Short Title: Pre-Emergence Weed Control with Three Herbicides

Pre-Emergence Control of Nine Invasive Weeds with Aminocyclopyrachlor, Aminopyralid, and Indaziflam

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There are an estimated 400 million ha of non-cropland in the US primarily designated as rangeland and pastureland, and there are over 300 invasive weeds found on these sites causing an estimated annual loss of $5 billion. Among the most invasive and problematic weeds are Dalmatian toadflax, diffuse knapweed, downy brome, and musk thistle. Currently, herbicides are the most common management strategy for broadleaf weeds and invasive winter annual grasses. Indaziflam, a new herbicide for invasive plant management in non-crop areas, is a cellulose-biosynthesis inhibitor capable of providing residual invasive winter annual grass control up to 3 years after treatment

(YAT). A field experiment was conducted to determine if residual Dalmatian toadflax and downy brome control of aminocyclopyrachlor, imazapic, and picloram could be extended by tank-mixing with indaziflam. Indaziflam tank-mixed with aminocyclopyrachlor, imazapic, and picloram provided increased Dalmatian toadflax (84 to 91%) and downy brome (89 to 94%) control 4 YAT, compared to treatments excluding indaziflam. Treatments without indaziflam controlled 50 to 68% of Dalmatian toadflax and <25% downy brome 4 YAT. Based on these results, a greenhouse dose-response experiment was conducted with aminocyclopyrachlor, aminopyralid, and indaziflam to compare pre-emergence control of nine common non-crop weeds. Averaged across species, aminocyclopyrachlor and aminopyralid GR_{50} values (herbicide concentration resulting in 50% reduction in plant biomass) were 29- and 52-times higher compared to indaziflam, respectively. These data suggest that indaziflam could be used for residual control of non-crop weeds, as a tank-mix partner with other foliar applied broadleaf herbicides.

Nomenclature: imazapic; indaziflam; picloram; aminocyclopyrachlor; aminopyralid; common mullein, *Verbascum thapsus* L.; common teasel, *Dipsacus fullonum* L.; curly dock, *Rumex crispus* L.; Dalmatian toadflax, *Linaria dalmatica* (L.) Mill.; diffuse knapweed, *Centaurea diffusa* Lam.; downy brome, *Bromus tectorum* L.; halogeton, *Halogeton glomeratus* (M. Bieb.) C.A. Mey.; marestail, *Conyza Canadensis* (L.) Cronquist; musk thistle, *Carduus nutans* L.

Key words: dose-response, Great Basin, invasive weed, invasive winter annual grass, rangeland, restoration.

Management Implications

Native plant communities that provide wildlife habitat and important ecosystem services are negatively impacted by invasive weeds. Many of these invasive weeds are prolific seed producers, which makes the soil seed bank the primary mechanism responsible for rapid re-establishment. Long-term control of many weed species has been difficult due to limited management options and budget constraints. Short-term control does not provide the time necessary for the re-establishment of the native plant community so there is often an open niche for re-establishment or secondary invasions to occur. Although herbicides are a commonly used management tool, there are limited herbicide options that provide the long-term control necessary to deplete the soil seed bank of invasive weed seed and allow recovery of co-occurring desired species. An herbicide with residual activity would be desirable for control of germinating seedlings, and while aminocyclopyrachlor, aminopyralid, and picloram have residual activity, their residual activity is less than indaziflam. The results presented here provide evidence that indaziflam could be used alone or in combination with broadleaf herbicides to potentially extend control up to 4 years after treatment (YAT). For invasive winter annual grasses such as downy brome, indaziflam could be applied alone preemergence; however, having limited post-emergence activity, indaziflam would need to be used in combination with other broadleaf herbicides to control actively growing rosettes in the fall or spring. Indaziflam's residual activity could provide the necessary time for desired co-occuring species to re-establish. Indaziflam represents an interesting opportunity to influence rangeland plant community assembly in areas affected by invasive species that dominate native rangelands primarily by their high propagule pressure. Indaziflam could be used in conjunction with other methods to shift the advantage from exotic invaders with high propagule pressure back toward natives and other desirable vegetation. Because indaziflam is a unique mode of action (cellulose biosynthesis inhibitor) for non-crop weed management, combining indaziflam with other modes of action in a single treatment could also be used for resistance management. Although additional research is necessary to verify these findings under field conditions, this study supports our previous indaziflam work with downy brome (Sebastian et al. 2016b).

Invasive weed management in non-crop areas (primarily rangeland and pastureland) remains a significant challenge throughout the US (Duncan et al. 2004; Evans and Young 1970; Kelley et al. 2013; Kyser et al. 2013; Mangold et al. 2013). Rangeland and pastures comprise about 42% (400 million ha) of the total land area in the US and in these areas, invasive plants can cause an estimated loss of $5 billion annually (Pimentel et al. 2005). Cultural practices contributing to the establishment and spread of invasive plants include disturbance and over-grazing by domestic livestock (Davies et al. 2016; Porensky et al. 2017), purposeful introduction for agriculture and horticulture, unintentional introduction via contaminated seed, and climate change (DiTomaso et al. 2010; Varanasi et al. 2016).

Invasive weeds that infest rangeland and other non-crop areas can have significant negative ecological impacts including depleting soil moisture and nutrients, reducing forage production, reducing plant diversity and community productivity, altering fire frequency, and reducing the value of recreational land (Beck et al. 2008; DiTomaso et al. 2010; Knapp 1996; Watson and Renney 1974; Whisenant 1990). Invasive weeds are frequently designated as noxious because of these impacts. Many of these invasive plants are prolific seed producers and exert high propagule pressures on invaded sites. Propagules can spread by multiple dispersal mechanisms including mechanical (vehicles and contaminated machinery), wildlife and livestock (ingested or coat hair entanglement), and human recreation (Sheley et al. 1999). Once established, several noxious weeds have extensive taproot systems allowing them to extract moisture and nutrients from deep within the soil profile (DiTomaso 2000; Gerlach and Rice 1996).

