Project Title: Pre- and post-release assessments of *Mecinus heydenii* and *Rhinusa brondelii*, candidate classical biological control agents for invasive toadflax (*Linaria* spp).

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Amount requested (FY13): $37,650.

Matching funds: PI salary (exceeds 25%).

Project leveraging: This project will leverage an anticipated 20% - 30% of additional sponsorship.

Project Goals and Supporting Objectives:

1. Determine how to optimally maintain and build up cultures of *Mecinus heydenii* and *Rhinusa brondelii* under quarantine growing conditions until approval is obtained to make field releases in North America (starter cultures initially received from CABI-Switzerland in spring/summer 2014).

2. Complete remaining host specificity testing for both candidate agents.

3. Assess potential of both agents to control their respective natural host: for *M. heydenii* – yellow toadflax, and for *R. brondelii* – Dalmatian toadflax.

4. Assess potential of both agents to control field-collected (naturally occurring) and manipulated hybrids (generated via known maternal and paternal crosses) of Dalmatian and yellow toadflax.

5. Develop and submit petitions for field release of *Mecinus heydenii* and *Rhinusa brondelii* with Swiss, Serbian, and Canadian collaborators.

6. Identify and prepare potential field release sites; characterize site biotic and abiotic attributes pre-release.

Project Justification/Urgency:

Yellow toadflax (YT) (*Linaria vulgaris* Mill.), and Dalmatian toadflax (DT) (*Linaria dalmatica* [L.] Mill.) (Plantaginaceae), are invasive Eurasian plants now established throughout continental North America (USDA, NRCS 2014; De Clerck-Floate and Turner 2013; De Clerck-Floate and McClay 2013). Introduced as an ornamental and medicinal plant to northeastern North America in the 1600s, YT's showy blossoms continued until recently to encourage anthropogenic spread through commercial nurseries and seed catalogs (Mack 2003; Lajeunesse 1999; Pennell 1935). DT was similarly introduced to North America as an ornamental, with the first record of New World cultivation anecdotally reported in 1894 (Alex 1962). DT has repeatedly escaped cultivation, becoming naturalized and widely distributed, and now infests rangelands, open forests, and transportation corridors throughout North America (Lange 1958; Robocker 1974; Nowierski 1992).

YT and DT are short-lived perennial species capable of propagating both vegetatively and sexually (Saner et al. 1995; Vujnovic and Wein 1997). DT typically occupies dry, rocky, uncultivated land, becoming aggressively invasive in response to fire (Jacobs and Sheley 2003) or other profound disturbance. YT occurs in a wide range of habitats including crop (Volenburg et al. 1999) and uncultivated land (Darwent et al 1975; Sutton et al. 2007). Naturally-occurring hybrid toadflax (HT) populations derived from cross-pollination between DT and YT was first confirmed from multiple sites in Montana; HT has subsequently been found at additional locations in Washington, Idaho and Colorado (Ward et al. 2009; Turner 2012; Boswell 2013).

Because invasive toadflaxes compete with surrounding vegetation on native grasslands (Lajeunesse et al. 1993), they have the potential to directly or indirectly impact rare plant species (Maron and Marler 2008). Appropriate preventative and/or control measures must be undertaken where disturbance has facilitated toadflax infestations; otherwise, both toadflax species are capable of significantly jeopardizing the floral biodiversity of invaded ecosystems, including threatened, endangered, and sensitive species (Phillips and Crisp 2001; Pauchard et al. 2003). Toadflax can replace valuable forbs and grasses, reducing the efficiency of grazing by wildlife, particularly on winter ranges (Grubb et al. 2002). When other forage plants are scarce, wildlife, birds and rodents will feed on toadflax, but it is not known to be heavily utilized by native wildlife species (Robocker 1970). In the Greater Yellowstone Ecosystem, YT is considered to be among the most invasive exotic plant species, and a significant threat to native biodiversity in open, human or naturally disturbed environments in protected areas of the Rocky Mountains (Pauchard et al. 2003).

