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***Berteroaincana* Seed Biology**

Effect of Herbicide Management Practices Used by Invasive Plant Managers on *Berteroa incana* (Hoary Alyssum) Seed Biology and Control

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Hoary alyssum (*Berteroa incana* L.) is a non-native invasive forb that is noxious in California, Idaho, Michigan, Montana, Oregon, Washington, Wyoming, Alberta, British Columbia, and Saskatchewan. Managing *B. incana* is difficult because it has an extended flowering period, during which plants simultaneously flower and produce seeds. Consequently, poorly timed herbicide applications may kill *B. incana* flowers but not prevent viable seed production. We examined how different herbicide management practices used by invasive plant managers affected *B. incana* seed production and viability the year of application as well as its population density one year after application. Professional invasive plant managers sprayed *B. incana* with various herbicides as part of their current management practices at six sites in southwestern Montana in summer 2016. We collected *B. incana* plants four weeks post-

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application for seed biology analyses. Across the six sites, non-sprayed *B. incana* produced 5 to 1,855 seeds per plant and averaged 429 seeds per plant. Seed production was reduced by 64 to 99% with seven of the 11 herbicide applications. *Berteroa incana* seed viability in non-sprayed areas averaged 53% and ranged from 36 to 73% across the sites. Nine of the ten herbicide applications used by invasive plant managers reduced seed viability 49 to 100%. Few of the herbicide management practices reduced *B. incana*'s population density the following growing season, suggesting that managers should expect reoccurring infestations at least one year after application. Our results show that invasive plant managers can reduce *B. incana* viable seed production even when spraying plants that have flowered and formed seed pods. However, sites may need to be monitored for additional years to treat reoccurring infestations.

Key words: Application timing, noxious weed, rangeland, seed production, seed viability.

Hoary alyssum (*Berteroa incana* L.) is an annual, biennial, or short-lived perennial mustard that is native to Eurasia (Warwick and Francis 2006). It was first observed in North America in 1893 in Ontario, Canada. Since then, it has been found in 39 U.S. states (EDDMapS 2017) and all Canadian provinces except Newfoundland and Prince Edward Island (Warwick and Francis 2006), spreading primarily through contaminated lawn and forage seed. Currently, it is listed as a noxious weed in California, Idaho, Michigan, Montana, Oregon, Washington, Wyoming, Alberta, British Columbia, and Saskatchewan (Parkinson et al. 2017). *Berteroa incana* is prevalent in disturbed areas such as trails, roadsides, and gravelly river banks, as well as pastures. In rangeland settings, *B. incana* can be toxic to horses if it comprises 30% or more of their diets (Geor et al. 1992). It also has relatively few palatable carbohydrates and proteins compared to native vegetation, making it undesirable forage (Hastings and Kust 1970). *Berteroa incana* propagates exclusively by seeds, which are produced concurrently with its flowers throughout the frost-free season (Warwick and Francis 2006).

Research on *B. incana* management is limited. In greenhouse experiments, fertilization and mowing were not effective at reducing *B. incana* (Stopps 2012). Although somewhat effective in cropping systems, tilling is not a practical option in pastures (Warwick and Francis 2006), and there are no biocontrol agents for *B. incana* (Parkinson et al. 2017). Additionally, there is limited information on chemical control of *B. incana* (Dutt et al. 1983; Kust 1969; Richardson and Zandstra 2005), and no studies have been conducted in rangeland or grass pasture.

Chemical control recommendations suggest that herbicide applications prior to *B. incana* flowering may be most effective (Jacobs and Mangold 2008). However, *B. incana* is hard to identify at its vegetative stage and many invasive plant managers do not treat the weed until it

has flowered (J Ansley and S LaMont, personal communication). Unfortunately, when plants are flowering and easier to identify, they may also be producing seeds. Understanding the effect of herbicide management practices on seed biology of flowering *B. incana* is critical to improving control of this weed. The objective of this research was to examine how current herbicide management practices used by invasive plant managers affected seed production and viability of flowering *B. incana* the year of application as well as population density the following year. We hypothesized that herbicides applied by invasive plant managers would reduce seed production and viability, but we were uncertain as to what extent. We also hypothesized that herbicide applications would decrease *B. incana* density one-year post-application.