This can result in rapid shifts in the dominant native plant communities (James et al. 1991).

Of the over 300 rangeland weeds in the US, downy brome (*Bromus tectorum* L.) and Dalmatian toadflax (*Linaria dalmatica* (L.) Mill.) have emerged as two of the most wide-spread and problematic, with average annual spread rates of 14% and 19%, respectively (DiTomaso 2000; DiTomaso et al. 2010; Duncan et al. 2004). Disturbance favors these particular invasive plants so they commonly invade degraded areas such as roadsides, abandoned crop fields, gravel pits, clearings, and overgrazed rangeland (Beck 2009). Downy brome, an invasive winter annual grass, has rapidly spread throughout many regions of the US displacing native vegetation and altering fire frequency and intensity (Knapp 1996; Whisenant 1990; Zouhar 2008). Duncan et al. (2004) estimated that over 22 million hectares of the western United States are infested with downy brome. Dalmatian toadflax, an escaped ornamental, is a short-lived herbaceous perennial plant (Alex 1962) that is most commonly found in semi-arid areas, on course textured, gravelly soils (Alex 1962; Robocker 1970). It is a self-incompatible species contributing to its high level of genetic variability (Kyser and DiTomaso 2013; Wilson and Turner 2005). Dalmatian toadflax produces large amounts of seed that can remain viable in the soil for approximately 10 years (Robocker 1970). Once established, high seed production along with aggressive vegetative propagation enables Dalmatian toadflax to spread rapidly and to dominate and persist (Wilson and Turner 2005). Other non-crop, broadleaf weeds that have major economic and ecological impacts include diffuse knapweed (*Centaurea diffusa* Lam.), musk thistle (*Carduus nutans* L.), curly dock (*Rumex crispus* L.), common mullein (*Verbascum thapsus* L.), halogeton (*Halogeton glomeratus* (M. Bieb.) C.A. Mey.), marestail (*Conyza canadensis* (L.) Cronquist), and common teasel (*Dipsacus fullonum* L.) (DiTomaso 2000; Duncan et al. 2004; Rose et al. 2009). There are currently limited management options that provide long-term control of these weeds.

Among the available control strategies for invasive weed control in non-crop areas (mechanical, cultural, biological, and chemical), herbicides are the primary method for controlling invasive weeds in non-crop areas (DiTomaso 2000; Mangold et al. 2013). Synthetic auxin or growth regulator herbicides such as aminocyclopyrachlor (Method®), aminopyralid (Milestone®), and picloram (Tordon®) are commonly recommended residual broadleaf herbicides, while imazapic (Plateau®) has been the primary herbicide for downy brome control (Kyser et al. 2013; Mangold et al. 2013; Sebastian and Beck 2004). Several other herbicides including glyphosate (Roundup®) and rimsulfuron (Matrix®) have been used for short-term downy brome control (Kyser et al. 2013). None of these herbicides have provided long-term control of invasive weeds when used alone, resulting in rapid re-infestations (DiTomaso et al. 2010; Mangold et al. 2015; Sebastian et al. 2012).

Lack of residual control and resulting seedling recruitment could be attributed to the chemical properties of these herbicides (Sebastian et al. 2012). Aminocyclopyrachlor, aminopyralid, imazapic, and picloram are all water-soluble herbicides (ability of an herbicide to dissolve in water) with values ranging between 2,200 and 207,000 mg L^{-1}. Another indicator of an herbicide's hydrophilicity or lipophilicity can be estimated by its Log K_{ow} (octanol/water partitioning coefficient). The herbicides mentioned above have a range of Log K_{ow} (pH 7) values (-2.87 to 1.18) which are characteristic of hydrophilic (water-soluble) compounds. Because aminocyclopyrachlor, aminopyralid, imazapic are water soluble, their leaching potential is high, ultimately decreasing the herbicide concentration available in the soil solution for plant uptake beyond the initial year of application (Oliveira Jr et al. 2013). A study conducted by Oliveira et al. (2013) also showed desorption hysteresis with aminocyclopyrachlor and picloram, suggesting the herbicide that is sorbed to soil is resistant to desorption and irreversibly bound to soils.

Another factor to consider for long-term control of invasive plants is the soil seed bank. The longevity of weed seeds in the soil for the species mentioned above are all >2 years (Burnside et al. 1996; Rector et al. 2006; Robocker 1970; Robocker et al. 1969; Sheley et al. 1998; Weaver 2001). Therefore, new herbicides should be evaluated that have decreased leaching potential and provide the soil residual control necessary to deplete the soil seed bank. Residual control for multiple growing seasons would also provide native perennial plants a competitive advantage for re-establishment (DiTomaso et al. 2010; Patrick and Wilson 1983; Rose et al. 2009).

