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Synthetic Auxin Herbicides Control Germinating Scotch Broom (*Cytisus scoparius*)

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Scotch broom is a large, nonnative shrub that has invaded forests and grasslands in 27 U.S. states. Without treatment, Scotch broom's persistent seedbank ensures a continuing source of regeneration after soil disturbance. In growth chamber studies, five rates of three synthetic auxin herbicides, aminocyclopyrachlor (AC), aminopyralid (AP), and clopyralid (CP), were compared for PRE control of Scotch broom. Cumulative 90-d emergence, mortality, and biomass of seedlings did not vary among herbicides, averaging 42% of seeds sown, 75% of emerged seedlings, and 9 mg seedling^{-1} for treated containers, respectively, versus 46%, 17%, and 26 mg seedling^{-1} for nontreated containers. Low rates of each herbicide (< 50% of the maximum labeled rate [MLR]) provided 60 to 80% control, whereas 100% MLR provided 69 to 89% control. Although the herbicides differed in cost per unit seedling mortality (AP < CP < AC), each demonstrated strong potential as a viable treatment for PRE control of Scotch broom.

Nomenclature: Aminocyclopyrachlor; aminopyralid; clopyralid; Scotch broom, *Cytisus scoparius* (L.) Link CYSC4.

Key words: Seedbank, seedling emergence, mortality, biomass.

Cytisus scoparius es un arbusto no-nativo y de gran tamaño que ha invadido bosques y pastizales en 27 estados de los Estados Unidos. Sin tratamiento, el persistente banco de semillas de *C. scoparius* asegura una continua fuente de regeneración después de la perturbación del suelo. En estudios en cámaras de crecimiento, cinco dosis de tres herbicidas auxinas sintéticas, aminocyclopyrachlor (AC), aminopyralid (AP), y clopyralid (CP), fueron comparados para el control PRE de *C. scoparius*. La emergencia acumulada a 90-d, la mortalidad, y la biomasa de plántulas no varió entre herbicidas, promediando 42% de las semillas sembradas, 75% de las plántulas emergidas, y 9 mg plántula^{-1} para contenedores tratados, respectivamente, versus 46%, 17%, y 26 g plántula^{-1} para contenedores no tratados. Dosis bajas de cada herbicida (<50% de la dosis máxima de la etiqueta [MLR]) brindaron 60 a 80% de control, mientras 100% de la MLR brindó 69 a 89% de control. Aunque los herbicidas difirieron en el costo por unidad de mortalidad de plántulas (AP < CP < AC), cada uno demostró un fuerte potencial como tratamiento viable para el control PRE de *C. scoparius*.

Scotch broom is a large, nonnative, leguminous shrub that has invaded forest and grassland sites in 19 eastern and 8 western U.S. states (Invasive Plant Atlas 2012). Native to western and central Europe, Scotch broom has nitrogen-fixing bacteria located in nodules on its roots and is capable of fixing up to 111 kg N ha^{-1} yr^{-1} in aboveground tissues and returning 17 kg N ha^{-1} yr^{-1} to the soil as leaf and stem litter fall (Watt et al. 2003). Introduced as an ornamental in the 1850s (Gilkey 1957), the species is a prolific seeder with individual shrubs producing from 100 to 14,000 seeds yr^{-1} (Bossard and Rejmánek 1994; Rees and Paynter 1997). Once the seeds are buried, their germination can be delayed for at least 5 yr (Bossard 1993; Bossard and Rejmánek 1994), resulting in seedbanks of 200 to 27,000 seeds m^{-2} (Rees and Paynter 1997; Sheppard et al. 2002). A variety of fire, herbicide, and mechanical treatments are effective for POST control of Scotch broom (Bossard and Rejmánek 1994; DiTomaso et al. 2006; Oneto et al. 2010). Hexazinone and metsulfuron are effective herbicides for PRE control of broom, whereas sulfometuron has demonstrated limited efficacy (Harrington 2009; Ketchum and Rose 2003). Both sulfometuron and metsulfuron can limit root development of Scotch broom seedlings, and thereby increase their susceptibility to mortality from soil drought (Harrington 2009). Prescribed fire also has been used successfully to reduce Scotch broom seedbanks (Bossard 1993; Haubensak et al. 2004; Herrera-

