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Proposal Transmittal Information

Date: October 30, 2015

To: Richard Reardon, National Program Manager
Biological Control
rreardon@fs.fed.us

RE: **Request for Proposals: Technology Development for the Biological Control of Invasive Native and non-Native Plants, Fiscal Year 2016**

Colorado State University submits the proposal entitled "Biological Control of Russian Knapweed: Mass-Rearing, Release, and Monitoring of the Gall Stem Wasp *Aulacidea acroptilonica*." Paul Ode is CSU's Principal Investigator.

When submitted under cover of this letter, the referenced proposal has gone through the University's standard review process. The appropriate program and administrative personnel of the institution involved in this application are aware of the sponsoring agency's guidelines and are prepared to enter into good faith negotiations to establish the necessary inter-institutional agreement(s). The institution makes all applicable assurances/certifications.

Project Period: 07/01/2016 – 06/30/2019
Budget: Federal request: $99,402 // required 50% CSU contribution: $99,500

Person to be contacted for administrative or contractual matters or arrangements:

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Colorado State University Office of Sponsored Programs
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Authorized for submission by:
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Thank you,

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xc: Paul Ode, Bioagricultural Sciences & Pest Management, 1177

Project Title: Biological control of Russian knapweed: mass-rearing, release, and monitoring of the gall stem wasp *Aulacidea acroptilonica*

Principal Investigators: Paul Ode, Colorado State University, Department of Bioagricultural Sciences and Pest Management, Campus Delivery 1177, Fort Collins, CO 80523-1177, 970-491-4127 (tel), paul.ode@colostate.edu; Dan Bean, Colorado Department of Agriculture, Palisade Insectary, Palisade, CO 81507, 970-464-7916 (tel), 970-464-5791 (fax), dan.bean@state.co.us.

BCIP Contacts:
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Amount Requested: Year 1 (2016): $33,813; Year 2 (2017): $35,204; Year 3 (2018): $30,385; **Total: $99,402**

Matching Funds: $99,500

Project Goals and Supporting Objectives

Project goals: The primary goal of this project is to implement a sustainable, classical biological control program of the Russian knapweed *Acroptilon repens* (L.) DC (Asteraceae) integrating the effects of two biological control agents. Russian knapweed is one of the most serious invasive weeds throughout the western US including many Forest Service lands. Two biological control agents, the gall midge *Jaapiella ivannikovi* Fedotova (Diptera: Cecidomyiidae) and the stem-galling wasp *Aulacidea acroptilonica* V.Bel. (Hymenoptera: Cynipidae), have been recently approved for release, yet biological control efforts have been hampered to a large degree by challenges in mass-rearing programs to produce sufficient numbers of midges and wasps for releases. Furthermore, we know little about how these two biological control agents interact in the field: do they complement one another or do they interfere possibly reducing the overall effectiveness of biological control efforts of this noxious weed. To address these challenges, we have the following three objectives:

Supporting objectives:

1. Increasing numbers of stem-galling wasps and gall midges, redistribution of both agents to Russian knapweed field sites throughout Colorado.
2. Examine the compatibility of control effected by the stem-galling wasp and the gall midge in the field.

Project Justification/Urgency:

Russian knapweed is one of the most serious invasive weeds in North America, especially infesting farm and rangeland throughout western North America, including many regions of Colorado (Fig. 1). Russian knapweed can grow on a wide range of soil types and moisture conditions and does particularly well in recently disturbed soils; it generally does not invade healthy, intact, native habitats (Zouhar 2001). Patches of Russian knapweed that do occur in diverse, otherwise native, vegetation tend to be smaller with much lower germination rates (Barosh et al., unpublished data). In part, this plant is difficult to control because it has an extensive root system through which it can propagate vegetatively. Furthermore, Russian knapweed is considered to be allelopathic (Stermitz et al. 2003), possibly contributing to its ability to grow in large, dense stands that can crowd out native vegetation. Similar to many invasive weeds, Russian knapweed stands are denser in invaded North America compared to native western Asia (e.g. Turkey; unpublished data cited in Djamankulova et al. 2008). Consequently, Russian knapweed is a stronger competitor than many native North American plants (Ni et al. 2010, Callaway et al. 2012). While seedling establishment appears to play a minor role in established clones, it is likely the primary means of colonizing new sites or sites at the periphery of an established patch (Djamankulova et al. 2008). Each ramet can produce upwards of 1200 seeds, which can remain viable in the seed bank for up to 5 years (Anderson 1968). Controlling seed production may prove to be crucial in slowing the spread of this noxious weed.