Indaziflam (Esplanade, Bayer CropScience) is a new herbicide with the potential to provide residual control of germinating seeds of annual, biennial, and perennial weeds. Previously, indaziflam has been used primarily for total vegetation management (e.g. roadsides, railroads, power substations, oil pads), weed control in turf, established citrus, grape, and tree nut crops (Brosnan et al. 2012; de Barreda et al. 2013; Jhala and Singh 2012; Kaapro 2012). Indaziflam is a cellulose-biosynthesis inhibitor (CBI) (Brabham et al. 2014; EPA 2010), representing a unique mode of action for non-crop areas with residual soil activity and broad spectrum preemergence (PRE) control

(Sebastian and Nissen 2016; Sebastian et al. 2016b; Sebastian et al. 2014). As previously mentioned, the range of water solubilities (2,200 to 207,000 mg L^{-1}) and log K_{ow} (-2.87 to 1.18) values of aminocyclopyrachlor, aminopyralid, imazapic, and picloram results in herbicide dilution in the soil profile and short-term soil residual activity; however, indaziflam is more lipophilic with water solubility of 3.6 mg L^{-1} and log K_{ow} of 2.8 (pH7). The recommended non-crop use rates are relatively low for indaziflam (73 to 102 g ai ha^{-1}), and comparable with imazapic (70 to 123 g ai ha^{-1}), aminocyclopyrachlor (70 to 140 g ae ha^{-1}), and aminopyralid (53 to 123 g·ae·ha^{-1}); however, picloram is recommended at higher use rates (140 to 1,121 g·ae·ha^{-1}). Indaziflam's residual downy brome (*Bromus tectorum* L.) control was evaluated by Sebastian *et al.* (2016b) and indaziflam treatments provided better residual downy brome control 2 and 3 years after treatment (YAT) compared to imazapic, glyphosate, and rimsulfuron. Indaziflam has not previously been evaluated for PRE control of other noxious weeds for use in non-crop areas. Indaziflam is currently restricted to sites not grazed by domestic livestock and further studies are needed to establish a grazing tolerance (personal communication; David Spak, Bayer CropScience, Research Triangle Park, NC.).

Based on previous field and greenhouse research, indaziflam appears to have several attributes that could be used to enhance invasive plant management; therefore, a field study was established to determine if tank-mix treatments combined with indaziflam provided longer residual Dalmatian toadflax and downy brome control than aminocyclopyrachlor, imazapic, and picloram applied alone. This would corroborate results presented by Sebastian et al. (2016b) that indaziflam applied alone increased residual downy brome control, while further evaluating the residual control on the seedlings of an additional invasive weed, Dalmatian toadflax. The second objective of this study was to conduct a greenhouse bioassay to compare pre-emergence control of nine additional weeds found on rangeland and other non-crop areas with aminocyclopyrachlor, aminopyralid, and indaziflam. These three herbicides all have relatively low recommended field use rates; therefore, this experiment allowed us to directly compare pre-emergence control of the nine species evaluated.

Materials and Methods

Herbicide Efficacy Field Trial and Experimental Design. In 2010 a field trial was conducted to evaluate the effectiveness of herbicides for long-term downy brome and Dalmatian toadflax control. The experiment was conducted at only one site; however, the results provide the framework for the subsequent greenhouse experiment. The field experiment was located in Longmont, CO, (lat 40°14'57.53"N, long 105°12'35.46"W) on Rabbit Mountain Open Space. Immediately before treatments were initiated (June 2010), visual percent canopy cover estimates were conducted across the study site to estimate pre-treatment cover of downy brome, Dalmatian toadflax, and native co-occurring species. The canopy cover of actively growing downy brome and Dalmatian toadflax at peak standing crop (June 2010) was approximately 85% and 30%, respectively. Perennial grasses (<10% canopy cover) included primarily western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love), and native forbs and sub-shrubs (~20% canopy cover) included Louisiana sage (*Artemisia ludoviciana* Nutt.), fringed sage (*Artemisia frigida* Willd.), common sunflower (*Helianthus annuus* L.), sulphur-flower buckwheat (*Eriogonum umbellatum* Torr.), and hairy goldenaster (*Heterotheca villosa* (Pursh) Shinners). The soil at the study site is Baller sandy loam (loamyskeletal, mixed, superactive, mesic Lithic Haplustolls), with 1.5% organic matter in the top 20 cm (USDA-NRCS 2014). The average elevation is 1,725 m (5,660 ft). Mean annual precipitation based on the 30-yr average (1981-2010) at the study site was 363 mm and the mean annual temperature was 9.1 C (Western Regional Climate Center 2013). Precipitation was close to the 30-yr average in 2010, 2011, and 2014. A statewide-drought occurred in 2012 and average total precipitation decreased 134 mm. In 2013, the site received above-average precipitation with an additional 110 mm above the 30-yr average (CoCoRaHS 2015).

Herbicide treatments (Table 1) were applied in summer at two application timings; 20 June 2010 when Dalmatian toadflax was in the flowering growth stage and 11 August 2010 during Dalmatian toadflax regrowth; however, no downy brome had emerged when these applications were made. Therefore, we considered these applications to be pre-emergence with respect to downy brome. Herbicide treatments were applied to different plots at the two application timings. The 13 herbicide treatments (including a non-treated) were applied to 3 by 9 m plots arranged in a randomized complete block design with four replications and are listed in Table 1. All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat fan nozzles at 187 $L \cdot ha^{-1}$ at 207 kPa. All treatments included 1% $v \cdot v^{-1}$ methylated seed oil.

Visual percent control evaluations were conducted in June of each year (2011-2014). Control evaluations were estimated by comparing visual estimates of Dalmatian toadflax and downy brome cover in the treated plots (using the entire 3 by 9 m plot area) compared with the non-treated plots. Plots with 0% canopy cover received a

100% control rating, while plots with 100% canopy cover received a 0% control rating. Perennial grass canopy cover estimates were also conducted the final year of the study (June 2014).