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Reddy et al. 2012). Recent developments of aminopyralid and aminocyclopyrachlor—synthetic auxin herbicides having soil activity at low rates (Claus et al. 2010; Jachetta et al. 2005)—prompted this research to determine their potential for PRE control of Scotch broom. The objective of the research was to compare emergence, mortality, and biomass of Scotch broom seedlings after PRE treatments of aminocyclopyrachlor, aminopyralid, and a similar compound, clopyralid, applied at a wide range of rates.

Materials and Methods

Seed Collection and Sowing. Scotch broom seeds were collected in 2009 and 2010 from different sets of mature plants in the Olympia, WA area. Collections were made in mid-July as soon as seed pods had begun to dehisce. Seeds were dried thoroughly at room temperature prior to being removed from their pods and then stored in sealed glass containers at 5 C. Emergence, mortality, and biomass responses of Scotch broom seedlings were studied in two 90-d growth series beginning in March and November 2010, respectively. To minimize potential losses in viability from cold storage, seeds from the 2009 and 2010 growing seasons were used in the March and November growth series, respectively. Seeds were sown in clear plastic containers (dimensions: 12 cm long by 17 cm wide by 6 cm deep) filled with forest soil from a site near Matlock, WA (47.206°N, 123.442°W). The soil, a very gravelly loamy sand of the Grove series (Dystric Xerorthent) formed in glacial outwash (U.S. Department of Agriculture–Natural Resources Conservation Service [USDA-NRCS] 2012), is especially prone to invasion by Scotch broom because of its typically low abundance of associated vegetation (Peter and Harrington 2012). Containers were filled with a fixed weight of field-moist soil that had been sieved to remove coarse fragments ($>$ 2 mm); the soil surface was tamped level, resulting in a 3-cm depth, and deionized water was applied to achieve field capacity (35% volumetric moisture content; Harrington 2009). Fifty nonstratified seeds were spread across the soil surface of each container and pressed in sufficiently to cover them with soil.

Herbicides and Rates. Three synthetic auxin herbicides were compared: (1) aminocyclopyrachlor (AC) formulated as Method® with a maximum labeled rate (MLR) of 280 g ae ha^{-1} (DuPont Company, Wilmington, DE 19803), (2) aminopyralid (AP) formulated as Milestone® with an MLR of 120 g ae ha^{-1} (Dow AgroSciences LLC, Indianapolis, IN 46268), and (3) clopyralid (CP) formulated as Transline® with an MLR of 560 g ae ha^{-1} (Dow AgroSciences LLC). Three studies were conducted either to compare a broad range of application rates for each herbicide (Studies I and II) or to compare all three herbicides in the same test environment (Study III). In Study I, conducted in the March growth series, five rates (0, 25, 50, 100, and 200% MLR) were replicated three times each in a comparison of AP and CP, for a total 30 containers. In Study II, conducted in the November growth series, the same five rates were replicated three times each for AC, for a total of 15 containers. In Study III, also conducted in the November growth series, three containers were assigned to each of three rates per herbicide corresponding to 0, 50, and 100% MLR, for a total of 27 containers. Each herbicide treatment was applied in deionized water via an adjustable pipetor (Oxford Macro-Set Transfer Pipetting System, model 890007, 5-ml capacity, Agora Science LTD, Oxford, U.K. OX2 9BG) to achieve an equivalent spray volume of 1870 L ha^{-1}. This spray volume (approximately 3.8 ml per container)—20 times that recommended for ground applications—was used to ensure uniform herbicide distribution within each container. Nonionic surfactant (R11®, Wilbur-Ellis Company, Walnut Creek, CA 94596) was added to each treatment at 0.5% v/v concentration.