Extensive infestations of rangeland with toadflax are expensive and complicated to manage (Lehnhoff et al. 2008). For cattle producers depending on public land grazing allotments, toadflax infestations can significantly diminish forage quantity and quality. Grazing animals on rangeland where toadflax and other invasive weeds are treated with herbicides may interfere or delay requirements for certified organic production, reducing profits through lost premiums for organically produced meat, while increasing production inputs. Application of costly herbicides has no guarantee of efficacy - chemical control of toadflax is often insufficient, difficult (e.g., rough natural terrains) or too damaging to native vegetation (Krick 2011; Sebastian and Beck 1992, -1989; Robocker 1968; Ferrell and Whitson 1987, -1988, -1989).

Both toadflax infestation and its chemical control have the potential to negatively impact the aesthetic and ecological values/services of vegetation communities. Reducing infestations and therefore the need for chemical treatments of invasive toadflax can increase the aesthetic values of such locales, attract greater wildlife diversity and thereby directly benefit local tourism. Due to health concerns and degradation of aesthetic quality, tourists and recreationists are likely to avoid areas where the appearance of vegetation is obviously altered by herbicide treatments. Similarly, tourists and other outdoor enthusiasts would also be more likely to visit areas perceived as 'natural' and 'pristine' vs. 'invaded' by visually distinct monocultures of yellow toadflax.

Reducing or eliminating herbicide applications would significantly benefit vegetation communities in toadflax invaded natural areas. Undesirable non-target impacts of herbicide applications may jeopardize plant community biodiversity beyond a legally or aesthetically acceptable level. Reducing the unintended non-target effects of herbicide application on non-weed vegetation directly increases habitat diversity and quality for pollinating insects. On a local scale, if herbicide applications eliminate large tracts of toadflax for the current growing season, crucial resources for pollinators supplied by toadflax are also abruptly lost.

Biological control allows for the gradual conversion of toadflax dominated areas to increased diversity and richness of other forb species capable of supplying the same necessary resources to pollinators. In the worst case scenario, herbicide applications can cause a conversion of mixed forb and grass vegetation communities to communities dominated by annual invasive grasses, thereby significantly altering local trophic interactions. Advantages of using classical biocontrol include sustainability, reducing supply and labor costs of repeated mechanical and chemical treatments while maintaining longer term control, reducing unintended and undesirable non-target impacts (Wilson et al. 2005; Sing and Peterson 2011).

Several exotic toadflax-feeding insect species are currently established in North America, the result of both intentional and adventitious introductions with host plant material (Sing et al. 2005). Three unintentionally introduced seed feeding beetles were the first toadflax specialists recorded in North America: *Brachypterolus pulicarius*, *Rhinusa antirrhini* and *Rhinusa neta* (Harris 1984). Agents screened for host specificity and approved for release before being intentionally introduced include a defoliating moth, *Calophasia lunula*; two root-boring moths, *Eteobalea intermediella* and *E. serratella*; a root galling weevil *Rhinusa linariae*; and a stem mining weevil, *Mecinus janthinus* (Harris 1963; De Clerck-Floate and Harris 2002; McClay and De Clerck-Floate 2002; Nowierski 2004; Wilson et al. 2005; De Clerck-Floate and McClay 2013; and De Clerck-Floate and Turner 2013).

Establishment and efficacy of insect biological control agents of exotic toadflaxes has varied considerably according to release host and location. Host preference for *L. vulgaris* over *L. dalmatica* (or vice versa) is evident in the field for certain established agent species (e.g., MacKinnon et al. 2005). Failure of agents to establish on or control the target weed(s) now seems plausibly explained by herbivore-host mismatches during initial North American releases. *Mecinus* populations successfully established on Dalmatian toadflax in North America are now known to be generally comprised of individuals from a previously cryptic, newly described Dalmatian toadflax-affiliated species, *M. janthiniformis* (Toševski et al. 2011).

Despite the presence of several exotic toadflax-feeding insect species in North America for more than 50 years, none have demonstrated proven, consistent efficacy under all environmental conditions and in all ecosystems/habitats where North American YT or DT currently thrive. Furthermore, recommendations for biological control of HT have not yet been developed, primarily because biocontrol attained with the best available agents, *M. janthinus* and *M. janthiniformis*, against their 'natural' hosts remains so inconsistent.

McClay and Hughes (2007) and De Clerck-Floate and Miller (2002) emphasize the ever-present, looming threat that climatic extremes may play in the failure of *M. janthinus* and *M. janthiniformis* to meet biological control expectations. Anecdotal reports in spring 2014 of high overwintering mortality across many *M. janthinus* and *M. janthiniformis* populations in Montana, for example, coupled with historically dense infestations of YT and DT provided further evidence of the need to assess additional species of candidate toadflax biological control agents.