Greenhouse Experiment: Comparing Aminocyclopyrachlor, Aminopyralid, and Indaziflam Preemergence Weed Control. Based on the results of the field research, we designed a greenhouse experiment to determine if the extended Dalmatian toadflax and downy brome control provided by indaziflam in the field was due to increased residual seedling control. This experiment was designed to compare indaziflam's pre-emergence efficacy with two herbicides commonly recommended for annual, biennial, and perennial weed control in non-crop areas (aminocyclopyrachlor and aminopyralid). Aminopyralid was used in this greenhouse bioassay in place of picloram because the average recommended use rate for indaziflam is comparable to the average aminopyralid use rate. This allowed for direct comparisons between herbicides on an active ingredient basis for aminopyralid, aminocyclopyrachlor, and indaziflam. The two species evaluated in the field experiment (Dalmatian toadflax and downy brome) were also included in the greenhouse experiment, along with seven additional species (diffuse knapweed, musk thistle, curly dock, common mullein, halogeton, marestail, and common teasel). Species were chosen because they are all commonly found on natural areas and open-spaces in Colorado, seed is readily available and grow well under greenhouse conditions, and they represent all the major growth habits (annual, biennial, and perennial).

For the greenhouse bioassay, seeds were collected in Larimer and Boulder County and stored at -4 C until planting. The 9 different species were planted separately at a constant depth of 0.5 cm in 13- by 9- by 6-cm plastic containers, filled with an Otero sandy clay loam field soil (Coarse-loamy, mixed (calcareous), mesic Aridic Ustorthents) with 3.9% OM and pH 7.7. Seeding densities were adjusted based on germination percentages from a preliminary greenhouse test, to reach a target density of 40 plants/pot. Plants were maintained in a greenhouse with a 25/20°C day/night temperature with natural light supplemented with high-intensity discharge lamps to give a 15-h photoperiod. Plants were sub-irrigated as needed and misted overhead daily to reduce soil crusting.

The greenhouse experiment was a completely randomized factorial design with seven herbicide rates and a non-treated with three replicates per treatment (rates (8) x replicates (3) x species (9) x herbicide (3) = 648). The experiment was conducted 10 December 2016 and repeated 16 February 2016. A preliminary greenhouse study was conducted for each herbicide and species to determine a range of doses that would best fit a logistic regression. It is not unusual for both preemergence and postemergence herbicides to provide control at lower than labeled rates in the greenhouse with ideal environmental conditions, so it was not surprising to us that herbicide doses for the regression analysis were much lower than recommended field use rates. Rates used in the dose-response are listed in Table 2. Herbicides were applied preemergence using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray nozzle (TeeJet Spraying Systems Co., Wheaton, IL) at 187 L·ha^{-1} at 172 kPa.

Plants were harvested at the soil surface approximately 4 to 5 WAT depending on the growth stage of each species. Weights were recorded after samples were dried for 5 d at 60 C. Percent dry weight reduction was calculated relative to the non-treated control plants for each treatment.

Data Analysis. For the herbicide efficacy field experiment, repeated measures analysis of variance (ANOVA) was used to determine the effects of herbicide treatments on long-term Dalmatian toadflax and downy brome control (2011-2014). Percent control data were first analyzed in SAS 9.3 using Proc MIXED, with year after treatment defined as the repeated measure (SAS Institute 2010). A Tukey-Kramer adjustment was performed and factors included in the model were treatment, timing, year, and all possible interactions. Dalmatian toadflax and downy brome control response variables were analyzed separately, and main effects and interactions were tested at the $\alpha = 0.05$ significance level. Before analysis, all response variables were arcsine square root-transformed to meet the assumption of normality. To determine herbicide impacts on residual Dalmatian toadflax and downy brome control, the significant treatment-by-year interaction was evaluated using the Proc GLIMMIX method and the LINES statement. This provided comparisons of least squares means across years ($P \leq 0.05$). Non-transformed means are presented in all figures.

Data from the greenhouse dose-response experiment were first analyzed using the PROC MIXED method in SAS 9.3 with treatment as a fixed effect and experiment and replicate as random effects (SAS Institute 2010). Based on a non-significant homogeneity of variance (ANOVA) and experiment-by-herbicide rate interaction, results from the repeated experiments were pooled. The treatment effect was significant, therefore, nonlinear regression in Graphpad Prism 7.00 (GraphPad Software, La Jolla California USA, www.graphpad.com) was used to describe the response of the nine weed species to aminocyclopyrachlor, aminopyralid, and indaziflam. The herbicide concentrations resulting in 50% reduction in plant biomass (GR_{50}) compared to the non-treated control were determined for each invasive weed species using four-parameter log-logistic regression. The equation used to regress herbicide concentration with percent reduction in plant dry biomass as compared to the non-treated control was:

$$Y = C + \left[\frac{(D - C)}{1+10^{(\mathrm{Log}GR_{50} - X) \cdot b}}\right] \quad [1]$$

where *C* and *D* represent the lower and upper limits of the dose-response curve, respectively, and *b* represents the slope of the best-fitting curve through the GR_{50} value. For curve fitting and GR_{50} estimation, the model was constrained to a maximum of 100 and minimum of 0. Mean separation of herbicide GR_{50} values were analyzed by Fisher's Protected LSD test at the 5% level of probability. The average recommended use rate for indaziflam ranges from 83 to 94% (73 and 102 g ai ha^{-1}) of the average recommended aminocyclopyrachlor (70 to 140 g ae ha^{-1}) and aminopyralid (53 to 123 g·ae·ha^{-1}); therefore, pre-emergence control was compared directly using GR_{50} - estimates.

Results and Discussion

Field Experiment.

