The Effect of Hexazinone, Sulfometuron, Metsulfuron, and Atrazine on the Germination Success of Selected *Ceanothus* and *Rubus* Species

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ABSTRACT: *This study documents the impact pre-emergent forestry herbicides have on germination of some selected seral woody competitors in the Pacific Northwest. Four commonly used pre-emergent soil-active herbicides (hexazinone, sulfometuron, metsulfuron, and atrazine) typically used for herbaceous weed control were applied at six rates over stratified seed of* Ceanothus velutinus *(CEVE) and* Ceanothus integerrimus *(CEIN) in a greenhouse efficacy trial. In addition, hexazinone and sulfometuron were applied over stratified* Rubus ursinus *(RUUR)* and Rubus parviflorus *(RUPA) seed and sulfometuron over stratified seed of* Rubus spectabilis *(RUSP) at the same six rates. Numbers of seed to successfully germinate and develop true leaves were counted over a 9 wk period immediately following herbicide application. The hexazinone treatments reduced germination and growth of CEVE, CEIN, and RUPA. The RUUR species was tolerant of the hexazinone herbicide at low rates but at higher rates was strongly affected. The sulfometuron treatments had less effect on survival probability than hexazinone but strongly reduced the average dry weight of plantlets of all species. Seedling dry weight decreased with increasing rate of both metsulfuron and atrazine. Increasing the metsulfuron rate reduced the probability for CEVE seedlings to survive but not CEIN. Finally, atrazine sharply reduced the plantlet survival and reduced dry weight of both CEVE and CEIN even at low rates.West. J. Appl. For. 17(4):194–201.*

Key Words: Vegetarian management, pre-emergent herbicides, wood competitors, seed germination.

Foresters interested in maximizing conifer productivity in the US Pacific Northwest often control both the woody and herbaceous weed components on reforestation sites for several years during conifer establishment. The established woody component is often controlled through mechanical site preparation treatments and/or foliage active herbicides. The herbaceous component is often controlled using pre-emergent soil–active herbicides such as sulfometuron, hexazinone, atrazine, and metsulfuron that persist at phytotoxic levels in the soil from 20–90 days. Hexazinone and atrazine are in the triazine chemical family and interfere with the photosynthetic system of plants, eventually depriving them of energy necessary to fix carbon (Boger and Sandmann 1989). Sulfometuron and metsulfuron are sulfonyureas. These deactivate acetolactate synthase, which is required for synthesis of essential branched side-chain amino acids (Boger and Sadmann 1989, Ahrens 1994). All four herbicides are effective on a variety of newly establishing germinants and have their best control efficacy if applied prior to development of true leaves (Kelpsas 1999).

The development of technologies and chemistry to control established woody vegetation has received a lot of scientific interest, as has initial control of herbaceous competition (Loucks et al. 1996). Less attention has been paid to controlling species of woody plants, such as many *Rubus* and *Ceanothus* species as they establish from seed. One of the potential advantages of applying soil active herbaceous herbicides is the collateral control of seedbanked perennial or woody germinants. This could be especially important on sites prone to quick invasion from woody perennials such as

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on burned sites with large seedbanks of *Ceanothus* spp. or in areas where invasion by *Rubus* spp. is common. For example, as many as 600,000 *C. intergerrimus* seedlings per hectare have been found following a fire (Conard et al. 1985). Monleon et al. (1999) demonstrated that as few as 6,000–15,000 *C. velutinus* seedlings per hectare at the time of planting can have significant long-term effects on Douglas-fir (*Pseudotsuga menziesii*) growth. Seedlings of *C. intergerrimus* and *C. velutinus* can dominate a site within 2 to 5 yr after a disturbance (Tirmenstein 1989, 1990) and can delay the dominance of conifers for decades (Zavitkovski and Newton 1969). Similarly, *Rubus spectabilis* Pursh, *R. ursinus* and *R. parviflorus* can quickly invade reforestation environments from seed and be significant competitors (Haeussler et. al. 1990, Stein 1995). Anecdotal observations suggest that commonly used herbaceous herbicides (hexazinone, sulfometuron, metsulfuron, and atrazine) decrease the germination success of *Ceanothus* and *Rubus* species, but to date we are unaware of any published research documenting these effects.

