Biological Control of Invasive Plants FY2014

1. Title: Host Specificity Testing of the Chondrilla crown moth, *Oporopsamma wertheimsteini*, for the Biological Control of Rush Skeletonweed

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3. Cooperators:
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4. Amount Requested:
We are requesting $62,530 (Yr1:$ 36,316, Yr2: $ 21,716, Yr3:$ 4,498) over three years; with cost share of $20,841.

5. Project Goal and Supporting Objectives:
Potential biocontrol agents of invasive weeds must undergo host specificity testing before they can be approved for use in the United States. Host specificity testing for the Chondrilla crown moth, *Oporopsamma wertheimsteini*, has been initiated and no-choice tests are nearly completed. Further tests are needed to delineate non-target feeding on several plant species, as well as testing supplemental plant species that have been difficult to grow or not available during the initial tests.

Our objectives are:

1) Complete host specificity studies of *Oporopsamma wertheimsteini*; and
2) Determine the role of plant volatiles in the selection of host plants by *Oporopsamma wertheimsteini.*

6. Project Justification/Urgency:
Rush skeletonweed (*Chondrilla juncea*) (RSW) (Asteraceae) is a widespread, invasive weed of Eurasian origin found through the semiarid areas of western United States. It is a long lived perennial with an extensive root system and, once established, outcompetes and replaces desirable forbs, grasses, and shrubs (Whitson et al. 1991, Holm et al. 1997). The worst RSW infestations are on private, National Forest, and BLM lands with reported infestations of over 6.2 million ac. (2.5 million ha) (Sheley et al. 1999). Infested land types include roadsides, railways, rangelands, pastures, grain fields, coastal sand dunes, and shaley hillsides in mountainous regions (Reed 1979; Sheley and Hudak 1995). Infestations of RSW continue to expand as much as 41,000 ha per year (Spollen and Piper 1995, Kinter et al. 2007), and this weed has recently spread to Arizona, British Columbia, Montana, Nevada, South Dakota, Utah and Wyoming. Because of the large area infested, high rate of dispersal, and difficulties in the use of herbicides (e.g., inaccessible infestations, variable susceptibility of populations to herbicides, low economic value), effective biological control is needed to economically combat RSW.

A biological control program was implemented for rush skeletonweed during the late 1970's in an attempt to manage infestations of this weed and limit its spread into new areas. Three agents were established in Idaho and other areas of the West but have only provided limited control of the weed (Supkoff et al. 1988; Julien and Griffiths 1998). One additional agent, the root-feeding moth *Bradyrrhoa gilveolella* (Lepidoptera, Pyralidae), was approved for release in 2001 and is currently established at a limited number of sites in Idaho and Oregon (Littlefield MSU per comm.). In the mid-1990's, a renewed effort to find and introduce a new complex of biocontrol agents to North America was undertaken. This was a cooperative project between the USDA Forest Service, Rocky Mountain Research Station, Montana State University, and the Idaho State Department of Agriculture (cost sharing program), Montana Department of Agriculture and USDA ARS European Biological Control Laboratory and other overseas cooperators. From 2001 to 2003, the USFS and cooperators also inventoried the other natural enemies of rush skeletonweed around the Black Sea, an area climatically similar to the Northern Rockies. To date, over 50 insects have been found attacking rush skeletonweed in this area, of which at least five appear to have potential as biological control agents (Markin 2002; Markin 2007).