Dalmatian Toadflax Control. At both application timings (June and August), the significant treatment-by-year interaction ($P<0.001$) was evaluated (Figure 1). All herbicide treatments except imazapic provided similar Dalmatian toadflax control 1, 2, and 3 YAT. The only treatments providing residual Dalmatian toadflax control above 80% 4 YAT were treatments including indaziflam (Figure 1). At the June and August application timings, aminocyclopyrachlor alone provided 50% and 55% Dalmatian toadflax control, while control with picloram was 68% and 64% 4 YAT, respectively. These same treatments tank-mixed with indaziflam resulted in 84 to 91% Dalmatian toadflax control 4 YAT. A previous study conducted by Sebastian et al. (2012) illustrated the importance of residual weed seedling control following the initial year of application. Dalmatian toadflax control with aminocyclopyrachlor was 90 to 97% 1 YAT; however, seedlings appeared in plots as early as 15 MAT, and there was limited control of those individuals (4 to 26%) 2 YAT. Without residual weed seedling control invasive weeds such as Dalmatian toadflax are able to re-establish via the soil seed bank.

Downy Brome Control. The treatment-by-year interaction ($P<0.001$) was more pronounced for downy brome than with Dalmatian toadflax, and there was no effect of application timing on herbicide efficacy ($P=0.830$). Compared to the non-treated plots, downy brome control with imazapic and indaziflam treatments were statistically similar at $P<0.05$ (84 to 99%) 1 YAT; however, residual downy brome control was greatly reduced for imazapic alone 2 YAT (61 to 64%). By 2014 (4 YAT), the downy brome population had recovered via the soil seed bank and imazapic control was less than 25% (Figure 1). Indaziflam treatments, however, provided significantly greater residual downy brome control 3 (91 to 96%) and 4 YAT (89 to 94%) compared to treatments not including indaziflam.

Response of Co-occurring Perennial Grasses. Visual estimates of perennial grass canopy cover (%) in 2014 revealed 46 ± 4% (mean ± SE) cover in non-treated plots. Averaged across the two application timings, picloram and aminocyclopyrachlor applied alone resulted in 65 ± 1% and 61 ± 3% perennial grass canopy cover 4 YAT, respectively. Imazapic and indaziflam treatments applied alone or in a tank-mix resulted in 55 ± 4% and 75 ± 2% perennial grass canopy cover, respectively. It is likely the indaziflam treatments providing increased residual control of downy brome and Dalmatian toadflax 4 YAT, resulted in increased perennial grass re-establishment.

Indaziflam has a low water solubility (3.6 mg L^{-1}) and high Log K_{ow} (2.8), meaning that all the herbicide is concentrated at the soil surface and is not diluted by leaching through the soil profile. Indaziflam has limited photodegradation, ~150 day soil half-life, and significantly greater relative potency than other pre-emergence herbicides (Sebastian et al. 2016a). These characteristics work in concert to provide long-term residual control (Sebastian et al. 2016b; Sebastian et al. 2014). These results support a new management concept, using indaziflam in combination with commonly recommended broadleaf herbicides (e.g. aminocyclopyrachlor and picloram), to significantly decrease weed seeds in the soil seed bank. This could greatly reduce weed seedling pressure in the years following initial treatments, providing the time necessary to facilitate the recovery of co-occurring species (Ball 2014; Harmoney et al. 2012). Reducing yearly applications to potentially every 4 years as these data suggest, would decrease herbicide costs, reduce the total amount of herbicide applied, minimize non-target impacts, and reduce the potential of shifting the native plant community with annual herbicide treatments (DiTomaso 2000).

Results from our field experiment established that indaziflam's control of germinating seeds provided residual Dalmatian toadflax and downy brome control 4 YAT. Based on these data, we hypothesized that indaziflam may also provide residual control of many other invasive weeds found in non-crop areas. This field experiment was used as a foundation for the subsequent greenhouse bioassay comparing the pre-emergence control of aminocyclopyrachlor, aminopyralid, and indaziflam.

Greenhouse Experiment. Dalmatian toadflax and downy brome control with aminocyclopyrachlor, aminopyralid, and indaziflam are presented in Figure 2. The GR_{50} estimates for downy brome showed that indaziflam was 125- and 99-times more active compared to aminocyclopyrachlor and aminopyralid, respectively ($P<0.0001$, Table 3). Similarly, indaziflam was 19- and 247-times more active on Dalmatian toadflax pre-emergence compared to aminocyclopyrachlor and aminopyralid, respectively ($P<0.0001$, Table 3). This is conformational evidence for the cause of extended weed control with indaziflam under field conditions for Dalmatian toadflax and downy brome compared to treatments without indaziflam (Figure 1).

The response of the seven remaining weed species to aminocyclopyrachlor, aminopyralid, and indaziflam are presented in Figure 2, and GR_{50} estimates are found in Table 3. Indaziflam was 106- ($P<0.0001$), 4- ($P<0.0001$), 9- ($P=0.0012$), and 5-times ($P<0.0001$) more active than aminopyralid on common mullein, diffuse knapweed, halogeton, and marestail, respectively; however, these two herbicides had similar activity on curly dock ($P=0.3421$) and musk thistle ($P=0.8674$) (Table 3). Aminopyralid was 2- and 9-times more active (lower GR_{50}) on common teasel compared to indaziflam and aminocyclopyrachlor, respectively ($P<0.0001$) (Table 3). Compared to aminocyclopyrachlor across all nine species, indaziflam was 3- to 145-times more active ($P<0.0001$, Table 3).