Growing Conditions and Measurements. The three studies were conducted in two controlled-environment growth chambers (Studies I and II: model G30, Controlled Environments, Inc., Winnipeg, Manitoba, Canada R3H 0R9; Study III: controller 6000, Enconair, Inc., Winnipeg, Manitoba, Canada R2W 3A8). Each growth chamber was programmed to provide a dark : light temperature regime (14 h : 10 h) of 15 : 20 C and a light intensity of approximately 50 μmol m^{-2} s^{-1} photosynthetic photon flux density—conditions found to promote broom germination (Bossard 1993; Harrington 2009). For each study, treatment replications were blocked according to upper, middle, and lower shelves within each growth chamber to control for potential variation in growing conditions. Containers were watered uniformly with deionized water every 4 to 6 d during the study to achieve field capacity based on container weight.

Counts of seedling emergence and mortality were conducted daily during the first week of each study and every 2 to 3 d thereafter through 90 d after sowing. Although the 90-d study duration did not provide a total assessment of seed viability because of Scotch broom's extremely protracted period of germination (Harrington 2009), it did provide a relative comparison of the herbicides and application rates under standardized conditions. Emergence of a seedling was recorded when either its epicotyl or hypocotyl was visually detectable. Mortality of a seedling was recorded when the vast majority of its tissues became necrotic and began to decompose. Dead seedlings were removed and discarded after each count. Emergence per container was expressed as a percentage of seeds sown ($n = 50$), and mortality was expressed as a percentage of 90-d emergence. At the end of each study, surviving seedlings were excavated, washed, and composited by container into a paper envelope. The biomass samples were dried to a constant weight in a forced-draft oven at 65 C and weighed to the nearest 0.001 g. In Study I, seedling biomass was separated into shoot and root components that were composited by container and dried and weighed separately.

Statistical Analysis. Data from each study were analyzed separately in SAS (SAS Institute Inc. 2008) with a significance level of $\alpha = 0.05$. The experimental design for each study was a randomized complete block with three replications per treatment. For each study, values of 90-d emergence, mortality, and biomass of Scotch broom seedlings were subjected to ANOVA in PROC MIXED to account for random effects of blocking and fixed effects of treatment. The models for Studies I and III tested for effects of herbicide, application rate, and their interaction, whereas the model for Study II tested only for the effect of AC rate. Scatter plots from each ANOVA revealed that the residuals had relatively homogeneous distributions when plotted against predicted values, and therefore, variable transformations were not considered necessary to meet statistical assumptions. For each study, the F tests from ANOVA indicated the absence of significant main effects of herbicide, as well as the absence of a significant interaction between herbicide and application rate for each of the response variables (described below). When significant main effects of rate were detected, variation in a given response variable was modeled as a polynomial function of rate for treated containers. The regression analysis was applied only to data from Studies I and II because each had four nonzero herbicide rates to enable fitting of the polynomial model and testing for lack of fit. For each of these two studies, the extra-sums-of-squares approach was applied in PROC REG to compare the following full model against potential reduced models (Neter et al. 1989):

$$Y = b_0 + b_1X + b_2X^2, \qquad [1]$$

where Y is emergence, mortality, or biomass of Scotch broom seedlings for a given container; b_0, b_1, and b_2 are regression parameters to be estimated; and X is application rate (% MLR). Reported values for the coefficient of determination (R^2) were adjusted for degrees of freedom (Draper and Smith 1981).

Results and Discussion

Visual Symptoms and Overall Findings. Each of the herbicides caused swelling of the hypocotyl and inhibited development of root hairs on Scotch broom seedlings as they emerged. Upon emergence, herbicide-treated seedlings displayed symptoms of epinasty that often resulted in desiccation and mortality. The vast majority of seedlings that emerged in herbicide-treated containers eventually died within the 90-d duration of each study. Thus, seedling biomass in herbicide-treated containers consisted of the most recent germinants that had not yet succumbed to lethal effects of a given herbicide, and hence, it was indicative of an earlier developmental stage of seedlings than that present in nontreated containers.