Materials and Methods

To determine the efficacy of *B. incana* herbicide management practices, we cooperated with invasive plant managers in southwestern Montana that sprayed *B. incana* between June and July 2016 at six sites located near Belgrade, Sheridan, or West Yellowstone, MT. Sites were located in right of ways, natural areas, or rangelands (Table 1). Managers selected herbicides that emulated their regular management practices and applied all herbicides according to label directions (Table 2). All herbicides were applied with a non-ionic surfactant. Managers recorded *B. incana* growth stage at time of herbicide application (i.e., vegetative or flowering). If flowering, they classified *B. incana* at an “early” or “late” flowering stage. If seed pods covered less than 50% of the average *B. incana* plant’s flowering stem, the infestation was described to be at an early flowering stage. If seed pods covered more than 50% of the average *B. incana* plant’s flowering stem, the infestation was described to be at a late flowering stage. In all cases, managers applied herbicides in the last two weeks of June or the first week of July when *B.*

incana was flowering (Table 1). *Berteroa incana* was at an early flowering stage at half of the sites and at a late flowering stage at the other half of the sites (Table 1). Managers also set aside a non-sprayed area at each site to serve as a control. The size of sprayed and non-sprayed areas varied within and among sites, but none were less than 28 m².

We collected *B. incana* plants at each site approximately four weeks after herbicide applications in 2016 to use for seed production and seed viability analyses. We followed a cluster sampling design by randomly locating five non-overlapping rectangular 0.1 to 12 m² sampling plots along a transect that we placed within each sprayed or non-sprayed area, leaving a 1 m buffer on each side of the sampling plots. Transect lengths differed between herbicide applications and sites, ranging from 7 to 176.5 m to adequately represent the area sprayed. Since we were characterizing the effect of herbicide applications on seeds, if a sampling plot was placed in an area with no *B. incana*, we randomly relocated it along the same transect. Each sampling plot was divided into four equally sized quadrats, 0.025 to 3 m², and we randomly selected two quadrats for sampling. If there were no *B. incana* plants within a quadrat, we randomly selected another quadrat within the same sampling plot. The variation in transect, sampling plot, and quadrat size was due to differences in the size of the sprayed and non-sprayed areas and the patchy distribution of *B. incana* at each site. All *B. incana* plants within the two selected quadrats were clipped at ground level, and each plant was placed in a separate bag. Due to the large number of plants collected within some quadrats, we randomly selected three plants from each quadrat to count all seeds and determine average seed production. If there were fewer than three plants growing in a quadrat, we used one or two plants to calculate seed production.

Seed viability was determined by conducting tetrazolium tests on seeds from all sites except TNC, where there were not enough seeds to perform tests. Seeds from the seed

production assessment were composited by site and herbicide application. Four replications of 110 seeds from each herbicide application were imbibed on blotter paper, wetted with deionized water, and placed within a sealed Petri dish for one to six hours to soften their seed coats. Then, we made a small nick along the edge of all seeds and placed them in a 1% solution of 2,3,5-triphenyltetrazolium chloride for 24 hours at ~21°C. We assessed the viability of 100 seeds from each replication following the protocol for the Brassicaceae Group 2 in the Tetrazolium Testing Handbook (Miller 2010).

We sampled population density of *B. incana* at all sites during mid-May to early-June 2017 to assess effectiveness of herbicide management practices the following year. We placed five 20 by 50 cm frames along the 2016 sampling transects used for seed production and seed viability analyses and measured the density of *B. incana* stems or rosettes within each frame. To avoid calculating density where *B. incana* had been cut in 2016 for our seed production and viability assessments, each sampling frame was placed adjacent to a 2016 sampling plot.