Averaging across all nine species, indaziflam was 29- and 52-times more active then aminocyclopyrachlor and aminopyralid, respectively. This indicates that indaziflam appears to provide increased seedling control of these invasive species compared to commonly recommended broadleaf herbicides. These data are consistent with the idea that the long-term residual control by indaziflam observed in the field (Figure 1) could be due to less dilution in the soil profile and increased relative potency (Christensen 1994; Ritz et al. 2006; Sebastian et al. 2016a) as compared to other broadleaf herbicides such as aminocyclopyrachlor and aminopyralid. Indaziflam could be tank-mixed with other herbicides commonly used for non-crop weed management (2,4-D, chlorsulfuron, clopyralid, dicamba, glyphosate, imazapyr, metsulfuron, triclopyr). This could extend weed control beyond the initial year of application, and provide multiple modes of action in a single application as a tool for resistance management (Lagator et al. 2013). Indaziflam has limited postemergence activity so, tank-mixing with herbicides evaluated in this study and those listed above would be needed to control established weeds. Indaziflam could then provide the residual activity necessary to control germinating seedlings that appear as early as the year after initial herbicide application (Sebastian et al. 2012).

Tank-mixing indaziflam with the suite of primarily broadleaf herbicides provides land managers with an opportunity to consider managing the soil seed bank of invasive weeds in non-crop areas. This could provide time for co-occurring species to respond with increased abundance, increasing the overall resistance and resilience of the dominant native plant community (Chambers et al. 2014). Unfortunately, sites that have been dominated by downy brome for many years may have a limited number of native perennial seeds in the soil seed bank, but unlike downy brome, some native species do establish a persistent seed bank (Thompson and Grime 1979). The establishment of a persistent or transient seed bank is highly species dependent. For example, one of the most important species in the Great Basin plant community, big sagebrush (*Artemisia tridentata*), does not form a persistent seed bank and relies on annual seed rain and appropriate environmental conditions to establish new individuals (Young and Evans 1989). Plants with persistent soil seed banks will be more likely to respond in an environment without downy brome competition; however, those species with transient seed banks could already be eliminated from a site (Humphrey and Schupp 2001).

Integrating indaziflam with other mechanical, cultural, and biological tools could also greatly increase the success of long-term management programs (DiTomaso 2000). Further tolerance studies should be conducted to determine any potential non-target impacts. For sites with limited co-occurring species, re-vegetation studies using various techniques including drill or broadcast seeding should be evaluated. In addition, the impact of indaziflam on long-term control of these key invasive weeds needs to be evaluated under field conditions and compared to treatments without indaziflam.

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Table 1. Herbicides and rates applied in evaluating the dose-response of eight annual, biennial, and perennial weed species.

Common name	Trade name	Rates applied[a] (g ai ha^{-1})	Application timing[b]	Manufacturer
Aminocyclopyrachlor	Method	57	June 2010	Bayer CropScience; Research Triangle Park, NC
Imazapic	Plateau	105	June 2010	BASF Specialty Products; Research Triangle Park, NC
Picloram	Tordon	227	June 2010	Dow AgroSciences, LLC; Indianapolis, IN
Aminocyclopyrachlor + Indaziflam	Method + Esplanade	57 + 58	June 2010	Bayer CropScience; Research Triangle Park, NC
Picloram + Indaziflam	Tordon + Esplanade	227 + 58	June 2010	Dow AgroSciences, LLC; Indianapolis, IN Bayer CropScience; Research Triangle Park, NC
Aminocyclopyrachlor	Method	57	August 2010	Bayer CropScience; Research Triangle Park, NC
Imazapic	Plateau	105	August 2010	BASF Specialty Products; Research Triangle Park, NC
Picloram	Tordon	227	August 2010	Dow AgroSciences, LLC; Indianapolis, IN
Aminocyclopyrachlor + Indaziflam	Method + Esplanade	57 + 58	August 2010	Bayer CropScience; Research Triangle Park, NC
Picloram + Indaziflam	Tordon + Esplanade	227 + 58	August 2010	Dow AgroSciences, LLC; Indianapolis, IN Bayer CropScience; Research Triangle Park, NC
Aminocyclopyrachlor + Imazapic	Method + Plateau	57 + 105	August 2010	Bayer CropScience; Research Triangle Park, NC BASF Specialty Products; Research Triangle Park, NC
Picloram + Imazapic	Tordon + Plateau	227 + 105	August 2010	Dow AgroSciences, LLC; Indianapolis, IN BASF Specialty Products; Research Triangle Park, NC

[a] All treatments included 1% v v^{-1} methylated seed oil.
[b] At the June 2010 and August 2010 application timings, Dalmatian toadflax was in the flowering and re-growth stages, respectively, while both application timings were preemergence for downy brome.

Table 2. Species, herbicides, and rates applied in greenhouse studies evaluating the dose-response of nine annual, biennial, and perennial weed species.

Common name	Scientific name	Rates applied (g ai ha^{-1})		
		Aminocyclopyrachlor	Aminopyralid	Indaziflam
Common mullein	*Verbascum thapsus*	0, 9, 18, 35, 70, 140, 210, 280	0, 1.8, 3.5, 7, 14, 28, 56, 112	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Common teasel	*Dipsacus fullonum*	0, 1, 2, 4, 9, 18, 35, 70	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Curly dock	*Rumex crispus*	0, 2, 4, 9, 18, 35, 70, 140	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Dalmatian toadflax	*Linaria dalmatica*	0, 1, 2, 4, 9, 18, 35, 70	0, 1.8, 3.5, 7, 14, 28, 56, 112	0, 0.05, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9
Diffuse knapweed	*Centaurea diffusa*	0, 4, 9, 18, 35, 70, 140, 280	0, 1.8, 3.5, 7, 14, 28, 56, 112	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Downy brome	*Bromus tectorum*	0, 9, 18, 35, 70, 140, 280, 560	0, 3.5, 7, 14, 28, 56, 112, 224	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Halogeton	*Halogeton glomeratus*	0, 2, 4, 9, 18, 35, 70, 140	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9
Marestail	*Conyza Canadensis*	0, 0.5, 1, 2, 4, 9, 18, 35	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9
Musk thistle	*Carduus nutans*	0, 1, 2, 4, 9, 18, 35, 70	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7

[a] All treatments were applied pre-emergence.

