Long-term forage dynamics in pastures sprayed with residual broadleaf herbicide: A test of legume recovery

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Miller, A. J., Bork, E. W., Hall, L. M. and Summers, B. 2015. **Long-term forage dynamics in pastures sprayed with residual broadleaf herbicide: A test of legume recovery**. Can. J. Plant Sci. **95**: 43–53. Legumes such as alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) are important components of northern temperate pastures where they increase forage productivity and quality, but are susceptible to decline when exposed to broadleaf herbicides. Little is known about the long-term sward responses following herbicide use in northern temperate pastures, including the recovery of legumes and their subsequent contribution to forage production. We established five field sites over 2 yr to assess changes in grass, legume, total forage (grass + legume) and other forb biomass, as well as the recovery of a common weed, dandelion (*Taraxacum officinale* Weber), for up to 3 yr following a single application of two broad leaf herbicides with residual properties (aminopyralid and aminocyclopyrachlor). The importance of defoliation and the legume seed bank in facilitating legume biomass recovery were also evaluated with mowing and legume overseeding, respectively, in a strip/split-split design. Both herbicides had similar functional impacts on sward composition based on peak annual biomass, reducing legume biomass by an average of 71–100% across the 3 yr, equivalent to 63.4–22.6 g m^{-2} from year 1 to year 3, respectively. Although grass biomass did not change significantly with herbicides, net reductions in total forage were limited to 6.8% (28 g m^{-2}) over the study, suggesting at least some ability of grasses to compensate for legume removal. Legume biomass was greater following overseeding and only in non-sprayed controls, but then decreased over time. Conversely, biomass of other forbs and cover of dandelion were lower shortly following herbicide application, only to reach levels similar to non-sprayed controls by the second growing season. Defoliation also influenced sward composition, favoring dandelion recovery following herbicide application. As both weed control and legume re-establishment are important objectives for livestock producers, the result of this study provides useful insight into the long-term impact of broad-leaf weed control on forage production in mixed swards of northern temperate pastures.

Key words: Forage biomass, herbicide decay, legumes, mowing, weed control

Miller, A. J., Bork, E. W., Hall, L. M. et Summers, B. 2015. **Dynamique à long terme des pâturages traités avec un herbicide résiduel contre les dicotylédones : essai sur le rétablissement des légumineuses**. Can. J. Plant Sci. **95**: 43–53. Les légumineuses comme la luzerne (*Medicago sativa* L.) et le trèfle blanc (*Trifolium repens* L.) jouent un rôle important dans les pâturages des régions tempérées du nord, car elles en augmentent la productivité de même que la qualité des fourrages. Cependant, elles peuvent décliner quand elles sont exposées aux herbicides contre les dicotylédones. On sait peu de choses sur la réaction à long terme des peuplements à l'application d'herbicides dans les pâturages des régions tempérées du nord, notamment sur le rétablissement des légumineuses et sur la contribution subséquente de ces dernières à la production de fourrages. Les auteurs ont recouru à cinq sites expérimentaux aménagés sur le terrain pendant deux ans pour évaluer les changements que subit la biomasse des graminées, des légumineuses, des fourrages (graminées + légumineuses) et des dicotylédones, ainsi que le rétablissement d'une mauvaise herbe commune, le pissenlit (*Taraxacum officinale* Weber), au cours des trois années qui ont suivi une seule application de deux herbicides contre les dicotylédones aux propriétés résiduelles (aminopyralid et aminocyclopyrachlor). Les chercheurs ont aussi évalué comment la défoliation et la réserve de semences des légumineuses facilitent le rétablissement de la biomasse de légumineuses, respectivement en tondant et en sur-ensemençant des parcelles aménagées en bandes et en double tiroir. Les deux herbicides ont un impact fonctionnel similaire sur la composition du peuplement, si l'on se fie à la biomasse maximale annuelle. En effet, ils ont réduit la biomasse des légumineuses d'en moyenne 71 à 100% au cours des trois années de l'étude, soit de l'équivalent de 63,4 à 22,6 g par m^2 de la première à la troisième année, respectivement. Bien que la biomasse des graminées n'ait pas changé de façon significative avec l'application des herbicides, la réduction nette de la production fourragère totale s'est limitée à 6,8% (28 g par m^2) durant l'étude, signe que les graminées parviennent à compenser dans une certaine mesure la disparition des légumineuses. Le sur-ensemencement entraîne une plus forte biomasse de légumineuses, mais uniquement sur les parcelles non traitées, biomasse qui diminue ensuite avec le temps. Inversement, la biomasse des dicotylédones et la couverture du pissenlit diminuent peu après l'application des herbicides pour atteindre un niveau semblable à celui des parcelles témoins non traitées à la deuxième période végétative. La défoliation influe aussi sur la composition du peuplement, favorisant la reprise du pissenlit après l'application de l'herbicide. Puisque la

Abbreviations: **a.i.**, active ingredient; **AMCP**, aminocyclopyrachlor; **AMP**, aminopyralid; **ANOVA**, analysis of variance; **EC**, electrical conductivity; **MAT**, months after treatment; **OM**, organic matter; **PPB**, parts per billion; **WAT**, weeks after treatment

lutte contre les mauvaises herbes et le rétablissement des légumineuses sont deux importants objectifs pour les éleveurs, les résultats de cette étude fournissent des renseignements précieux concernant les répercussions à long terme de la lutte contre les dicotylédones sur la production fourragère des peuplements mixtes dans les pâturages des régions tempérées du nord.