This article details the results of a carefully controlled laboratory trial designed to evaluate, for the first time in a controlled environment, the effectiveness of four herbicides at six rates on the germination success of *C. velutinus, C. intergerrimus, R. spectabilis, R. ursinus, and R. parviflorus*. The herbicide rates tested encompass the range of rates commonly used by forest managers to control herbaceous vegetation in Pacific Northwest forests west of the Cascades.

Materials and Methods

Seed Stratification

Viable seed for *C. intergerimus* (CEIN), *C. velutinus* (CEVE), *R. spectabilis* (RUSP), *R. ursinus* (RUUR), and *R. parviflorus* (RUPA) were purchased from seed collectors specializing in native plants. *Ceanothus* spp. seed was scarified by adding the seed to water of 170°F, cooling it overnight, and then keeping the seed in a cool moist environment for 90 days (Reed 1974). The *Rubus* spp. seed was put in warm moist conditions for 90 days followed by 90 days in moist cool conditions (Brinkman 1974).

Experimental Design

The study consisted of 13 independent experiments, each evaluating the effect of a single herbicide at six rates on an individual plant species. Each experiment utilized a completely randomized design with eight replications of the six herbicide rates (Table 1). Each replication (experimental unit) consisted of a $2 \times 3 \times 2$ in. pot filled with a sandy loam soil. A total of 48 pots were used for each independent experiment resulting in a combined total of 624 individual pots for all 13 experiments. The rates ranged from a no herbicide control (treatment 1) to nearly double normal operational rates (treatment 6). Field operational rates generally fall within categories four and five.

Stratified seed for a single species were placed on the surface of each of the 48 pots designated for a single herbicide and covered with a thin layer of gravel to hold the seed in place. More seed of some species were planted than others depending on the size of seed, expected viability, and availability. Ten seeds of CEVE and CEIN, eight of RUSP and six of RUUR and RUPA were used per pot. Due to seed limitations RUUR and RUPA were tested with hexazinone and sulfometuron. *Rubus spectabilis* was tested only with hexazinone.

The herbicide treatments were applied via a backpack gas-powered boom sprayer on August 16, 1997. All the pots for a given herbicide rate were placed within a 1.86 m^2 area. The amount of herbicide necessary to cover the 1.86 m^2 area based on the rates shown in Table 1 was added to a 500 ml solution, and the entire solution was sprayed over the area. This required several passes of a spray wand driven by a pressure regulated gas powered backpack sprayer. Once the herbicide treatment was applied, the pots were moved to a greenhouse and watered three times daily by a light mist irrigation system that kept the pots moist throughout the experiment.

Measurements

The number of seeds/pots that had germinated and were alive was recorded twice a week for 9 wk following the herbicide treatments. Only seed with exposed cotyledons were included in this count. The number of live germinants/pot with true leaves was also recorded at these timings. At the end of 9 wk, all the live plantlets were harvested. Care was taken to keep the root systems of germinants intact, and all the plantlets from each pot were dried together and weighed. This weight was divided by the number of surviving plantlets to provide an estimate of average plant weight.

Analysis

Binomial logistic regression was used to analyze the number of plantlets to survive the full 9 wk at the differing rates using PROC GENMOD in the SAS 7.0 statistical package. Logistic regression was used because of highly skewed data and the high incidence of zeros in the database. The number of plantlets surviving was the dependent variable and the rate of herbicide the independent. This analysis technique allowed us to model the probability for plantlets to survive to week 9 at the differing rates of herbicides

Table 1. Herbicide rates tested.

	Hexazinone		Sulfometuron		Metsulfuron		Atrazine	
Treatment	kg a.i./ha	oz a.i./ac	kg a.i./ha	oz a.i./ac	kg a.i./ha	oz a.i./ac	kg a.i./ha	oz a.i./ac
1	0	0	0	0	0	0	0	0
2	0.56	8	0.05	0.75	0.01	0.15	1.12	1
3	1.12	16	0.1	1.5	0.02	0.3	2.24	2
4	1.68	24	0.15	2.25	0.03	0.45	3.36	3
5	2.24	32	0.2	3	0.04	0.6	4.48	4
6	3.36	48	0.3	4.5	0.08	1.2	5.60	5

(Ramsey and Schafer 1997). Analyses were run independently for each herbicide, and species combination examined. In addition, linear regression was used to model the average weight of surviving plantlets at the end of the experiment using PROC GLM in the same software package. A log transformation was required to meet assumptions of equal variance in this analysis, and data reported are back-transformed.