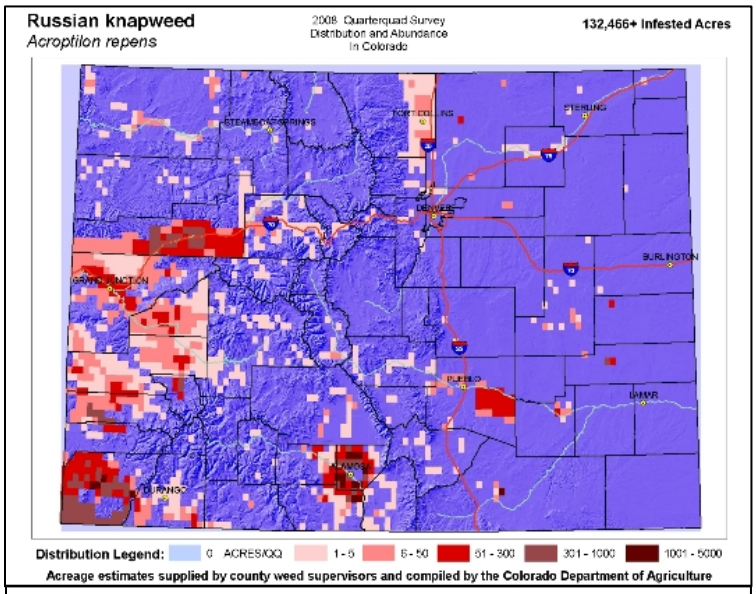

Fig. 1. Infestation densities of Russian knapweed in CO

Biological control agents:

By themselves, mechanical removal and use of herbicides to control Russian knapweed over vast areas of infestation that generally have low economic value are impractical and unsustainable (Jones & Evans 1973, DiTomaso 2000). Biological control, either alone or in conjunction with these other control strategies, is therefore viewed as a promising option. Two biocontrol agents (a gall-forming midge [*Jaapiella ivannikovi* Fedotova (Diptera: Cecidomyiidae)] and stem-galling wasp [*Aulacidea acroptilonica* V.Bel. (Hymenoptera: Cynipidae)]) have been recently approved for release (USDA APHIS 2008, 2009). However, it is unclear whether release of one or the other species or of both species is the best approach. Both agents are highly restricted to *A. repens*. *Jaapiella ivannikovi* adults lay up to 15 eggs at the apical or lateral meristems; upon hatching the larvae form a gall, which prevents the meristem from producing a flower. *Jaapiella ivannikovi* has approximately 4 generations per

year. Because the midge is multivoltine, young knapweed shoots are susceptible to attack throughout the growing season. *Aulacidea acroptilonica*, on the other hand, is univoltine. Adults emerge in early spring. Females lay their eggs in the stems below the apical meristem (Djamankulova et al. 2008). *Aulacidea acroptilonica* larvae aestivate, then overwinter as third instars, and pupate the following spring. Because the wasp is univoltine, only young shoots early in the spring are attacked.

Both gall-forming insects have been shown to reduce growth, aboveground biomass, and seed output of Russian knapweed. In their native Uzbekistan, *J. ivannikovi* reduces shoot length by 10-15%, aboveground biomass by 20-25%, and seed output by 90-95% and attack by *A. acroptilonica* reduces shoot length by 20-25%, aboveground biomass by 25%, and seed output by 75% (Djamankulova et al. 2008). Similar results have been documented in Wyoming (Collier et al. 2006, 2007). Maximum wasp gall densities in the field in Uzbekistan are up to 12 galls per shoot and 400 galls per 100 shoots; maximum midge gall densities in the field are up 15 galls per shoot but less than 10% of shoots harbor midge galls in the most heavily attacked populations (Djamankulova et al. 2008).

