Project Title:
Assessing the potential for *Mecinus janthinus* (*ex Linaria vulgaris)* to improve the efficacy and increase the implementation of yellow toadflax biological control.

Principal Investigators:

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Cooperators and Other Participating Institutions:

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Amount Requested: FY10: $24,871; FY11: $21,290; FY12: $21,290; total: $67,451.

Project Leveraging: The proposed project would benefit significantly from labor and funds invested in field activities completed during last year's preliminary field season: weevils were collected and released on new insectary sites in MT (5) and CO (1); monitoring transects were set up and read at all new MT release sites; 8 monitoring transects were established and read in the area where *M. janthinus* was field-collected from yellow toadflax; and several scouting trips were made to determine the initial extent of weevil establishment. This project would also build on the results of USFS FHP STDP R1-2006-01: Developing guidelines to assist land managers with implementation and assessment of biological control of Dalmatian and yellow toadflax using *Mecinus janthinus*; STDP R4-2009-02: Utilizing *Mecinus janthinus* insectaries on co-mingled stands of Dalmatian and yellow toadflax and their hybrids to improve yellow toadflax biocontrol; and STDP R4-2010-01: Post-wildfire inundative releases of *Aphthona* spp. flea beetles and *Mecinus janthinus* as biological "herbicides" on leafy spurge and Dalmatian toadflax. The CO Department of Agriculture Palisades Beneficial Insectary, a regionally recognized center for biocontrol implementation in the West, currently has a dedicated 0.5 FTE technician supporting *Linaria* biocontrol projects; this is expected to continue for at least another 5 years, given the critical nature of the toadflax invasion in CO.

Project Goals and Supporting Objectives:

- Project goals:
  - deliver a safe, sustainable, effective and predictable biocontrol tool for operational integrated management of yellow toadflax, an invasive weed that is currently expanding unchecked due to a lack of effective biological and chemical management tools;

- facilitate FHP program delivery by developing and disseminating effective and accessible mass-rearing protocols for *Mecinus janthinus* (*ex Linaria vulgaris)*
- Supporting objectives:
  - supply agents to establish insectaries in MT and CO locations where yellow toadflax is a pervasive management problem;
  - assess biocontrol releases to better understand environmental limitations on, and ways to optimize, successful establishment of this agent;
  - using standardized protocols monitor biocontrol releases to determine the efficacy of *M. janthinus* (*ex Linaria vulgaris)* under a range of MT and CO field conditions;
  - provide regional tech transfer guidance to increase successful implementation of yellow toadflax biocontrol.

<u>Project Justification/Urgency</u>:

Yellow toadflax, *Linaria vulgaris* Miller (Plantaginaceae) (USDA, NRCS 2010), a short-lived perennial herb of Eurasian origin (Saner et al. 1995; Zilke and Coupland 1954) imported for ornamental, textile dying and medicinal purposes by early American settlers. Naturalized in eastern U.S. colonies by 1671 (Josselyn 1672; Mack 2003), yellow toadflax was considered a significant and fairly common agricultural weed as early as 1849 (Darlington 1849; Leighton 1970; Mack 2003). In 1866 Illinois' first State Entomologist, Benjamin Dann Walsh, suggested importing herbivorous insects for domestic management of exotic weeds (Sheppard 2004; Klatt 2009). Influenced by the pervasive and persistent nature of infestations in the eastern U.S. at the time, Walsh proposed yellow toadflax as the first U.S. classical weed biological control management target (Klatt 2009). Yellow toadflax continues to have an extensive and expanding distribution, occurring in all continental U.S. states, and is currently classified as noxious in eight states and five Canadian provinces (Harris and McClay 2005; USDA, NRCS 2010). Yellow toadflax invasion of minimally disturbed native plant communities at high elevations in the northern and central Rocky Mountains has recently been reported; studies of yellow toadflax in Colorado wilderness areas have indicated that invasion more commonly occurs at sites with greater existing species richness, possibly because such sites have higher levels of soil fertility (Sutton et al. 2007).

A classical biological control program against invasive toadflax (*L. vulgaris* and *L. dalmatica*) was initiated in the 1960s (Wilson et al. 2005). Although several agents have since successfully established in North America, none to date have had significant impact on *L. vulgaris*. The most effective agent known against Dalmatian toadflax is *Mecinus janthinus* Germar, which has become widely established and has contributed to the decline of this weed in the U.S. and Canada (Sing et al. 2008). Repeated attempts to establish *M. janthinus* on North American yellow toadflax have been unsuccessful to the point that USDA-APHIS-PPQ currently recommends not releasing this agent on yellow toadflax (Larry Skillestad, *pers. comm.*).