Efforts have already been collaboratively reinitiated by the US and Canada to find new toadflax agents. The yellow toadflax shoot-galling weevil *Rhinusa pilosa* was the first of these newly identified species to be promoted by the International Toadflax Biological Control Consortium as an effective and safe candidate agent. Notification was received in 2013 that TAG supported the permit petition to make environmental releases of *R. pilosa*; the permit application remains under review by USDA APHIS PPQ. The opportunity to eventually release and assess the impact of two new candidate biological control agents, the Dalmatian toadflax stem galling weevil *Rhinusa brondelii*, and the yellow toadflax stem mining weevil *Mecinus heydenii*, may help to fill gaps in YT and DT biological control by meeting challenges posed by the environmental extremes found in invasive toadflax's adopted North American range.

Significant progress has been made in developing a successful program for toadflax biocontrol. In 2009 PI (Sing) and collaborators discovered established populations of *Mecinus janthinus* on yellow toadflax in western Montana. These have since been widely redistributed and are beginning to build up to collectable numbers in Idaho and Colorado. Experiments conducted in spring-summer 2014 with the stem mining weevil *Mecinus heydenii* showed differential levels of host acceptance and suitability for a range of parental (yellow and Dalmatian toadflax) and hybrid toadflax genotypes. Weevils placed on YT (the so-called 'natural' host) produced a high number of adult progeny. HT derived from manipulated crosses with YT as the maternal parent generated nearly as many live adult offspring as pure YT hosts. HT derived from manipulated crosses with DT as the maternal parent produced very few progeny. Field collected and verified HT from three disjunct locations yielded highly variable results: Site 1 plants produced many adult progeny; plants from Site 2 produced very few live adults; and plants from Site 3 appeared to be unsuitable, with no progeny produced. The successful addition of *Rhinusa brondelii* and *Mecinus heydenii* to the suite of agents attacking YT, DT and HT would increase the odds of sustained biological control of these weeds, which persist and invade such a wide range of habitats under broad environmental conditions.

Approach:

Source of *Rhinusa brondelii* and *Mecinus heydenii*: Insects originally sourced from field plots and greenhouse-reared colonies in northern Serbia at the Institute for Plant Protection and Environment (Zemun, Serbia) were received from CABI and are currently being reared in the quarantine facility at Montana State University, Bozeman, MT. Agents sourced from the Serbian colonies are of known identity and are free of natural enemies. Insects being reared in the MSU quarantine facility will be securely caged and inspected frequently for emergence of parasitoids, inquilines, or other unwanted arthropods, and for disease symptoms that may indicate the presence of entomopathogens. Unwanted arthropods or infected individuals will be immediately removed and destroyed through submersion in ethyl alcohol or autoclaving.

Maintaining and increasing cultures of *Mecinus heydenii* and *Rhinusa brondelii* under containment growing conditions: Insects received in late spring and summer 2014 will need to be retained in quarantine until approval is obtained for field release, which could take 1 or more years. *Mecinus heydenii* adults received in spring 2014 were immediately placed on a range of test plants in the quarantine greenhouse; by mid-summer a significant number of F1 adults began to emerge. All above-ground portions of each of the large test plants was inspected and carefully dissected to enumerate live/dead of all weevil life stages. Live adults were then transferred to new host plants. *Rhinusa brondelii* was received in late summer 2014 as non-active, non-feeding summer aestivating adults. Rearing protocols provided by European and Canadian collaborators are now being used for continuous culture of both insect species in an attempt to artificially force the agents to transition to overwintering aestivation in small rearing cages that are being retained in a plant growth chamber. Insects must be transferred weekly to fresh cages provisioned with washed sand (aiding in within-cage moisture regulation), small strips of corrugated cardboard (for harborage), and sprigs of fresh host plant material maintained in cubes of floral foam. This approach is being used for the first time by the PI of this proposal, so some trial and error and room for improvement in executing existing rearing protocols are expected. Every attempt will be made to streamline rearing to make it as efficient, successfully productive and cost-effective as possible.