Results

The hexazinone treatments reduced germinant survival and growth of CEVE, CEIN, and RUPA. The RUUR species was more tolerant of the hexazinone herbicide at low rates but at higher rates was strongly impacted. The sulfometuron treatments tended to have a less pronounced impact on the number of individuals of all species to survive 9 wk after planting than hexazinone but strongly reduced the average dry weight of plantlets of all species. Metsulfuron and atrazine were only tested against the *Ceanothus* species. Seedling dry weight decreased with increasing rate of both metsulfuron and atrazine. Increasing the metsulfuron rate reduced the probability for CEVE seedlings to survive but not CEIN. Finally, atrazine sharply reduced the probability of plantlets to survive and reduced dry weight of both CEVE and CEIN even at low rates.

The mean number of seedlings to germinate and to develop true leaves at each sampling period was calculated and plotted over time for each combination of herbicide and species examined, resulting in 26 individual graphs. Examination of these graphs revealed three generalized germination-rate patterns. To aid in the presentation of these results we will refer to these germination patterns as Type I, II, and III (Figure 1). In the Type I pattern, seed at all herbicide rates germinate at roughly the same pace, but after 16 days, those treated with herbicide, especially the higher rates, showed a marked decline in the mean number of live germinants. Type II is typified by a slow gradual increase in number of live germinants in those treatments receiving any herbicide treatment through the duration of the experiment. Type III is typified by a quick increase in number of live germinants that stabilize after about day 23 and stay the same throughout the remainder of the experiment. Two true leaf development patterns were also observed that will be referred to as Type A and B. In Type A, there are few seedlings that produce true leaves, with the exception of the check treatment, through the duration of the experiment. In Type B there is a general increase in numbers of seedlings with true leaves over time, and those treatments receiving smaller doses of herbicide have more seedlings with true leaves than those receiving higher doses.

Hexazinone

Seeds of all species tested started to germinate within a week of planting, and the numbers increased until around day 22 following a Type-I response (Figure 1). Germination number increased slightly thereafter for CEVE, CEIN, and RUPA in the control treatment. For CEVE, CEIN, and RUPA, counts of live germinants dropped precipitously for all rates of hexazinone after day 22. This drop was not as obvious for RUUR.

The development of true leaves for CEVE, CEIN, and RUPA, followed a Type-A pattern (Figure 1). Development of true leaves in the control replications increased rapidly until around day 22 and then increased only slightly. Even at low rates of herbicide, very few plantlets developed true leaves, and the few that did tended to be at the lowest herbicide rates. Development of true leaves followed a different pattern for the RUUR plantlets. This species exhibited what we have defined as a Type-B pattern in which development of true leaves continued to increase throughout the study period but at lower rates for increasing rate of herbicide (Figure 1).