For each study, the main effects of herbicide and the interaction of herbicide and application rate were not significant for 90-d emergence ($P \geq 0.084$), mortality ($P \geq 0.183$), and biomass ($P \geq 0.217$). Both emergence and mortality averaged lower for seeds collected in 2009 (Study I) than for those collected in 2010 (Studies II and III) (Table 1).

Table 1. Seedling emergence, mortality, and biomass of Scotch broom (standard errors in parentheses) resulting from PRE applications of selected synthetic auxin herbicides in three 90-d growth chamber studies.

Study[a]	Herbicide	Emergence[b]	Mortality	Biomass
		%	%	mg
I	Aminopyralid	26 (1.8)	69 (4.5)	5 (0.8)
	Clopyralid	32 (2.2)	65 (5.3)	7 (1.1)
	Nontreated check	35 (1.5)	4 (1.8)	23 (4.4)
II	Aminocyclopyrachlor	43 (2.7)	86 (2.7)	7 (0.9)
	Nontreated check	39 (4.4)	15 (4.4)	20 (1.3)
III	Aminocyclopyrachlor	38 (2.3)	82 (5.9)	8 (1.8)
	Aminopyralid	43 (2.0)	79 (5.6)	12 (2.5)
	Clopyralid	46 (4.2)	64 (7.4)	8 (0.5)
	Nontreated check	46 (1.6)	17 (5.2)	26 (3.0)

[a] Values presented are the average for each herbicide applied at 25, 50, 100, and 200% of the maximum labeled rate (MLR) for Studies I and II and applied at 50 and 100% MLR for Study III. The MLR for aminocyclopyrachlor, aminopyralid, and clopyralid are 280, 120, and 560 g ae ha^{-1}, respectively.

[b] Within a given study, means for each variable did not vary significantly among herbicides ($P > 0.05$). Means for the nontreated check and those from Study II are shown for comparison.

Seedling Emergence. *Study I.* In a comparison of AP and CP applied at five rates, emergence averaged 29% for treated containers versus 35% for nontreated containers (Table 1). Main effects of application rate were significant ($P = 0.042$). Emergence declined from 31 to 23% as rate increased from 25 to 200% MLR (Figure 1). The polynomial model for predicting emergence from rate did not have significant lack of fit ($P = 0.557$).

Study II. In a comparison of AC applied at five rates, main effects of rate were not significant ($P = 0.196$) and emergence averaged 43% across all nonzero rates (Figure 1).

Study III. In a comparison of all three herbicides applied at three rates, emergence averaged 42% for treated containers versus 46% for nontreated containers (Table 1). Emergence did not vary significantly among rates ($P = 0.119$) (Figure 1).

Results of the three studies, including trends in the data with increasing rate (Figure 1), suggest that each of the herbicides caused a slight reduction in emergence.

Seedling Mortality. *Study I.* Seedling mortality averaged 67% in containers treated with either AP or CP versus 4% in nontreated containers (Table 1). Main effects of application rate were significant

