RMRS Weed Biocontrol Research:
Past Accomplishments, Current Status, and Future Challenges

RMRS Weed Biontrol Team
Accomplishments Report
Fiscal Years 2007-2011

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Cover Photo:
Infestation of Yellow Toadflax (*Linaria vulgaris*). Credit: Larry Howery.

Inset Photos:

Yellow toadflax plant. Credit: Michael Shephard, USFS.

Toadflax Stem Weevil (*Mecinus janthinus*). Credit: *Biological Control of Invasive Plants in the United States.*

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Introduction

This report has the primary objective of documenting the accomplishments of weed biocontrol researchers at the Rocky Mountain Research Station (RMRS) over the last five fiscal years. RMRS weed biocontrol scientists perform basic and applied research directed at the mission of helping to stem the tide of massive invasion of exotic invasive plants in the Interior West. A five-year timeframe (Fiscal Year 2007-2011) serves as a timely anniversary for showcasing the high productivity of individual scientists involved in this research effort.

During this period, the classical biocontrol program at the Bozeman Forestry Sciences Laboratory has been greatly expanded with the aid of increased line item funding, the augmentative biocontrol program at the Provo Shrub Sciences Laboratory has grown through influxes of extramural funding, a biocontrol program at the Albuquerque Forestry Sciences Laboratory has been initiated, and ecological research in support of the weed biocontrol effort has continued at the Missoula Forestry Sciences Laboratory.

The research products generated by the station's weed biocontrol program are outlined by individual scientists in the accomplishment reports that follow. They clearly show a strong dual emphasis. First, there is an emphasis on the publication of first-rate science in respected peer-reviewed outlets, but second, and perhaps more importantly, there is an emphasis on the timely provision of weed biocontrol application information and tools to managers on the ground.

Given the products, personnel and facilities at RMRS, it is clear that the weed biocontrol program is on track to becoming a flagship program within RMRS and across Forest Service Research and Development. Such a flagship program will garner national and international attention, attract new partners and new sources of extramural funding, and most importantly, generate new knowledge and tools that will have a significant positive impact on addressing the invasive weed problem.

Because this research program addresses significant threats posed by invasive weeds and delivers critical information to managers to aid in controlling invasions, a secondary objective of this report is to draw attention to the relevance, quality and performance of this program in the broader context of R&D themes, products and programs. Why is this program relevant? Weed invasion rates are increasing due to global trade, accidental species introductions, and human immigration and travel patterns. The rapid spread of invasive species affects farming, ranching, water management and tourism, reduces economic land values, increases fire risk and damage in many ecosystems and bioregions, impairs wildlife and fish habitats, and jeopardizes many native species.

Not only does Forest Service have a major role to play in developing and delivering knowledge to mitigate these threats, it also has an ethical responsibility to prevent and control the spread of invasive species and to repair the damage caused by invasions on National Forests and Grasslands, on other public lands, on state and private lands and in the international arena. Invasive species do not stop their spread at jurisdictional or international boundaries.

Now more than ever and despite government budget restrictions, it is time to heed the growing problem of invasive species - before it is too late. To defend our nation's lands against this threat, it is necessary to invest in the following areas:

- important research and development,
- partnerships between managers and researchers,
- communication within our agency across areas, regions and stations,
- outreach about this problem.

In R&D and at RMRS, investments are needed in new and continuing research to develop weed biocontrol options, to design improved biocontrol solutions for existing problems, to move rapidly on new and pending invasive species problems, and to monitor consequences of biocontrol releases, especially in the Interior West.

Background

Weed biocontrol research at the RMRS was initiated in 1986 with a focus on classical biocontrol. It has grown since that time into a multifaceted program involving Station scientists at several locations and with a significant cross-program component. The classical biocontrol program is still the centerpiece, but the research agenda has expanded to include augmentative biocontrol approaches for important grass weeds that are intractable from a classical biocontrol perspective. In addition, the program has developed strong cross-disciplinary links both within the Station and with outside collaborators in order to examine the consequences of both classical and augmentative biocontrol in a broader ecological context.

The expertise and accomplishments of RMRS biocontrol scientists ensure a continued leadership role in both classical and augmentative weed biological control research. A 2005 review recommended that the classical biocontrol program at the Bozeman Forestry Sciences Laboratory should not only be continued but expanded, based on the quality and relevance of delivered products and the ongoing need to find new agents and to optimize implementation with approved agents. As a result, the roster of Station researchers already engaged in weed biocontrol-related investigations was augmented in 2008 with two additional, dedicated classical weed biocontrol research specialists.

Newly upgraded infrastructure at the RMRS Bozeman weed biocontrol research facility will allow these researchers and their collaborators to make both theoretical and applied contributions to the discipline equal to those of top weed biocontrol research programs world-wide. Classical weed biocontrol research at the Bozeman facility is enhanced by on-site greenhouses, growth chambers and laboratory facilities housing specialized diagnostic equipment and instruments. In addition, the Bozeman facility is uniquely linked through past and present collaborative research ties to the world-class insect, plant and pathogen quarantine facility located on the nearby Montana State University campus. The opportunity for RMRS researchers to conduct cutting-edge classical weed biocontrol research is considerably enhanced by access to the MSU quarantine facility, which provides a containment level available at only five total locations in the United States.

Existing infrastructure at the Shrub Sciences Laboratory in Provo in support of augmentative biocontrol research includes high-quality greenhouse, growth chamber and laboratory facilities. The capacity to carry out innovative biocontrol research at the Shrub Sciences Laboratory is enhanced by strong ties with collaborators at nearby Brigham Young University, who provide expertise in diverse disciplines.

In the past, classical weed biocontrol research as a discipline focused primarily on screening and testing new agents for release; consequently, host-specificity studies were emphasized. More recently, conceptual and practical advances in plant-consumer interactions, plant pathology and physiology, insect physiology, plant, insect and pathogen genetics, biogeography and systematics, and trophic and community ecology are now being adapted and exploited in both classical and augmentative weed biocontrol research. The breadth of professional expertise on the RMRS biocontrol team uniquely positions RMRS to significantly advance the science of weed biological control by integrating complex ecologically-driven research approaches into the development and delivery of improved weed biological control tools to our customers.

Stakeholder demand for weed biocontrol research products in the intermountain West has been historically strong. The high level of support for the RMRS weed biocontrol program became apparent in the provision of funding allocated through the USDA Forest Service's Research and Development Washington Office as a line-item to RMRS expressly for the maintenance of staff, infrastructure, and resources in support of a classical weed biocontrol research program at the Bozeman Forestry Sciences Laboratory. RMRS research findings and products have ensured the continuation of existing in-state and regional classical weed biocontrol programs, and also the expansion of the customer base as new weed problems emerge. Users of biocontrol research in the management community have repeatedly shown their strong support both for the proven methods of classical biocontrol and for the more long-term effort to find augmentative biocontrol solutions to major grass weed problems.

A major challenge for the RMRS biocontrol program in the future is the availability of prioritized, stable funding. In particular, to fully capitalize on the staff and infrastructure investments made at the Bozeman laboratory, the Station will need to find ways to provide sufficient operating funds and staff to permit the effective leveraging of funds from the scientific community in the form of grants, and from the management community in support of on-the-ground efforts. The augmentative biocontrol program at Provo has received little direct operating support, but the scientific advances there have largely been possible because a well-trained, permanent, full-time technician is dedicated to this project. This kind of staffing continuity is key to long term productivity.

To guarantee long term viability of the weed biocontrol program, the team has set some tasks for the coming year which include renewing its vision and goals regarding the direction of weed biocontrol research for the next five years, and using the team as a vehicle for proposal development, internal and external communication, and outreach to potential collaborators and partners. This includes an evaluation of how team vision and goals complement and build on the efforts of other biocontrol research groups and therefore makes a uniquely valuable contribution. By conceptually integrating the biocontrol efforts at the different RMRS laboratories, a coherent program of team efforts and self-directed individual studies will emerge to show major conceptual and practical advances to the basic and applied science of weed biocontrol. RMRS is presently well-positioned to become an important go-to organization in this arena. It is therefore imperative that the program be nurtured, and funded, to the maximum extent possible, in these difficult times.

The Weed Biocontrol Research Team

Dr. Jack Butler is a Research Ecologist for the Grassland, Shrubland and Desert Ecosystems Science program at the RMRS Forest and Grassland Research Laboratory in Rapid City, South Dakota. His research involvement with weed biocontrol was primarily as a member of TEAM Leafy Spurge, where he worked on community-level responses to leafy spurge biocontrol. He is currently working with sickleweed, a newly emerging invasive threat on the western Great Plains. Dr. Butler also administered expansion of the Bozeman biocontrol effort over the past five years.

Dr. George Markin (retired 2008) is a Research Entomologist who participated in the development and release of classical biocontrols for numerous forest weeds over his long career at the Bozeman Forestry Sciences Laboratory. His work gave RMRS credibility over the long term with forest managers and helped build a client base for the current biocontrol

research effort. Dr. Markin continues to do volunteer work for the Grassland, Shrubland and Desert Ecosystem Science Program.