In spring 2009 access was granted for the first time in seven years to yellow toadflax biocontrol releases sites made on private land in Powell County, MT. Releases of *M. janthinus* (*ex L. dalmatica*) had been made in the late 1990s by Dr. Robert Nowierski, then a Montana State University biocontrol specialist, at the request of the Blackfoot Challenge, a watershed land stewardship organization. In 2001 two local sites also received releases from a population of *M. janthinus* collected from yellow toadflax in Alberta, Canada and supplied to MSU by Dr. Alec McClay. A small but viable population of *M. janthinus* was discovered feeding on the stunted residual yellow toadflax plants at one of the original release sites during the 2009 site evaluations (the other site had no remaining yellow toadflax plants). An extensive survey encompassing an overall area of approximately 60 square miles revealed that low densities of the weevil were feeding on all yellow toadflax patches inspected at multiple sites throughout Powell County. It was evident on all inspected sites that the insects had overwintered, indicating weevil movement away from the original release points and establishment of multiple self-distributing colonizers (Sing, et al., *in preparation*).

Yellow toadflax infests fields, overgrazed pastures, rangeland, waste areas, and roadsides throughout western North America. Region 1, 2 and 4 Forest Service land managers universally attest to the unsatisfactory results and high cost of chemical control of this weed. Negative environmental and ecological impacts of yellow toadflax infestations primarily center on displacement of desirable plant species and forage quantity and quality reduction. Yellow toadflax is highly competitive for resources; floral diversity is diminished when it displaces desirable and/or native species in rangeland and forest habitats. Dual modes of reproduction enhanced by high seed production characterizing this opportunistic ruderal species allow it to rapidly spread and persist in newly colonized sites. Yellow toadflax is considered an important crop-weed species in certain regions, such as the small grain production region in northern Alberta (Baig et al. 1999; Harker et al. 1995; Darwent et al. 1975; O'Donovan and McClay 1987; O'Donovan and Newman 1989), and has specific crop associations with commercial peppermint and strawberry production (Volenberg et al. 1999; McClay and De Clerk-Floate 2002). Yellow toadflax infestations reduce effective available grazing land because cattle must become acclimated to feeding on its unpalatable foliage (Jeanneret and Schroeder 1992; Lajeunesse et al. 1993). Livestock may avoid feeding on yellow toadflax altogether if bioactive secondary compounds, including alkaloids, flavonoids, triterpenoids, steroids and iridoid glucosides are increased or concentrated when plants are drought stressed (Hua et al. 2002; Burrows and Tyrl 2001).

Although laboratory testing by Swiss and American research groups independently confirmed Dalmatian and yellow toadflax to be equally preferred hosts for *M. janthinus* under greenhouse or garden growing conditions, this species is reported to have established on yellow toadflax only at the Montana and Alberta sites mentioned above. While several other agents have been approved for release, or are in the process of being screened for release against yellow toadflax, *M. janthinus* (*ex L. vulgaris*) presently represents the greatest promise for effective yellow toadflax biocontrol.

<u>Approach</u>:

Description and sequence of project activities*:*

1. Consult with local CO and MT land mangers to identify <u>potential</u> yellow toadflax biological control release sites. Narrow those down to <u>suitable</u> release/insectary sites based on environmental and ecological factors, agent permit status, and weed management needs and plans. We will scout for appropriate release sites in 2010 for all sites needed for the duration of the project.
2. Set up monitoring transects - pre-release – on all selected release/insectary sites using a standardized protocol. Transect data will be used to assess the impact of the agent on the target weed at individual and population levels, and on wider plant community dynamics. We will set up monitoring transects in 2010 on all sites needed for the duration of the project.

