

Project Title: Initiating a new yellow toadflax (*Linaria vulgaris*) biocontrol program with the stem galling weevil *Rhinusa pilosa*.

Principal Investigator:

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Cooperators and Other Participating Institutions:

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Amount requested (FY13): \$27,015.

Matching funds: PI salary: \$7,168 (exceeds 25%).

Project leveraging: This project will leverage an anticipated 20% - 30% of additional sponsorship.

Project Goals and Supporting Objectives:

1. Import *Rhinusa pilosa* from established collaborators at CABI-Switzerland and/or Agriculture and Agri-Food Canada (Lethbridge Alberta) into containment facility at Montana State University (Bozeman, Montana).
 - a. submit updated 526 form to USDA APHIS-PPQ; secure appropriate red-and-white shipping labels
 - b. make transportation arrangements, including bonded agent to hand-carry agents through U.S. Customs at the port of entry
2. Maintain continuous greenhouse culture of high quality host plants.
3. Set-up and maintain research/cultivation resources (plants) and space in MSU quarantine facility.
4. Assess the health and confirm the identity of adult emerging weevils.
5. Build-up quarantine population of *Rhinusa pilosa*.
6. Identify and characterize potential field release sites:
 - a. pre-release biotic and abiotic site attributes such as plant community composition, target weed density, etc. (ideally, for at least one field season before and two or more after releases are made)
 - b. maintain communication with local resource managers/land owners

7. Release the stem galling weevil, *Rhinusa pilosa* (Gyllenhal) as a classical biological control agent for yellow toadflax (*Linaria vulgaris* Mill.).
8. Monitor local yellow toadflax populations to determine if and how the agent is affecting target weed
 - a. vigor/competitiveness
 - b. population density
 - c. population age/stage composition
 - d. spread
9. Assess ecological interactions of *Rhinusa pilosa* with onsite fauna and flora.

Project Justification/Urgency:

Yellow or common toadflax, *Linaria vulgaris* Mill. (Plantaginaceae), is an erect perennial forb 15-80 cm tall with linear pale green to blue-green leaves. Yellow toadflax's flowers are zygomorphic (petals of two or more shapes, sizes and colors) and snapdragon-like, 25-33 mm long and pale-yellow to yellow, with an orange bearded throat and yellow spur. Introduced as an ornamental and medicinal plant to northeastern North America in the 1600s, yellow toadflax's showy blossoms continued until recently to encourage anthropogenic spread through commercial nurseries and seed catalogs (Mack 2003; Lajeunesse 1999; Pennell 1935). Unintentional dissemination, e.g., as a crop seed contaminant, in baled hay, along railway corridors, and in ships' ballasts, has further contributed to the comprehensive invasion of North America by this weed (USDA, NRCS 2012; Mitich 1993; Saner et al. 1995).

Invasive exotic toadflaxes tolerate a broad range of environmental conditions and soil types (Saner et al. 1995; Vujnovic and Wein 1997; De Clerck-Floate and Harris 2002; McClay and De Clerck-Floate 2002). Yellow toadflax has successfully established in North American rangelands, grasslands, scrub and shrublands, and to varying degrees in agricultural areas (croplands, fields, and pastures), riparian zones, along roads, in dunes, and on disturbed and cultivated land (Alex 1962; Coupland et al. 1963; Robocker 1974; Darwent et al. 1975; Morishita 1991; Jacobs and Sheley 2003; Pauchard et al. 2003; D'Antonio et al. 2004; Sutton et al. 2007; Dodge and Fulé 2008). Commonly associated with summer-moist, coarse soils, yellow toadflax has successfully spread into high mountain valleys and parks, including forested rangelands, and is tolerant of sub-arctic conditions (Saner et al. 1995). Once established, invasive toadflaxes have the potential, like many weeds, to spread into adjacent non-disturbed areas (re: yellow toadflax invasions of undisturbed alpine areas of White River National Forest - Hal Pearce and Sarah Ward, personal observation; Zilke and Coupland 1954).