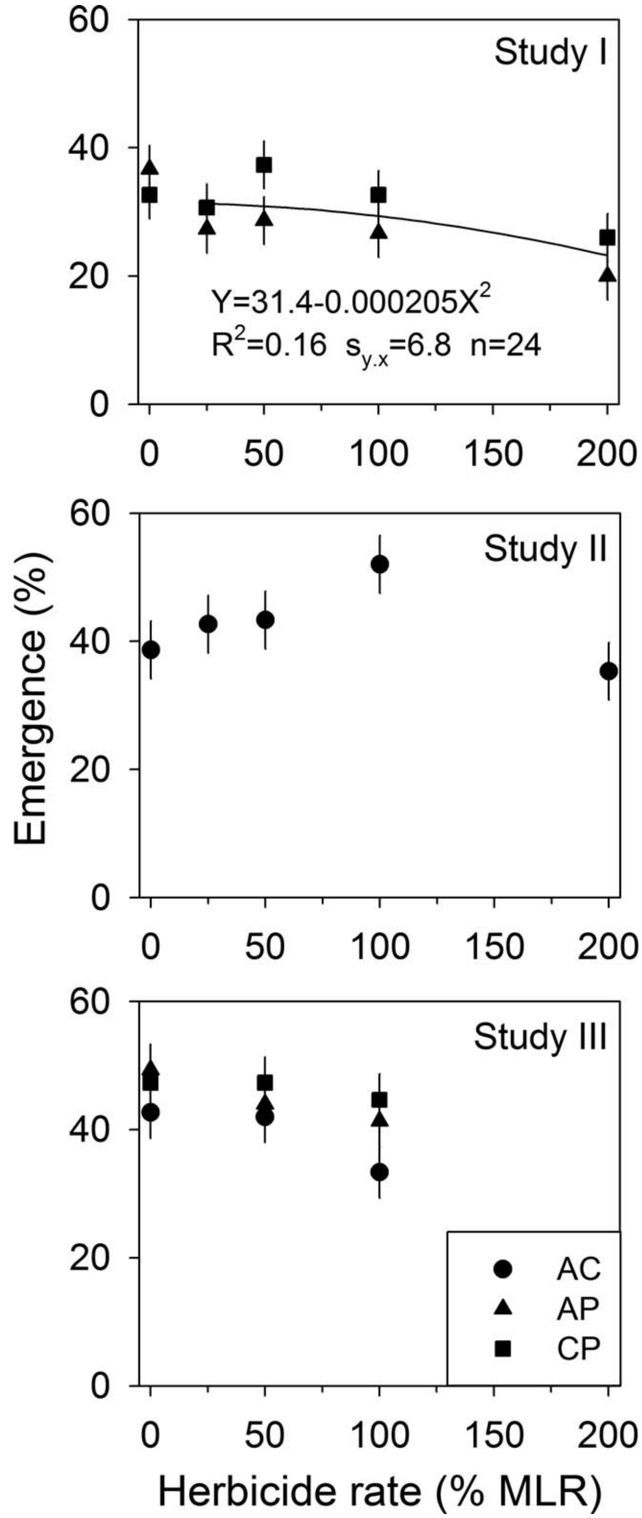

Figure 1. Seedling emergence (± standard error) of Scotch broom as affected by the percentage of maximum labeled rate (MLR) of aminocyclopyrachlor (AC; MLR = 280 g ae ha^{-1}), aminopyralid (AP; MLR = 120 g ae ha^{-1}), and clopyralid (CP; MLR = 560 g ae ha^{-1}) in three 90-d growth chamber studies. Means for the nontreated check (0% MLR) are shown for comparison.