Mots clés: Biomasse fourragère, désagrégation des herbicides, légumineuses, tonte, lutte contre les mauvaises herbes

Maintenance of legumes in pastures is an important goal for livestock producers striving to maximize forage quality and production. Many northern temperate pastures are composed of a mix of cool-season grasses and legumes, a combination that imparts several ecological and economic benefits (Vogel et al. 1983). Legumes are known for their nitrogen-fixing properties and ability to aid nutrient cycling in agro-ecosystems (Kunelius et al. 1982; Seguin 2001). The transfer of biologically fixed nitrogen to neighboring grasses increases overall production, reducing the need for fertilizers, which are otherwise necessary to maintain productivity in cool-season swards (Olsen et al. 1981; Vogel et al. 1983; Popp et al. 2000). In the process, legumes reduce the cost of forage production.

The production benefits of legumes are numerous, including that they have greater crude protein concentration than grasses, are palatable to livestock, and can increase forage intake and individual animal performance (Groya and Sheaffer 1981; Kunelius et al. 1982; Kunelius and Campbell 1984; Merou and Papanastasis 2009). Inclusion of a legume can also stabilize forage production both seasonally and annually, a result of differences in the timing of yield contributions between grasses and legumes (Groya and Sheaffer 1981; Seguin 1998; Sleugh et al. 2000; Katepa-Mupondwa et al. 2002). Additional benefits of grass–legume swards include reduced erosion, greater community recovery after disturbance, increased resistance to weed invasion, and greater stand longevity compared with grass or legume monocultures (Sleugh et al. 2000; Sanderson et al. 2005). Despite these benefits, the maintenance or restoration of pasture legumes can be problematic when legumes decline or are eradicated with agronomic practices (Grekul and Bork 2004; Enloe et al. 2007).

In many jurisdictions, weed control is a legal obligation. In Alberta for example, producers must control noxious weeds, destroy prohibited noxious weeds, and take measures to prevent their spread (Province of Alberta 2008). These weeds are often broadleaf species (*Cirsium arvense* L., *Ranunculus acris* L., *Sonchus arvensis* L., etc.) that can be reduced in pastures through the application of broadleaf herbicides (Grekul et al. 2005), in turn increasing forage production (Bork et al. 2007). The disadvantage of herbicide use is that legumes are typically decreased or eliminated (Grekul and Bork 2004; Enloe et al. 2007). This places producers in a difficult position, as they are legally mandated to control weeds, but in doing so may risk reducing pasture productivity and forage quality through legume loss.

Prompt re-establishment of legumes following herbicide application is therefore an important goal of livestock producers. Legumes depend on favorable conditions for germination, emergence and growth, including the presence of herbicide residues in soil within tolerable levels (Renz 2010). The “legume withdrawal period”, defined as the time between herbicide application and successful legume establishment, is dependent on herbicide degradation rates, which in turn, vary with the residual properties of herbicides, soil and environmental conditions. Herbicide decay rates generally increase with soil organic matter content and associated microbial activity (Veeh et al. 1996; Picton and Farenhorst 2004), high soil temperatures that assist in biochemical breakdown (Walker and Zimdahl 1981; Veeh et al. 1996), greater soil moisture (Walker and Zimdahl 1981; Parker and Doxtader 1983), and higher soil pH (Loux and Reese 1992; Aichele and Penner 2005). In addition to herbicide degradation, environmental factors and vegetative competition play key roles in regulating legume recovery, with varied success across climatic regimes (Gist and Mott 1956; Vough and Marten 1971; Mikkelson and Lym 2011).

Recommendations for legume reseeding are limited for areas treated with aminopyralid (AMP) and aminocyclopyrachlor (AMCP), two bioactives developed for broadleaf weed control. At present, data are either unavailable on expected recovery of legumes (AMCP), or a bioassay is recommended prior to seeding (AMP) [United States Environmental Protection Agency (USEPA) 2010; Dow AgroSciences 2012]. Soil dissipation studies for AMP indicate a terrestrial half-life of 34.5 d, and a laboratory half-life between 31.5 and 533.2 d (USEPA 2005). The half-life for AMCP is between 22 and 126 d in field dissipation studies (USEPA 2010). Field trials in Colorado directly comparing the two bioactives found half-lives of 28.9 and 32.5 d for AMP and AMCP, respectively (Lindenmayer 2012). It is reasonable to assume that herbicide degradation will slow in northern regions with colder average temperatures and shorter growing seasons, thereby extending half-lives and impeding legume recovery.

The Central Parkland is a productive region of Alberta that has been subject to abundant agricultural development (Natural Regions Committee 2006). The region contains fertile Black Chernozemic soils with high organic matter and favorable precipitation (350–450 mm yr^{-1}, falling mostly during the growing season), factors that combine to create an agro-ecosystem well suited for forage production (Soil Classification Working Group 1998; Natural Regions Committee 2006). However, these conditions also correspond with a short growing season and cool temperatures (Natural Regions Committee 2006). Initial studies on sensitivity of legume seedlings to AMP and AMCP in the Parkland indicated that alfalfa and white clover were equally sensitive to both products (Miller 2013). Seedlings did not establish when herbicides were applied at rates emulating the amount of herbicide expected to be present after 0, 1, 2, and 3 half-lives. At 0.0625 times the recommended rate, a level emulating residue after 4 half-lives had passed, herbicide residual activity allowed for only 40% survival of legume seedlings (Miller 2013). In parallel long-term trials, withdrawal intervals of 23 and 26 mo after spraying were needed to maximize plant densities of white clover and alfalfa, respectively (Miller 2013).

