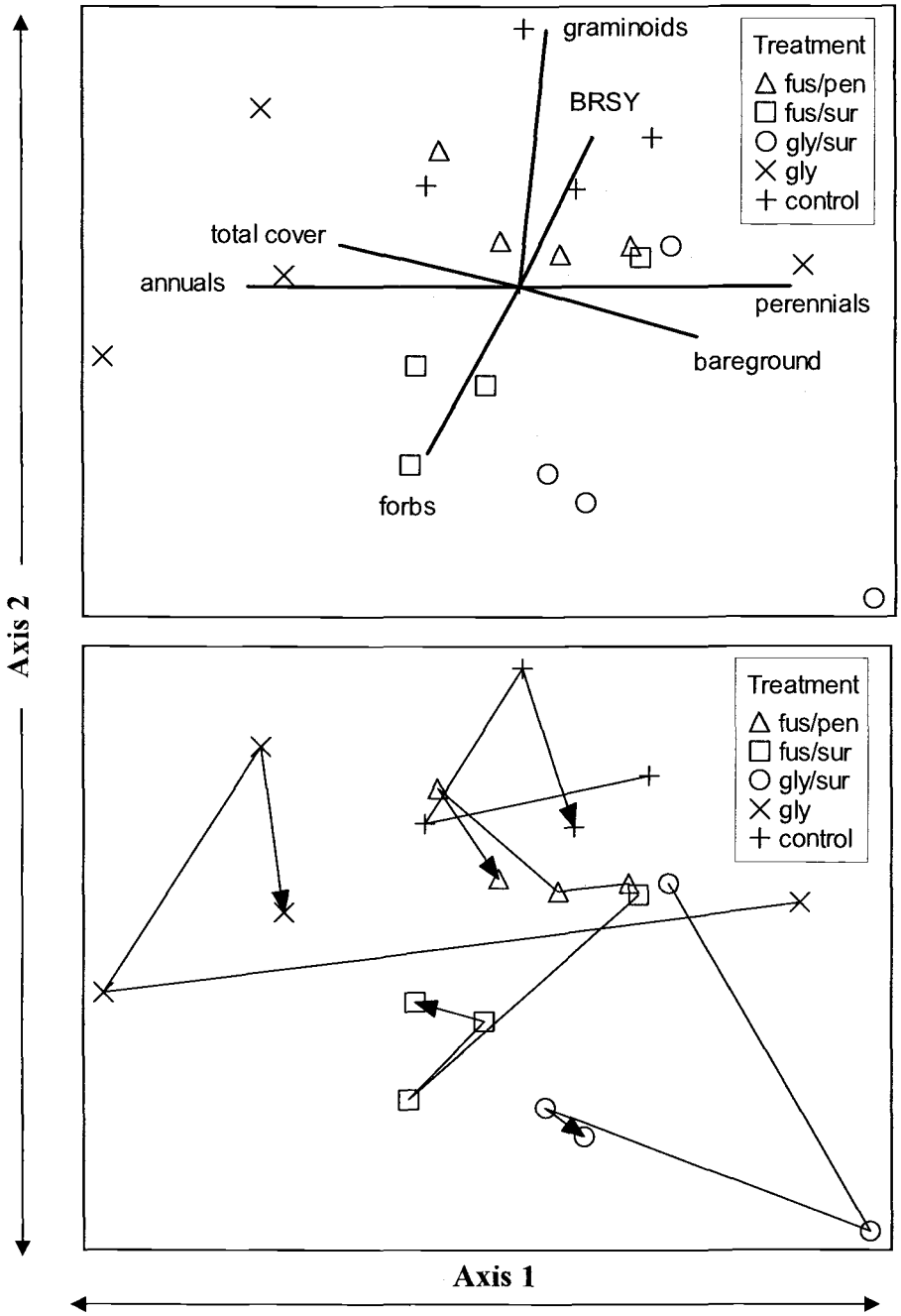

Figure 3-1. NMS ordinations of plots in species space for Butterfly Meadows data. The upper ordination displays the significant community descriptors in relation to the ordination scores of the plots, an r^2 of 0.4 was used as a cutoff for including a particular vector. The lower ordination displays successional vectors for the same treatments as above, with lines connecting repeatedly measured plots over four growing seasons with the arrow head marking the final year (2006). Each point represents the plot averages for the treatment. Included in the ordinations are the controls (+'s), September application of fusilade at a low rate with pendulum (triangles), September application of fusilade at a medium rate with surflan (squares), September application of glyphosate and surflan (circles), and August application of glyphosate (X's). BRSY is an abbreviation for *Brachypodium sylvaticum*.

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Chapter 4 : Conclusion

Herbicides can play two seemingly opposing roles in plant conservation. Herbicides can be used in a restoration context to release native species from competitive inhibition by non-native, invasive species, or on the other hand, herbicide drift threatens native plant communities along roadsides and agricultural fields. With less than 1% of Oregon's Willamette Valley prairie remaining, conservation and restoration of prairie remnants has become an important management objective.

One aim of this thesis was to incorporate native species into EPA's phytotoxicity tests in order to improve our understanding of how non-target herbicide exposure shapes rare Willamette Valley plant communities. The second aim of this thesis was to describe how different herbicide combinations and dates of applications could be used to restore a degraded upland prairie of the Willamette Valley.

Findings from the improved vegetative vigor tests indicate that native plants can be easily grown for phytotoxicity tests and that these tests provide valuable ecological information regarding the relative susceptibility of native plants to various herbicides that crop species do not afford. The EC values generated from this study are a useful means to compare the relative susceptibility of plant species to the same herbicide, or the relative effects of different herbicides to the same species. From an ecological perspective the differential sensitivity of non-crop plants to herbicides suggests that plant communities that are exposed to low concentrations of herbicides could transition from biologically diverse communities to communities dominated by herbicide tolerant species. The negative indirect effects that a shifting resource base has on declining wildlife populations is of great concern, especially considering the magnitude of the area to which herbicides are applied worldwide.

Differential responses to herbicide can also be used in a restoration context to target introduced species or avoid harming native species. The four year study conducted at Butterfly Meadows showed how herbicides can be used to quickly change the trajectory of a plant community. September applications of glyphosate were most effective at controlling the invasive species *Brachypodium sylvaticum*. There was some evidence that the soil-active preemergent herbicide Surflan provided extended control of the invasive grass since it targeted newly germinating seedlings.

Although none of the treatments harmed the preexisting vegetation, there was minimal regeneration by native species following release from competition by *B. sylvaticum.* This suggests that the native plants at this site are recruitment limited and may require seeding or outplanting treatments in order to bolster their fecundity. Due to the fact that upland prairies are highly fragmented and have shrunken in size so dramatically, it is possible that many species are suffering from low reproductive output due to inbreeding depression.

Managing Butterfly Meadows in order to regain its ecological integrity will require a multi-year, integrated strategy. Multiple herbicide treatments will be required in order to reduce the initial cover of *B. sylvaticum*, control plants that were tolerant to the first application, and control subsequent recruitment of the weed through the seedbank. In order for the native plant community to resist future invasions and redevelop into a dynamic native dominated community, seeding or outplanting events will surely be required due to the limited pool of propagules.

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