<u>Plant-mediated (indirect) interactions between midge and wasp</u>:

Competition is often presumed to be rare among biocontrol agents of weeds because plants provide ample resources and several niches for multiple species of herbivores (Denoth et al. 2002). However, while poorly documented for weed biological control programs (Milbrath & Nechols 2014), plant-mediated indirect interactions among herbivores are widely appreciated to be an important force in the broader ecological literature (Denno et al. 1995, van Veen et al. 2006, Denno & Kaplan 2007).

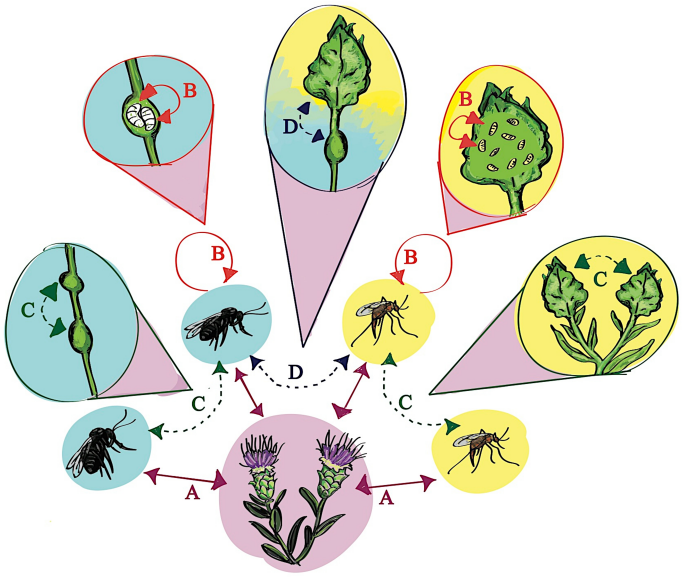

Figure 2. Direct and plant-mediated indirect interactions among the gall midge (yellow), gall wasp (blue), and Russian knapweed (pink). A: direct interactions between the wasp or midge and Russian knapweed; B: direct interactions among developing insect larvae with a wasp or midge gall; C & D: plant-mediated indirect interactions between two galls of the same (C) or different (D) species. (illustration by T. Barosh)

Gall-forming insects form particularly intimate relationships with their host plants. Gall formers divert nutrients from flowers, seeds, and overall plant growth (Harris & Shorthouse 1996). Gall insects may therefore directly affect reproductive output of the plant and indirectly influence the plant's ability

to compete with other plants. As such, two gall-forming insects attacking the same plant may be expected to interact with one another as they modify their host plant's physiology and feed off the plant's photosynthates. In this way, insect galls act as metabolic sinks and compete with other plant metabolic sinks such as developing fruits or meristematic tissues.

Virtually nothing is known about how the two biological control agents of Russian knapweed, *J. ivannikovi* and *A. acroptilonica*, interact, either directly or indirectly, in the field (Fig 2). Yet, such information is vital if we are to predict the outcome of releasing one versus both species. If the impact of both species simultaneously attacking Russian knapweed is additive or even facilitative (as suggested by our preliminary data, Fig 3), then releasing both agents together

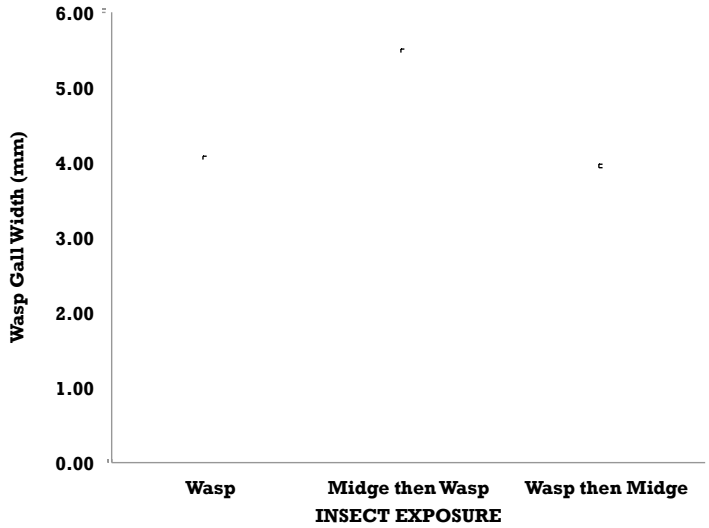

Fig. 3. Facilitation of wasp gall growth by midge gall.