For seed production, seed viability, and population density, we analyzed each site separately because of the variation in herbicide management practices. Herbicide applications at each site were analyzed separately by comparing to the non-sprayed control. We compared the effects of herbicide applications on *B. incana* seed production within each site using Poisson generalized linear mixed models (GLMMs), with random effects for sampling plot and quadrat nested within sampling plot to account for our cluster sampling design (Dobson and Barnett 2008). We examined the effects of herbicide applications on *B. incana* seed viability within each site using contingency tables and Fisher's exact tests, which compared the overall proportion of nonviable seeds to viable seeds for each herbicide application (Agresti 2013). We assessed the effects of herbicide applications on *B. incana* population density within each site using Quasi-

poisson generalized linear models (GLMs), using log-area of our sampling frame (size= 0.1m²) as an offset to enable statements about plants on a m² basis (Dobson and Barnett 2008). We conducted these analyses using R Software (R Core Team 2016) and packages “plyer” and “PropCIs”.

Results and Discussion

Seed production. All herbicide management practices at Edward’s Peninsula, Stoddard’s Point, TNC, Airport, and Tower reduced seed production (Table 3). At sites where herbicides were spot-sprayed when *B. incana* was at an early flowering stage (Edward’s Peninsula, Stoddard’s Point, and TNC), the reduction in seed production ranged from 93 to 99% (Table 3). Specifically, at Edward’s Peninsula, metsulfuron + aminopyralid reduced seed production from 101 seeds per plant in the non-sprayed area to 7 seeds per plant, a 93% reduction. At Stoddard’s Point, the same herbicide reduced seed production from 512 seeds per plant in the non-sprayed area to 3 seeds per plant, a 99% reduction. At TNC, metsulfuron + picloram + 2,4-D reduced seed production from 5 seeds per plant in the non-sprayed area to <1 seed per plant, a 98% reduction.

At two sites where herbicides were broadcast sprayed at a late flowering stage (Airport and Tower), seed production decreased 64 to 98% (Table 3). At Airport, aminocyclopyrachlor + chlorsulfuron + 2,4-D reduced seed production from 1,855 seeds per plant in the non-sprayed area to 662 seeds per plant, a 64% reduction. The same herbicide application at Tower reduced seed production from 92 seeds per plant in the non-sprayed area to 2 seeds per plant, a 98% reduction. At Airport, aminopyralid + metsulfuron + 2,4-D reduced seed production from 1,855 seeds per plant in the non-sprayed area to 155 seeds per plant, a 92% reduction. The same

herbicide application at Tower reduced seed production from 92 seeds per plant in the non-sprayed area to 4 seeds per plant, a 98% reduction.

At another site where herbicides were broadcast sprayed at a late flowering stage (Brown Ranch), three of the four herbicide management practices did not affect seed production: 2,4-D + fluroxypyr + dicamba + metsulfuron, metsulfuron + 2,4-D, and imazapic applications averaged 26, 41, and 20 seeds per plant, respectively, compared to 11 seeds per plant in the non-sprayed area. Conversely, chlorsulfuron + 2,4-D increased *B. incana* seed production from 11 seeds per plant in the non-sprayed area to 195 seeds per plant, nearly a 20-fold increase. This increase in seed production was surprising, but it may have been due to grass injury from chlorsulfuron (Derr 2012). A loss in grass vigor could have reduced competition between grasses and *B. incana* with a resulting increase in *B. incana* seed production.

Across our six sites, non-sprayed *B. incana* produced 5 to 1,855 seeds per plant and averaged 429 seeds per plant (Table 3). Seed production also varied greatly among individual plants within sites. For example, at Airport one individual produced 319 seeds while another produced 5,632 seeds. Seed production of non-sprayed plants in our study was lower than that reported by Reichman (1988) and Stevens (1932), the only other studies we found that reported *B. incana* seed production. Stevens (1932) reported 2,530 seeds per plant whereas Reichman (1988) recorded a range of 116 to 2,640 seeds per plant. The differences between these studies and ours could be due to study location (Montana versus Minnesota and North Dakota) and the nature of the studies. Our study took place in naturally-occurring populations of *B. incana* whereas Reichman (1988) transplanted *B. incana* into disturbed soil with varying degrees of competition from other species of plants and Stevens (1932) selected a single plant growing with

little competition. Our study adds to the body of knowledge regarding *B. incana* seed biology, suggesting that seed production can be highly variable both within and across sites.

