

1. **Project Title:** “Chem herding”: Adapting existing semiochemical delivery technologies to improve the precision of weed biocontrol agents

2. **Principal Investigator:**

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3. **Cooperators, Collaborators, and Other Participating Institutions:**

- Nancy Gillette – USDA FS PSW Research Station, Berkley, CA
- Sharlene Sing – USDA FS RM Research Station, Bozeman, MT
- Kevin Delaney, John Gaskin, Mary Mayer – USDA ARS NPARL, Sidney, MT
- Liz Hebertson – USDA FS Forest Health Protection, Ogden, UT

4. **Amount Requested (yearly and total), Project Leveraging**

Annual request- \$15,000; Total request- \$15,000; Matching funds- \$3,900 (26%)

Project leverages approximately \$10,000 in biological control sponsorship to a new target invasive

5. **Project Goals and Supporting Objectives:**

1. Determine the release rate of pheromone from three controlled release formulations impregnated with *Diorhabda carinulata* (Desbrochers) – (Dc hereafter) pheromone formulations
2. Determine the release rate of host plant volatiles from three controlled release formulations impregnated with Dc attractive plant compounds  
\*All determinations will be conducted in the laboratory under controlled conditions and under field conditions.
3. Show proof of concept using formulations at a saltcedar infestation where Dc is known to be established.

6. **Project Justification/Urgency:**

A key criticism of management with biological agents has been that once released, biocontrol agents establish inconsistently and spread unpredictably. A technological advance such as controlled release formulations might demonstrate considerable value in helping to 1) direct and 2) retain biocontrol agents in targeted locations. In the long term, controlled release of pheromones might be utilized to discourage agent movement into specific sites (such as native species conservation areas), via facilitating strong retention.

As background, the reputation of weed biological control may have been significantly altered in recent years following reports of widespread defoliation of saltcedar by a weed biocontrol agent, the flea beetle *Diorhabda elongata* species group (Stromberg et al. 2009; Sogge et al. 2008). Publicized efforts to causatively link the defoliation of saltcedar with the degradation and loss of critical nesting habitat of the southwest willow flycatcher has posed new challenges to research and implementation activities. These challenges may be met, in part, using controlled release technology.

Three types of commercial products will be tested for development: Hercon® flakes, ISCA® technologies SPLAT™ wax based formulations (De Lame et al. 2007; Stelinski et al. 2007), and Scentry® sprayable impregnated hollow microfibers (Trumble and Baker 1984; Stelinski et al. 2008). These can all be used to deliver pheromone, but primarily under a mating disruption paradigm, although Trumble and Baker (1984) shows proof of concept for microfibers using a trapping comparison. Both the flake and wax based formulations are used commercially in forest applications for gypsy moth mating disruption, while the sprayable microfibers are used only in crop and orchard settings. However, the primary markets for the products remain agricultural and almost all are designed to 'mislead' insects. The produced development of wax, flake and fiber formulations for the long term increase of local abundance of as biological control agents using a combination of aggregation pheromone and host plant attractants has not been attempted.

As one detailed example – given primarily because of demonstrated multiple uses as carrier, Hercon® Environmental developed a proprietary laminated polymer technology to produce biodegradable flakes (Bio-Flake® and MicroFlake®) that can be used as a carrier for a range of timed-release, non-toxic/low toxicity insect pheromones, attractants, and repellents, and low risk insecticides. The Hercon® flake pheromone delivery system is thought to cause negligible negative environmental and human health impacts, especially in comparison with conventional control and application methods. As an example, the application rate for Gypsy moth mating disruption pheromone is approximately two 1/32" x 3/32" flakes per square foot, or ¼ cup of biodegradable flakes per acre. These flakes can be embedded with aliphatic pheromones that are not reducing immediate threats from undesirable chemical leachates in the environment. Equally viable technology may also be available in the form of wax-based controlled release media and impregnated microfibers.

Hercon® flakes are now primarily utilized to disseminate pest insect semiochemicals (Gillette et al. 2009a, b) to protect valuable tree species by effective dispersal of anti-aggregation pheromones. However, *Tamarix* spp. folivores, Dc, are motivated to orient in the landscape in response to chemical cues from conspecifics (aggregation pheromone; Cossé et al. 2005) and host plants compounds (food + likely source of conspecifics; Cossé et al. 2006). Manipulation of Dc via flakes embedded with behaviorally active compounds has potential for controlling agent movement.