While surveying for an additional population of *Bradyrrhoa gilveolella* in Armenia, we were able to obtain one of these promising agents: the Chondrilla root crown, *Oporopsamma wertheimsteini* (Lepidoptera: Tortricidae). This moth is distributed throughout Iran, Central Asia, Asia Minor, the Balkans, and in favorable localities (sandy grasslands) in Hungary, Slovakia and Slovenia (Fazekas 2009; Fazekas and Lescar 2009). The moth has one generation per year and has two quiescent periods, a pupal aestivation during the summer and winter hibernation as an egg or first instar larva. Larvae feed within a silken tube attached at the root crown of the plant. Larvae may kill small diameter plants and weaken larger thicker rooted plants (Hasan and Wapshere 1977; Christofaro, BBCA per. com.; Littlefield, MSU unpublished data). Hasan and Wapshere (1977) reported an 80% decline of rush skeletonweed during the early summer at sites in Azerbaijan where the moth was present. *Oporopsamma wertheimsteini* has been reported as monophagous on *Chondrilla juncea* in its native range (Hasan and Wapshere 1977; Fazekas 2009; Fazekas and Lescar 2009). Preliminary host specificity tests were carried out by the Australians in the 1970s supported this narrow host specificity; however the moth was not considered for release since it was not climatically adapted to the warmer Australian rush skeletonweed infestations (Hasan and Wapshere 1977) and further testing was terminated. The moth appears better adapted to climates with hot, dry summers and cold winters; and habitats with lighter, sandy soils (Hasan and Wapshere 1977; Fazekas 2009; Fazekas and Lescar 2009); such as those found in the Intermountain West region of the U.S. In 2010 a collection of *O. wertheimsteini* was acquired from Armenia by the Russian Biocontrol Group in St. Petersburg. No-choice host tests were conducted at the Montana State University Containment Facility in Bozeman, MT. Fifty seven plant species plus several cultivars and three *C. juncea* biotypes were tested in 2011- 2012. Feeding and larval development was observed in two North American species, *Krigia biflora* and *Micoseris lindleyi*, and three introduced species: *Scorzonia lacinata*, *Sonchus arvensis*, and *Tagopogon dubius*, but was very marginal when compared with that of the *C. juncea* controls. Additional tests including open field choice/ oviposition tests are needed to assess the potential risk to these non-target plants. We speculate that the ovipositional host range by the adult moths is restricted to *C. juncea*. To further delineate this attraction to potential hosts, the use of plant volatiles as attraction or ovipositional cues will also be investigated.

7. Approach:

1) Complete host specificity studies of *Oporopsamma wertheimsteini*

Both no-choice and open field choice tests will be conducted. Pupal *O. wertheimsteini* will be obtained from cooperators from Armenia to supplement our existing laboratory colony at MSU. No-choice tests will be conducted at the Montana State University Containment Facility, Bozeman, MT. Five first instar larvae will be transferred to tests plants. A minimum of 10 replications per plant species will be conducted. Tests plants will consist of species that have been difficult to grow e.g. *Crepis*, *Microseris*, *Lygodesmia*, etc., additional replications of previously tested plants e.g. *Stokesia laevis*, *Nothocalais troximoides*, different lettuce cultivars, etc., and additional species of *Krigia* and representatives of other Asteraceae tribes. In all approximately 16 plant species will be tested or retested; supplementing the previous host tests. Plants will be grown in 480 ml plastic cups with opened, domed lids to contain any mobile larvae. A rush skeletonweed biotype 1 (Banks, Idaho) control will be maintained for each series of tests (if conducted over several time periods). Plants will be harvested upon the completed development of larvae on rush skeletonweed controls or upon the death/ senescence of the plant. The number of feeding tubes, live or dead larvae and/or pupae will be recorded. Pupae will be maintained until adult emergence. Adult weight and fecundity of females will be recorded.

Open field choice tests will be conducted in two separate sites in Armenia or in southern Russia by the Russian Biocontrol Group. The following plant species will be initially tested: rush skeletonweed (local biotype), North American natives: *Krigia biflora* and *Micoseris lindleyi*, and introduced species: *Scorzonia lacinata*, *Sonchus arvensis,* and *Tagopogon dubius*. Ten replicates (20 total) per plant species will be planted at each field site in a randomized complete block design. Plants will be spaced a minimum of 2 m apart to reduce dispersal of larvae from damaged plants. Adults or infested plants with pupal *Oporopsamma* will be placed systematically throughout plots during autumn. Plants will be maintained and harvested the following June or at time of death and dissected for the presence of feeding tubes, and live or dead larvae or pupae. AVOVA will be conducted to determine difference among sites and treatments. Open field tests may need to be repeated based on successful plant establishment or overwintering, and any additional plant species added to the design based on no-choice tests.

To determine if plants other than *C. juncea* are utilized by *Oporopsamma* in its native range, locally occurring Asteraceae plants at the Armenian study/ collection sites will be inspected for the presence of the moth. Herbarium specimens will be taken for identification and all living larvae will be fixed in ethanol or by any other methods suitable for morphological identification or further molecular taxonomy analysis.