Seed viability. When averaged across five sites where we tested seed viability (TNC was not assessed because of too few seeds), 53% of non-sprayed seeds were viable and ranged from 36 to 73% (Table 3). All herbicide management practices decreased *B. incana* seed viability compared to non-sprayed seeds at their respective sites, except the chlorsulfuron + 2,4-D application at Brown Ranch where viability increased (Table 3). Where *B. incana* was spot-sprayed at an early flowering stage (Edward's Peninsula and Stoddard's Point), herbicide applications reduced average seed viability 93 to 99%. Specifically, metsulfuron + aminopyralid reduced average seed viability at Edward's Peninsula from 73% in the non-sprayed area to <1% (Table 3). At Stoddard's Point, the same herbicide application reduced average seed viability from 36% in the non-sprayed area to 2.5%.

Where *B. incana* was broadcast sprayed at a late flowering stage (Airport, Tower, and Brown Ranch), herbicide applications reduced average seed viability 49 to 100%, with the exception of the chlorsulfuron + 2,4-D application at Brown Ranch. At Airport, aminopyralid + metsulfuron + 2,4-D reduced average seed viability from 71% in the non-sprayed area to 8.3%. At Tower, the same herbicide application reduced average seed viability from 46% in the non-sprayed area to 4.2%. At Airport, aminocyclopyrachlor + chlorsulfuron + 2,4-D reduced average seed viability from 71% in the non-sprayed area to 10.5%. At Tower, the same herbicide application reduced average seed viability from 46% in the non-sprayed area to 0%. At Brown Ranch, 42% of seeds in the non-sprayed area were viable. Metsulfuron + 2,4-D reduced seed viability to 9.7%; 2,4-D + fluroxypyr + dicamba + metsulfuron reduced seed viability to 21%; and imazapic reduced seed viability to 5.3%. However, chlorsulfuron + 2,4-D increased seed

viability to 84% which was two times higher than seed viability in the non-sprayed area. This result parallels seed production, where chlorsulfuron + 2,4-D also led to an increase in the number of seeds produced.

The reduction in seed viability across all sites and all herbicide management practices except the chlorsulfuron + 2,4-D application at Brown Ranch was promising given that applications occurred when *B. incana* was flowering and seed pods were present. This is encouraging news for invasive plant managers who are unable to identify *B. incana* before it flowers or do not have the resources to spray multiple times per season due to the weed's prolonged flowering period. Our results support other studies that showed herbicide applications during flowering and early seed formation can impede production of viable and germinable seeds across a variety of weedy species (Asghari and Evans 1992; Mashhadi and Evans 1986; McDaniel et al. 2002; Nurse et al. 2015). Because *B. incana* propagates only by seed (Warwick and Francis 2006), controlling viable seed production is critical for effective management.

Population density one year after herbicide application. In our analysis of population density at four sites (Edward's Peninsula, Stoddard's Point, TNC, and Brown Ranch), the herbicide application of metsulfuron + aminopyralid at Edward's Peninsula and Stoddard's Point was the only herbicide management practice that resulted in a reduction in *B. incana* population density the following growing season (Table 3). At Edward's Peninsula, metsulfuron + aminopyralid reduced population density from 3,280 plants m⁻² in non-sprayed areas to 816 plants m⁻² in spring 2017. At Stoddard's Point, the same herbicide application reduced population density from 596 plants m⁻² in non-sprayed areas to 32 plants m⁻². The only other herbicide application that differed from non-sprayed areas occurred at Brown Ranch, where we observed a higher population density of *B. incana* in the area where chlorsulfuron + 2,4-D was applied compared to

the non-sprayed area (2,436 v. 180 plants m⁻²; Table 3). None of the other herbicide management practices at Brown Ranch and TNC affected *B. incana* population density. We were unable to statistically analyze population density data from two sites (Airport and Tower) due to zero-inflated data.