Our goal here was to document long-term forage biomass responses in northern temperate pastures following broadleaf herbicide application. Specific objectives were: (1) to indirectly quantify the degradation of two herbicide bioactives [aminopyralid (AMP) and aminocyclopyrachlor (AMCP)] applied at recommended field rates by assessing their effect on legume biomass at varied intervals following herbicide application, (2) to evaluate the role of environmental factors (specifically light and moisture) on legume biomass recovery, and (3) to document long-term pasture community dynamics, including forage biomass and weed abundance, following spraying, and link those responses to herbicide type, environmental conditions, mowing and legume overseeding.

MATERIALS AND METHODS

Study Sites

Field trials were conducted from May 2010 to September 2012 in five separate fields located within 100 km of Edmonton, Alberta, across the Central Parkland. Study sites were hayfields of various ages, internally uniform in topography and relatively homogenous in plant composition, with an initial legume component of 10–30% by cover. Hayfields rather than pastures were used to avoid the confounding effects of livestock grazing.

Three sites were established in May 2010 near Stony Plain, Fort Saskatchewan and the University of Alberta St. Albert research station, with an additional two sites established in June of 2011 near the towns of Chipman and Millet (Table 1). Sites differed slightly in sward composition, but were generally dominated by a combination of orchard grass (*Dactylis glomerata* L.), smooth brome (*Bromus inermis* Leyss), Kentucky bluegrass (*Poa pratensis* L.), quackgrass [*Elytrigia repens* (L.) Beauv.], timothy (*Phleum pratense* L.), and intermediate wheatgrass (*Thinopyrum intermedium* Host). Growing season precipitation and temperature data for all sites during the study period indicated above-average precipitation occurred in 2011, with minor variation in precipitation the other years (Miller 2013). Temperatures closely followed the long-term norms for all sites.

Experimental Design and Treatments

All sites consisted of a strip/split-split plot randomized block design, with four replicate blocks per site. Mowed main plots (6×12 m) were randomly assigned to half of each block, which, in turn, were crossed with herbicide treatments (3×12 m) oriented perpendicularly to the mowed treatments. Herbicide bioactives (AMP and AMCP) were randomly assigned to subplots, and applied at either full field rates (1 × treatment: 120 g a.i. ha^{-1} of AMP, or 60 g a.i. ha^{-1} of AMCP), or maintained as an untreated control (0 × treatment), one for each herbicide. Within each mowing × herbicide subplot, three seeding sub-subplots were established (2×3 m), including overseeding with alfalfa or clover, and a natural recovery treatment with no seeding.

Site Preparation

Sites were initially mowed to 10 cm to reduce all vegetation to a uniform height, and facilitate seeding and spraying. Sites were then raked (either by hand or mechanically) to remove excess litter, and sub-subplots seeded by hand at a rate of 16 kg ha^{-1} with either white Dutch clover (Common #1, seed from Viterra, Fort Saskatchewan, AB, www.viterra.ca) or alfalfa (cv. Algonquin, Certified #1, seed from Viterra, Fort Saskatchewan, AB, www.viterra.ca). Plots were then hand raked to ensure good seed to soil contact. Germination testes were 87.2% for alfalfa and 91.7% for clover.

Seven to ten days after seeding, herbicides were applied using a 2-m hand-held boom, equipped with Air Bubble Jet 110010 nozzles (ABJ Agri Products, Brandon, MB, www.abjagri.com), mounted on a CO_2 backpack sprayer, delivering 100 L of herbicide solution ha^{-1} applied 50 cm above the plant canopy. Spraying occurred 2010 Jun. 21 at the St. Albert, Stony Plain, and Fort Saskatchewan sites, 2011 Jun. 22 at Millet, and 2011 Jul. 02 at Chipman. Spraying treatments were randomized across repetitions, with a 50-cm buffer between sprayed and control plots. Mowing was repeated within mowed strip plots every 4 wk throughout the growing season (May to September) each year.

Field Measurements

Biomass was harvested from a randomly located 50×50 cm area within each non-mowed sub-subplot during peak growth each year, typically between mid-July and mid-August. We did not harvest biomass within mowed plots due to the obvious influence of mowing on standing biomass. Vegetation was clipped to a 2-cm height, sorted to grass, legume (alfalfa or white clover), and other (non-leguminous) forbs, dried to a constant mass and weighed. To assess changes in weed composition, the canopy cover of dandelion (*Taraxacum officinale* Web.), the most prevalent weed at each site, was recorded prior to herbicide application in all subplots, and again at the time of harvest in each growing season, to the nearest 1%.

Light measurements (μm m^{-2} s^{-1}) were recorded monthly for each subplot using a 1-m AccuPAR model

Table 1. Physical characteristics of each study site, as sampled in May 2010 or 2011[z]. All sites are level with negligible slope

Site	Location/ lat.–long.[y]	Soil type	Texture	Available N (NO_3+NH_4) (mg kg^{-1})	OM (%)	pH	EC (µs cm^{-1})
Fort Saskatchewan	53°47′18″ N, 113°20′38″ W	Solodized Solonetz	Silt Loam	5.5	9.0	7.1	482.0
Glenpark	53°10′38″ N, 113°24′42″ W	Gleyed Eluviated Black Chernozem	Silty Clay Loam	39.2	60.5	7.9	2580.0
Chipman	53°44′29″ N, 112°31′43″ W	Eluviated Black Chernozem	Sandy Loam	14.3	9.0	7.1	319.5
St. Albert	53°41′34″ N, 113°38′5″ W	Eluviated Black Chernozem	Silty Clay	7.8	16.4	8.4	467.0
Stony Plain	53°27′17″ N, 114°8′12″ W	Eluviated Black Chernozem	Sandy Loam	4.7	8.6	7.3	206.5

[z] Values represent the average of 10 soil cores collected at 0- to 30-cm depth.