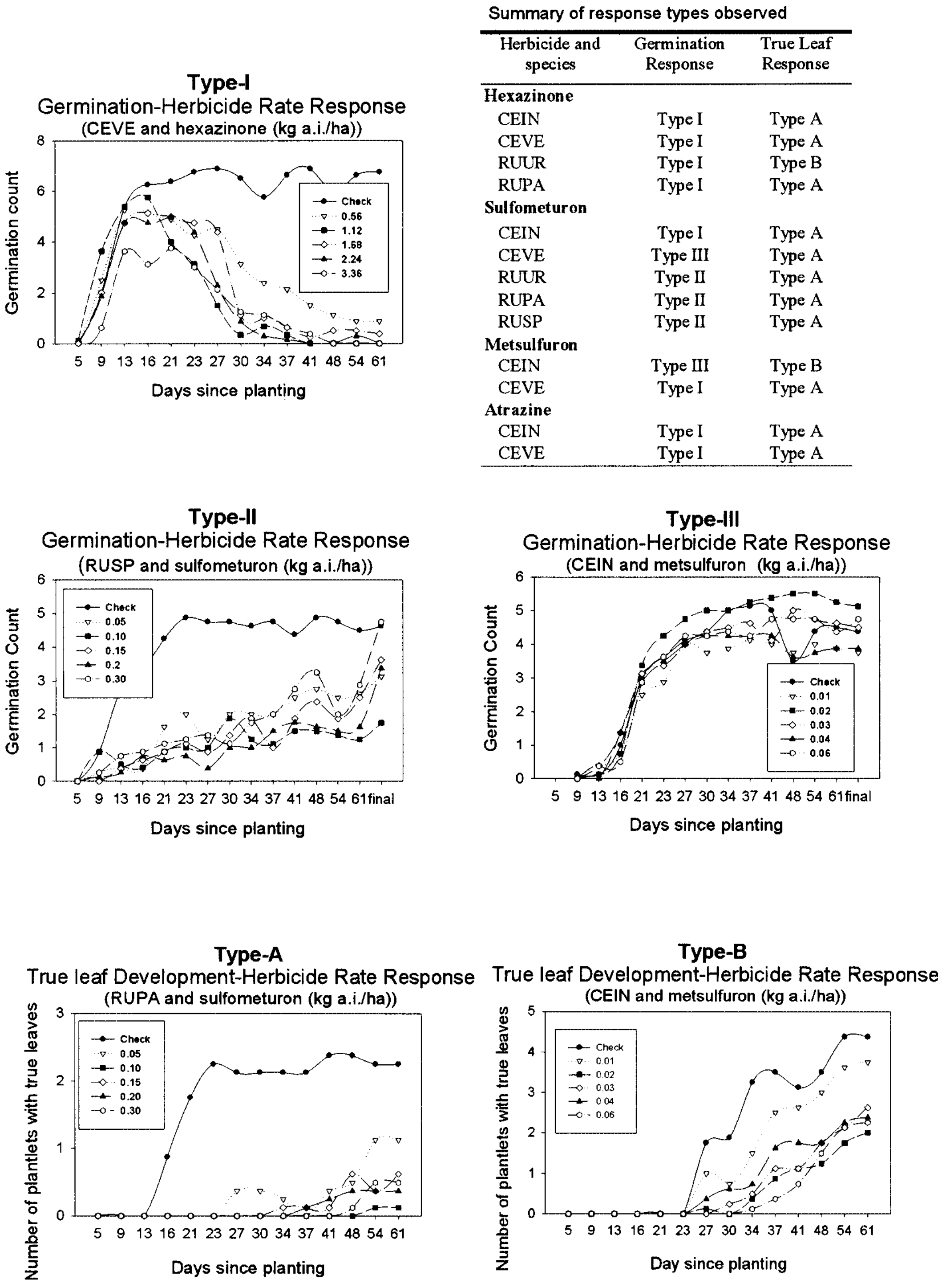
Of the few CEVE and CEIN plantlets to survive 9 wk, those in the low to moderate hexazinone treatments had leaves that were thinner and reduced compared to the controls. In addition, the root systems were visibly reduced not only in length but also in fibrosity. Hexazinone had less of a visual effect on RUPA and RUUR. For RUUR, most surviving plantlets developed true leaves, and rate effects were not obvious. The leaves of RUPA appeared to decrease in size with increased rate. For both *Rubus* spp., the root systems were markedly reduced from that observed in the controls.

The probability of surviving 9 wk for all species was strongly influenced by increasing rate of hexazinone (Figure 2). Of the two *Ceanothus* spp., CEVE was most susceptible to hexazinone, with the 0.56 kg a.i./ha rate resulting in a 20% chance of survival and dropping to less than 10% at the 1.12 kg a.i./ha rate. For CEIN it was not until rates approached 1.68 kg a.i./ha before survivorship potential dropped below 10%. Of the species treated with hexazinone, RUUR was the most resistant. Hexazinone rates of over 2.24 kg a.i./ha were required to reduce survivorship potential to below 10%. *Rubus parviflorus* was much more susceptible to hexazinone, and a rate of only 1.68 kg a.i./ha resulted in less than a 10% chance of survival.