Our work found that few of the herbicide management practices that invasive plant managers were using for *B. incana* in southwestern Montana reduced population density the following growing season, suggesting that managers should expect reoccurring infestations at least one year after application. Although we did not differentiate *B. incana* density across life history stages when sampling in 2017, many of the observed individuals were seedlings or small rosettes that we suspect emerged from seeds in the soil seed bank after herbicide applications in 2016. *Berteroa incana* can build up large seed banks in the soil as demonstrated in a field study in Canada where up to 132 million *B. incana* seeds ha⁻¹ were found in the soil; those seeds remained viable for two years (Stopps 2012). Regardless of *B. incana*'s ability to accumulate an impressive seed bank (Stopps 2012), even a small number of viable seeds may be adequate to sustain an infestation. For example, a life history analysis of spotted knapweed (*Centaurea stoebe* L.), which like *B. incana* reproduces only via seed production, showed that as little as 1% and 20% of seeds entering the seed bank in the fall went on to emerge as seedlings that fall and the following spring, respectively (Jacobs and Sheley 1998). If invasive plant managers are reducing but not completely eliminating viable seed production, *B. incana* may persist.

Conclusions and management implications. This study was conducted to determine the impacts of current management practices used by invasive plant managers on *B. incana* seed biology and control. Consequently, we were unable to control some variables that could have affected our results. For example, Brown Ranch was grazed by cattle while other sites were not.

Differential grazing pressure could have affected plant competition and subsequent *B. incana* growth, as well as interacted with some herbicide applications to increase seed production and viability. Additionally, all managers that treated their *B. incana* infestations at early flowering stages spot-sprayed, whereas all managers that treated their infestations at late flowering stages broadcast sprayed. It is possible that the more consistent reductions in seed production observed when herbicides were applied at early flowering stages were influenced by application method. Finally, we were unable to make direct comparisons between herbicide active ingredients and application methods because our study was cooperator-driven, and cooperators used herbicides that fit their individual management needs.

Products containing the active ingredients metsulfuron or 2,4-D are currently recommended for use on *B. incana* (Parkinson et al. 2017; Prather et al. 2016). Of the 11 herbicide applications used by invasive plant managers in southwestern Montana, seven contained metsulfuron and eight contained 2,4-D. Since managers chose their herbicides, there were often tank mixes used that contained multiple active ingredients. Future studies could examine these recommended herbicides in isolation to determine which is more effective. Our work highlights that a reduction in viable seeds is possible with several different herbicide applications, even though applications occurred when *B. incana* was flowering and seed pods were present. However, few herbicide management practices resulted in a reduction in *B. incana* population density the following year, suggesting that invasive plant managers may need to monitor and re-treat *B. incana* populations for several years if there is an established *B. incana* seed bank. Managers may benefit from mapping infestations while *B. incana* is flowering so that infestations can be easily located in the vegetative stage and herbicide applications can be more effectively applied in subsequent years.

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333 Table 1. Site information for six sites where invasive plant managers sprayed *Berteroa incana* in southwestern Montana in 2016.

	Edward's	Stoddard's				
	Peninsula	Point	TNC	Airport	Tower	Brown Ranch
Location	West	West	West	Belgrade, MT	Belgrade, MT	Sheridan, MT
	Yellowstone,	Yellowstone,	Yellowstone,			
	MT	MT	MT			
Application date	7/5/2016	7/7/2016	6/24/2016	6/20/2016	6/20/2016	6/29/2016
<i>Berteroa incana</i>	Early	Early	Early	Late	Late	Late
flowering stage at application						
Method of application	Spot-spray	Spot-spray	Spot-spray	Broadcast spray	Broadcast spray	Broadcast spray
Site description	Natural area	Right-of-way	Natural area	Right-of-way	Right-of-way	Rangeland

334

335 Table 2. Herbicide management practices applied by invasive plant managers at six sites in southwestern Montana where their effects
 336 on *Berteroa incana* seed production and viability in 2016 and population density in 2017 were evaluated.