Dr. Susan Meyer is a Research Ecologist for the Grassland, Shrubland and Desert Ecosystems Science Program at the Shrub Sciences Laboratory in Provo, Utah. Her research emphasis is on the restoration of native plant communities on rangelands that have been invaded by annual grass weeds such as cheatgrass, red brome, and medusa head. This has led her to tackle the problem of biocontrol of these destructive invaders using augmentative strategies with naturally occurring fungal plant pathogens.

Yvette Ortega is an Ecologist for the Wildlife and Terrestrial Ecosystems Program at the Forestry Sciences Laboratory in Missoula, Montana. Her main interest is community interactions and how they are influenced by environmental context. She has applied this focus to a range of problems from forest fragmentation to invasive species. Current research addresses efficacy of biological control under differing climate scenarios, mechanisms underlying plant invasions, and impacts of plant invasion on songbirds via food chains.

Dr. Dean Pearson is a Research Ecologist and Deputy Program Manager for the Wildlife and Terrestrial Ecosystems Program at the Forestry Sciences Laboratory in Missoula, Montana. His research focuses on community ecology with an emphasis on biological invasions and invasives management, including biological control. His work on invasives management has lead to overview and keynote addresses on this subject as far abroad as South Korea. His work in biocontrol has directed the field toward increased emphasis on efficacy of control agents and is featured in recent biocontrol textbooks and reviews.

Dr. Rosemary Pendleton is a Research Ecologist for the Grassland, Shrubland and Desert Ecosystems Science Program at the Forestry Sciences Laboratory in Albuquerque, New Mexico. who works primarily at the community ecology level in the desert grassland-shrubland interface. She has recently become involved with a study on cheatgrass fungal endophytes, some of which may have potential as biocontrol candidates for annual grass weeds.

Dr. Justin Runyon is a Research Entomologist for the Grassland, Shrubland and Desert Ecosystems Science Program at the Forestry Sciences Laboratory in Bozeman, Montana. He is involved in the search for and the evaluation, approval, and monitoring of exotic natural enemies (usually insects) for the suppression of invasive weeds. One goal of his research is to understand and predict the outcomes of biocontrol introductions through application of the principles of chemical ecology of plant-insect interactions to classical biological control of weeds.

Dr. Sharlene Sing is a Research Entomologist for the Grassland, Shrubland and Desert Ecosystem Science Program at the Forestry Sciences Laboratory in Bozeman, Montana, where she works on improving the safety and efficacy of classical insect biocontrol of invasive weeds such as Dalmation toadflax, saltcedar, and Russian olive, and on the integration of classical weed biocontrol in an Integrated Pest Management context.

Biocontrol Team Accomplishments Summary

Over the five-year period (Fiscal Year 2007-2011), scientists on the RMRS weed biocontrol team have produced a total of 44 peer-reviewed publications and 25 publications in symposium proceedings and other outlets targeting the management community.

The team has leveraged over $2.5 million dollars in extramural funding over this period, and has continued or initiated collaborative work with at least eight different universities (Brigham Young University, Colorado State University, Gonzaga University, University of Idaho, University of Montana, University of Nevada Reno, University of Washington, and Washington State University), at least seven sister agencies (USDA-ARS, USDA-APHIS, USDI-BLM, USDI-NPS, DOD-Idaho Army National Guard, USFS-SPF, and USFS-NFS), at least four international research groups (CABI Switzerland, BBCA-Rome, Russian Biocontrol Group, and the University of Naples), and numerous NGO and state-level agencies.

These statistics demonstrate the existence of an active and vigorous research effort that has the potential to coalesce into a nationally prominent and valuable program. Its recognition and value can only increase with care and encouragement by the Forest Service.

The progress profiles that comprise the remainder of this report provide more detailed information on biocontrol team accomplishments arranged by individual scientist and project.

Project Title:	**Biological Control of Leafy Spurge**
Lead Science Program:	**Grassland, Shrubland and Desert Ecosystems (GSD)**
Principal Investigator:	**Jack Butler**

Describe progress made on Plan of Work
The majority of my research in classical biocontrol of invasive plants came my involvement with a unique research and demonstration project (**T**he **E**cological **A**rea-wide **M**anagement of Leafy Spurge; working title was TEAM Leafy Spurge; www.team.ars.usda.gov), which was funded and administrated by the Agricultural Research Service, Sidney, MT. The objective TEAM Leafy Spurge was to develop and evaluate an integrated pest management strategy for leafy spurge specific to large portion of the Great Plains. I was part was part of the biological control assessment effort that was charged with documenting leafy spurge population dynamics in response to control by flea beetles (*Apthona* sp.), determining the role of selected site characteristics on establishment and persistence of the beetles, evaluating the response of resident plants to control of leafy spurge, and evaluating potential non-target impacts on a species closely related to leafy spurge. The last paper of my research was published in 2010.

Describe significant accomplishments and/or products delivered since project initiation
Numerous informal field demonstrations and tours associated with TEAM Leafy Spurge research were conducted in addition to more formal events: Spurgefest I (1999) and Spurgefest II (2001). Each event was 3-days in length and featured a TEAM Leafy Spurge (TLS) symposium, tours of TLS research and demonstration sites, hands-on flea beetle collecting, and beetle distribution (http://www.team.ars.usda.gov/index2.html). The program culminated in a TEAM Leafy Spurge Symposium held during the Annual Meeting of the Society of Range Management, Salt Lake City, UT, January 2004 and a special issue of *Rangeland Ecology and Management* published in 2006. **Butler, J.L.,** M.A. Parker, and John T. Murphy. 2006. Efficacy of flea beetle control of leafy spurge in Montana and South Dakota. Rangeland Ecology and Management. 59:454-461. Wacker, S.D. and **J.L. Butler.** 2006. Potential impact of two *Aphthona* spp. on a native, nontarget Euphorbia species. Rangeland Ecology and Management. 59:468-474. **Butler, J.L.** and S.D. Wacker. 2010. Lack of native vegetation recovery following biological control of leafy spurge. Rangeland Ecology and Management. 63:553-563.

Describe key research to be completed and products to be delivered in the next 3 years
• I recently completed a study on the biology and ecology of sickleweed (*Falcaria vulgaris*), a new invasive plant in the Northern Great Plains. The current product is a MS thesis that I directed and was completed in May 2010. I anticipate 2 publishable manuscripts from this research. In addition, the study has been extended to include an evaluation of the population genetics of sickleweed. Such information is preparatory to evaluating the potential of using biocontrol agents to control sickleweed.

Describe key partnerships and funding leveraged (if applicable)
• The Agricultural Research Service funded and administered TEAM Leafy Spurge. In addition to providing considerable technical and logistical support, I received $209,645 in financial support from the ARS during my involvement with TEAM Leafy Spurge. Other key partnerships included the University of Wyoming, Montana State University, North Dakota State University, and South Dakota State University; the Dakota Prairie, National Park Service, and numerous states, county, and local invasive plant managers.

Notes and Considerations on this Project's Progress
The leafy spurge biocontrol effort is complete. Travel restrictions could be a factor in completing the sickleweed project.

Project Title	Classical Biocontrol of Forest Weeds
Lead Science Program	Grassland, Shrubland and Desert Ecosystems (GSD)
Principal Investigator	George Markin

Describe progress made on Plan of Work
George Markin was a research entomologist at the Bozeman lab from 1995-2008 specializing in finding, testing, and introducing biocontrol agents to control forest weeds. He retired in 2008, but continues to serve as a volunteer in Bozeman and continues weed biocontrol activities. Products from his work as a RMRS scientist and volunteer continue to this day. This report summarizes his publications since first receiving RMRS Biocontrol funding in 2006. George is currently coordinating the 2011 XIII Weed Biocontrol Symposium in Hawaii, and he still presents three to five formal presentations annually on biocontrol at interagency and professional entomological and biocontrol meetings.

Describe significant accomplishments and/or products delivered since project initiation
Publications: 1. **Markin, G.P.** & Littlefield, J. 2006. Biological control of tansy ragwort in Montana: status of work as of November 2006. 2. Coombs, E.M., **Markin, G.P.** & Andreas, J. 2008. Release and establishment of the Scotch broom seed beetle *Bruchidius villosus* in Oregon and Washington, USA. Pp. 516-520 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK. 3. Friesen, R.D., Causton, C.E. & **Markin, G.P.** 2008. Status of biological control of banana poka, *P. mollissima* (aka *P. tarminiana*) in Hawaii. Pp. 669-675 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK. 4. Hill, R.L., Ireson, J., Sheppard, A.W., Gourlay, A.H., Norambuena, H., **Markin, G.**, Kwong, R. & Coombs, E. 2008. A global view of the future for biological control of gorse, *Ulex europaeus* L. Pp. 680-686. In: *Proceedings of the XII International Symposium on Biological Control of Weeds*.

(Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

5. Kashefi, J., **Markin, G.P.** & Littlefield, J. 2008. Field studies of the biology of the moth *Bradyrrhoa gilveolla* (Treitschke) (Lepidoptera: Pyralidae) as a potential biocontrol agent for *Chondrilla juncea*. Pp. 568-572 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

6. Lecheva, I., Karova, A., & **Markin, G.P.** 2008. The insect fauna of *Chondrilla juncea* L. (Asteraceae) in Bulgaria and preliminary studies of *Schinia cognate* (L.) (Lepidoptera: Noctuidae) as a potential biological control agent. Pp. 301-305 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

7. Littlefield, J.L., **Markin, G.P.,** Puliafico, K.P. & DeMeij, A.E. 2008. The release and establishment of the tansy ragwort flea beetle in the northern Rocky Mountains on Montanan. Pp. 573-576 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

8. **Markin, G.P.** 2008. *Ulex europaeus* L. common gorse. Pp. 1140-1142. *The Woody Plant Seed Manual*. Eds.: F.T. Bonner & R.P. Karrfalt. USDA Forest Service Agriculture Handbook 727.