[y] Lat.–Long. represent exact coordinates of site.

LP-80 PAR/LAI Ceptometer light wand (Decagon Devices Inc., Pullman, WA, http://www.decagon.com/), and averaged for use in analysis relative to each treatment. Soil moisture was recorded on a monthly basis using an ML2X-DeltaT moisture probe (ML2X-ThetaProbe soil moisture sensor, DeltaT Devices, Cambridge, UK, http://www.delta-t.co.uk/). Moisture readings were taken a minimum of 7 d after precipitation.

Soil samples were collected for each site prior to treatment to provide baseline information on physical characteristics. Soil cores, 5 cm in diameter, were taken at depths of 0–15 and 15–30 cm, from 10 points evenly spaced along a W pattern, as outlined by Thomas (1985). Samples were pooled within a depth class and analyzed for texture (percent sand, silt and clay), organic matter (%), pH, electrical conductivity (salinity) (µS cm^{-1}), and available nitrogen (NH_4+NO_3 mg kg^{-1}), using the methods outlined by Carter and Gregorich (2008). Finally, soil pits were dug at each site to identify the soil type (Table 1).

Analysis

The relationship between herbicide application and legume recovery, as represented by legume biomass, total forage biomass (i.e., legumes + grass biomass), and the biomass of grasses and other forbs, was explored using a split-split plot analysis of variance. A repeated measures design was incorporated to account for variance and covariance associated with multiple measurements taken on the same experimental unit (i.e., subplot) over time. Biomass responses were assessed using the general linear mixed models (GLIMMIX) procedure of SAS software version 9.2 to evaluate significance of main effects (i.e., herbicide type and rate, legume seeding and sampling time since spraying) and their interactions ($P \leq 0.05$). For all significant effects, a Tukey's HSD post hoc test was used to compare means and minimize risk of a type one error.

Prior to analysis, biomass of each vegetation component (forb, grass, legume, and total forage) was tested for normality using the Kolmogorov–Smirnov test in Proc UNIVARIATE (SAS Institute Inc. 2008) and found to be non-normal ($P < 0.10$). Although efforts to transform the data were unsuccessful, the GLIMMIX procedure is relatively robust to non-normal data (SAS Institute Inc. 2008). A normal/Gaussian distribution was used for data analysis. While non-parametric procedures were considered, the loss of explanatory power associated with these techniques was deemed undesirable (L. Goonewardene 2012, personal communication, AFNS Department, Faculty of ALES, University of Alberta, Edmonton, AB). Data were analyzed with site as a random factor to provide a generalized test of treatments and ensure broadly applicable results.

To evaluate the relationship between legume biomass recovery and environmental factors (light and moisture), correlations were performed to identify significant associations, their magnitude and directionality. Correlations were done for grass, legume, total forage (grass + legume) and forb biomass, against the environmental variables. Prior to correlation, both vegetation and environmental data were tested for normality, and found to be non-normal ($P < 0.10$), with transformations unable to attain normality. Thus, a Kendall's Tau rank correlation was used to assess the association between variables. Environmental correlations were limited to the control (0 ×), non-mowed and non-seeded treatments to remove the obvious confounding effects of other treatments on legume abundance.

To investigate changes in sward composition and herbicide efficacy over time, cover of dandelion, the most ubiquitous weed, was evaluated. Significant treatment effects and interactions were analyzed using a mixed model analysis of variance with a repeated measures design. Only non-seeded treatments were used to prevent confounding effects of seeding on dandelion cover. Dandelion cover was initially tested for normality using the Kolmogorov–Smirnov statistic, found to be non-normal ($P < 0.10$), and therefore subject to an arcsine transformation. Transformed data were subsequently run with the MIXED procedure in SAS software version 9.2, and a Tukey's HSD test used to minimize risk of a type one error in post hoc mean comparisons.

RESULTS

There were no significant differences in responses between sites, and sites were therefore combined for further analysis.

Biomass Responses

Legume biomass varied significantly in response to herbicide rate and seeding, both of which also interacted with sampling time since spraying, along with a 3-way interaction among these factors (Table 2). Herbicide rate effects on legume biomass were evident in all 3 yr, with plots treated with herbicide (1 ×) consistently yielding less legume ($P < 0.001$) than those non-treated (0 ×) (Fig. 1). However, legume biomass also declined throughout the 3 yr of the study in both 0 × and 1 × herbicide treatments, with greater absolute reductions in non-sprayed plots (Fig. 1). Averaged across treatments, plots seeded to alfalfa and clover produced 1.6-fold (31.7 g m^{-2}) and 1.7-fold (34.2 g m^{-2}) greater ($P < 0.05$) legume biomass, respectively, in comparison to non-seeded plots (20.0 g m^{-2}). Herbicide rate by seeding effects were limited to the first sampling period, at which time increases in legume biomass occurred only in non-sprayed (0 ×) plots seeded to either alfalfa or clover (Fig. 2). No differences in legume biomass were observed between plots sprayed with different types of herbicide ($P \geq 0.10$).