Linaria vulgaris has dual modes of reproduction. New infestations are typically attributed to sexual reproduction (i.e., originating from dispersed seeds), while patch expansion is facilitated primarily through vegetative reproduction (Nadeau et al. 1992). The densest infestations of yellow toadflax form from root buds arising on rhizomes that radiate laterally (underground), up to 0.9 m from a substantial (~1.2 m) taproot. Plants produce 75-694 shoots (typically 90-100) in one growing season and 200-250 by the second year. Patches with radii of 1.01-2.01 m have developed from first-year seedlings in one growing season. The diameter of established patches can increase annually by 1.22 m (Beck 2001; CDFR 2008; Zouhar 2003). Yellow toadflax flowers from mid-May to September; the annual average of 1,500-30,000 seeds/plant mature between July and October, depending on location and elevation (Saner et al. 1995). Seed dispersal from dried capsules can continue through the winter. Seed dispersal mechanisms include wind, water, animals (including people), vehicles, and movement of

substrates. Seeds can float on water for extended periods. Shoot phenology within a patch can simultaneously range from vegetative to flowering to seed set, thereby significantly increasing management complexity.

Because invasive toadflaxes compete with surrounding vegetation on native grasslands (Lajeunesse et al. 1993), they have the potential to directly or indirectly impact rare plant species in these habitats (Maron and Marler 2008). Unless appropriate preventative and control measures for noxious weeds are undertaken during the urban interface and forest restoration efforts, noxious weeds could cause serious impacts to the biodiversity of these ecosystems and to threatened, endangered, and sensitive species (Phillips and Crisp 2001). Toadflaxes can replace valuable forbs and grasses, reducing the efficiency of grazing by wildlife, particularly on winter ranges (Grubb et al. 2002). When other forage plants are scarce, wildlife, birds and rodents will feed on toadflax, but it is not known to be heavily utilized by any native animal species (Robocker 1970). In the Greater Yellowstone Ecosystem, *L. vulgaris* is considered to be among the most invasive exotic plant species, and a significant threat to native biodiversity in open, human or naturally disturbed environments in protected areas of the Rocky Mountains (Pauchard et al. 2003). Toadflax is fire-adapted and if it is qualitatively unique to the invaded ecosystem, it has the potential to alter the fire regime (Zouhar 2003).

Extensive infestations of rangeland with yellow toadflax are expensive and complicated to manage. For cattle producers depending on public land grazing allotments, yellow toadflax infestations can significantly diminish forage quantity and quality. Grazing animals on rangeland where yellow toadflax and other invasive weeds are treated with herbicides may interfere or delay requirements for certified organic production, reducing profits through lost premiums for organically produced meat, while increasing production inputs. Application of costly herbicides has no guarantee of efficacy - chemical control of yellow toadflax is often insufficient, difficult (e.g., rough natural terrains) or too damaging to native vegetation (Krick 2011).

Both toadflax infestation and its chemical control have the potential to negatively impact the aesthetic and ecological values/services of vegetation communities. Reducing infestations and therefore the need for chemical treatments of yellow toadflax can increase the aesthetic values of such locales, attract greater wildlife diversity and thereby directly influence tourism. Due to health concerns and degradation of aesthetic quality, tourists and recreationists are likely to avoid areas where the appearance of vegetation is obviously altered by herbicide treatments. Similarly, tourists and other outdoor enthusiasts would also be more likely to visit areas perceived as 'natural' and 'pristine' vs. 'invaded' by visually distinct monocultures of yellow toadflax.

Reducing or eliminating herbicide applications would significantly benefit vegetation communities in yellow toadflax invaded natural areas. Undesirable non-target impacts of herbicide applications may jeopardize plant community biodiversity beyond a legally or aesthetically acceptable level. Reducing the unintended non-target effects of herbicide application on non-weed vegetation directly increases habitat diversity and quality for pollinating insects. On a local scale, if herbicide applications eliminate large tracts of yellow toadflax for the current growing season, crucial resources for pollinators supplied by yellow toadflax are also abruptly lost. Biological control would allow for the gradual conversion of yellow toadflax dominated areas to increased diversity and richness of other forb species capable of supplying the same necessary resources to pollinators. In the worst case scenario, herbicide applications can cause a conversion of mixed forb and grass vegetation communities to communities dominated by annual invasive grasses, thereby significantly altering local trophic interactions.