9. Markin, G.P. & Littlefield, J.L. 2008. Biological control of tansy ragwort (*Senecio jacobaeae*, L.) by the Cinnabar moth, *Tyria jacobaeae* (CL) (Lepidoptera: Arctiidae), in the northern Rockies. Pp. 583-588 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

10. Puliafico, K.P., Littlefield, J.L., **Markin, G.P.** & Schaffner, U. 2008. Field and laboratory observations of the life-history of the Swiss biotype of *Logitarsus jacobaeae* (Coleoptera: Chrysomelidae). Pp. 200-205 In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

11. Volkovitsh, M.G., Dolgovskaya, M.Y., Reznik, S.Y., **Markin, G.P.,** Cristofaro, M., & Tronci, C.

2008. *Sphenoptera foveola* (Buprestidae) as a potential agent for biological control of rush skeletonweed, *Chondrilla juncea*. Pp. 227-231. In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK.

12. Littlefield, J.L., **Markin, G.P.,** Kashefi, J., & Prody, H.D. 2008. Habitat analysis of the rush skeleton weed root moth, *Bradyrrhoa gilveolella* (Lepidoptera: Pyralidae). Pp. 60. In: *Proceedings of the XII International Symposium on Biological Control of Weeds*. (Eds: M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, & B.G. Rector) CAB International Wallingford, UK. Abstract only.

13. Gunderson-Izurieta, S., **Markin, G.P.,** Reedy, N. & Frieberg. 2009. Southern Idaho student "bug crews": weeds, youth, and biocontrol in the rangelands of Idaho. Rangelands 31:36-40.

14. **Markin, G.P.** 2009. *Passiflora mollissima* (HBK) Bailey (Passifloraceae). Pp. 319-331. *Biological Control of Tropical Weeds using Arthropods*. Eds.: R. Muniappan, G.V.P. Reddy, & A. Raman. Cambridge University Press.

15. Birdsall, J. & Markin, G. 2010. Biological Control of Yellow Starthistle (*Centaurea solstitialis*) in the Salmon River Canyon of Idaho. Pp. 462-469. *Invasive Plant Science and Management*. Vol. **3**:4.

16. **Markin, G.P.** & Horning, C.J. 2010. Discovery of a gall-forming midge, *Asphondylia pilosa* Kieffer (Diptera: Cecidomyiidae), on Scotch broom (*Cytisus scoparius* (L.) Link) (Fabaceae). Pp. 260-263. In: *Journal of the Kansas Entomological Society*. Vol. 83:3.

17. Progar, R.A., **Markin, G.,** Milan, J., Barbouletos, T. & Rinella, M.J. 2010. Inundative release of *Aphthona* spp. flea beetles (Coleoptera: Chrysomelidae) as a biological "herbicide" on leafy spurge in riparian areas. Pp. 242-248. In: *Journal of Economical Entomology*. Vol. 103: 2.

Project Title	Annual Brome Biocontrol with the Seed Bank Pathogen Black Fingers of Death
Lead Science Program	Grassland, Shrubland, and Desert Ecosystems (GSD)
Principal Investigator	Susan Meyer

Describe progress made on Plan of Work

Since formal initiation of this work in summer 2007, we have completed the plan of work for our 3-year Joint Fire Sciences-funded study and filed our final report online. We anticipate completion of our 4-year NRI-funded study in December 2011. We were able to obtain sufficient evidence for the feasibility of using black fingers of death (*Pyrenophora semeniperda*) for annual brome seed bank control in this relatively short period of time to justify filing a patent application through the US Forest Service. This emerging biocontrol technology has implications for weed control in both rangeland and intensive agricultural applications that could extend in concept well beyond annual grass weeds.

Describe significant accomplishments and/or products delivered since project initiation

- We have demonstrated proof of the concept in field inoculation trials at multiple sites that this pathogen can be used to control persistent seed banks of annual bromes.
- We have developed a culture collection of >700 strains fingerprinted using ITS, SSR and SNP markers.
- We have filed an application to patent our concepts and technology for annual brome biocontrol.
- We have published nine peer-reviewed papers and four proceedings papers on this pathosystem.
- We have produced an online annotated draft genome sequence for *Pyrenophora semeniperda.*
- List of publications since 2006:

Allen P.S., **Meyer S.E**.,Foote K. 2010. Induction and release of secondary dormancy under field conditions in *Bromus tectorum.* Extended abstract. In: Pendleton R.L., Meyer S.E., and Schultz B.S., eds. Proceedings of Seed Ecology III: International Society for Seed Science Third Conference on Seeds and the Environment, Salt Lake City, Utah, June 20-24, 2010. P. 2-3. Avaliable online at TREESEARCH.

Beckstead J. , **Meyer S.E.,** Street L.S., Allen P.S. 2010. Effect of fire on a seed bank pathogen and on seeds of its host *Bromus tectorum* . In: Pendleton R.L., Meyer S.E., and Schultz B.S., eds. Proceedings of Seed Ecology III: International Society for Seed Science Third Conference on Seeds and the Environment, Salt Lake City, Utah, June 20-24, 2010. P. 10-11. Avaliable online at TREESEARCH.

Beckstead J., **Meyer S.E.**, Molder C.J., and Smith C. 2007. A race for survival: can *Bromus*

tectorum seeds escape *Pyrenophora semeniperda* caused mortality by germinating quickly? Ann. Bot. 99:907-914.

Beckstead J., **Meyer S.E.,** Connolly B.M., Huck M.B., and Street L.E. 2010. Cheatgrass facilitates spillover of a seed bank pathogen onto native grass species. J. Ecology 98:168–177.

Beckstead, J., Street LE, **Meyer SE,** and Allen PS. 2011. The effect of fire on the cheatgrass seed pathogen *Pyrenophora semeniperda*. Rangeland Ecology and Management 64:148-157.

Boose D., Harrison S.L., Clement S., and **Meyer S.E.** 2011. Population genetic structure of the seed pathogen *Pyrenophora semeniperda* on *Bromus tectorum* in western North America. Mycologia 103:85-93.

Dooley, SR and Beckstead, J. 2010. Characterizing the interaction between a fungal seed pathogen and a deleterious rhizobacterium for cheatgrass control. Biological Control 53: 197-203.

Meyer S.E., Quinney D. Nelson D.L., and Weaver J. 2007. Impact of the pathogen *Pyrenophora semeniperda* on *Bromus tectorum* seedbank dynamics in North American cold deserts. Weed Research 47: 54-62.

Meyer S.E., Smith D.C. and Beckstead J. 2007. *Bromus tectorum* seed banks: impact of the pathogen *Pyrenophora semeniperda.* Extended abstract. Turner S., Merritt D., Clarke S., Coommander L,. and Dixon K. eds. Proceedings of Seed Ecology II: International Society for Seed Science Second Conference on Seeds and the Environment, Perth, Australia, September 12-17, 2007. P. 59.

Meyer S.E., Beckstead J., Allen P.S., and Smith D.C. 2008. A seed bank pathogen causes seedborne disease: *Pyrenophora semeniperda* on undispersed grass seeds in western North America. Can. J. Plant Path. 30:525-533.

Meyer, SE, Nelson, DL, Beckstead, J and Clement S. 2008. Cheatgrass biocontrol using indigenous fungal pathogens. P. 61-67. In: Kitchen, Stanley G.; Pendleton, Rosemary L.; Monaco, Thomas A.; Vernon, Jason, comps. Proceedings—Shrublands under fire: disturbance and recovery in a changing world; 2006 June 6–8; Cedar City, UT. Proc. RMRS-P-52. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Meyer SE, Stewart TE, and Clement S. 2010. The quick and the deadly: growth versus virulence in a seed bank pathogen. New Phytologist 187:207-216.

Stewart, TE, **Meyer SE,** and Allen PS. 2009. First report of *Pyrenophora semeniperda* in Turkey and Greece. Plant Disease Reporter 93:1351.

Describe key research to be completed and products to be delivered in the next 3 years

- Develop improved bulk inoculum production technology and test it in bioassays and field studies.
- Begin the quest for an industry partner to work jointly on developing a commercially viable product.
- Begin work on the molecular genetic regulation of virulence, with the goal of selecting or breeding more effective strains for biocontrol.
- Expand field studies to more completely and explicitly address potential threats to non-target species.
- Examine the potential for seed bank biocontrol with this pathogen for other known annual grass weed hosts, such as medusahead wildrye.

- Prepare a series of papers based on existing unpublished data sets, including studies on genomics, host range, seed banks, field inoculation trials, and population genetic structure, for submission to peer-reviewed journals.

Describe key partnerships and funding leveraged (if applicable)
• The research team includes scientists, students and support staff from USFS, Brigham Young University, and Gonzaga University. • Formal funding was initiated in 2007 (USDA CSREES NRI $390K, Joint Fire Sciences $355K). • Additional funding was provided in 2010 by USFS-SPF ($67K) and the Nature Conservancy ($15K). • Field study partners currently include USDI BLM (Spokane, Ely, West Desert Districts, Parashant NM) and USDI USFWS (Hanford Reach NM).