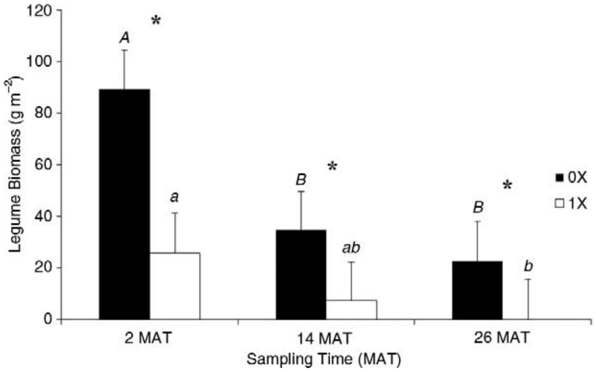

Fig. 1. Mean (±SE) legume biomass (g m^{-2}) measured at peak growth during each of 3 successive years, within the 0 × and 1 × herbicide plots. Within a herbicide rate, sampling time means with different letters differ based on a Tukey HSD test ($P \leq 0.05$). Within sampling times, pairs of herbicide rates with an asterisk differ ($P \leq 0.05$).

Grass biomass did not respond to the experimental treatments (Table 2), but increased significantly over the study period from an average of 312.5 (±46.1) g m^{-2} in year 1, to an average of 437.4 (±46.6) g m^{-2} in year 3. Total forage biomass (i.e., legumes and grasses combined) varied significantly in response to the main effects of herbicide rate and sampling time only (Table 2), with no higher level interactions. Control (0 ×) treatments had an average total forage yield of 410.3 (±46.1) g m^{-2}, while 1 × treatments were lower ($P < 0.05$) with an average yield of 382.3 (±46.0) g m^{-2}. Similar to grass biomass, total forage increased ($P < 0.05$) during the study from 356.7 (±46.7) g m^{-2} in year 1 year, to 449.9 (±46.7) g m^{-2} in year 3.

The biomass of non-leguminous forbs responded significantly to rate of herbicide, sampling time and their interaction (Table 2). Herbicide rate effects on forbs were only apparent during the first sampling period (year 1), when average forb biomass in the 1 × treatment (7.0 ± 5.9 g m^{-2}) was below ($P < 0.0001$) that of the 0 × treatment (29.6 ± 5.9 g m^{-2}) (Fig. 3). Forb biomass in

Table 2. Summary of F-statistic and significance (*P* values) associated with various vegetation biomass (g m^{-2}) responses to herbicide type and rate, legume seeding treatment, and various sampling times after spraying, as well as all associated interactions. Data are pooled over five study sites. Times represent 2–26 mo after spraying

	Grass biomass		Legume biomass		Total forage biomass (grass + legume)		Other forbs	
Treatment effect	F stat	*P* value	F stat	*P* value	F stat	*P* value	F stat	*P* value
Sampling time (Time)	27.0	**< 0.0001**	27.8	**< 0.0001**	15.6	**< 0.0001**	6.11	**0.002**
Herbicide rate (Rate)	2.22	0.14	75.9	**< 0.0001**	6.02	**0.015**	21.3	**< 0.0001**
Seeding (Seed)	0.73	0.48	3.39	**0.03**	0.45	0.64	2.00	0.14
Herbicide type (Herb)	0.30	0.58	2.71	0.10	0.87	0.35	0.37	0.54
Rate × Time	2.33	0.10	6.09	**0.003**	2.16	0.12	8.28	**0.0003**
Seed × Time	0.53	0.71	2.47	**0.04**	0.10	0.98	1.01	0.40
Herb × Time	0.61	0.54	0.03	0.97	0.67	0.51	1.05	0.35
Herb × Rate	0.08	0.78	0.03	0.86	0.26	0.61	0.29	0.59
Rate × Seed	0.28	0.76	2.14	0.12	0.07	0.94	1.23	0.29
Herb × Seed	2.31	0.10	1.63	0.20	1.18	0.31	0.40	0.67
Rate × Seed × Time	1.24	0.29	2.46	**0.05**	1.01	0.40	0.63	0.64
Herb × Seed × Time	1.14	0.34	1.02	0.40	1.73	0.14	0.27	0.90
Herb × Rate × Time	1.17	0.31	0.91	0.40	1.25	0.29	0.23	0.79
Herb × Rate × Seed	0.60	0.55	1.11	0.33	0.11	0.90	0.32	0.73
Herb × Rate × Seed × Time	0.41	0.80	0.38	0.82	0.39	0.82	0.55	0.70

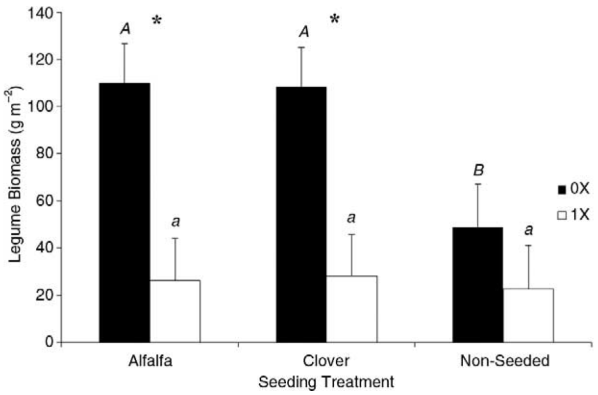

Fig. 2. Legume seeding by herbicide rate effects on mean (±SE) legume biomass (g m^{-2}) measured during the first growing season (2 MAT). Within a rate, means with different letters differ based on a Tukey HSD test ($P \leq 0.05$). Within a seeding treatment, paired rates with an asterisk differ ($P \leq 0.05$).

0 × treatments also decreased ($P = 0.0006$) between year 1 and year 2, with no difference between the final 2 yr ($P = 0.64$).