would be justified as we expect that control of Russian knapweed would be enhanced compared to releases of only one of the two biological control agents. However, if only one agent (midge or wasp) is primarily responsible for Russian knapweed control or if the midge and the wasp compete with one another reducing overall control of Russian knapweed, then it is prudent to release only the most effective agent. This assessment depends on a firm understanding of how the midge and wasp interact with their host plant as well as how they directly or indirectly interact with each other. Therefore, the central aim of our proposal to explore the mechanisms underlying the interactions among the two gall-forming herbivores and their host plant. Although accidentally introduced over 120 years ago, only recently has biological control been used as a management tool to control Russian knapweed. While troubling from a management perspective, the fact that large areas of land infested with Russian knapweed are currently not controlled and have not been subjected to biological control efforts presents an excellent opportunity to conduct manipulated, experimental releases of combinations of both gall-forming biological control agents as well as their use in conjunction with mechanical and chemical control.

Approach:

1. *Increasing numbers of stem-galling wasps and gall midges, redistribution of both agents to Russian knapweed field sites throughout Colorado.* One of the challenges of mass-rearing *A. acroptilonica* is the fact that it is a univoltine species. Furthermore, unlike *J. ivannikovi*, *A. acroptilonica* galls produce only one or two wasps making propagation much more labor intensive. These factors necessarily slow down the rate at which we can produce large numbers of this wasp for release and distribution to land managers throughout the region. We are currently working on ways to speed up production of the wasp. Much of our efforts will focus on shortening the diapause period by exploring the minimum length of time that wasps galls need to spend at low temperatures. This will require extensive greenhouse space in which to grow plants to expose to gall wasps (see budget request) and growth chamber space (to which we have access) set a cool temperature (0-5˚C). We will also use the outdoor gardens of Russian knapweed at the Palisade Insectary to propagate gall stem wasps. These gardens are approximately 3000 m^2 in size and are irrigated to promote continual knapweed growth from spring through fall. Knapweed plants will be mown every six weeks to promote stem regrowth, which is attractive to ovipositing stem galling wasps. Prior to mowing, stems that have been galled by the wasps during the previous round of infestation will be harvested and stored in a cold chamber to induce diapause.

   Using these techniques we hope to be able to propagate wasps for distribution and release throughout the plant growing season. At a minimum, we would like to produce 5000 to 10,000 wasp galls for distribution and release. Using a combination of greenhouse-reared, garden-reared (described above), and field-collected gall midges, we have successfully collected and redistributed nearly 4400 midge gall for redistribution to 87 field sites throughout Colorado in 2014. We plan to continue these approaches to increase production up to 10,000 galls per year for distribution to interested landowners throughout the region.

2. *Examine the compatibility of control effected by the stem-galling wasp and the gall midge in the field.* While both the stem-galling wasp and the gall midge have both been approved for release, surprisingly little information exists about their compatibility in the field in terms of suppressing Russian knapweed infestations. We will establish 80 monitoring sites for Russian knapweed throughout the Front Range, the Arkansas Valley, the San Luis Valley, and the western slope of Colorado– all areas suffering moderate to severe infestations of Russian knapweed (Fig. 1). Midges alone will be released at 20 randomly selected sites, wasps alone at another 20 sites, both midges and wasps will be released at 20 sites, and the remaining 20 sites will serve as controls. We will set up sites using a rangeland weeds monitoring protocol that measures stem density of the knapweed near the release point as well as knapweed densities within a hectare surrounding the release point. This protocol also includes measurements of other plant densities in the plot including native and introduced species. We will monitor each site at least once a year for the three year duration of the proposed project. We also intend to continue monitoring at least select sites at five and ten years post release.