Project Title	Exploring Cheatgrass Biocontrol with the Head Smut Pathogen
Lead Science Program	Grassland, Shrubland and Desert Ecosystems (GSD)
Principal Investigator	Susan Meyer

Describe progress made on Plan of Work

The objective of this research was to learn enough about the *Ustilago bullata-Bromus tectorum* pathosystem to determine whether this pathogen has potential as a safe and effective mycoherbicidal biocontrol agent against cheatgrass. We have completed extensive studies on the ecology and ecological genetics of this pathosystem, and much of this research is either already published or in preparation for submission. We have determined that this organism is unlikely to be effective as a cheatgrass biocontrol agent. Specific requirements for high infection during the narrow infection window at the time of seedling emergence are not regularly met in the field in environments where cheatgrass is a major problem. Numerous field inoculation trials over a seven-year period have failed to result in significant control. We do not anticipate further biocontrol work with this organism.

Describe significant accomplishments and/or products delivered since project initiation

Describe key research to be completed and products to be delivered in the next 3 years

- A peer-reviewed manuscript on the classical genetics of virulence in the *Ustilago bullata - Bromus tectorum* pathosystem will be submitted for publication.
- A peer-reviewed manuscript on host-specificity of *Ustilago bullata* strains from *Bromus tectorum* will be submitted for publication.
- A peer-reviewed manuscript on the comparative physiological ecology of teliospore dormancy and germination for the cheatgrass pathogens *Ustilago bullata* and *Tilletia fusca* (chestnut bunt) will be submitted for publication.
- An annotated draft genome sequence for *Ustilago bullata* will be published online.

Describe key partnerships and funding leveraged (if applicable)

- Original funding for this project came from USDA CSREES NRI for the period 1998-2003 ($375K)
- This project was funded during the period 2000-2009 by the Idaho Army National Guard ($700K)
- Funding for the *Ustilago bullata* genome sequencing project is through the Faculty Undergraduate Mentoring Program at Brigham Young University (2010-2011; $20K)

Project Title	**Understanding the Causes and Consequences of Cheatgrass Die-offs in the Great Basin**
Lead Science Program	**Grassland, Shrubland and Desert Ecosystems**
Principal Investigator	**Susan Meyer**

Describe progress made on Plan of Work
Cheatgrass die-offs are unexplained cheatgrass stand establishment failures over sometimes large areas. For an annual plant such as cheatgrass, this equates to an apparent 'die-off' for at least a year. While the main thrust of this project is to understand the die-off phenomenon and its implications for rangeland health and restoration, it could lead to the identification of potential new cheatgrass biocontrol agents. This project received its first formal funding in FY2011, and the funds are just now being put into place. However, our earlier small-scale studies on this phenomenon positioned us well for obtaining the current funding through an invited proposal from the BLM.

Describe significant accomplishments and/or products delivered since project initiation
• Completed a field study on cheatgrass seed bank dynamics in relation to die-offs. • Completed a greenhouse investigation on the possible role of soilborne pathogens in die-offs. • Submitted an invited proposal for a 5-year study to the BLM and received funding for the first phase of the study.

Describe key research to be completed and products to be delivered in the next 3 years
• Carry out a landscape-scale spatiotemporal analysis of die-off patterns in two geographic areas using remote sensing technology, and use this analysis to infer possible die-off causal agents. • Carry out precision seeding experiments on and off die-offs with both with cheatgrass and with native species that would be used in restoration seedings, to determine whether die-off causal agents are active in die-offs of different ages and whether they impact seeded native species. • Carry out field and laboratory manipulative experiments with candidate soilborne pathogens isolated from die-off soils, to determine whether they are capable of causing stand failure, and if so, under what sets of environmental conditions. • Examine possible die-off causal agents, including insect herbivory, seed predation, and soilborne, airborne and animal-vectored plant disease, using survey techniques. • Present findings to land management groups and prepare manuscripts for publication.

Describe key partnerships and funding leveraged (if applicable)
• Principal investigators on this project include scientists from RMRS, Brigham Young University, Gonzaga University, and the University of Nevada-Reno. Land management cooperators include the BLM West Desert District, Utah and the BLM Winnemucca District, Nevada. • Funding for the project is currently coming from the BLM (Idaho State Office). We have received $275K to carry out the first phase of the research.

Project Title	Efficacy of Weed Biological Control
Lead Science Program	Wildlife and Terrestrial Ecosystems (WTE)
Principal Investigator	Dean Pearson, Yvette Ortega

Describe progress made on Plan of Work
The focal plan of work for this team has been advancing the science of biocontrol by applying ecological principals to improve biocontrol theory and application. Work by this team has changed the paradigm in weed biocontrol. Prior to this research, the emphasis in biocontrol was on host-specificity for minimizing impacts on nontarget species. Work by this team has definitely shown that host-specific biocontrol agents can have severe nontarget effects through food web interactions (e.g., Pearson and Fletcher 2008), even though host-specific agents were previously presumed safe. By applying food-web theory, this team has shown that protecting against indirect nontarget effects requires high efficacy of the agent. Our work is bringing about a profound shift in biocontrol, toward an emphasis on agent efficacy, which is simultaneously leading toward greater safety and success of biocontrol. This team has since gone on to explore the mechanisms underlying agent-host interactions to understand how environmental context influences biocontrol outcomes. This work is beginning to come to fruition (Ortega et al. in review). This team has developed a conceptual framework for weed management, including biocontrol (Pearson and Ortega 2009). This team has also made efforts to change biocontrol policy to improve funding for efficacy and post-release studies in biocontrol (Maron et al. 2010). The team is undertaking significant biogeographical research in the native and multiple invaded ranges for dozens of weed species to better understand causes of biological invasions and improve their management (recently initiated studies).

Describe significant accomplishments and/or products delivered since project initiation
Contributions to biocontrol by this team include 14 publications, mostly in major scientific journals, and dozens of seminars for scientists, managers, and the public across much of the US. This work has also garnered several awards and extensive national and international press (e.g., http://www.rmrs.nau.edu/wildlife/invasives/press.php). Publications from FY 2007 to FY 2011:

1. **Ortega, Y. K., Pearson, D. E.,** Waller, L. P., Sturdevant, N. J., Maron, J. M. Population-level compensation impedes biological control of an invasive forb and indirect release of a native grass. Ecology tentatively accepted
2. Maron, J. L., **D. E. Pearson,** S. M. Hovick, W. P. Carson. 2010. Funding needed for assessments of weed biological control. Frontiers in Ecology and the Environment 8:122-123.
3. **Pearson, D. E.,** and **Y. K. Ortega**. 2009. Managing invasive plants in natural areas: moving beyond weed control, pp 1-21, in (ed.) R.V. Kingley, Weeds: Management, Economic Impacts and Biology. Nova Publishers, NY
4. **Pearson, D. E.,** and R. M. Callaway. 2008. Weed biocontrol insects reduce native plant recruitment through second-order apparent competition. Ecological Applications 18:1489-1500.
5. **Pearson, D. E.,** and R. J. Fletcher, Jr. 2008. Mitigating exotic impacts: restoring native deer mouse populations elevated by an exotic food subsidy. Ecological Applications18 (2):321-334.
6. **Pearson, Dean E.;** Ragan M. Callaway. 2006. Biological control agents elevate hantavirus by subsidizing deer mouse populations. Ecology Letters 9(4): 443-450.

Describe key research to be completed and products to be delivered in the next 3 years

- Secondary invasion in invasive species management (including biocontrol). Cross-program collaborative literature review. (DE Pearson, YK Ortega, JB Runyon, JL Butler – to be submitted for publication 2012).
- Long-term effects of climate change (drought) and the biocontrol agent, *Cyphocleonus achates*, on spotted knapweed and its competitive dominance over bluebunch wheatgrass. (DE Pearson, YK Ortega, NJ Sturdevant – to be submitted for publication 2013).
- Numerous additional cross-program collaborative reviews and several studies could be completed over the next three years, but funding for this work was terminated.

Describe key partnerships and funding leveraged (if applicable)

Funding & Partnership Summary 2007-2011:

- State and Private Forestry – provides consultation and funding ($9,500).
- RMRS Competitive Research Initiative. $127,000.
- Bitterroot Ecosystem Management Research Project $66,800
- Wildlife and Terrestrial Ecosystems $51,500
- RMRS Biocontrol Program funding $141,600
- University of Montana – contributes greenhouses and common garden sites.

Notes and Considerations on this Project's Progress
• This effort has been steadily building momentum over the years and was beginning to integrate into a more synergistic program of work with cross-program efforts involving GSD through the RMRS biocontrol funding (e.g., secondary invasion work under future products). However, RMRS Biocontrol funding for this work was terminated. Hence, future products are dependent on soft funding and uncertain.