Dandelion Responses

Dandelion cover did not differ between the 0 × (15.7% ± 5.4) and 1 × (16.3% ± 5.4) treatments ($P = 0.82$) prior to herbicide application. After spraying, dandelion cover varied with herbicide rate (F = 5.12; $P = 0.02$), sampling time (F = 4.79; $P = 0.009$) and their interaction (F = 10.87; $P < 0.0001$). Total dandelion cover 1 yr after herbicide application was lower ($P < 0.05$) in the 1 × treatment (8.3% ± 5.4) than the 0 × treatment (18.1% ± 5.4), but recovered in sprayed plots by the final year (14.1% ± 5.6) to levels similar ($P = 0.78$) to those found in plots not receiving herbicide (14.2% ± 5.6).

Dandelion also responded to mowing (F = 34.71; $P < 0.0001$) and its interaction with sampling time (F = 24.74; $P \leq 0.0001$). While dandelion was similar between mowed (15.1% ± 5.4) and non-mowed (17.0% ± 5.4) plots prior to the initiation of treatments ($P = 0.99$), dandelion in non-mowed plots declined ($P < 0.05$) during years 2 and 3 to levels below ($P < 0.05$) that of adjacent mowed plots. One year after mowing started, mowed and non-mowed plots had 17.1% (±5.4) and 9.3% (±5.4) dandelion cover, respectively, a pattern that became increasingly evident in the final year when mowed and non-mowed plots had 24.7% (±5.6) and 3.7% (±5.6) cover, respectively.

Biomass–Environment Relationships

Environmental effects were assessed only in non-sprayed plots to avoid the confounding effects of herbicide application. All biomass responses were positively correlated with soil moisture readings, regardless of sampling time (Table 3). Grasses were particularly correlated with soil moisture in June, while the biomass of legumes and other forbs were more correlated with late season (August) soil moisture. Not surprisingly, total forage biomass, and grass biomass in particular, were negatively associated with light availability (μm m^{-2} s^{-1}) averaged over the growing season. Mowing increased light availability to the understory by 49%, from an average of 243.1 (±6.6) μm m^{-2} s^{-1} in non-mowed plots, to 478.3 (±6.6) μm m^{-2} s^{-1} in mowed plots. Despite this, legume biomass remained unrelated to light availability (Table 3).

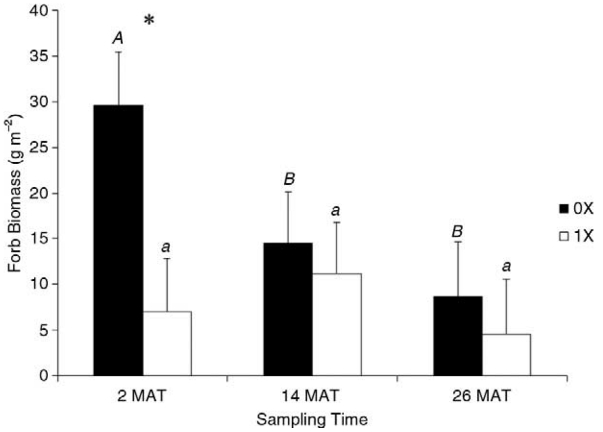

Fig. 3. Mean (±SE) non-leguminous forb biomass (g m^{-2}) measured at peak growth during each of 3 sampling years within the 0 × and 1 × herbicide plots. Within a herbicide rate, sampling time means with different letters differ based on a Tukey HSD test ($P \leq 0.05$). Within sampling times, pairs of herbicide rates with an asterisk differ ($P \leq 0.05$).

DISCUSSION

Although various factors played a role in the recovery of legume biomass, herbicide application was the main driver regulating this process, and reduced legume biomass irrespective of other factors. Marked reductions in legume biomass observed with herbicide application were also similar for both AMCP and AMP. Effects of additional factors (i.e., seeding) on legume biomass were more subtle when interpreted in the context of herbicide exposure. In the absence of herbicide, however, seeding became the primary determinant of legume abundance.

Forage Responses

Legume biomass did not vary with bioactive identity, indicating AMP and AMCP had functionally similar impacts on legume re-establishment and associated forage responses. AMP and AMCP have similar chemistries, being pyrimidine carboxylic acids with an auxinic mode of action that selects for broadleaf plants, and are degraded in the soil by microbial activity (Dow AgroSciences 2005; DuPont 2009). Further, both are translocated from leaves and roots to meristematic tissues, where they mimic auxin hormones and cause leaf cupping, loss of apical dominance, unregulated epinastic growth and death (Bussan and Dyer 1999; USEPA 2005).

Table 3. Correlation coefficients (Kendall's Tau-b statistic) indicating the relationship of soil moisture (%) and light (μm m^{-2} s^{-1}) with legume, grass, total forage and other forb biomass (g m^{-2}) within non-seeded, non-mowed, non-sprayed control (0 ×) plots

Component	Response	June soil moisture	August soil moisture	Average soil moisture	Light availability
Biomass	Legume	+0.086*	+0.16**	+0.14**	−0.08
	Grass	+0.16***	+0.10*	+0.15**	−0.22***
	Total forage^z	+0.24***	+0.20***	+0.25***	−0.25***
	Other forbs	+0.21***	+0.24***	+0.24***	−0.43

^{z}Total forage includes grass and legume biomass.
*, **, *** $P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively.