3. Annually sustainably collect individuals from a genetically distinct population of the toadflax stem mining weevil *Mecinus janthinus* (Coleoptera: Curculionidae) discovered in 2009 to have established and self-distributed on yellow toadflax throughout Powell County, MT.
4. Annually prepare and ship weevils overnight express to local collaborators.
5. Annually make and document releases of *M. janthinus ex L. vulgaris* at selected and prepared sites in CO and MT to establish insectaries for future collection and redistribution.
6. Annually collect monitoring transect data and samples on all release/insectary sites, regardless of apparent establishment status, according to established protocols.
7. Determine if the Powell County, MT weevils can successfully establish and proliferate at additional intra- and inter-state locations.
8. Annually survey area around each release/insectary to document the annual extent and rate of spread *M. janthinus ex L. vulgaris* populations.
9. Annually at the end of the field season enter the data so that monitoring transect data can be analyzed to determine the biocontrol efficacy of *M. janthinus ex L. vulgaris* under the range of field conditions at all project insectaries in CO and MT.
10. Annually analyze monitoring transect data to assess for potential changes in plant community composition correlated with the implementation of yellow toadflax biocontrol at all project insectaries in CO and MT.
11. Annually report results of analysis and field observations to FHP as required.
12. Annually attempt to collect and distribute releases of *M. janthinus ex L. vulgaris* to additional states in R1, 2, and 4 as the supply of weevils permits.
13. Annually increase the implementation yellow toadflax biocontrol through scientific and management presentations, popular press and tech transfer articles, and field tours and workshops.

Methods*:*

Initial plot characterization. The number of new release monitoring plots established annually during the course of the study will be dictated by the availability of weevils from the Powell County, MT source population. Our goal will be to establish at least 5 new insectaries per state for each year of the study. Site parameters recorded at the time of release, including GPS coordinates; slope; slope position (valley bottom, slope toe, midslope, plateau); aspect; soil surface and subsurface texture; estimated extent of infestation at the time of release; and size of the initial release (total number of insects) will facilitate objective comparisons between successful and unsuccessful releases over the projected three year study period.

Plot design. Our monitoring protocol was designed to detect post-release changes in the abundance of the biocontrol target or the insect biocontrol agent; to determine where and when biocontrol impact expands away from the agent release points; and to assess biocontrol-correlated impacts on the wider vegetation community. The overall sampling design will employ a 0.88 ha monitoring macroplot (Figure 1A), to be established at and centered on each agent release point (ARP), and will consist of: 1) sixteen $1m^2$ vegetation sampling subplots (Figure 1B); 2) four $5m^2$ agent sampling subplots (Figure 1B), and 3) six 50m vegetation and agent nested frequency transects that originate 2.5m from the ARP (Figure 1A).

Insect sampling. Mecinus janthinus presence and abundance will be estimated on each 10 x 10m core plot and from all 1.0 m^2 frequency frames. Adult foliar feeding injury will be assessed according to damage rating classes (Schat 2008). All of the previous year's toadflax stems in each sample quadrat will be collected, labeled and dissected in the lab where the following *M. janthinus* population variables will be recorded: number of empty pupation chambers with emergence holes, number of empty pupation chambers without emergence holes, number of live and dead adults, pupae, and larvae. Exit holes are easily distinguished and remain obvious throughout the season of emergence, allowing estimates of weevil emergence in each plot (Schat 2008). These sites will be visited in subsequent years and the same data will be collected.

Vegetation sampling. Observations will be timed to occur when the target population is in peak flower. The following toadflax data, reported for ‘mature’ (likely to attain reproductive status in the present growing season) vs. ‘immature’ (likely to remain pre-reproductive throughout the present growing season) stems, will be recorded from each of the sixteen $1m^2$ quadrats surrounding the ARP: number of stems; shortest, tallest and estimated mean stem height; and mean foliar damage rating. Transect samples will be taken using 26 nested frequency frames, beginning at 0m and thereafter at two meter intervals to total 156 transect plots at each release. Weed data will be collected using a nested frequency frame. Percent cover of yellow toadflax, grasses, all forbs other than yellow toadflax, woody species, litter, rock and bare soil will also be recorded from each $1m^2$ core and transect sample point quadrat.

1A. **1B.**

Figure 1. A. Macroplot design: core plot with radiating transects for frequency sampling.
B. Core plot detail: sixteen $1m^2$ subplots for stem density counts (center); 10 m^2 insect sampling plot.