Project Title	**Effect of endophytic fungi on cheatgrass (*Bromus tectorum* L.) growth and fecundity**
Lead Science Program	**Grassland, Shrubland and Desert Ecosystems (GSD)**
Principal Investigator	**Rosemary Pendleton**

Describe progress made on Plan of Work

Over 700 fungal endophytes were isolated from 55 cheatgrass populations across western North America. Taxa were characterized via ITS sequences and morphology. In both greenhouse and field trials, select endophytes either increased or decreased the fecundity and biomass of cheatgrass. Endophyte effects were often population-dependent. In the New Mexico population, inoculation with an endophytic isolate of *Morchella* significantly increased cheatgrass fecundity 16-35%. Fire-adapted endophytic mutualisms likely contribute to the maintenance of community domination by cheatgrass, providing a positive feedback mutualism. In contrast, endopytic *Sporormiella,* a dung fungus, decreased fecundity of New Mexico cheatgrass plants. Current research seeks to enhance their relative abundance in cheatgrass-infested fields through the use of prescriptive grazing. Three dung-fungus inoculation techniques were tested with leaf inoculation proving most effective.

Describe significant accomplishments and/or products delivered since project initiation

- Over 700 fungal endophytes were isolated from 55 cheatgrass populations across western North America and characterized via ITS sequences and morphology.

Presentations:

- Joint Meeting of the Mycological Society of America and of the International Symposium on Fungal Endophytes of Grasses. 7th International Symposium on Fungal Endophytes of Grasses. Lexington, KY: June 2010
- Northern Rockies Invasive Plant Council. 2nd Invasive Species in Natural Areas Conference Coeur d'Alene, ID: October 2010

Manuscripts:

- Carta, L.K., A.M. Skantar, Z.A. Handoo, and M.A. Baynes. Supplemental description of *Paraphelenchus acontioides* Taylor and Pillai, 1967 (Tylenchida: Paraphelenchidae) with ribosomal DNA trees, and a morphometric compendium of females of the species of *Paraphelenchus*. Nematology. *(accepted*)

Describe key research to be completed and products to be delivered in the next 3 years

- Locate suitable sites and install enclosures for grazing trials
- Conduct choice trials with sheep to determine if there is a preference for endophyte-infected cheatgrass.

Manuscripts:

- Baynes, M.A., G. Newcombe, L. Dixon, L. Castlebury, and K. O'Donnell. A novel plant-fungal mutualism associated with fire. Science (*not yet submitted; out for pre-submittal review).*
- Baynes, M.A., D.A. Russell, G. Newcombe, L.K. Carta. Nematodes influence endophytic community assemblage in cheatgrass (*Bromus tectorum* L.) (*working on submittal to Microbial Ecology by end of year*)
- Baynes, M.A., **R. Pendleton**, and G. Newcombe. Effects of select endophytes on growth and fecundity of cheatgrass (*Bromus tectorum* L.): field trials. (*in progress*)

Describe key partnerships and funding leveraged (if applicable)
University of Idaho in-kind contributions: salary, land, fencing, sheep >$20,000 annually USDA-ARS US National Fungus Collections: sequencing of 300+ isolates $15,000 Institute of Microbiology Fungal Reference Centre: sequencing of 30+ isolates $1,500

Notes and Considerations on this Project's Progress
This was begun as a three year project. Second year funding was reduced (FY11) and third year funding (FY12) is highly questionable.

Project Title	Advancing biocontrol of weeds through chemical ecology
Lead Science Program	Grassland, Shrubland and Desert Ecosystems (GSD)
Principal Investigator	Justin Runyon

Describe progress made on Plan of Work

This goal of this work is to improve biocontrol of invasive plants by examining the chemical ecology of agent-weed interactions to better understand why some agents successfully reduce populations of weeds whereas others do not. Plant-insect chemical ecology has contributed much to agriculture, but has not previously been applied to biocontrol of weeds.

This research encompasses several projects including (1) measuring the role of costly induced plant defenses in successful biocontrol, (2) identifying and comparing characteristics of plant-insect interactions between successful and unsuccessful agents, and (3) examining chemically-mediated interactions between multiple biocontrol agents on a shared weed species. All of these projects are underway, but progress has been stalled by the renovation of the Bozeman Lab. However, the renovation will be complete in August 2011 and will provide a top-notch facility in which to conduct this research. I have spent much time working to purchase the required supplies and equipment; some of the larger purchases include: (1) gas chromatograph and mass spectrometer, (2) walk-in growth chamber, (3) in-house plant-insect volatile collection system, (4) portable volatile collection systems for use in the field, and (4) an ultra-low freezer to properly store samples. A collaborator at Montana State University was identified and a JVA established to better complete this work.

Consultations, identification of field sites, and collection of field data was conducted in 2009-2011 in the Gallatin, Kootenai (MT) and Boise (ID) National Forests. Successful rearing of greenhouse colonies of insects and cultivation of plants have been determined and studies will be completed with the addition of laboratory data to be added once the Bozeman renovation is complete.

Describe significant accomplishments and/or products delivered since project initiation

I was hired in September 2008 and was directed to spend much of the first year wrapping up previous research (data analysis, writing, etc.). Four peer-reviewed papers on the chemical ecology on invasives resulted from this work:

1. **Runyon, Justin B.,** Mark C. Mescher, Consuelo M. De Moraes. 2006. Volatile Chemical Cues Guide Host Location and Host Selection by Parasitic Plants. Science 313: 1984-1967 (Refereed).
2. Mark C Mescher, **Justin B Runyon**, and Consuelo M De Moraes. 2006. Plant Host Finding by Parasitic Plants A New Perspective on Plant to Plant Communication. Plant Signal Behav. 1(6): 284–286.

3. **Runyon, Justin.** 2008. The cingulum: A unique structure of some Dolichopodidae. Fly Times 41: 3.
4. **Runyon, Justin B.,** Mark C. Mescher, and Consuelo M. De Moraes. 2008. Parasitism by *Cuscuta pentagona* Attenuates Host Plant Defenses against Insect Herbivores. Plant Physiology 146: 87–995.
5. **Justin B. Runyon.** 2008. New Species of Long-Legged Flies (Diptera: Dolichopodidae) from Central Pennsylvania. Proceedings of the Entomological Society of Washington 110(2):363-373.
6. Weaver, D. K., M. Buteler, M. L. Hofland, **J. B. Runyon**, C. Nansen, L. E. Talbert, P. Lamb, and G. R. Carlson. 2009. Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. *Journal of Economic Entomology* 102: 1009-1017. (Refereed).
7. **Runyon, J. B**., J. F. Tooker, M. C. Mescher, and C. M. De Moraes. 2009. Parasitic plants in agriculture: chemical ecology of germination and host location as targets for sustainable control: a review, pp. 123-136. *In* E. Lichtfouse (ed.) *Organic Farming, Pest Control and Remediation of Soil Pollutants,* Sustainable Agriculture Reviews 1. (Refereed).
8. **Runyon, J. B.,** M. C. Mescher, and C. M. De Moraes. 2010. Plant defenses against parasitic plants show similarities to those induced by herbivores and pathogens. *Plant Signaling and Behavior* 5: 929-931. (Refereed).
9. **Runyon, J. B.,** M. C. Mescher, G. W. Felton, and C. M. De Moraes. 2010. Parasitism by *Cuscuta pentagona* sequentially induces JA and SA defence pathways in tomato. *Plant, Cell and Environment* 33: 290-303. (Refereed).

Additional contributions include at least a dozen seminars for scientists and managers about this work.

Describe key research to be completed and products to be delivered in the next 3 years

- Complete and publish research on role of induced chemical defenses in successful biocontrol of houndstongue.
- Complete and publish results of chemically-mediated changes induced in host weeds from first set of biocontrol agents (this will probably be done by weed species starting with rush skeletonweed then tansy ragwort; other invasive species will follow).
- A literature review on the underappreciated application of chemical ecology to biocontrol of weeds has begun and should be submitted in 2012.

Describe key partnerships and funding leveraged (if applicable)

- Gallatin National Forest – provides field sites and consultation
- Forest Health Protection (Regions 1 and 4) – provides field sites and consultation
- Montana State University – provides access to facilities and consultation
- National Park Service – provides field sites and research permits

- USDA-APHIS – provides consultation and permits

Notes and Considerations on this Project's Progress
Renovation of the Bozeman lab has prevented the completion of much of this work. Because of the renovation the scientist has been unable to set up a functional lab or use equipment purchased with biocontrol dollars. All research requiring use of the labs and equipment has been delayed until the renovation is completed in August 2011.

Project Title	Classical Biocontrol of Invasive Plants
Lead Science Program	Grassland, Shrubland and Desert Ecosystems (GSD)
Principle Investigator	Justin Runyon

Describe progress made on Plan of Work
This report covers non-chemical ecology research on classical biocontrol of several invasive plants, most notably rush skeletonweed and tansy ragwort. This work has two main components: (1) foreign exploration and testing for new biocontrol agents, and (2) establishment and evaluation of already approved agents. A network of domestic and foreign partners has been established to work on exploration and evaluation of potential biocontrol agents for rush skeletonweed. This work is being conducted in Armenia, Russia, and Turkey as well as in the quarantine facility at MSU and focuses on several root-feeding insects. A root crown-feeding moth is presently undergoing evaluation in the MSU quarantine, whereas a root-boring beetle is undergoing scrutiny in eastern Europe by cooperators. This overseas work has depended on RMRS Biocontrol funding to date. However, RMRS funding was terminated and future progress is dependent on soft funding and uncertain (finding soft dollars to support foreign work in this economic climate is extremely difficult). Several projects are underway evaluating the efficacy and establishment of several approved biocontrol agents of rush skeletonweed and tansy ragwort. One project examines the single and combined efficacy of two biocontrol insects of tansy ragwort in Montana (i.e. what is the best recipe of agents to use and where?); a second examines the biology and efficacy of a newly established root-feeding moth of rush skeletonweed in Idaho. With the loss of RMRS biocontrol funding, I am presently searching for funding to complete these projects.