The range of applications for utilizing controlled release formulations to optimize weed biological through the semiochemical manipulation of agents seems quite broad. The goal of this proposal would therefore be to perform a thorough study of the application of this semiochemical technology using the chrysomelid Dc as a very good model. The following represent longer term objectives that could be used as a subsequent starting point (Yr. 2 and beyond) for evaluating the potential use of controlled release formulations in weed biocontrol applications using the optimal system (determined in Yr. 1):

1. Determine if pheromone impregnated media can be used to facilitate the intentional dispersal of locally abundant DC populations to aggregate in nearby

uncolonized or under-colonized *Tamarix* spp. stands in two locations ( for example, the Arkansas River, CO and Shoshone River, WY)

2. Develop host plant volatile impregnated media for co-attraction of Dc
3. Determine if these media can be used to retain newly-released populations of Dc in the same locations
4. Monitor post-application numbers of adults, immatures and defoliation by Dc.
5. Monitor subsequent year populations of Dc at target sites
6. Determine if pheromone impregnated media could be used for monitoring for the presence of Dc in areas where establishment is unknown or questionable

## **7. Approach**

- 1. Develop and prototype formulations - pheromone.** Synthesize gram scale amounts of Dc pheromone- (2*E*, 4*Z*)-2,4-heptadien-1-ol (Petroski, 2003), which has been demonstrated to be a single component of insect-produced volatiles that traps equivalently to a paired semiochemical combination (Cossé et al, 2005). Provide this material for incorporation into flake, wax, and fiber formulations. Determine pheromone release rates at biologically relevant temperatures from 20°C through 40°C using laboratory volatile collection apparatus. A local field deployment will allow for collection of a weekly sample of product release rates. Amounts remaining and release rates will be compared to laboratory data. Monitoring of on-site temperatures and weather conditions will allow for this assessment.
- 2. Develop prototype formulations – host plant attractants.** We will prepare solutions for the impregnation of flakes and fibers using four commercially available high purity volatiles identified in Cossé et al. 2006. Commercially available (*Z*)-3-hexanal will shortpath-distilled from triacetin using a gentle Kugelrohr process. The fact that four compounds in differing amounts play a role in host plant attraction can be addressed by developing individual preparations for each compound and combining to view collective release rates at selected temperatures in the laboratory and field (see above). For the wax preparation, it may be possible to impregnate the matrix with all volatiles or by developing separate formulations for each.  
We will employ standard techniques used in our chemical ecology laboratory to quantify compound release rates from flakes, fibers and wax. These techniques match those described in Piesik et al. 2007, but will use both fully humidified and dry air.
- 3. Field design – deploying formulations (proof of concept).** We will use a nearby site at Lovell, WY to conduct pairwise trials of formulation driven aggregations of adult insects using treatment and control preparations of each formulation, each separated by 200 m - across 3 replicates per location. Replicates will be separated by 250 m. A stand of *Tamarix* comprising approximately five mature individuals will be treated in all cases. Temperature and weather data will be monitored on-site.
- 4. Field design – array and monitoring.** Consultative expertise in monitoring at target field sites will be provided by collaborators from USDA, ARS in Sidney, MT.

A standard monitoring protocol will be used. This has been developed and is used by all Dc research collaborators.

- 5. Data management and analysis.** The data collected cover a wide variety of biotic and abiotic variables. A pasted document describing the procedure is below. However, for the purposes of proof of concept, analysis appropriate for comparing local abundance of adult insect numbers will be used. It is anticipated that a mixed-model anova will be used for parameters of specific interest, where possible. Some data will probably require non-parametric analysis. The data collected will be part of a candidate graduate student summer project and will be published in a scientific journal and in a FHP numbered publication, where appropriate.

## **SALT CEDAR BIOCONTROL MONITORING DATA**

(INFORMATION CURRENTLY COLLECTED TWICE PER SUMMER)

**Marked Branches (N, S, E, & W):** Potential predators, leafhoppers, number of *Diorhabda* eggs; larvae and adults

**Combined *Diorhabda*, leafhopper and other Herbivore Damage:**

0%, 1-10%, 10-50%, 51-95%, 96-100%

**Branch Data (N, S, E, & W):** Primary Dead? (Y or N), New primary marked? (Y or N), Length of live primary, number of live secondary branches  $\geq 1$  cm

**Plant Volume:** Height (3 measurements around tree to top of live growth)

**Plant Morphology & Condition:** Densimeter readings (3x)- 0-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%; Green Foliage- 0-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%; Senescing- 0-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%; Dead- 0-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%

**Reproductive Status:** Flowers (Y or N), If Y - Buds? Open? Seed?

**Whole Tree *Diorhabda* Count (for larvae and adults, count all if  $\leq 100$  or select category 100-300, 300-600, 600-1000, 1000+):** Eggs (Present or Absent), Larvae, Adults

## **Other Data Collected**

**Canopy cover-understory vegetation quadrants:** forbs, grasses, plant litter, bare soil (none 0%, minor 1-10%, moderate 10-50%, severe 50-95%, complete 95-100%)

**Overall extent of saltcedar infestation:**  $\leq 5$  ac, 5-100 ac, 100-1000 ac,  $>1000$  ac

**General Topography:** Level, slight slope, moderate slope, steep slope, or hilly

**Soil type:** Gravel/cobble, sand, sandy loam, loam, silt loam, clay loam, clay

**Aspect:** North, South, East, West, Northwest, Northeast, Southwest, Southeast

**Probability of Flooding:** Very low, low-moderate, high

**Native (pre-infestation) plant communities at site if known:**

**Latitude, Longitude, Elevation**

**8. Expected Products and Outcomes:** (products and how they will be used)

The results of the proposed study will determine if existing controlled release formulation technology is feasible and relevant for weed biological control applications. Laboratory and field assays and a field bioassay associated with the study will demonstrate the feasibility of the technology.