Statistical analysis*:* Analysis of variance (ANOVA) and longitudinal regression analyses (Singer and Willett 2003) will be used to test for year-to-year reductions in the density and height of yellow toadflax stems according to reproductive class. The nested frequency data will assess annual changes in yellow toadflax frequency across entire macroplots by comparing total frequencies between years (and their confidence intervals). Agent spread from the release point will be evaluated by 1) comparing the frequency of insects in “inner” (i.e., near the release point) and “outer” frequency frames among years through contingency table chi^2 analyses, and 2) through distance methods spatial analysis (Perry 1998).

Expected Products and Outcomes:

- Provide a reliable, simplified protocol for establishing insectaries for the purpose of producing populations of *Mecinus janthinus* with an increased acceptance of yellow toadflax hosts.
- Disseminate the results of this research at scientific meetings, via workshops at selected locations and at annual meetings for both land managers and weed coordinators, and through on-line media and conventional extension materials.

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Education:
Doctor of Philosophy. 2002. Montana State University. Land Resources and Environmental Sciences.
Master of Science. 1997. McGill University. Natural Resource Sciences.
Bachelor of Arts. 1984. Dalhousie University. English.

Employment:
09/2008 – present: Research Entomologist. Evaluation of the efficacy and identification of the potential non-target impacts associated with classical weed biological control. Selection and testing of candidate agents for classical weed biological control. Facilitation of classical weed biocontrol implementation. USDA Forest Service – RMRS.
04/2006 – 09/2008: Assistant Research Professor. Development and application of methods to determine efficacy and non-target impacts associated with weed biological control; comparative economic and ecological risk assessment of biological control. Montana State University - LRES.
01/2002 – 10/2005: Research Entomologist (Post-doc). Weed biological control; ecology and management of weeds in post-burn environments. USDA Forest Service – RMRS.

Major Committees: USDA APHIS Technical Advisory Group (09/2008 – present); USDA-CSREES Regional Project W-2185 (2003-present); National Strategy and Implementation Plan for Invasive Weeds (2003-04); Montana Interagency Weed Biological Control Committee (2002-present); International Toadflax Biocontrol Consortium (2007-present).

Select publications:

Ward, S.M., C.E. Fleischmann, M.F. Turner and **S.E. Sing**. 2009. Hybridization between invasive populations of Dalmatian toadflax (*Linaria dalmatica*) and yellow toadflax (*Linaria vulgaris*). Invasive Plant Science and Management 2(4): 369-378.

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Peterson, R.K.D., **S.E. Sing**, and D.K. Weaver. 2005. Differential physiological responses of Dalmatian toadflax, *Linaria dalmatica* (L.) Miller, to injury from two insect biological control agents: implications for decision-making in biological control. Environmental Entomology 34: 899-904.

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Wilson, L.M., **S.E. Sing**, G.L. Piper, R.W. Hansen, R. DeClerck-Floate, D.K. MacKinnon and C.B. Randall. 2005. Biology and Biological Control of Dalmatian and Yellow Toadflax. USDA Forest Service, Forest Health Enterprise Technology Team, Morgantown, VA. FHTET-2005-13.

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EDUCATION

Ph.D.	**West Virginia University,** Morgantown, WV 26506.
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B.S.	**West Virginia University,** Morgantown, WV 26505.

Additional Academic Study

Survival of trees after fire injury.
Use of semiochemicals to manage bark beetles.
Biocontrol of introduced invasive weeds.

Professional Experience

2008-present Research Entomologist, PNW Research Station, LaGrande, OR
2003-2008 Research Entomologist, PNW Research Station, Corvallis, OR
2000-2003 USDAFS, Forest Health Protection Entomologist
1996-2000 Postdoctoral Research Associate Oregon State University

Selected Publications

Progar, R.A., N. Sturdevant, and M.J. Rinella. 2010. Trapping Douglas-fir Beetle (*Dendroctonus pseudotsugae*) with Pheromone Baited Multiple-Funnel Traps to Reduce Tree Mortality. Western Journal of Applied Forestry. (In review).