At a zero hexazinone rate, average plantlet dry weight of CEIN was greater than CEVE. Dry weight of both species dropped with increasing hexazinone rate (Figure 2). A hexazinone rate of 1.68 kg a.i./ha reduced the dry weight of CEIN by 59% and a 3.36 kg a.i./ha rate reduced CEIN dry weight by 84%. *Ceanothus velutinus* dry weight was reduced by 74% and 93% at the 1.68 and 3.36 kg a.i./ha rates, respectively. Seedling weight dropped similarly with increasing hexazinone rate for both genera tested. Both *Rubus* species had dry weights 50% or less at the 1.68 kg a.i./ha rate and less than 25% at the 3.36 kg a.i./ha rate than when no herbicide was applied.

Sulfometuron

If treated with sulfometuron at any rate, surviving CEVE plantlets tended to be very small and generally consisted of little else but cotyledons and a short root (less than 3 cm versus roots over 21 cm in the controls). Treated CEIN plantlets had more pronounced true leaves and longer roots (up to 9cm) than CEVE although they still appeared stunted compared to the controls. Similarly, the *Rubus* species devel-



Summary of response types observed

Herbicide and species	Germination Response	True Leaf Response
Hexazinone		
CEIN	Type I	Type A
CEVE	Type I	Type A
RUUR	Type I	Type B
RUPA	Type I	Type A
Sulfometuron		
CEIN	Type I	Type A
CEVE	Type III	Type A
RUUR	Type II	Type A
RUPA	Type II	Type A
RUSP	Type II	Type A
Metsulfuron		
CEIN	Type III	Type B
CEVE	Type I	Type A
Atrazine		
CEIN	Type I	Type A
CEVE	Type I	Type A

Figure 1. Examples of general response patterns of germination count (Type-I, II, and III) and true leaf development (Type A and B) observed over the 9 wk assessment period and summary of patterns observed for each herbicide and species tested.

oped shorter root systems at higher rates of sulfometuron and in many cases at the highest rates consisted of nothing more than cotyledons with virtually no visible root.

Germination of CEVE followed a Type-I pattern with counts peaking around day 22 and counts in treatments that received even light doses of herbicide dropping quickly thereafter. Few CEVE plantlets survived the full 9 wk. Germination of CEIN under all rates of sulfometuron increased rapidly until day 22 and then leveled off with no consistent differences in germination count throughout the study period, a Type-III response (Figure 1). Germination counts for the three *Rubus* species followed a Type-II response with germination counts in the controls increasing rapidly until day 22 and then leveling off. *Rubus* seed in the herbicide treatments had germination counts that increased at a slower rate. By the end of the 9 wk period, the counts nearly equaled that of the untreated controls for some treatments, while others were