Specific products would include:

1. Pheromone and host plant attractants that have been incorporated in controlled release technology as effective lures.
2. The deployment of these products in a proof- of-concept field demonstration of efficacy using model weed and biocontrol agent systems.
3. A strategy to develop for other target weed biological control systems

**9. Literature Cited**

Cossé, A.A., R.J. Bartelt, B.W. Zilkowski, D.W. Bean and E.R. Andress. 2006. Behaviorally active green leaf volatiles for monitoring the leaf beetle, *Diorhabda elongata*, a biocontrol agent of saltcedar, *Tamarix* spp. *Journal of Chemical Ecology* 32: 2695-2708.

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Gillette, N.E., C.J. Mehmehl, J.N. Webster, S.R. Mori, N. Erbilgin, D.L. Wood, J.D. Stein. 2009b. Aerially applied methylcyclohexenone-releasing flakes protect *Pseudotsuga menziesii* stands from attack by *Dendroctonus pseudotsugae*. *Forest Ecology and Management*. 257: 1231–1236.

Gillette, N.E., J.D. Stein, D.R. Owen, J.N. Webster, G.O. Fiddler, S.R. Mori and D.L. Wood. 2006. Verbenone-releasing flakes protect individual *Pinus contorta* trees from attack by *Dendroctonus ponderosae* and *Dendroctonus valens* (Coleoptera: Curculionidae,

Scolytinae). *Agricultural and Forest Entomology*. 8: 243–251.

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Also:

ARS project website: “Pheromone and host odor attractants for managing *Diorhabda* spp.: Biological control agents of saltcedar” available at:  
[http://www.ars.usda.gov/research/publications/Publications.htm?seq\\_no\\_115=200970](http://www.ars.usda.gov/research/publications/Publications.htm?seq_no_115=200970)

Budget – 1yr

○ Salary - Student labor -	\$11,000
○ Fringe benefits @ 10% -	\$1,100
○ Supplies - synthetic compounds, field items	\$2,000
○ Travel – Weaver and student to local assay and WY bioassay sites	\$900
_____ Foregone MSU Off-Campus F&A (IDCs) @ 26% -	(\$3900)
<b>TOTAL</b>	<b>\$15,000</b> (\$3,900)

Timetable - assuming Federal FY end 9/30 2012

June 2011:                      Synthesize compounds

June and July 2011:	Impregnate formulations
June-August 2011:	Begin laboratory and field assessment of longevity and release rates; concomitant proof of concept field trial
September 2011:	Prepare data for analysis
January 2012:	Conduct replicated laboratory assay of release rates
June 2012:	Field assessment of longevity and release rates; concomitant field trial
August-September 2012:	Prepare publication and write report

**David Weaver**  
**Associate Professor**

**Education**

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

**Professional Experience**

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

**Select Publications**

- Buteler, M., D.K. Weaver, P.L. Bruckner, G.R. Carlson, J.E. Berg, P.F. Lamb. 2010. Identification of winter wheat cultivars suitable as trap crops for the wheat stem sawfly based on screening using agronomic traits and semiochemical production. *Canadian Entomologist* 142: 222-233.
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**Nancy Gillette** (formerly Nancy Rappaport)  
**Research Entomologist**

**Education**

- B.A. Fine Arts, University of California, Berkeley, 1969
- Ph.D., Forest Entomology, University of California, Berkeley, 1987

**Professional experience**

- 11/88-4/90: Entomologist, PSW Research Station. Berkeley, CA (includes post-doc at Institut National de la Recherche Agronomique, Orléans, France, 1989)
- 4/90-6/90: Acting Assistant Station Director for Research Planning, PSW Research Station, Berkeley, CA
- 6/90-5/91: Entomologist, PSW Research Station, Berkeley, CA
- 5/91-11/95: Research Entomologist, PSW Research Station, Albany, CA
- 11/95-12/96: Interagency liaison, USDA Forest Health Protection/US EPA Biopesticide and Pollution Prevention Division, Washington, D.C.
- 1/97-present: Research Entomologist, PSW Research Station, Albany, CA

**Select Publications** (Note: Rappaport = Gillette)

- Zhang, L.W., N.E. Gillette, and J.H. Sun. 2007. Electrophysiological and behavioral responses of *Dendroctonus valens* to non-host volatiles. *Annals of Forest Science* 64: 267-273.
- Erbilgin, N., N.E. Gillette, S. Mori, J.D. Stein and D.L. Wood. 2007. Acetophenone as an antiattractant for western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Scolytidae). *Journal of Chemical Ecology* 33: 817-823.
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**Sharlene Sing**  
**Research Entomologist**

**Education**

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

**Professional experience**

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

**Selected publications**

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