Approach:

Source of *Rhinusa pilosa*: Insects will be sourced from a field plot and greenhouse-reared colony in northern Serbia at the Institute for Plant Protection and Environment (Zemun, Belgrade). Shipped *Rhinusa pilosa* will be received and reared in quarantine facilities in Canada (AAFC, Lethbridge Research Centre, Insect Microbial Containment Facility, Lethbridge, Alberta) and in the U.S.A. (Montana State University Quarantine, Bozeman, Montana). Agents thus sourced from the Serbian colony will be of known identity and will be expected to be free of natural enemies. However as an extra precaution, the shipped weevils will be reared a generation on its known host plant species (*Linaria vulgaris*) in regulator-inspected and certified quarantine facilities in Canada (AAFC, Lethbridge Research Centre, Insect Microbial Containment Facility, Lethbridge, Alberta) and the U.S.A. (Montana State University Quarantine, Bozeman, Montana). During rearing in respective quarantine facilities, insects will be securely caged and inspected frequently for emergence of parasitoids, inquilines, or other unwanted arthropods, and for disease symptoms that may indicate the presence of entomopathogens. Unwanted arthropods or infected *R. pilosa* will be immediately removed and destroyed through submersion in ethyl alcohol or autoclaving.

Intended sites for initial release, timing of release, and methods to be used: Initial U.S. releases are planned for an outdoor nursery (caged plots of yellow toadflax) within a large chain-link fenced garden study area at the USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Laboratory on the campus of Montana State University in Bozeman, MT, and in secure sites on Forest Service and privately-owned lands in west central Montana, also where establishment can be closely monitored.

Releases will be made of spring-emerged adults, timed to coincide with the growth phenology of *L. vulgaris* shoots most optimal for agent reproduction at the chosen sites. *Rhinusa pilosa* females prefer actively-growing shoots that have emerged approximately 10 cm above ground in the early spring (April-May). Both female and male weevils will be released at the same time in either caged or open situations. The number of adults released per site will depend on the availability of lab-reared weevils, but if possible, initial releases will be about 100 adults/site. Experiments during the initial release stage are being planned to determine optimum release strategies for increasing establishment success.

Post-release monitoring: The anticipated time of initial releases will be early spring (April-May) and will be dependent on the phenological development of *L. vulgaris* at chosen release sites. Young, actively-growing shoots should be approximately 10 cm above ground for optimum acceptance by female weevils and gall induction. Initial monitoring will assess establishment, local spread and impact of *Rhinusa pilosa*.

a) **Establishment:** Monitoring will take place yearly in mid-late summer (June-August) for galls and in spring (April-May) for emerged adults, for up to 3-4 years after initial releases to ascertain establishment. To generally determine presence of the insect, release patches of toadflax will be carefully inspected during a slow walk-through (e.g., minimum of 30 minutes to 1 hour depending on patch size) by two or more people familiar with the insect and its galls. Gall and/or adult density will be determined by randomly selecting 30-100 *Linaria vulgaris* stems within a known area (e.g., within a caged plot) or systematically along transects laid through a release patch (e.g., every 1 m), and the number of galls or encountered adults recorded (see galls in photos below). Other data, such as position of the galls within shoots, or gall size may be recorded at this time. A subsample of galls will be collected and dissected under a microscope in

the laboratory to determine the stage of weevil development, the incidence of any mortality and gall growth abnormalities, and/or if any parasitoids or other arthropod gall inhabitants are present. A sample of late summer galls also may be collected for laboratory rearing of inhabitants to evaluate level of parasitism or attack by inquiline if required.

b) **Local spread:** Once establishment has been confirmed, monitoring of *R. pilosa* spread at the patch level will commence. The point or restricted area of initial release within a yellow toadflax patch will be designated as the center of two crossing, perpendicular transects, each 30-100 m long depending on the size of the patch. At regular intervals along each transect, permanent quadrats (e.g. 50 X 20 cm) will be established and monitored yearly for presence and number of galls or adult weevils during the seasonal periods previously mentioned. Location of each quadrat relative to the center and transect identifier also will be recorded for determining the rate and direction of *R. pilosa* spread within the patch.

c) **Impact:** The monitoring protocols have yet to be developed, but the same permanent quadrats established to monitor spread could be used to determine the impact of *R. pilosa* on host plants and any responses of the local plant community post release. In addition to conducting a yearly gall count within each quadrat, stems could be randomly selected and measured for height or incidence of flowering, and percent cover of *L. vulgaris*, other invasive plants, and native plant species recorded. Although non-target attack is unanticipated, any plants within the Plantaginaceae also will be inspected for galls during the impact assessments. Additional field experiments may later be designed

Required Documentation:

A petition for field release of the yellow toadflax stem galling weevil *Rhinusa pilosa* was submitted by the PI of this proposal and co-authors for review by the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in March 2012. The Proposed Plant List for Host Specificity Testing of Candidate Biological Control Agents of Invasive Non-native *Linaria* (Plantaginaceae) was submitted by the PI of this proposal and co-authors for review by the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in July 2011.