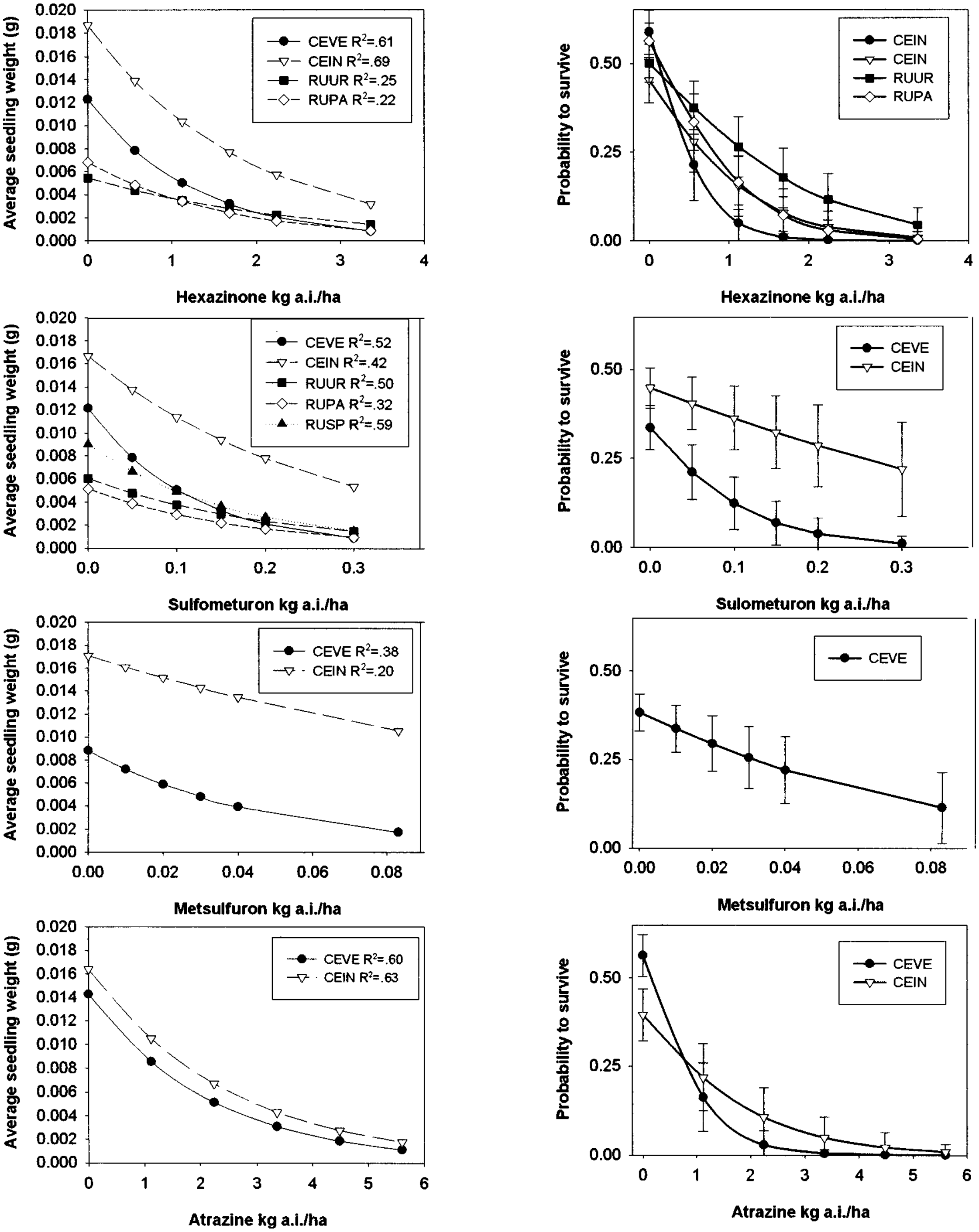


Figure 2. Regression curves for average weight of seedlings treated (left column) with different rates of herbicides are illustrated for the four herbicides examined (back transformed from a ln transformation). In addition curves generated for the probability of a seedling (right column) to survive when treated with different rates of herbicide are illustrated by herbicide examined (back-transformed from a logit transformation). Error bars represent standard errors, and curves for only those species whose probability for survival was significantly influenced by the herbicide are shown.

only half of that of the control. Sulfometuron applications delayed the development of true leaves in *every* species until approximately day 40 when most of the surviving plantlets began to develop true leaves. This was a Type-A response.

Sulfometuron rate did not influence the survivorship potential of any of the three *Rubus* species examined (Figure 2). *Ceanothus velutinus* survivorship was reduced below 10% at rates greater than 0.16 kg a.i./ha. *Cenaothus intergerrimus* survivorship potential was less affected and continued to be greater than 25% even at the highest sulfometuron rate applied, 0.315 kg a.i./ha.

Increasing rate of sulfometuron decreased the average dry weight of plantlets for all species examined (Figure 2). At 0.16 kg a.i./ha of sulfometuron, dry weight had been reduced by 74, 43, 51, 58, and 60% for CEVE, CEIN, RUUR, RUPA, and RUSP, respectively. At 0.315 kg a.i./ha, dry weight had been reduced for the same species by 93, 69, 76, 83, and 84%, respectively.