Expected Products and Outcomes:
We expect to develop mass-rearing protocols that allow the production of 5,000 to 10,000 midge and wasp galls per season for delivery to land managers and landowners and to redistribute to additional knapweed sites where biological control has not previously been attempted as well as sites where establishment was poor (possibly due to previous low agent release numbers). We also expect to determine whether biological control of Russian knapweed is more effective (as measured by reduction in stem density, reduction in seed production) when either midges or wasps are introduced as single species releases or together. We expect this information will be invaluable as part of a broader Russian knapweed management plan. Long-range monitoring is essential to detect establishment of the midge and wasp populations as well as effects of these agents on the reduction in knapweed infestation densities. We fully recognize that it may take some time to generate sufficient wasp and midge material to conduct all the proposed releases. We also recognize that it may take several years (beyond the three years funding requested in this proposal) to document effects of interactions between midges and wasps. Therefore, we are committed to securing future funding to carrying out long-term monitoring of these field sites beyond the duration of the requested funding.

Timetable:
Year 1 (2016): Establish monitoring field sites throughout Colorado, begin mass-rearing protocols for the stem-galling wasp and continue efforts to maximize production of the gall midge. Conduct pre-release stem density counts of knapweeds at each site. Conduct releases of midges and wasps at as many sites as our supplies permit.

Year 2 (2017): Continue to establish monitoring field sites (if not completed in Year 1) and continue to mass-produce both stem-galling wasp and gall midge, focusing on ways to improve production numbers and especially determining the conditions to speed up diapause in the stem-galling wasp. Continue to conduct releases of midges and wasps at the different monitoring sites.

Year 3 (2018): Continue monitoring field sites, collecting information on knapweed stem densities and flower production. Continue mass-rearing efforts and distribution of agents to landowners and land managers.

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Education:
PhD 1994, MS 1990, University of Wisconsin-Madison, Entomology
BA 1986, Earlham College, Biology

University of California–Davis	Entomology	1994–1996
Arizona State University	Zoology	1996-1997
Leiden University (The Netherlands)	Evolution and Ecology	1997-1998
Texas A&M University	Entomology	1998-1999
USDA–ARS BIRL (Newark, DE)	Entomology	1999–2002

Appointments:
2011 Associate Professor, Dept. Bioag. Sci. Pest Management, Colorado State University
2008 Assistant Professor, Dept. Bioag. Sci. Pest Management, Colorado State University
2002 Assistant Professor, Department of Entomology, North Dakota State University

Recent Select Publications:

Ode PJ, Johnson SN, Moore BD. 2014. Atmospheric change and induced secondary metabolites – are we reshaping the building blocks of multi-trophic interactions? Current Opinion in Insect Science 5: 57-65. http://dx.doi.org/10.1016/j.cois.2014.09.006

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Recent Professional Experience

2005-present	Director, Biological Pest Control Program and Manager, Palisade Insectary, Colorado Department of Agriculture. State Biocontrol Specialist, Colorado. Affiliate Faculty, Dept. Bioagricultural Sciences and Pest Management, Colorado State University, Ft. Collins
2000-2005	Research Associate, Department of Vegetable Crops, University of California, Davis
1990-2000	Research Associate and Lecturer, Department of Biology, UNC-CH
1988-1990	Lecturer, Department of Biology, UNC-Chapel Hill

Education

Ph.D.	1983	University of Wisconsin, Madison, Entomology, Zoology minor
M.S.	1978	University of Wisconsin, Madison, Entomology
B.A.	1975	University of California, Santa Cruz, Biology, with highest honors

Selected Recent Publications

Hultine, K.R., Bean, D.W., Dudley, T.L., and Gehring, C. (2015) Species introductions and their cascading impact on biotic interactions in desert riparian ecosystems. Integrative and Comparative Biology, doi:10.1093/icb/icv019.