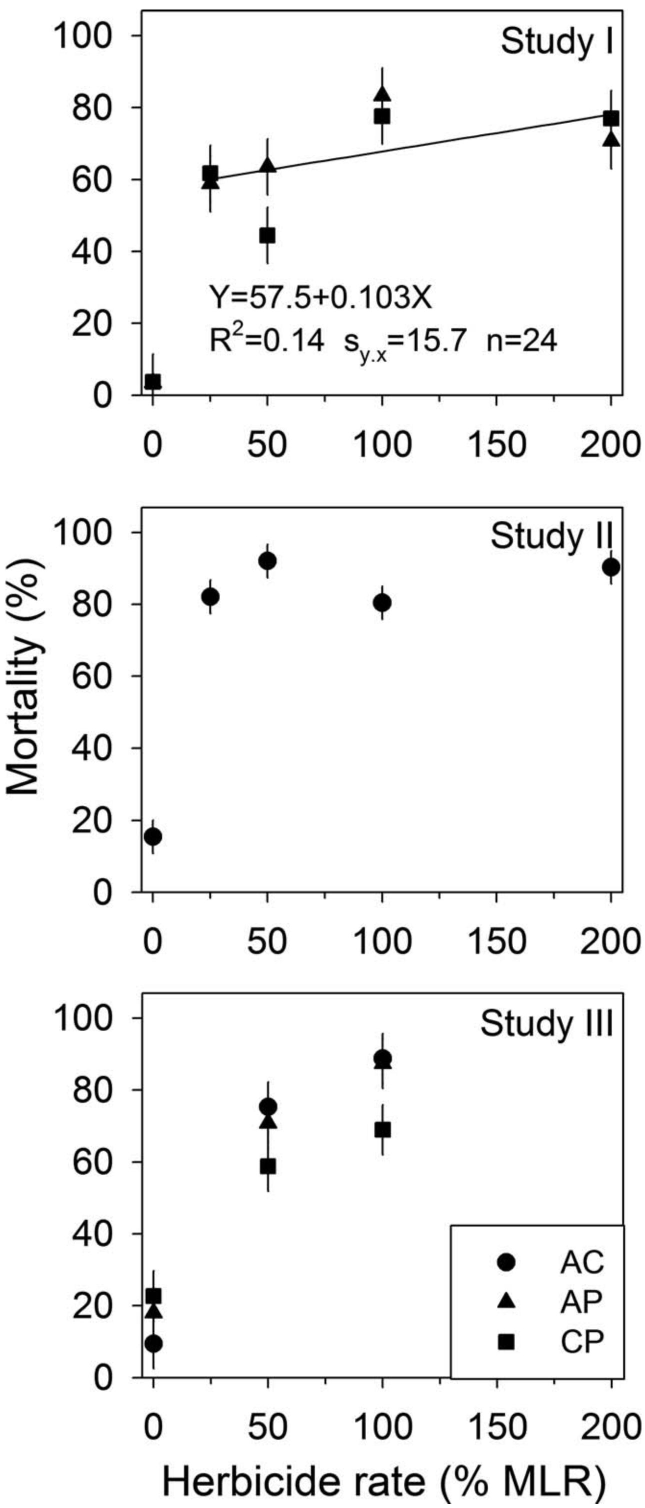

Figure 2. Seedling mortality (± standard error) of Scotch broom as affected by the percentage of maximum labeled rate (MLR) of aminocyclopyrachlor (AC; MLR = 280 g ae ha^{-1}), aminopyralid (AP; MLR = 120 g ae ha^{-1}), and clopyralid (CP; MLR = 560 g ae ha^{-1}) in three 90-d growth chamber studies. Means for the nontreated check (0% MLR) are shown for comparison.

($P < 0.001$). Mortality increased linearly from 60 to 78% as rate increased from 25 to 200% MLR (Figure 2); however, the final model had significant lack of fit ($P = 0.038$).

Study II. Seedling mortality averaged 86% in AC-treated containers versus 15% in nontreated containers (Table 1). Although main effects of herbicide rate were significant ($P < 0.001$), the regression analysis failed to identify any significant relationship between mortality and AC rate for treated containers (Figure 2).

Study III. Main effects of herbicide rate were significant ($P < 0.001$) in a comparison of all three herbicides applied at three rates. Seedling mortality averaged 17, 68, and 82% for 0, 50, and 100% MLR, respectively (Figure 2).

The vast majority of seedlings present for the final counts of emergence and mortality in herbicide-treated containers were recent germinants that already had begun to display signs of swelling, epinasty, and necrosis, thus indicating that significant levels of soil activity from the three herbicides were still present 90 d after treatment. The half-lives in soil for AC, AP, and CP have been estimated to be 50, 30, and 40 d, respectively (Iowa State University Agronomy Extension 2012; Sullivan 2011). This suggests that soil activity sufficient to cause mortality of Scotch broom seedlings existed throughout the 90-d duration of each study, especially for containers that received the higher application rates.

Seedling mortality data from Study III at 60 and 90 d after treatment were combined with herbicide cost data (Bruce Alber, Wilbur-Ellis Company, personal communication) to rank treatments according to their cost per unit of seedling mortality and to identify potential effects of differences in herbicide half-life (Table 2). On an acid-equivalent basis, herbicide toxicity to Scotch broom seedlings was ranked as AP > AC > CP. Despite having the shortest half-life (30 d), AP at 100% MLR achieved a high level of control at one of the lowest costs (87% control at \$0.54 per unit mortality). The other herbicides either had a similar level of control at higher cost (AC at 100% MLR: 89% control at \$1.29 per unit mortality) or a lower level of control at similar cost (CP at 50% MLR: 59% control at \$0.56 per unit mortality).