Metsulfuron

Only the two *Ceanothus* species were tested with metsulfuron. After 9 wk, the surviving CEIN seedlings were only slightly visibly affected by the metsulfuron treatment. Root length at the highest rate was slightly less than in the controls, and some leaves appeared to be slightly more lanceolate than at lower rates. The CEVE seedlings were more visibly affected by the metsulfuron, with root lengths being considerably shorter when the herbicide was applied. Few CEVE individuals developed true leaves at even low rates and leaves in the highest rate treatments tended to more lanceolate.

The germination count for CEIN followed a Type-III response with the germination count increasing rapidly until day 22 and then staying the same or increasing slightly with time at all rates (Figure 1). No differences in germination count by rate were apparent. In contrast, the germination count for CEVE followed more of a Type-I response although the decline in germination count was not as precipitous as illustrated in Figure 1, and counts at higher rates tended to be lower than with low rates.

Development of CEIN true leaves followed a Type-B response with the number of individuals developing true leaves continually increasing with time, and higher rates having a lower percentage of plantlets with true leaves than lower rates (Figure 1). Development of true leaves for CEVE followed a Type-A pattern with few plantlets outside of the control developing true leaves and these not until late into the study.

The probability for survival decreased for CEVE with increasing rate but not for CEIN (Figure 2). However, for CEVE the probability for survival was not lowered below 10% even at the highest metsulfuron rate.

Average seedling weight for both species was negatively influenced by metsulfuron rate for both species. As with hexazinone and sulfometuron, CEIN was less severely impacted than CEVE. *Ceanothus intergerrimus* weight was reduced by 16% with 0.03 kg a.i./ha of metsulfuron and by 65% with 0.084 kg a.i./ha. *Ceanothus velutinus* was reduced by 66 and 80% at the two rates, respectively.

Atrazine

Only the *Ceanothus* species were treated with atrazine. Live seedlings of both species at week 9 were extremely stunted and consisted of cotyledons and very short roots. If present, true leaves were highly reduced. This was especially true at the 2.24 kg a.i./ha and higher rates.

Germination counts for both species exhibited a Type-I response (Figure 1). Few plantlets survived to the final harvest in any but the control treatments. True leaf development followed a Type-A response for both species, with very few seedlings producing true leaves. Few survived for 9 wk in any but the control treatment.

For both species, atrazine applications strongly reduced the probability of seeds to germinate and survive through the duration of the study (Figure 2). Fewer than 10% of seeds for both species survived at the 2.24 kg a.i./ha rate.

Average seedling dry weight was reduced by 79 and 74% when treated with 3.36 kg a.i./ha of atrazine for CEVE and CEIN, respectively. At the 5.6 kg a.i./ha rate, dry weight was reduced by 90 and 89%, for CEVE and CEIN, respectively.

Discussion

The four herbicides tested reduced the establishment potential of both *Ceanothus* species in the greenhouse setting. Similarly, the hexazinone and sulfometuron herbicides reduced the establishment potential of the *Rubus* species. This is in contrast to the ability of these herbicides to control established plants of most, but not all of these species under field conditions. Applications of sulfometuron at operational forestry release rates on RUSP generally result in mortality, but both RUUR and RUPA suffer only variable intermediate damage (Williams et al. 1994). RUUR is moderately sensitive to hexazinone, while no information was found relating to the sensitivity of RUPA. Applications of hexazinone and atrazine were ineffective at controlling established *Ceanothus* spp. individuals while metsulfuron causes variable intermediate damage (Boyd et al. 1985, Williams et al. 1994). We are unaware of any information on the influence of sulfometuron on this genus.

Influence of Chemical Family

The impact of the herbicides tested varied by species at the lower rates. At rates approaching operational treatments, large reductions in the probability to survive when treated with hexazinone and atrazine (both in the Triazine chemical family) were found and plantlet dry weight reductions occurred for all herbicide-species combinations tested. Germination counts over time resulted in some distinct and consistent patterns. Hexazinone and atrazine treatments tended to result in a Type-I response, while in most cases sulfometuron and metsulfuron (in the sulfonylureas chemical family) resulted in Type-II or Type-III responses. The hexazinone and atrazine herbicides always resulted in a Type-A pattern of true leaf development, and for the sulfometuron and metsulfuron herbicides a Type-A or B occurred.