2) Determine the role of plant volatiles in the selection of host plants by *Oporopsamma wertheimsteini*

To better understand host plant selection by *O. wertheimsteini*, we will compare volatile production by several host and nonhost plant species. Plant volatiles – airborne chemical compounds released by plants – are known to be important cues that signal plant identity and location to foraging herbivores. Plants we will examine include three genotypes of rush skeletonweed, nonhosts in which no feeding occurred, plants in which some feeding occurred (e.g., *Krigia biflora*, *Micoseris lindleyi*, *Scorzonia lacinata*, *Sonchus arvensis*), and several agriculturally importance species (lettuce cultivars, etc.). Volatiles will be collected in a greenhouse using portable volatile collection systems (Volatile Assay Systems, Rensselaer, NY). Each plant species will be enclosed in a clear Teflon® bag (American Durafilm Co., Holliston, MA) and air pulled out through a side port (0.5 liters min^{-1}) through volatile traps containing the adsorbent HayeSep-Q® (Restek, Bellefonte, PA). Volatile emissions will be collected for 1 hr and eluded from traps with 200 µl of dichloromethane and analyzed using an Agilent 7890A gas chromatograph coupled with a 5975C mass spectrometer. Quantifications will be made relative to internal standards using ChemStation software (Agilent Technologies, Wilmington, DE), and identifications of compounds confirmed by comparing retention times and mass spectra to commercial standards.

We will focus on differences in the amount or presence/absence of volatile compounds and explore the behavioral activity of individual compounds in bioassays. Using these results we can better assess the potential risk to non-target species, and strengthen the petition for use of *O. wertheimsteini.* These findings may also explain the pattern of feeding by *O. wertheimsteini* seen in our trials – and could identify compounds or types of compounds that are most likely to be important and useful for predicting host selection by this and other biocontrol agents generally.

8. Expected Products & Outcomes:

1) Complete host specificity studies of *Oporopsamma wertheimsteini*.
    - Assess the risk the potential feeding *Oporopsamma wertheimsteini* on non-target plants;
    - If this potential risk is low or negligible, prepare a petition (= biological assessment) for the field release of *O. wertheimsteini* to be submitted for review by TAG (Technical Advisory Group on the Biological Control of Weeds) & USDA-APHIS; and
    - Prepare a manuscript on the biology and hosts specificity of *O. wertheimsteini* for publication in a scientific journal.

2) Determine the role of plant volatiles in the selection of host plants by *Oporopsamma wertheimsteini*.
    - To further characterize the risk to non-target plants based on chemical attraction of *Oporopsamma* females to potential host plants;
    - Determine if these plant volatiles could be used as a monitoring tool should the moth be released; and
    - Prepare a manuscript regarding the plant volatile research.

Budget:

Category	Year 1	Year 2	Year 3	Total
Salaries: Quarantine technician 2 mo. @ $2920.33/mo. (conducting no-choice tests & maintaining insect colonies	5,841	5,841	0	$11,682
Salaries: Research assistant hourly 200 hr. @ 15.90/hr. (collecting & maintaining plants or insect colonies, assisting with drafting TAG petition or manuscripts)	3,180	3,180	3,180	$9,540
Benefits: Quarantine technician ~40.7%	2,377	2,377	0	$4,754
Benefits: Student/hourly: 10%	318	318	318	$954
Supplies: GC operation, chemicals, cups, cages, etc.	2,000	1,000	0	$3,000
Contracted services: Overseas collection, open field tests collection & shipping	20,000	6,500	0	$26,500
Travel: Per diem, lodging, vehicle rental, gas	600	500	0	$1,100
Rent: Quarantine greenhouse/lab space fees no choice tests & insect rearing	2,000	2,000	1,000	$5,000
Total USFS	$36,316	$21,716	$4,498	$62,530
MSU Match 25% (=33.33%): Salary (Littlefield) & ICDs not received	$12,104	$7,238	$1,499	$20,841
TOTAL PROJECT COST	$48,420	$28,954	$5,997	$83,371

Timetable:

	Spring 2014	Summer 2014	Autumn/ Winter 2014	Spring 2015	Summer 2015	Autumn/ Winter 2015	Spring 2015	Summer 2015	Autumn 2015
Objective 1 Host tests	Collect & grow tests plants	Setup open field tests; collect pupae for host tests	Release moths at open field tests; No-choice tests at MSU	Harvest open field tests; Additional no-choice tests at MSU	Setup open field tests & maintain plants if needed	Release moths at open field tests; Harvest no-choice tests	Harvest open field tests if required	Prepare TAG petition & journal manuscript	
Objective 2 Volatiles		Collect volatiles	Collect volatiles	Analyze volatiles	Analyze volatiles	Analyze volatiles			

References:

Fazekas, I. 2009. Distribution of *Oporopsamma wertheimsteini* (Rebel, 1913) and *Pelochrista subtiliana* (Jäckh, 1960) in Hungary (Lepidoptera: Tortricidae). Acta Naturalia Pannonica, 4 (2): 113–120.