These characteristics suggest AMP and AMCP have comparable withdrawal periods in treated soils, leading to indistinguishable patterns of legume re-establishment, which was indeed the case here, as supported by a parallel investigation that estimated 4 half-lives (about 200 d during the growing season) were needed to allow legume establishment (Miller 2013). Published half-lives for the bioactives tested here are wide ranging, at 31.5–533.2 d for AMP, and 22–126 d for AMCP, based on pre-registration studies conducted by the Environmental Protection Agency (USEPA 2005, 2010). Lindenmayer (2012) estimated half-lives of 28.9 and 32.5 d for AMP and AMCP, respectively, in Colorado. Moreover, similar control of Canada thistle (*Cirsium arvense* L.) was obtained in the forementioned study with AMP and AMCP, reinforcing the functional similarity of these bioactives in our study. However, AMCP has provided more effective residual control of spiny amaranth (*Amaranthus spinosus* L.) and kudzu [*Pueraria montana* (Lour.) Merr.] (Edwards 2010; Minogue et al. 2011), which presumably is an indicator of longer residual control. Variation in bioactive response among studies may be due to differences in bio-indicator plants (i.e., their sensitivity to AMP and AMCP herbicide), or differences in climatic regimes, as the aforementioned studies were conducted in the southeastern United States.

Differences among studies highlight variation in herbicide degradation across climatic regimes. Both bioactives tested are broken down by microbial activity; a process influenced by soil organic matter content, moisture, temperature and pH levels. Herbicide degradation rates generally increase with organic matter content and associated microbial activity (Veeh et al. 1996; Picton and Farenhorst 2004), warm soil temperatures (Walker and Zimdahl 1981; Goetz et al. 1990), greater soil moisture (Walker and Zimdahl 1981; Parker and Doxtader 1983), and higher soil pH (Aichele and Penner 2005). Different combinations of these factors will further alter decay rates.

Herbicide application removed legumes independent of seeding and mowing. In contrast, while legume seeding briefly increased legume abundance in non-sprayed plots, by the second growing season legumes were outcompeted by the vigorous grass community. Legume biomass in mixed legume–grass swards has been shown to decline over time regardless of agronomic practices (Groya and Sheaffer 1981; Rioux 1994; Sanderson et al. 2005). We postulate that the decline in legumes was a response to more vigorous grass plants in the absence of mowing competing heavily with legumes for light and moisture (Groya and Sheaffer 1981; Rioux 1994; Sanderson et al. 2005). Legume biomass was positively associated with soil moisture (Table 3), suggesting the latter may have been particularly important for facilitating legume establishment. Numerous studies, including this one, have shown legume biomass decreases over time, and may necessitate periodic reseeding, even in non-sprayed areas (Groya and Sheaffer 1981; Peterson et al. 1994). Although herbicide application and seeding treatments had significant initial effects on legume abundance, there must be other factors, competition and defoliation in particular, that regulate legume survival and abundance over time in mixed swards (Groya and Sheaffer 1981; Kunelius et al. 1982; Peterson et al. 1994). For legumes sensitive to light availability such as white clover (Frame 2005), decreases in their abundance may also reflect shading from neighboring grasses in the absence of repeated defoliation during the growing season. While we expected a positive relationship to manifest between legume biomass and light, and that this may be facilitated by mowing, this did not occur in the present study (Table 3).

Forb biomass (excluding legumes) also experienced a temporary decline following herbicide application, particularly immediately after spraying [2 months after treatment (MAT)]. Rapid recovery of forb biomass the following year is attributed to both degradation of herbicide residues, and the abundant seed bank of these pastures, which is typically dominated by disturbance adapted forbs (Harker et al. 2000). The increase in forb biomass during year 2 suggests herbicide residues had degraded sufficiently to allow for some forb re-establishment. Agricultural lands are prone to plant invasions because of resource availability (moisture, light, nutrients and space) associated with anthropogenic disturbance (Daehler 2003). The abundance of weed seeds in the seed bank of pastures in central Alberta has been noted to range between 207 and 580 plants m^{-2}, although weed abundance is also a localized characteristic (Harker et al. 2000). Even the lowest density (207 plants m^{-2}) documented suggests there is ample opportunity for forb establishment, and helps explain why forb biomass recovered so promptly from herbicide application.

Both grass and total forage biomass increased over the study period, regardless of herbicide application. These increases may reflect the fact that our harvest regimes allowed swards to reach peak biomass, and earlier defoliation in the growing season generally reduces biomass (DeBruijn et al. 2010). Moreover, the increase in grasses may reflect the decline in competition from legumes or other forbs, including weeds (e.g., Grekul et al. 2005), such as dandelion reported here in the absence of mowing. In the case of compensatory responses to legume removal, it is likely that grasses utilized available resources resulting from broadleaf plant removal (Wing-To and Mackenzie 1971; Groya and Sheaffer 1981; Sanderson et al. 2005), or benefited from a ‘‘flush’’ of available nitrogen associated with the decay of roots from affected plants (particularly legumes). Although we did not detect a significant increase in grass biomass from herbicides, the trend of increased grass biomass may have at least partly offset the decline in legume biomass following spraying, providing some degree of stability in total forage yield. Consequently, our results suggest the penalty of legume loss incurred due to herbicide application (about 6.8% over the course of the study) is not as large as expected, at least based on legume removal alone. Nevertheless, the slow return of legumes in the long-term may still lead to production decreases once nutrients become limiting, and we did not examine forage quality differences associated with the herbicide treatments.

The lower total forage productivity observed here following spraying is consistent with other studies showing herbicide application decreases productivity of mixed swards after legume removal, with decreases in crude protein yield as well as biomass (McLeod 2011). Thus, the decision of when and whether to spray pastures for weed control will depend on the magnitude of weed impacts on forage production (Grekul and Bork 2004) and the associated urgency of weed control.