Expected Products and Outcomes: Significant progress has been made recently in developing a successful program for yellow toadflax biocontrol. In 2009 PI (Sing) and collaborators discovered established populations of *Mecinus janthinus* on yellow toadflax in western Montana. These have since been widely redistributed and are beginning to build up to collectable numbers in Idaho and Colorado. The successful addition of *Rhinusa pilosa* to the suite of agents attacking yellow toadflax would increase the odds of sustained biological control of this weed, which persists and invades such a wide range of habitats under broad environmental conditions.



Sharlene E. Sing
Research Entomologist

Education

- B.A. English, Dalhousie University, 1984
- M.Sc. Natural Resource Sciences, McGill University, 1997
- Ph.D., Land Resources and Environmental Sciences, Montana State University, 2002

Professional experience

- 09/2008 – present: Research Entomologist, USFS RMRS, Bozeman, MT
- 04/2006 – 09/2008: Assistant Research Professor, Montana State University, Bozeman, MT
- 01/2002 – 10/2005: Research Entomologist (Post-doc), USFS RMRS, Bozeman, MT

Professional service

- RMRS Civil Rights Committee – representative for Bozeman lab
- supervisor, minority interns – summer weed biocontrol experiential internships at RMRS/MSU summer 2012
- TAG (Technical Advisory Group – Biological Control Agents of Weeds) – member representing national interests of US Forest Service on policy and reform, review of petitions and test lists for candidate weed biocontrol agents/programs
- editing duties: Proceedings of XIII ISBCW; organizing editor, petition for field release of the candidate yellow toadflax biocontrol agent *Rhinusa pilosa* and test plant list for potential *Linaria* biocontrol agents
- peer review for journals, research proposals, etc.
- adjunct faculty member and graduate student committee member: Montana State University and Colorado State University
- stakeholder group weed biocontrol advisor/collaborator: GYCC (Greater Yellowstone Coordinating Committee) – weeds subcommittee; Elkhorns Cooperative Wildlife Management Area- weeds working group; Montana Weed Control Association – weed biocontrol committee

Selected publications

Yun Wu, Y., T. Johnson, S.E. Sing, S. Raghu, G. Wheeler, P. Pratt, K. Warner, T. Center, J. Goolsby, and R. Reardon (eds). 2012. Proceedings of the XIII International Symposium on Biological Control of Weeds. Waikoloa, Hawaii, United States, September 11-16, 2011. U.S. Forest Service Morgantown, WV. (*in press*)

Runyon, J.B., J.L. Butler, M.M. Friggens, S.E. Meyer, and S.E. Sing. 2012. Invasive species and climate change (Chapter 7). In: Finch, Deborah M., ed. Climate change in grasslands, shrublands, and deserts of the interior American West: a review and needs assessment. Gen. Tech. Rep. RMRS-GTR-285. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 97-115.

De Clerck-Floate, R., A. Gassmann, S.E. Sing, I. Toševski. 2012. Petition for Field Release of *Rhinusa pilosa* (Gyllenhal, 1838) (Coleoptera: Curculionidae) Against Yellow Toadflax (*Linaria vulgaris* Mill.: Plantaginaceae) in Canada and the U.S.A. 92 p. (submitted to USDA APHIS PPQ for review March 2012).

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- Sing, S.E. and R.K.D. Peterson. 2011. Assessing risks for established invasive weeds: Dalmatian (*Linaria dalmatica*) and yellow (*L. vulgaris*) toadflax in North America. *International Journal of Environmental Research and Public Health*. 8: 2828-2853.
- Schat, M., S.E. Sing, R.K.D. Peterson, F.D. Menalled and D.K. Weaver. 2011. Growth inhibition of Dalmatian toadflax, *Linaria dalmatica* (L.) Miller, in response to herbivory by the biological control agent *Mecinus janthinus* Germar. *Journal of Entomological Science*. 46(3): 232-246.
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- Schleier, J.J., S.E. Sing and R.K.D. Peterson. 2008. Regional ecological risk assessment for the introduction of *Gambusia affinis* (western mosquitofish) into Montana watersheds. *Biological Invasions* 10: 1277-1287.
- Sing, S.E. and R.T. Arbogast. 2008. Predator density and time of addition influence the suppression of bruchids infesting stored grain legumes by the predatory bug *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae). *Environmental Entomology* 31: 131-142.
- Sing, S.E. and R.T. Arbogast. 2008. Predatory response of *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) to bruchid pests of stored food legumes. *Entomologia Experimentalis et Applicata* 126: 107-114.
- Jacobs, J. and S.Sing. 2008. Ecology and management of diffuse knapweed (*Centaurea diffusa* Lam.). USDA NRCS Invasive Species Technical Note No. MT-20. 12 pp.
- Schat, M., S.E. Sing and R.K.D. Peterson. 2007. External rostrum characters for differentiation of sexes in the biological control agent *Mecinus janthinus* (Coleoptera: Curculionidae). *The Canadian Entomologist* 139: 354-357.
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- Jacobs, J.S. and S.E. Sing. 2007. Ecology and management of saltcedar (*Tamarix ramosissima*, *T. chinensis* and *Tamarix ramosissima* x *T. chinensis* hybrids). USDA NRCS Invasive Species Technical Note No. MT-13. 12 pp.
- Jacobs, J.S. and S.E. Sing. 2007. Ecology and management of hound's tongue (*Cynoglossum officinale* L.). USDA NRCS Invasive Species Technical Note No. MT-8. 7 pp.
- Jacobs, J.S. and S.E. Sing. 2006. Ecology and management of yellow toadflax [*Linaria vulgaris* (L.) Mill.]. USDA NRCS Invasive Species Technical Note No. MT-6. 9 pp.
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David Weaver
Associate Professor