Triazine family herbicides, which include hexazinone and atrazine, interfere with the electron transfer from photosystem-I to photosystem-II, depriving plants of energy necessary to fix carbon (Boger and Sandmann 1989). Plants probably die due to the resultant production of reactive photo-products that damage cells (Ahrens 1994). Thus, plants treated with triazine herbicides use stored starch reserves contained in the seed to initially expand their cotyledons and produce a short root but then quickly die when their reserves are expended, yielding the Type-I pattern we observed.

The sulfonylureas herbicides, which include sulfometuron and metsulfuron, bind and deactivate the plant enzyme acetolactate synthase, which is involved in the synthesis of branched side-chain amino acids (Boger and Sandmann 1989). This, in turn, suppresses or stops plant growth by arresting cell division in the meristems (Ahrens 1994). Treatment with sulfonylurea herbicides resulted in the Type-II or III germination count response pattern in which germinating seedlings expand their initial tissues (cotyledons and initial tap root). Upon absorption of the herbicide they can no longer grow and do not die (Type-II response). In other instances the process of germination is slowed and plants are slower to expand their cotyledons with germination being slower as herbicide rate increased, a Type-III response. Both herbicide families resulted in similar patterns of true leaf development. Few plantlets develop true leaves prior to day 40 when those seedlings that are still alive begin to produce true leaves. The onset of leaf production suggests that metabolization of the herbicides to less toxic metabolites within the plant was keeping ahead of absorption of herbicide allowing the plants to resume normal physiological function.

Management Implications

Under field conditions it is unlikely that plantlets treated with even low to moderate rates of any of the herbicides would survive much past a few weeks due to alternate wetting and drying. Plantlets in the greenhouse were kept constantly moist and in a humid environment. Plantlets developing in the field would be facing repeated periods of low humidity and quickly drying soils. The inability to quickly grow effective root systems would cause most treated plantlets of both genera to desiccate and die with the first period of slight to moderate moisture stress. In many cases in the greenhouse, we had live plantlets 40 days after sowing that consisted of little more than reduced cotyledons with little or no root, yet they remained alive because moisture stress was nonexistent. These same plantlets would have quickly perished in field situations.

The sandy loam soil used and the procedures maintained in this study make direct herbicide rate comparisons to field situations problematic. It is likely that at similar application rates germinating seedlings in the field would receive herbicide doses greater than those experienced in this greenhouse study because the leaching potential under field conditions would be much less than in the greenhouse. We used a sandy loam soil that has a lower cation exchange capacity and less organic matter than many forest soils west of the Cascade Range in the Pacific Northwest. Many Pacific Northwest forest soils are typically fine in texture and high in organic matter (Franklin and Dyrness 1973).

The early benefits of herbaceous weed control on establishing conifers have been demonstrated across a range of sites in the Pacific Northwest (Cole and Newton 1987, Newton and Preest 1988, Monleon et al. 1999, Rose et al. 1999). Our results are the first we are aware of in the Pacific Northwest to demonstrate that common herbicides used to control herbaceous weeds have the potential to concurrently control woody germinants. By preventing the development of a competitive shrub community, greater resources for planted trees are made available. This ultimately will ensure better stocking and quicker crown closure by preventing the negative competitive effects associated with shrub competition (Howard and Newton 1984, Chan and Walstad 1987, Peterson et al. 1988, Harrington and Tappeiner 1991, Wagner and Radosevich 1991). In addition, early removal of woody competitors such as CEVE or RUSP may potentially reduce reforestation costs by eliminating the need of future herbicide release treatments that target these problematic species later in the development of the stand.

More experiments of this kind are needed to provide foresters with better insight into the mechnanisms underlying vegetation control by forestry herbicide applications. Most herbicide efficacy field trials poorly assess the true potential of an herbicide treatment to control all the potential species on a site. Shrubby seral competitors have a wide variety of stratification regimes necessary for successful germination. It is not possible to be definitive about the amount of seed-banked seed in a plot or its level of stratification at the time herbicide applications are made. In addition, providing exact doses of herbicide over a large area is difficult to achieve in most field trials. Thus, although field efficacy trials can provide some course level information, carefully controlled greenhouse trials provide needed insight into the potential to control weed species with a given herbicide at a given dose.

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