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Hasan, S., and A. J. Wapshere. 1977. Biology and host specificity of the Chondrilla crown moth, *Oporopsamma wertheimsteini* (Rebel) (Lepidoptera: Tortricidae). Bull. Ent. Res. 67:619-625.

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Whitson, T. D., L. C.Burrill, S. A. Dewey, D. W. Cudney, B. E. Nelson, R. D. Lee, and R. Parker (eds). 1991. Weeds of the West. Western Society of Weed Science, Laramie, WY.

<u>A short (not more than one-page) curriculum vitae for each principal investigator</u>: See attachments.

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EDUCATION

University of New Hampshire, Durham, NH	Forestry B.S.F.	1975
University of New Hampshire, Durham, NH	Entomology B.S.	1975
University of Idaho, Moscow, ID	Entomology M.S.	1980
University of Wyoming, Laramie, WY	Entomology Ph.D.	1986

ACADEMIC APPOINTMENT

1988–Present, Montana State University, Dept. of LRES, Bozeman, MT, Research Scientist/Quarantine Director
1995-1998, University of Wyoming, Dept. of Plant, Soil and Insect Sciences, Laramie, WY, Research Associate III
1992-1995, University of Wyoming, Dept. of Plant, Soil and Insect Sciences, Laramie, WY, Graduate Assistant

SYNERGISTIC ACTIVITIES

My position has both a service and a research component. As Quarantine Director I am responsible for the maintenance and operation of the Biological Containment Facility for the importation of weed feeding organisms. I also develop risk assessment documents for field release of exotic organisms for biological control and work closely with state and regulatory personnel, regional consortium groups, and local land managers for the importation, release, establishment and monitoring of various biological agents. My primary research emphasis is biological control of weeds, including the determination of host specificity, bionomics, and field ecology of potential agents. Through these projects I work with several co-investigators and cooperators from various private, county, state, tribal, federal, and international agencies or organizations. My outreach activities included presentations and workshops on the use of biocontrol, as well as participation in various invasive weed task force groups developing and implementing weed management plans.

PERTINENT PUBLICATIONS

Littlefield J., J. Kashefi, A. deMeij, and J. Birdsall. 2012. A petition for the field release of the gall mite *Aceria drabae* (Acari: Eriophyidae) for the biological control of hoarycress in North America. TAG Petition 012-03. 77 pp.

Littlefield J., J. Birdsall, L. Wilson, G. Grosskopf, R. DeClerck-Floate, and S. Turner. 2009. A petition for the field release of the gall wasp Aulacidea subterminalis (Hymenoptera: Cynipidae) for the biological control of invasive hawkweeds in North America. TAG Petition 09-02. 83 pp.

Littlefield, J. L., G. P. Markin, J. Kashefi, and H. Prody. 2008. Habitat analysis of the rush skeletonweed Root moth, *Bradyrrhoa gilveolella* (Lepidoptera: Pyralidae). XII International Symposium on the Biological Control of Weeds. April 22-27, 2007. La Grande Motte, France.Pp.60. April 2007, La Grande Motte, France. Pp.573-576.

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Education

Ph.D.	Pennsylvania State University, Entomology, May 2008.
M.S.	Montana State University, Entomology, May 2001.
B.S.	University of Virginia's College at Wise, Biology and Mathematics, 1998.

Professional Employment

2008-present	Research Entomologist, USDA Forest Service, Rocky Mountain Research Station
2008-present	Adjunct Faculty, Dept. of Plant Sciences & Plant Pathology, Montana State University
2001-2003	Research Associate, Department of Entomology, Montana State University

Selected Publications

Birdsall, J. B., W. McCaughey, and J. B. Runyon. 2012. Roads impact the distribution of noxious weeds more than restoration treatments in a lodgepole pine forest in Montana, USA. *Restoration Ecology* 20: 517-523.

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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.

Piesik, D., D. K. Weaver, J. B. Runyon, M. Buteler, G. E. Peck, and W. L. Morrill. 2008. Behavioral responses of wheat stem sawflies to wheat volatiles. *Agricultural and Forest Entomology* 10: 245-253.

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