The presence of at least some legume biomass in all treatments, even natural recovery plots exposed to herbicide (see Fig. 2), supports the ongoing potential for legume re-establishment over time. However, greater legume biomass resulted from legume overseeding, a result paralleled by other studies (Decker et al. 1969; Olsen et al. 1981; Schellenberg et al. 1994; Muto and Martin 2000; Carr et al. 2005; Mortenson et al. 2005). Trace legume recovery in non-seeded subplots likely occurred from the existing seed bank, which often contains legumes. Hard-seeded legumes have persistent longevity in the seed bank, but after seeding require longer for emergence (Lewis 1973) and eventual contribution to forage production. In general, although legume establishment is more favorable under conventional establishment (i.e., seeding into fallow) due to reduced competition, factors important for small seeded legumes (Rioux 1994), our results suggest interseeding of legumes into pastures can be used as well, and would have the benefit of avoiding the loss of forage during the establishment period.

Legume establishment with overseeding requires a favorable environment for germination, seedling emergence and survival, and in some instances can lead to population levels comparable to seeding with a mechanical drill (Bryan and Prigge 1990; Schleuter 2011). When combined with vegetation suppression, such as herbicide application or defoliation (via mowing or grazing), legume productivity is known to increase (Bowes and Zentner 1992; Seguin 1998; Muto and Martin 2000). Alfalfa has been successfully interseeded into grass-dominated rangeland and increased total forage biomass by 42–143% (Mortenson et al. 2005). Interseeded alfalfa does not establish well in bromegrass sod; however, compared with conventional planting (Rioux 1994), suggesting that legume re-establishment may depend on the suppression of competing vegetation (Cuomo et al. 2001), a finding corroborated by a parallel study to the current investigation (Miller 2013).

Weed Dynamics

Although herbicide application initially decreased dandelion cover, this species recovered by the second growing season to levels of the non-sprayed control and pre-spraying condition. This suggests that the bioactives tested (AMP and AMCP) do not have lasting residual effects on this weed, and control can be expected at recommended application rates for less than two growing seasons. The application of AMP at the same rate as in this study (120 g a.i. ha^{-1}) has been shown to reduce dandelion cover by 50 and 70% in Canada thistle infested and native tallgrass prairie communities in Minnesota, during the year of application (Almquist and Lym 2010). However, dandelion recovered to pre-treatment levels by 22 MAT (presumably the third growing season), corroborating our findings on dandelion responses to AMP. This indicates that for effective control of dandelion in grazed pasture, herbicides would have to be reapplied every second growing season, a process that could be relatively costly, and which would also result in a pasture devoid of legumes (Miller 2013).

Mowing appeared to be as important as herbicide in regulating the abundance of dandelion. Increases in dandelion under mowing were expected as this weed has the ability to tolerate and even thrive under high defoliation (Mølgaard 1977). Moreover, this species likely benefited from abundant light throughout the growing season within this treatment, which would allow dandelion plants to form well-developed, wide-spreading rosettes capable of suppressing neighboring plants (Godoy et al. 2011).

In contrast, the pronounced reductions in dandelion within non-mowed plots, both sprayed and non-sprayed, was attributed to increased competition from grasses (Mølgaard 1977). This, in turn, likely reflects the single defoliation regime at peak growth used to assess forage responses, which is known to maximize grass productivity (DeBruijn et al. 2010). Dandelion establishment in grasslands is only possible when grass cover and associated competition are low, with open spaces between plants enabling light infiltration through neighboring vegetation. Dandelion plants tend to be smaller and less vigorous in areas with strong grass competition (Mølgaard 1977), explaining why dandelion decreased over time in our non-mowed treatments. Light levels in the non-mowed treatments were generally below the light compensation point for this species [243.1 ($\pm$6.6) μm m^{-2} s^{-1} in non-mowed plots, vs. $\sim$300 μm m^{-2} s^{-1} light compensation point], accounting for why dandelion decreased in the absence of mowing (Godoy et al. 2011). Competitive vegetation and crop residues can shade out dandelion, reducing their germination and emergence (Derksen et al. 2002), and highlight the need to maintain a competitive grass stand to suppress this weed, such as also found under high-intensity–low-frequency defoliation (DeBruijn and Bork 2006). Collectively, these results suggest that successful dandelion control by herbicide application should not be expected in areas with heavy grazing or defoliation and, consequently, herbicide application in conjunction with conservative defoliation may allow for the greatest sustained decrease in dandelion.

SUMMARY AND CONCLUSIONS

A lack of information exists regarding withdrawal periods for legumes to broadleaf herbicides with residual properties, AMP and AMCP in particular, and post-spraying forage sward responses within northern temperate grasslands. We addressed this deficiency by documenting the effects of two broadleaf herbicides on overall sward dynamics over time as herbicide residues degrade. While legumes increased following overseeding in non-sprayed plots, herbicide treatments consistently lowered legume biomass throughout the study, leading to a modest net decline of about 7% total forage. Therefore, we conclude that seeding legumes after herbicide treatment may not be effective in restoring legumes even 26 MAT. Seeding should be delayed until herbicide residue has sufficiently dissipated. Although herbicides reduced the predominant weed, dandelion, in the year of application, this decline did not persist in the second growing season, and instead abundance of this species was more influenced by mowing than herbicides in the long-term. Our results provide clarification on the fundamental trade-off associated with legume removal and weed control in mixed forage swards of northern temperate pastures, and should lead to improved pasture management capable of balancing weed control with the maintenance of forage production. Knowledge of long-term pasture sward dynamics following herbicide application, legume seeding and other disturbances (e.g., grazing/mowing) will aid decisions regarding weed control and legume conservation, and provide a better understanding of how to optimize net forage availability with broadleaf herbicide use.

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