Hultine, K.R., Dudley, T.L., Koepke, D.F., Bean, D.W., Glenn, E.P. and Lambert, A.M. (2015) Patterns of herbivory-induced mortality of a dominant non-native tree/shrub (*Tamarix* spp.) in a southwestern US watershed. Biological Invasions, DOI 10.1007/s10530-014-0829-4

Nagler, P.L., S. Pearlstein, E.P. Glenn, T.B. Brown, H.L. Bateman, D.W. Bean and K.R. Hultine (2014) Rapid dispersal of saltcedar (*Tamarix* spp.) biocontrol beetles (*Diorhabda carinulata*) on a desert river detected by phenocams, MODIS imagery and ground observations. Remote Sensing of Environment 140: 206-219.

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Dudley TL, Bean DW, Pattison RR, Caires A (2012) Selectivity of a biological control agent, *Diorhabda carinulata* (Chrysomelidae) for host species within the genus *Tamarix*. Pan Pacific Entomologist 88:319–341

Bean, D.W., P. Dalin and T.L. Dudley (2012) Evolution of critical day length for diapause induction enables range expansion of *Diorhabda carinulata*, a biological control agent against tamarisk (*Tamarix* spp.). Evolutionary Applications 5: 511-523

Dudley, T.L. and D.W. Bean (2012) Tamarisk biocontrol, endangered species risk and resolution of conflict through riparian restoration. BioControl 57: 331-347

Budget and Scope of Work:

The PI's research group will be responsible for overseeing and conducting the releases and monitoring the outcomes of control of Russian knapweed populations by *Aulacidea acroptilonica* throughout the state (Objectives 2 and 3).

The co-PI's group will be responsible for improving the mass-rearing protocols for *A. acroptilonica* and developing effective release technologies that maximize establishment success. Furthermore, the co-PI's group will facilitate releases and monitoring efforts in the western part of the state. The co-PI's group is not requesting any financial support from this proposal.

CSU Budget Justification

The majority of the requested funds would support salaries in this labor-intensive project. Figures are adjusted by 4% each year to accommodate inflation, unless otherwise noted.

Salaries ($78,798):

1. PhD student ($69,449): We are requesting stipend support for a PhD student in years 1, 2, and 3 at $1,854/month. The PhD student will be actively involved in field work (releases and monitoring) and analyses for all years of the project.
2. PI ($9,349): We are requesting 0.5 month summer salary (based on a monthly salary of $9,166) for the PI in Years 1 and 2 of the project. The PI has a nine-month tenured appointment and the extra support would allow him to work on this project during the summer to complement the time used during the academic year.

Fringe benefits ($7,555):

Fringe benefits are calculated at CSU estimated rates. GRA: 7.29% in Year 1, 7.39% in Year 2, and 7.48% in Year 3; Academic Faculty: 25.73% in Year 1 and 26.06% in Year 2.

Fringe will be charged at the actual rate in effect when the expense is incurred.

Travel ($3,247):

We request $1,040 per year to partially defray domestic travel expenses for the PhD student and the PI to field sites around the state (2,000 miles/yr. at $0.52/mi).

Materials and supplies ($1,561):

We are requesting $500 per year to cover materials required by this project (e.g. pots, cages, soil for greenhouse rearing). These rearing supplies are necessary to develop mass-rearing techniques and implementation of a mass-rearing program for *A. acroptilonica*.

Equipment use fees ($8,241):

We are requesting $2,640 per year to cover greenhouse rental charges (475 sq ft at $220/month) incurred during the three years of this project. Greenhouse space is necessary to develop mass-rearing techniques and implementation of a mass-rearing program for *A. acroptilonica*.

Total direct costs ($99,402)

Matching costs ($99,500)

Matching costs include:

1) .88 month of the PI's salary per year plus fringe 25.73% Y1, 26.06% Y2, 26.40% Y3): $31,744
2) Indirect costs on CSU's cost share amount, plus unrecovered indirect costs on the federal request, which are calculated at CSU's federally negotiated rate of 51% MTDC in Year 1 and 52% MTDC in years 2 and 3 of modified total direct costs: $16,405 + $51,351 =$67,756