Assessing the potential of *M. heydenii* and *R. brondelii* to control hybrid toadflax: Experiments conducted in spring-summer 2014 with the stem mining weevil *Mecinus heydenii* showed differential levels of host acceptance and suitability for a range of parental (YT or DT) and hybrid toadflax genotypes. These whole plant tests will be repeated with *M. heydenii* and similar tests will begin with *R. brondelii* once the insects finish over-wintering aestivation in spring 2015.

Intended sites for initial release, timing of release, and methods to be used: Initial U.S. releases are planned for an outdoor nursery (separate caged plots of YT and DT) within a large chain-link fenced garden study area at Forestry Sciences Laboratory on the MSU campus, and in secure sites on Forest Service and privately-owned lands, also where establishment can be closely monitored. Releases will be made of spring-emerged adults, timed to coincide with the growth phenology of host plant shoots most optimal for agent reproduction at the chosen sites. Both female and male weevils will be released at the same time in either caged or open situations. The number of adults released per site will depend on the availability of quarantine-reared weevils, but if possible, initial releases will be made at a rate of approximately 100 adults/site. Experiments during the initial release stage are being planned to determine optimum release strategies for increasing establishment success.

Post-release monitoring: The anticipated time of initial releases will be early spring (April-May) and will be dependent on the phenological development of host plants at chosen release sites. Initial monitoring will assess establishment, local spread and impact of *Rhinusa brondelii* and *Mecinus heydenii*.

Required Documentation: Petitions for field release of the DT stem galling weevil *Rhinusa brondelii* and the YT stem mining weevil *Mecinus heydenii* will be completed and submitted by the PI of this proposal and co-authors for review by the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in 2015. A test plant list for invasive *Linaria* developed by the PI of this proposal and co-authors was approved by the Technical Advisory Group for Biological Control Agents of Weeds (TAG) and USDA-APHIS PPQ.

Expected Products and Outcomes:

1) Complete and submit petitions for *Rhinusa brondelii* and *Mecinus heydenii*.

2) Develop lab/greenhouse rearing methods that can be used to mass rear and sustain long term continuous cultures of weed biocontrol agents under quarantine conditions.

3) Develop sound recommendations for biological control of hybrid toadflax.

Budget Request FY15	
Professional technical support - salary: (GS-5 equivalent $28,000 x 0.25 FTE)	$7,000
Professional technical support - benefits: (35% x $7,000)	$2,450
Student technical support – during school year: (8 months x 4 weeks = 32 weeks part-time) (32 x 10 hrs/week = 320 hours) ($12.50/hr + 10% benefits = $13.75) (320 hours x $13.75 =$4,400)	$4,400
Student technical support – during summer: (4 months x 4 weeks = 16 weeks full-time) (16 x 40 hrs/week = 640 hours) ($12.50/hr + 10% benefits = $13.75) (640 hours x $13.75 =$8,800)	$8,800
Contracted services: overseas collection, open field tests, rearing and shipping	$10,000
Greenhouse supplies: potting soil, plant pots, fertilizer	$200
quarantine facility rent (Montana State University) $300/month x 12 months	$3,600
Field supplies:	$200
Field vehicle mileage (2,500 miles x $0.40/mi)	$1,000
TOTAL	$37,650

Proposed TIMETABLE:

May 2014: receive field collected *M. heydenii* in MSU quarantine; ramp up host plant production via clones of genetically characterized plants

May - July 2014: begin assessment of in-season feeding and colonization; begin mass rearing *M. heydenii*; begin whole plant tests against range of toadflax genotypes (quarantine)

July - August 2014: dissect adult *M. heydenii* weevils from large test plants; transfer progeny to fresh potted plants; continue mass-rearing

September - October 2014: receive *R. brondelii* in MSU quarantine; transition both agent species to overwintering aestivation cultures in growth chamber; fabricate cages; transfer agents to fresh cages every 2-7 days; continue mass rearing plants

November 2014: dissect adult *M. heydenii* weevils from second round of large test plants; transfer progeny to fresh potted plants; continue mass-rearing host (=food) plants

November 2014 - April 2015:	keep weevils alive! finish writing petitions and submit
May 2015:	identify potential field release sites; continue mass rearing for 2014 releases
May 2015 – beyond:	continue mass-rearing agents until approval for release is granted

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