Table 2. Herbicide costs, mortality of Scotch broom seedlings, and cost per unit mortality for PRE applications of selected synthetic auxin herbicides at 60 and 90 d after treatment (DAT).

Herbicide	Rate	Cost[a]	DAT	Mortality[b]	Cost per unit mortality
	g ae ha^{-1}	\$ ha^{-1}	d	%	\$ per %
Aminocyclopyrachlor	140	57.51	60	42	1.37
			90	75	0.77
	280[c]	115.02	60	49	2.35
			90	89	1.29
Aminopyralid	60	23.65	60	23	1.03
			90	71	0.33
	120[c]	47.30	60	45	1.05
			90	87	0.54
Clopyralid	280	32.95	60	31	1.06
			90	59	0.56
	560[c]	65.90	60	42	1.57
			90	69	0.95

[a] Herbicide costs were calculated based on the appropriate amount of the following products to achieve the application rates used in the research: Perspective® (\$162.26 kg^{-1}) for aminocyclopyrachlor (39.5% by weight), Milestone® (\$92.46 L^{-1}) for aminopyralid, and Transline® (\$42.27 L^{-1}) for clopyralid.

[b] Mortality is expressed as a percentage of emergence at 90 d based on observed responses from Study III.

[c] Maximum labeled rate.

Seedling Biomass. *Study I.* Biomass of Scotch broom seedlings averaged 6 and 23 mg for treated (AP or CP) and nontreated containers, respectively (Table 1). Main effects of rate were significant ($P < 0.001$). Biomass declined from 9 to 5 mg as rate increased from 25 to 200% MLR (Figure 3). The final model for predicting biomass from rate did not have significant lack of fit ($P = 0.887$). The magnitude of reductions in biomass from treatment with either AP or CP was similar for shoots and roots (data not shown). In contrast, Harrington (2009) observed greater reductions in root biomass than in shoot biomass of broom seedlings 90 d after treatment with either sulfometuron or metsulfuron, and the observed increases in seedling mortality from experimentally imposed soil drought were attributed to this differential biomass response.

Study II. Biomass of Scotch broom seedlings averaged 7 and 20 mg for AC-treated and nontreated containers, respectively (Table 1). Although main effects of AC rate were significant ($P < 0.001$), the regression analysis failed to identify any significant regression relationship between biomass and rate for treated containers (Figure 3). Biomass ranged from 5 to 9 mg for treated containers.

Study III. Biomass of Scotch broom seedlings averaged 9 and 26 mg for treated (AC, AP, or CP) and nontreated containers, respectively (Table 1). Main effects of rate were significant ($P < 0.001$), and biomass averaged 26, 11, and 8 mg for 0, 50, and 100% MLR, respectively (Figure 3).

Each of the synthetic auxin herbicides in the research provided effective PRE control of germinating Scotch broom seedlings. Low rates ($\leq$ 50% of the MLR) provided 60 to 80% control, whereas 100% MLR provided 69 to 89% control. These findings have three important implications to vegetation management in the western United States. First, clopyralid is used commonly for herbaceous weed control in forestry because it is safe to apply over seedlings of Douglas fir (*Pseudotsuga menziesii* var. *menziesii*) and other western conifer species. Findings from this research confirm field observations that operational treatments of clopyralid reduced regeneration of Scotch broom from existing seedbanks (Harrington and Schoenholtz 2010). Secondly, aminopyralid has been used successfully in restoration of native plant communities (Almquist and Lym 2010; Samuel and Lym 2008), because it controls many nonnative broadleaf species, yet it is tolerated by a wide variety of native grass, forb, and shrub species (Halstvedt et