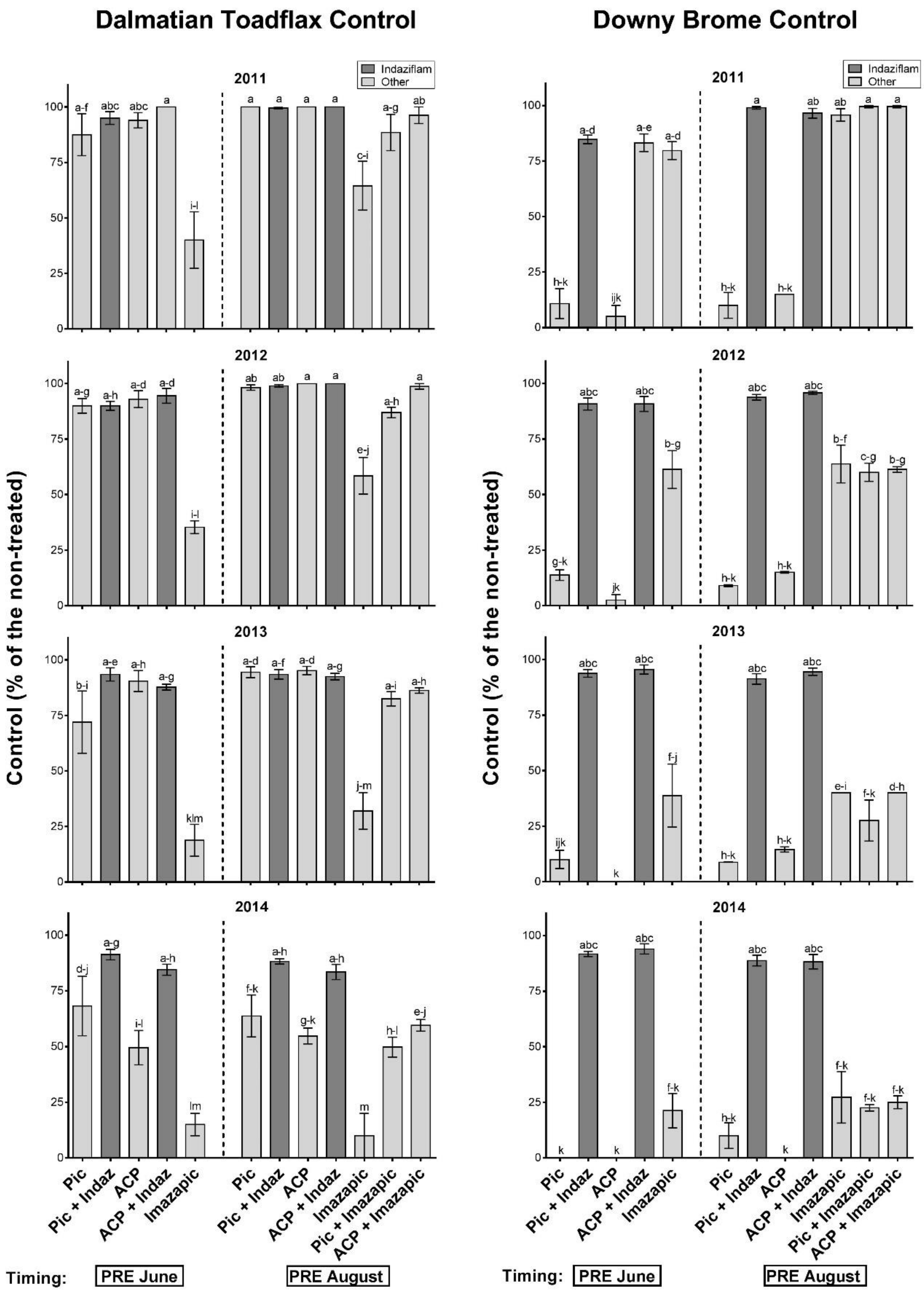
Table 3. Aminocyclopyrachlor, aminopyralid, and indaziflam rates resulting in 50 percent growth reduction of nine common invasive weeds found on non-cropland. Values were calculated using log-logistic regression[b]

Weed (common name)	GR_{50}[a] (g ai ha^{-1}) ± SE			GR_{50} ratio	
	Aminocyclopyrachlor ($g \cdot ai \cdot ha^{-1}$)	Aminopyralid ($g \cdot ai \cdot ha^{-1}$)	Indaziflam ($g \cdot ai \cdot ha^{-1}$)	Aminocyclopyrachlor/ Indaziflam	Aminopyralid/ Indaziflam
Common mullein	3.05 ± 0.02 b	7.45 ± 0.05 c	0.07 ± 0.01 a	45	106
Common teasel	6.89 ± 0.01 c	0.75 ± 0.02 a	1.33 ± 0.08 b	5	1
Curly dock	21.3 ± 0.03 b	1.25 ± 0.08 a	1.10 ± 0.07 a	19	1
Dalmatian toadflax	1.16 ± 0.02 b	14.8 ± 0.03 c	0.06 ± 0.05 a	19	247
Diffuse knapweed	6.20 ± 0.06 c	2.50 ± 0.03 b	0.58 ± 0.03 a	11	4
Downy brome	56.4 ± 11.08 b	38.5 ± 9.09 b	0.39 ± 0.02 a	145	99
Halogeton	1.04 ± 0.11 b	3.11 ± 0.02 c	0.36 ± 0.02 a	3	9
Marestail	2.09 ± 0.01 c	0.80 ± 0.07 b	0.17 ± 0.03 a	12	5
Musk thistle	1.25 ± 0.09 b	0.31 ± 0.07 a	0.33 ± 0.06 a	4	1

[a] Herbicide dose resulting in 50% dry biomass reduction.
[b] GR_{50} values within each weed (row) followed by the same lower case letter are not significantly different at the 5% level of probability.