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Ph.D.	1983	University of Wisconsin, Madison, Entomology, Zoology minor
M.S.	1978	University of Wisconsin, Madison, Entomology
B.A.	1975	University of California, Santa Cruz, Biology, highest honors
2005-present		State Biological Control Specialist, Manager, Palisade Insectary and Director, Biological Pest Control Section, Colorado Department of Agriculture. Affiliate Faculty, Dept. Bioagricultural Sciences and Pest Management, CSU, Ft. Collins
2000-2005		Research Associate, Department of Vegetable Crops, UC Davis
1988-2000		Research Associate and Lecturer, Department of Biology, UNC-CH
1986-1988		Research Associate, USDA ARS, Insect Attractants, Behavior and Basic Biology Research Laboratory, Gainesville, FL
1983-1986		Research Associate, Depts of Ag Chem and Entomology, OSU, Corvallis, OR
1975-1982		Research Assistant, Department of Entomology, University of Wisconsin, Madison, WI-

Recent Publications

Dalin, P., D.W. Bean, T. Dudley, V. Carney, D. Eberts, K.T. Gardner, E.N. Jones, D.J. Kazmer, J. Michels, S. O'Meara, D.C. Thompson & E. Hebertson. (2010) Seasonal adaptations to day length in four ecotypes of the leaf beetle *Diorhabda elongata* inform selection of biocontrol agents against saltcedar (*Tamarix* spp.). Environ. Entomol. (in press)

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Dudley, T.L., Dalin, P. & Bean, D.W. (2006) Status of biological control of *Tamarix* spp. in California. In: M.S. Hoddle & M.W. Johnson (Eds.) Proceedings of the Fifth California Conference on Biological Control. Riverside, CA. pp.137-140.

W. L. Bruckart, III, F. M. Eskandari, M. C. Becktell, D. Bean, J. Littlefield, A. L. Pilgeram, D. C. Sands, and M. C. Aime. (2006) *Puccinia acroptili* on Russian Knapweed in Colorado, Montana, and Wyoming. Plant Disease 90: 971.

Cossé, A.A., Bartelt, R.J., Zilkowski, B.W., Bean, D.W. and Petroski, R.J. (2005) The aggregation pheromone of *Diorhabda elongata*, a biological control agent of saltcedar (Tamarix spp.): identification of two behaviorally active components. J. Chem. Ecol. 31:657-670

Project Budget FY10	Requested FY10	Other-Source FY10
Seasonal employees 1-GS-4, 5 pp 1-GS-4, 10 pp	$ 5,680 (MT) $11,360 (CO)	GS-4, 5 pp (MT) $ 5,680 Progar $10,000 Sing $10,000
Travel Vehicle (3 months @ $285/month) Mileage (5000 miles @ $0.36/mile) Per diem (10 pp @ $414/pp/person)	$ 855 (CO) $ 1,800 (CO) $ 4,140 (CO)	Vehicle (MT) $ 855 Mileage (MT) $ 1,800 Per diem (MT) $ 4,140
Supplies (nets, coolers, icepacks)	$ 500 (CO)	Supplies (MT) $ 500
SUBTOTAL	$24,335	$32,975
Overhead RMRS (2.2%)	$ 536	
FY10 Total	**$24,871**	**$32,975**
Project Budget FY11	**Requested FY11**	**Other-Source FY11**
Seasonal employees 1-GS-4, 5 pp 1-GS-4, 5 pp	$ 5,680 (MT) $ 5,680 (CO)	Progar $10,000 Sing $10,000
Travel Vehicle (2 x 3 months @ $285/month) Mileage (5000 miles @ $0.36/mile) Per diem (2 x 5pp @ $414/pp/person)	$ 1,710 (CO + MT) $ 3,600 (CO + MT) $ 4,140 (CO + MT)	
Supplies (2 x $500)	$ 1,000 (CO + MT)	
SUBTOTAL	$20,810	$20,000
Overhead RMRS (2.2%)	$ 480	
FY11 Total	**$21,290**	**$20,000**
Project Budget FY12	**Requested FY12**	**Other-Source FY12**
Seasonal employees 1-GS-4, 5 pp 1-GS-4, 5 pp	$ 5,680 (MT) $ 5,680 (CO)	Progar $10,000 Sing $10,000
Travel Vehicle (2 x 3 months @ $285/month) Mileage (2 x 5000 miles @ $0.36/mile) Per diem (2 x 5 pp @ $414 /pp/person)	$ 1,710 (CO + MT) $ 3,600 (CO + MT) $ 4,140 (CO + MT)	
Supplies	$ 500 (CO)	
SUBTOTAL	$20,810	$20,000
Overhead RMRS (2.2%)	$ 480	
FY12 Total	**$21,290**	**$20,000**
Project Totals FY10-12	**$67,451**	**$72,975**

<u>Timetable</u>: see Approach - *Description and sequence of project activities* (above).

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