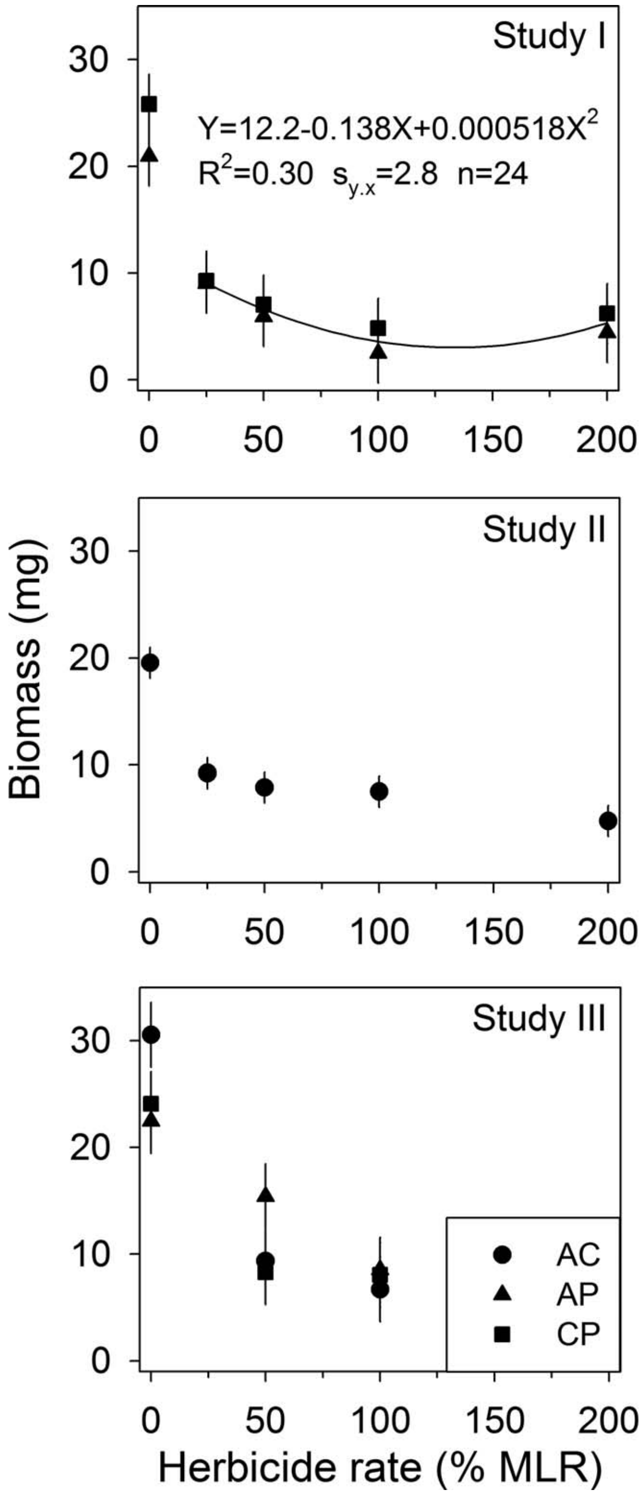

Figure 3. Seedling biomass (± standard error) of Scotch broom seedlings as affected by the percentage of maximum labeled rate (MLR) of aminocyclopyrachlor (AC; MLR = 280 g ae ha^{-1}), aminopyralid (AP; MLR = 120 g ae ha^{-1}), and clopyralid (CP; MLR = 560 g ae ha^{-1}) in three 90-d growth chamber studies. Means for the nontreated check (0% MLR) are shown for comparison.

al. 2010), especially when applied at low rates (Harrington et al. 2014). Results of this research indicate that low rates of aminopyralid will provide the added benefit of controlling germinating Scotch broom. Thirdly, depending on application rate and time since treatment, the herbicides varied in cost per unit of seedling mortality, with a general ranking of AP < CP < AC. Two of the herbicides, AP and CP, are currently labeled for forestry uses, and findings from this research indicate strong potential for AC in similar applications.

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