Describe significant accomplishments and/or products delivered since project initiation
Graduate students supported with RMRS biocontrol funds: Maier, Crystal A. 2010. Taxonomic studies in the metallic wood-boring beetle family (Coleoptera: Buprestidae: Sphenoptera and Chalcophora). Master's of Science in Entomology Thesis, Montana State University, Bozeman. xxvi +219 pp. Crystal's work resolved several outstanding taxonomic issues in this group that includes potential biocontrol beetles of rush skeletonweed. Crystal presented an award-winning poster at the 2009 Entomological Society of America meeting in Reno, NV. A paper from her RMRS-supported work is forthcoming.

A related publication from long-term data on the impacts of forest restoration treatments and roads on invasive plant distribution was recently published:

Birdsall, J. and W. McCaughey, and **J.B. Runyon**. 2011. Roads impact the recruitment and distribution of noxious weeds more than restoration treatments in a lodgepole pine forest in Montana, USA. *Restoration Ecology,* DOI: 10.1111/j.1526-100X.2011.00781.x

Describe key research to be completed and products to be delivered in the next 3 years

- Finish data collection and publish results on efficacy of a newly established rush skeletonweed root moth in Idaho
- Publish research on landscape scale management of leafy spurge along the Smith River corridor in Montana (with George Markin and Jennie Birdsall)
- Complete biology and management manual for tansy ragwort (funded by Forest Health Technology Enterprise Team)

Describe key partnerships and funding leveraged (if applicable)

- Russian Biocontrol Group (St. Petersburg)
- Biotechnology and Biological Control Agency (BBCA, Rome, Italy)
- Montana State University and quarantine (JVAs with Dr. Jeff Littlefield and Dr. Michael Ivie)
- Montana Department of Agriculture. Over the past 3 years we have obtained $25,000 from Montana Department of Agriculture's Border Project to support this work.
- FHP (Regions 1 and 4)
- BLM (Idaho)
- National Park Service

Notes and Considerations on this Project's Progress

Foreign exploration for biocontrol agents is collaborative and lengthy process, typically taking several years of research to get the most promising agents into a US quarantine facility for further testing.

Project Title	**Improving efficacy and safety to optimize and increase implementation of successful classical weed biological control on western US rangelands and forests.**
Lead Science Program	**Grassland, Shrubland and Desert Ecosystems (GSD)**
Principal Investigator	**Sharlene Sing**

Describe progress made on Plan of Work
• Long-term monitoring plots and transects on a wide range of biological control release sites have been established and data has been collected from them over multiple years. Monitoring is being conducted to: 1) evaluate the efficacy and developing recommendations for improving biological control of Dalmatian, yellow and hybrid toadflax invading burned or otherwise disturbed rangeland; 2) determine the potential for integrating biological control and herbicide treatments to manage invasive toadflax; 3) evaluate vegetation community responses following biological control of invasive toadflax; and 4) determine, through a meta-analysis of monitoring plot/transect data, if there are specific and consistent biotic and abiotic site characteristics that are correlated with successful establishment of *Mecinus janthinus*. • Naturally- occurring hybridization between Dalmatian (*Linaria dalmatica*) and yellow (*L. vulgaris*) toadflax has been confirmed following field observations and sample collections, manipulated plant crosses and molecular diagnoses. Herbivorous insects respond to their host plants both behaviorally (in terms of host location and acceptance) and biologically (in terms of reproductive success). Further related investigations will help us understand if shortfalls in realized biological control of exotic toadflax can be conclusively attributed to differential responses of toadflax biocontrol agents to host plant genotype. • RMRS led a collaborative effort to develop a host plant test list for screening candidate agents of invasive toadflax that was recently submitted for review to USDA APHIS PPQ. The test list provides guidance to foreign and domestic researchers participating in host specificity testing of candidate biological control agents. Agents are identified and collected from their native range, then preliminarily tested through a cooperative partnership between members of the North American Toadflax Biocontrol Consortium and the foreign exploration experts at CABI Switzerland. • RMRS conducted host specificity tests in the Montana State University quarantine facility on *Rhinusa pilosa*, the yellow toadflax stem-galling weevil, to ascertain response to naturally-occurring and manipulated hybrids of Dalmatian x yellow toadflax. Information

regarding candidate agent response to the genetic types of the target weed is required by USDA APHIS PPQ in applications for permits to release new biocontrol agents. Test insects were supplied by CABI and by our consortium partners at Agriculture and Agri-Food Canada, in Lethbridge, Alberta, Canada. Guidance on permit applications and hand-carrying agents into the country provided by APHIS PPQ Montana, and use of the MSU quarantine facility have been essential to the success of this work.

- Provided tech transfer support in terms of annual workshops to demonstrate biocontrol agent collection techniques and monitoring methods with federal, state and private stakeholders. Our program has also collected *M. janthinus* from yellow toadflax (one of few known sites where the agent has become established on this target weed species in North America); adult weevils have been subsequently shipped directly or through APHIS PPQ to cooperators throughout Montana, Colorado, Utah, Oregon, Idaho, Minnesota, North and South Dakota, and Wyoming.
- RMRS provides national agency representation on the Technical Advisory Group (TAG) to USDA-APHIS-PPQ on Biological Control of Weeds. TAG members are charged with representing the collective interests of their specific federal agency during APHIS-PPQ's review of petitions for the release of new weed biocontrol agents. In that context, TAG members are required to become well-informed on agent and target weed biology and ecology, and to evaluate the safety of releasing agents against potential immediate and longer range risks to U.S. agriculture and to consider wider ecosystem consequences of releasing the agent.

Describe significant accomplishments and/or products delivered since project initiation

Peer-reviewed publications:

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.

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

outstanding paper 2009)

Schleier, Jerome J., III; **Sing, Sharlene E.**; Peterson, Robert K. D. 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, Sharlene E.; Arbogast, Richard T. 2008. Predatory response of *Xylocoris flavipes* to bruchid pests of stored food legumes. *Entomologia Experimentalis et Applicata*. 126: 107-114.

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.

Pariera-Dinkins, C.L., S.K. Brumfield, R.K.D. Peterson, W.E. Grey, and **S.E. Sing**. 2007. Dalmatian toadflax (*Linaria dalmatica*): New host for cucumber mosaic virus. Weed Technology 21: 41-44.

Jacobs, J.S., **S.E. Sing** and J.M. Martin. 2006. Influence of herbivory and competition on invasive weed fitness: observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environmental Entomology 35: 1590-1596.

Sing, S.E., R.K.D. Peterson, D.K. Weaver, R.W. Hansen, and G.P. Markin. 2005. A retrospective analysis of known and potential risks associated with exotic toadflax-feeding insects. Biological Control 35: 276-287.

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.

Extension publications:

Jacobs, Jim; **Sing, Sharlene**. 2009. Ecology and management of tansy ragwort (*Senecio jacobaea* L.). Invasive Species Technical Note MT-24. Bozeman, MT: U.S. Department of Agriculture, Natural Resources Conservation Service. 13 p.

Jacobs, Jim; **Sing, Sharlene**. 2008. Ecology and management of diffuse knapweed (*Centaurea diffusa* Lam.). Invasive Species Technical Note No. MT-20. Bozeman, MT: U.S. Department of Agriculture, Natural Resources Conservation Service. 12 p.

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 houndstongue (*Cynoglossum officinale* L.). USDA NRCS Invasive Species Technical Note No. MT-8 January 2007. 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 November 2006. 9 pp.

Jacobs, J.S. and **S.E. Sing**. 2006. Ecology and management of Dalmatian toadflax (*Linaria dalmatica* [L.] Mill.). USDA NRCS Invasive Species Technical Note No. MT-3 July 2006. 9 pp.

Peterson, R.K.D. and **S.E. Sing**. 2007. Releasing biocontrol agents: Risk assessment and overdue reform. International Organization for Biological Control of Noxious Animals and Plants - Nearactic Regional Section Newsletter. Volume 29, Number 2 Summer 2007.

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. 116 pp.

Proceedings:

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Describe key research to be completed and products to be delivered in the next 3 years

- Determine potential biocontrol efficacy of naturally-occurring, host-associated yellow toadflax (YT), Dalmatian toadflax (DT) or YT x DT hybrid toadflax (HT) biotypes of the toadflax stem mining weevil *Mecinus janthinus* (*MJ*-YT; *MJ*-DT; *MJ*-HT) against infestations of toadflax populations by examining behavioral responses (host finding, feeding, mating, oviposition) on a range of host plant genotypes.
- Determine invasive potential of Dalmatian x yellow toadflax hybrids by measuring and comparing phenotypic traits contributing to invasiveness in F1, F2, BC1 and field-collected hybrid plants and the two parent species grown in a common garden study replicated in Bozeman, MT and Fort Collins, CO.
- Determine if hybrid toadflax susceptibility to specific herbicides is similar to levels reported for the relatively susceptible parent species Dalmatian toadflax vs. the highly tolerant parent species yellow toadflax.
- Develop chloroplast and SSR markers to unambiguously delineate yellow, Dalmatian and hybrid toadflax.
- Participate through partnerships with international biocontrol consortia in the identification and host specificity testing of candidate agents for invasive toadflax, oxeye daisy and Russian olive.