Site	Common name	Trade name ^a	Rates applied g ai ha ⁻¹	Carrier volume L ha ⁻¹
Edward's Peninsula	metsulfuron + aminopyralid	Escort ^{® b} + Milestone ^{® c}	42, 171	374
Stoddard's Point	metsulfuron + aminopyralid	Escort ^{® b} + Milestone ^{® c}	42, 171	374
TNC	metsulfuron + picloram + 2,4-D	Escort ^{® b} + Tordon ^{® c} + 2,4-D	42, 770, 1,987	281
Airport	aminopyralid + metsulfuron + 2,4-D	Opensight ^{® c} + 2,4-D	150, 1,057	561
	aminocyclopyrachlor + chlorsulfuron + 2,4-D	Perspective ^{® d} + 2,4-D	155, 1,057	561
Tower	aminopyralid + metsulfuron + 2,4-D	Opensight ^{® c} + 2,4-D	150, 1,057	561
	aminocyclopyrachlor + chlorsulfuron + 2,4-D	Perspective ^{® d} + 2,4-D	155, 1,057	561
Brown Ranch	2,4-D + fluroxpyr + dicamba + metsulfuron	E-2 ^{® e} + Escort ^{® b}	1,664, 42	234

metsulfuron + 2,4-D	Escort ^{® b} + 2,4-D	42, 1,057	234
imazapic	Plateau ^{® f}	165	234
chlorsulfuron + 2,4-D	Telar ^{® b} + 2,4-D	53, 1,057	234

337 ^a No manufacturer provided for 2,4-D because invasive plant managers used varying formulations

338 ^b DuPont, Wilmington, DE, www.dupont.com

339 ^c Dow AgroSciences, Indianapolis, IN, www.dowagro.com

340 ^d Bayer, Research Triangle Park, NC, www.bayercropscience.us

341 ^e Nufarm, Alsip, IL, www.nufarm.com

342 ^f BASF, Research Triangle Park, NC, www.bettervm.basf.us

343 Table 3. *Berteroa incana* seed production and seed viability in 2016, and population density the following year in 2017 at six sites in
 344 southwestern Montana. Stars next to individual herbicide applications indicate the level of significance when compared to the control
 345 within a single site (*= p-value <0.05; **= p-value <0.01; ***= p-value<0.001).

		2016	2017	
Site	Herbicide applications	Average seed production seeds per plant (SE)	Average seed viability % (SE)	Average population density plants m ⁻² (SE)
Edward's Peninsula	control	101 (72-142)	73 (72-74)	3,280 (2,767- 3,888)
	metsulfuron + aminopyralid	7 (5-11) ***	0.8 (0.3-1.2) ***	816 (587-1,135) **
Stoddard's Point	control	512 (221-1,186)	36 (33-38)	596 (483-735)
	metsulfuron + aminopyralid	3 (1-7) ***	2.5 (1.9-3.1) ***	32 (13-79) *
TNC ^a	control	5 (3-8)	NA	218 (140-338)
	metsulfuron + picloram + 2,4-D	0.09 (0.04-0.20) ***	NA	14 (2-80)
Airport ^b	control	1,855 (1,334-2,581)	71 (69-74)	NA

	aminopyralid + metsulfuron + 2,4-D	155 (111-215) ***	8.3 (7.3-9.2) ***	NA
	aminocyclopyrachlor + chlorsulfuron + 2,4-D	662 (476-922) *	10.5 (10-11) ***	NA
Tower ^b	control	92 (42-200)	46 (42-50)	NA
	aminopyralid + metsulfuron + 2,4-D	4 (2-9) *	4.2 (3.6-4.9) ***	NA
	aminocyclopyrachlor + chlorsulfuron + 2,4-D	2 (1-5) **	0 (0) ***	NA
Brown Ranch	control	11 (5-25)	42 (38-45)	180 (108-300)
	2,4-D + fluroxpyr + dicamba + metsulfuron	26 (12-61)	21 (18-24) ***	6 (0-100)
	metsulfuron + 2,4-D	41 (18-93)	9.7 (7.5-12) ***	10 (1-88)
	imazapic	20 (9-45)	5.3 (4.4-6.1) ***	12 (2-88)
	chlorsulfuron + 2,4-D	195 (86-443) *	84 (83-85) ***	2,436 (2,118- 2,802) ***

346 ^a Unable to conduct viability tests at TNC because too few seeds produced

347 ^b Unable to analyze population density at Airport and Tower with Quasi-poisson generalized linear models due to excess zeros with
348 certain herbicide applications.