Education

- Ph.D., Entomology, McGill University, Montreal, Québec, Canada, 1990
- B.S., Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada, 1984

Professional Experience

- Associate Professor of Entomology, Montana State University, 2002 - present
- Research Associate Professor, Department of Entomology, Montana State University, 1999 – 2002
- Research Assistant Professor, Department of Entomology, Montana State University, 1997 - 1999
- Research Entomologist (Post-doc), USDA-ARS, CMAVE, Gainesville, FL, 1994 - 1997
- Research Entomologist (Post-doc), USDA-ARS, SPIRDL, Savannah, GA, 1992 - 1994
- Postdoctoral Research Associate, Department of Entomology, Montana State University, 1990 – 1992

Select Publications

- D. Piesik, D. Pańka, M. Jeske, A. Wenda-Piesik, K. J. Delaney, and D. K. Weaver. In Press. Volatile induction of infected (*Fusarium* spp.) and neighboring uninfected barley and wheat may influence attraction/repellence of a cereal herbivore. *Journal of Applied Entomology*. Early view: <http://dx.doi.org/10.1111/j.1439-0418.2012.01742.x>
- Buteler, M. and D. K. Weaver. 2012. Host selection by the wheat stem sawfly in winter wheat and the potential role of semiochemicals mediating oviposition preference. *Entomologia Experimentalis et Applicata*. 143: 138-147
- D. Piesik, D. Pańka, K. J. Delaney, A. Skoczek, R. Lamparski, and D.K. Weaver. 2011. Cereal crop volatile organic compound induction after mechanical injury, beetle herbivory (*Oulema* spp.), or fungal infection (*Fusarium* spp.). *Journal of Plant Physiology*. 168: 878-886.
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Budget Request FY13	
Technical support - salary: (GS-5 equivalent \$28,000 x 0.5 FTE)	\$14,000
Technical support - benefits: (35% x \$14,000)	\$4,900
Student field assistant 12h X \$11.55/h [\$10.50 + 10% benefits]= \$139 10 field days @ \$139	\$1,390
Per diem – technical support and student field assistant \$139 x 2 x 5 field days	\$1,390
International shipping (including bonded broker fee)	\$1,200
Greenhouse supplies: potting soil, plant pots, fertilizer	\$500
Field supplies: Bioquip outdoor cage 6 x 6 x 6', 32 X 32 mesh Lumite (\$333.70 x 4)	\$1,335
Field supplies: Trimble Juno hand-held GPS	\$1,500
Field vehicle mileage (2000 miles x \$0.40/mi)	\$800
TOTAL	\$27,015

TIMETABLE

April - May 2013:	Receive field collected <i>R. pilosa</i> at quarantine facilities
May - July 2013:	Begin mass rearing of <i>R. pilosa</i> on <i>L. vulgaris</i> (quarantine)
July - August 2013:	Begin field cage releases; continue mass-rearing
September - October 2013	Assessment of in-season feeding and colonization; continue mass-rearing
September 2013 - April 2014:	Analyze data and prepare reports; continue mass rearing
March - April 2014:	Assess overwintering success at field sites; continue mass rearing for 2014 releases
May 2014 – beyond	New field cage releases and assessment of overwintering success; publications (post-funding efforts)

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