Describe key partnerships and funding leveraged (if applicable)

Funding for collaborative research from non-RMRS sources:

Sarah Ward (Colorado State University, Dept. Crop and Soil Sciences):

7/2010-12/2011($25, 061) USDA CSREES Western Regional IPM: Assessing long-term impacts of yellow toadflax invasion.
07/2008 - 06/2011 ($84,000) Colorado Dept. of Agriculture: Invasive potential of toadflax hybrids in Colorado.

07/2007 - 06/2010 ($16,000) Colorado Agricultural Experiment Station: Hybridization between invasive populations of Dalmatian toadflax and yellow toadflax

David Weaver (Montana State University, Department of Land Resources and Environmental Sciences):

05/2008-09/2009 ($31,080) Montana Noxious Weed Trust Fund: Survey of arthropods on invasive toadflax.

05/2009-09/2010 ($18,200) Montana Noxious Weed Trust Fund: Evaluation of invasive toadflax, identified hybrids and arthropod fauna.

05/2010-09/2011 ($43,500) Montana Noxious Weed Trust Fund: Efficacy of new yellow toadflax biological control agents.

05/2011-09/2012 ($39,250) Montana Noxious Weed Trust Fund: Optimizing establishment of new yellow toadflax biocontrol agents.

- 05/2011-09/2012 ($25,000) Montana Noxious Weed Trust Fund: Identifying and testing candidate agents for Russian olive biocontrol.

Tracy Sterling (Montana State University, Department of Land Resources and Environmental Sciences):

07/2011-07/2012 ($28,000) USFS Forest Health Protection Special Technology Development Program: STTRIP: student technology transfer research internship program.

Dan Bean (Colorado State Department of Agriculture):

07/2010-12/2012 ($65,955 total - $40,065 to Bean in a JVA, details below) USFS Forest Health Protection Special Technology Development Program: Assessing the potential for *Mecinus janthinus* (*ex Linaria vulgaris*) to improve the efficacy and increase the implementation of yellow toadflax biological control.

David Weaver and **Sarah Ward**:

01/2012-12/2014 ($415,604 – pending) USDA NIFA AFRI: Insect agents for hybrid weed biocontrol: The influence of host plant genotype and semiochemicals on specificity of host biotypes of a toadflax stem mining weevil.

David Weaver, **Dan Bean** and **Nancy Gillette** (USFS PSWRS Berkley, CA):

05/2011-09/2012 ($15,000) USFS Forest Health Protection Special Technology Development Program: “Chem herding” – adapting existing semiochemical delivery technologies to improve the precision of weed biocontrol agents.

Rob Progar (USFS PNWRS LaGrande, OR) and **Joseph Milan** (BLM Boise, ID):

06/2009-09/2011 ($133,246 total - $128,000 to Sing) USFS Forest Health Protection Special Technology Development Program: Solving the toadflax puzzle – utilizing *Mecinus*

janthinus insectaries on co-mingled stands of Dalmatian and yellow toadflax and their hybrids to improve yellow toadflax biocontrol.
06/2010-09/2012 ($97,000) USFS Forest Health Protection Special Technology Development Program: Post-wildfire releases of *Aphthona* spp. flea beetles and *Mecinus janthinus* as "biological herbicides" on leafy spurge and Dalmatian toadflax.

Collaborative research supported through RMRS Joint Venture Agreements:

Jennifer Andreas - Washington State University (07-JV-11221609-276; 08/13/2007-08/01/2012) ($50,455-Markin; $5,000-Sing).

- Title: Studies of the gall mite, *Aceria genistae*, as a potential biological control agent of Scotch broom.

Summary: The establishment of *Aceria genistae*, an adventitiously introduced eriophyid gall mite of the weedy shrub Scotch broom (*Cytisus scoparius*), was initially reported from the Tacoma, WA area in 2005. This discovery was followed up by a regional survey to determine the full extent of the mite's western North American distribution. Host specificity was also assessed to satisfy regulatory requirements for anticipated inter-state distribution of the mite. Protocols for transferring the mite to uncolonized areas were also developed. Uncertainties arising with regard to the mite's identity have necessitated investigation beyond the scope of the original project. The cooperator has been very effective in involving molecular and morphological taxonomists to resolve this issue. Further field collections and observations were planned for 2010-2011 following field reports of galls on a non-target host (and species of concern), *Lupinus densiflorus*. Following a discussion of logistical and regulatory issues related to monitoring and evaluating *L. densiflorus* individuals affected by putative *A. genistae* s.l. on Victoria Island, British Columbia, Canada, Canadian researchers volunteered to assist the cooperator with the collection of samples and field data, and offered to provide quarantine space in their Lethbridge, Alberta, Canada facility, if necessary. The source of galls on the non-target host cannot be determined until taxonomic experts can provide an accurate method for distinguishing between multiple closely related legume galling mites established in the study area. Final results of this collaborative research are therefore pending.

Dan Bean – Colorado Department of Agriculture (10-JV-11221632-254; 07/31/2010-12/31/2012) ($40,065-Sing; pass through USFS Forest Health Protection).

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

Summary: Funds were provided to RMRS through a USFS Forest Health Protection competitive grant to facilitate FHP's program delivery by objectively evaluating the post-release efficacy and operational feasibility of using classical biological control to manage

yellow toadflax on forested and range lands with a biotype of the toadflax stem mining weevil, *Mecinus janthinus*, that had recently become established on yellow toadflax in western Montana. The cooperator manages a beneficial insectary facility for the Colorado Department of Agriculture and therefore provides considerable technology transfer expertise for improving understanding of the biology of *Mecinus janthinus* (*ex Linaria vulgaris)*, a potential biocontrol agent for yellow toadflax, and for developing and disseminating effective mass-rearing protocols for this agent. Field monitoring at release sites throughout Montana and Colorado will help us to determine if, under a wide range of field conditions, *M. janthinus* ex. *L. vulgaris* functions as 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.

CABI Switzerland (10-IJ-11221632-226; 07/07/2010-08/13/2012) ($30,000-Sing).

- Title: Identification, collection, rearing and initial host-specificity screening for potential weed biological control agents of the invasive, exotic weeds yellow and Dalmatian toadflax, and oxeye daisy.

Summary: CABI Europe-Switzerland (CABI E-CH) is a world leader in biological control research with an impressive international track record of discovering and safety testing biocontrol agents and assisting overseas collaborators with many aspects of initial associated technology transfer. CABI E-CH has been working on invasive weed biological control projects since 1947 and has played a vital role in the development of an International Code of Conduct on the use of biological control. A classical biological control program for yellow and Dalmatian toadflax was initiated in the 1980s in response to the continuous rise in toadflax-infested public and private forest and rangeland acres throughout western North America. CABI E-CH has since collected and screened ten potential biological control agents from the weeds' native range. Between 1991 and 2001, following extensive testing, five of these candidate agents proved to be sufficiently host-specific to be approved for release in North America. The stem mining weevil *Mecinus janthinus* was first released in 1994 and is now successfully controlling Dalmatian toadflax populations in northwestern U.S and Canada. However, an equally effective biological control agent is still needed for yellow toadflax. The North American toadflax biocontrol consortium currently prioritizes CABI E-CH research goals and supports their work through participating member organizations such as the Ministry of Forest and Range, British Columbia; the Wyoming Biological Control Steering Committee; the Californian Department of Food and Agriculture; USDA-APHIS-CPHST; and more recently, the U.S. Forest Service.

A classical biological control project for oxeye daisy (*Leucanthemum vulgare*) has been underway since 2008. An informal survey of Forest Service invasive species managers and botanists in the northwestern U.S. suggests that oxeye daisy is a compelling target for classical biological control. Oxeye daisy is highly competitive, readily invading large tracts in remote and pristine locales, and as a result significantly reduces the quality and quantity of forage while posing a considerable threat to native species biodiversity. Exploration of management alternatives such as biocontrol are warranted due to the high cost and unsatisfactory outcome of conventional, herbicide-based management of oxeye daisy. Following an extensive literature review and native range field surveys by CABI E-CH researchers, several potential biological control agents have now been prioritized for further evaluation. CABI E-CH has already established a rearing colony and begun host-specificity and target impact tests with one species, a root-mining moth, *Dichrorampha aeratana*. In addition, CABI E-CH has coordinated a comprehensive collection of plant samples for the purposes of gaining a better understanding of the genetic variation of oxeye daisy in both the native and introduced ranges, and of the taxonomic status of closely related test plant species. The U.S. Forest Service's interest in this agreement is to facilitate the development and delivery of the scientific knowledge, technology, and tools that will increase the implementation of safe and effective classical biological control of invasive toadflax and oxeye daisy.

CABI scientists will survey the native range to find and collect candidate agents, develop rearing protocols and increase knowledge of their biology and ecology. Following extensive and rigorous testing, CABI will provide essential documentation via their research results that will support petitions submitted to USDA APHIS PPQ for approval to make field releases of the agent(s) in North America. The release of safe and effective classical weed biological control agents will make a significant contribution to sustainable and cost-effective integrated management programs for yellow and Dalmatian toadflax, and oxeye daisy invading public and private forested and range land.