Figure 1. Dalmatian toadflax and downy brome control represented as a percent of non-treated plots 1, 2, 3, and 4 YAT. Application timings were June and August 2010. At the June and August application timings, Dalmatian toadflax were in the flowering and re-growth stages, respectively; however, both timings were prior to downy brome emergence (PRE). Letters indicate differences among herbicide treatments across both timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: aminocyclopyrachlor (ACP, 57 $g \cdot ai \cdot ha^{-1}$), imazapic (105 $g \cdot ai \cdot ha^{-1}$), indaziflam (Indaz, 58 $g \cdot ai \cdot ha^{-1}$), picloram (Pic, 227 $g \cdot ai \cdot ha^{-1}$), non-treated.

Figure 2. Response of nine invasive species found in non-crop areas to aminocyclopyrachlor, aminopyralid, and indaziflam. Dose response curves were fit using four parameter log-logistic regression. Mean values of six replications are plotted. Vertical lines represent the herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) for each species and herbicide.



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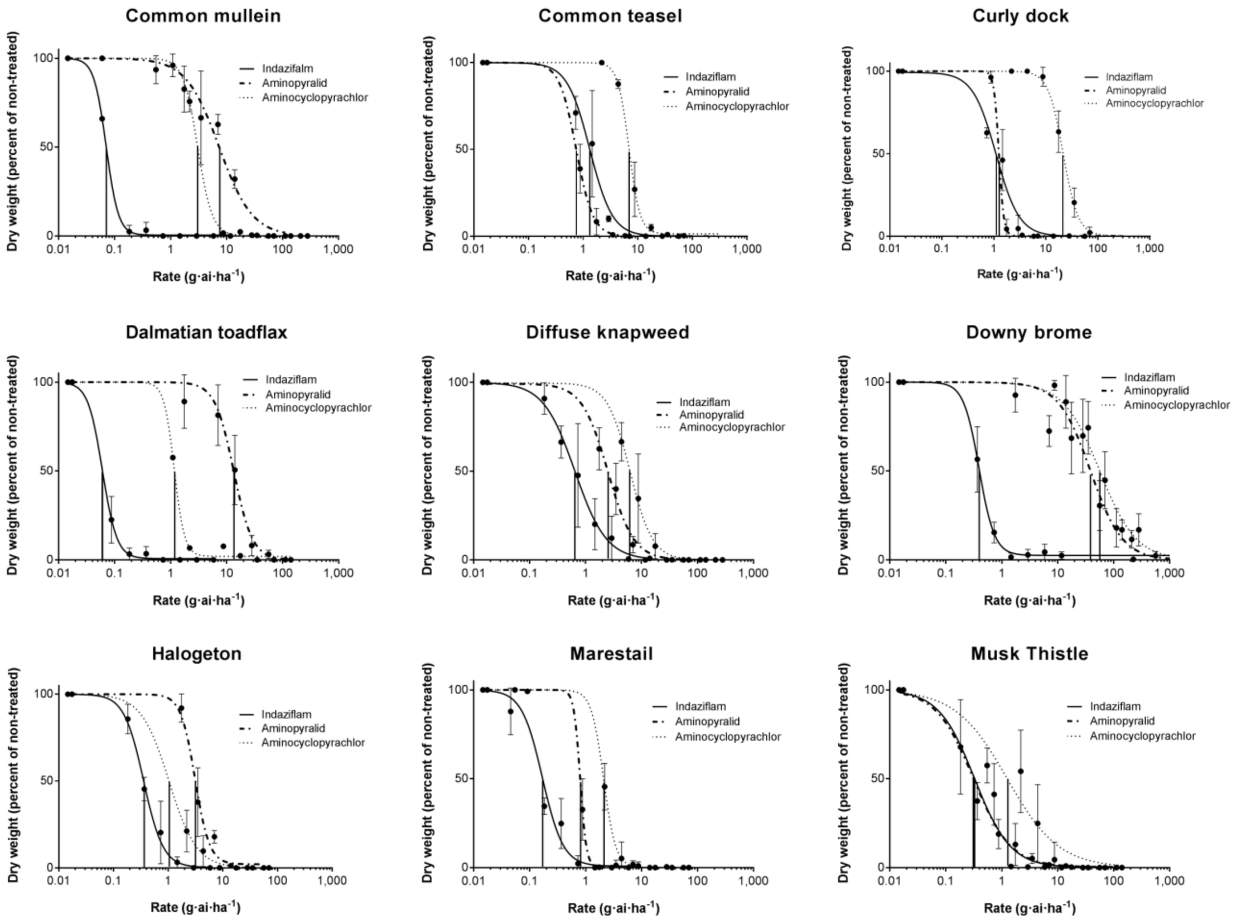
Common mullein
Common teasel
Curly dock
Dalmatian toadflax
Diffuse knapweed
Downy brome
Halogeton
Marestail
Musk Thistle
Indazifalm
Indaziflam
Aminopyralid
Aminocyclopyrachlor
Dry weight (percent of non-treated)
Rate (g·ai·ha⁻¹)
0.01
0.1
1
10
100
1,000
0
50
100

Figure 2.