Fritzi Grevstad - University of Washington (07-JV-11221609-281; 07/16/2007-09/30/2011) ($138,848-Markin; $25,000-Sing).

- Title: Determining the suitability of candidate biocontrol agents for gorse.

Summary: Gorse is a spiny evergreen shrub introduced into North America from Europe in the late 1800s that is presently listed as a class B noxious weed in Washington, Oregon, California, and Hawaii. It is most problematic in the Pacific Coastal zones, where it forms dense, impenetrable thickets in dunes, coastal bluffs, riverbeds, recreational lands, and forests. It out competes native plants and impedes access for humans and larger wildlife. In cleared areas, gorse grows faster than most timber trees and must be completely

removed from a site before planting new trees. Where it grows in the understories of established forests, gorse interferes with cultural operations, increasing pruning and thinning costs. The sharp stiff spines on larger plants make it intolerable as fodder for cattle as well as wild ungulates. Fields that are densely infested with gorse can no longer be used for foraging. With a high oil content in its leaves and stems, gorse is a severe fire hazard in shrub lands, forests, and residential communities. In 1936, the entire town of Bandon, Oregon was decimated by a gorse-fueled wildfire. The economic cost of gorse due to losses in production of pastures and timberland to be $997,000 per year within the state of Oregon alone. This does not take into account the immeasurable losses due to recreational, wildlife, and aesthetic impacts. The unique ocean spray meadows frequently invaded by gorse in Washington, Oregon, and California are home to many federally threatened and endangered animals including the Oregon silver-spot (*Speyeria zerene hippolyta*), the Mission Blue (*Icaricia icarioides missionensis*), the San Bruno elfin (*Callophrys mossii bayensis*), the Point Arenas mountain beaver (*Aplodontia rufa nigra*), and the western snowy plover (*Charadrius alexandrinus nivosus*). In Canada, gorse threatens the unique Garry Oak ecosystem on southern Vancouver Island. Control of gorse is particularly difficult because it spreads rapidly and re-sprouts from seeds and roots following cutting, fire, or chemical treatment. Seeds remain viable for 30 years in the soil, with one report of viability after 70 years. The plant frequently infests sensitive habitats or rugged terrain where herbicide applications and other treatments are difficult or restricted. Although traditional control methods have been used successfully at some sites, they have been too costly to apply at a regional scale and gorse has continued to spread. Two promising biological control insects, previously introduced into Hawaii, New Zealand, and Australia for gorse control, have not yet been introduced to the mainland United States. These are *Agonopterix umbellana* (formerly *A.ulicitella*), a tip feeding moth and *Sericothrips staphylinus*, a sap feeding thrips. Much of the required host specificity testing for these insects has already been completed in the earlier biocontrol programs. However, additional testing of native and economically important North American plant species is required to ensure that these insects would be safe to release here. The primary purpose of this project was to complete host specificity testing for these two insects and to prepare a petition to release them as biological control agents for review by the Technical Advisory Group (TAG) to USDA-APHIS-PPQ on Biological Control of Weeds.

Bob Peterson – Montana State University (11-JV- *agreement number not assigned yet*; 08/30/2011-12/31/2013) ($35,812-Sing).

- Title: Influence of toadflax genotype and biocontrol agent biotype on plant primary physiological responses: A mechanistic approach to improving agent screening and enhancing impact.

Summary: The efficacy of classical biological control of invasive weeds by insect herbivores can be inconsistent and unpredictable, especially when compared to herbicide treatments or other conventional weed control strategies. Improving the overall success and safety of weed biocontrol depends on adopting a more mechanistic approach to identifying and screening candidate agents. Understanding how herbivores affect plants and under what conditions injury to individual plants translates into population level impacts can help to identify insect species that are more likely to be effective as weed biological control agents. Previous collaborative work by RMRS and MSU confirmed that specialized herbivory by approved classical weed biocontrol agents differentially affects primary physiological responses of the exotic invasive rangeland weed Dalmatian toadflax, *Linaria dalmatica* (L.) Miller, according to herbivore treatment density and species or age-stage correlated feeding injury. Herbivorous insects are known to influence plant population dynamics by affecting plant growth, root-to-shoot ratios, reproductive rates, and primary physiology. Plant gas exchange processes such as photosynthesis, transpiration, and respiration represent a subset of plant primary physiological processes. Understanding how insect injury influences these parameters is important because such primary processes determine plant growth, development, and, ultimately, fitness.

The impact of stem mining injury on photosynthesis has been examined through plant growth chamber and greenhouse studies on agricultural species, and field studies in feral systems. The results of a previous collaborative RMRS-MSU study showed that the photosynthetic rates of injured plants were 17 to 39% lower (depending on field site and year) than uninjured plants in a replicated, but un-manipulated study of the effects of larval stem mining by the toadflax stem-mining weevil, *Mecinus janthinus* Germar, on the photosynthetic rate of *L. dalmatica*. Garden and field experiments were subsequently conducted to evaluate the impact of four levels of herbivore pressure by *M. janthinus* on gas exchange in *L. dalmatica*. Photosynthesis, conductance, and transpiration were measured throughout the growing season. Both garden and field experiments indicated that plant gas exchange rates were affected by increasing herbivore treatment density and age-stage correlated feeding injury, suggesting that further investigation of primary physiological impairment with intensified herbivore pressure is warranted.

Anecdotal reports suggest that hybridization among *Linaria* species, fairly common in their European native range, apparently occurs less frequently in invasive North American populations. Hybridization between yellow and Dalmatian toadflax has now been confirmed from multiple field sites in Montana and Idaho via molecular diagnostic techniques. Host plant hybridization potentially generates unanticipated incompatibilities between biocontrol agents and their target plants. Novel hybrid host genotypes arising in the invaded range have no shared evolutionary history with biocontrol agents from the

native range. Targeting the geographical area of origin of an invasive plant in the search for candidate biocontrol agents assumes that plant genotypes to which specialist insect herbivores are locally adapted will also be found in invading populations. Host plant hybridization in the invaded range violates this assumption and may explain biocontrol failure where agents have performed poorly on hybrid forms of their target host plant. Hybridization has the potential to alter host plant morphology, physiological processes, phenology, physical and chemical defenses, and habitat tolerances and presents both adaptive challenges and benefits for specialized herbivores such as weed biocontrol agents.

The overall objective of the proposed study is to experimentally determine how the intensity of age-stage linked herbivore pressure, i.e., adult weevil folivory vs. larval stem mining, separately or in combination, influences measureable correlates of host plant primary metabolism. The potential for differential physiological responses to these herbivore treatments, as influenced by host plant genotype (parental species *L. dalmatica* and *L. vulgaris* vs. hybrids), will be comprehensively evaluated. Additionally, impacts of herbivory by divergent, genetically distinct host-associated biotypes of *M. janthinus* (*MJ*-DT vs. *MJ*-YT) on the primary physiology of *L. dalmatica*, *L. vulgaris* and their hybrids will be compared and contrasted.

Sarah Ward – Colorado State University (10-JV-11221632-225; 07/07/2010-08/31/2012) ($29,620-Sing).

- Title: Evaluation of invasive potential and management options for hybrid toadflax populations.

Previous collaborative work has confirmed that invasive populations of yellow toadflax and Dalmatian toadflax are spontaneously hybridizing, and that hybrid toadflax populations are developing at sites co-invaded by these introduced species. Further work is needed to determine the frequency and composition of these hybrid toadflax populations, and how their presence affects biocontrol and chemical management options for toadflax invasion on public lands. A specific goals of the collaborative project will be to determine the invasive potential of Dalmatian x yellow toadflax hybrids by measuring and comparing phenotypic traits contributing to invasiveness in F1, F2, BC1 and field-collected hybrid plants and the two parent species grown in a common garden study replicated in Bozeman, MT and Fort Collins, CO. Garden and field experiments will be conducted to determine if hybrid toadflax susceptibility to specific herbicides is similar to levels reported for the relatively susceptible parent species Dalmatian toadflax vs. the highly tolerant parent species yellow toadflax. Clear identification of the management target is essential for effective prescription of control measures; therefore, the cooperator's research group

is currently developing chloroplast and SSR markers that will be used to unambiguously delineate yellow, Dalmatian and hybrid toadflax.

Notes and Considerations on this Project's Progress

Productivity associated with planned basic research (agent behavioral responses to host plant genotype) has been significantly reduced by facilities renovations that have been delayed, indefinitely postponed or cancelled.

Delayed facilities renovations have necessitated the cancellation of planned research with outside collaborators and the loss of potential probe data may have reduced funding success.

Poor condition of existing greenhouse facility has delayed and ruined experiments.

Foreign travel restrictions in FY2011 forced the cancellation of planned molecular diagnostics training and native range agent and host plant observations and collections with a world renowned toadflax biocontrol research collaborator in Serbia. Prospects for rescheduling this trip are uncertain. Methods learned and information gathered during this trip would have greatly enhanced self-sufficiency of the project and reduced reliance on and therefore funding outlay to North American collaborators for routine diagnostics.

Attendance and participation in local, national and international conferences has significantly enhanced collaborative research opportunities, and has been essential in the development of new research projects.