1. **PROGRESS REPORT:** Cooperative Agreement 16-CA-11420004-065

2. **PERIOD UNDER REPORT:** June 27 - November 30, 2016

3. **TITLE:** Assessing Population Sizes, Biocontrol Potential and Mass Production of the Root Boring Moth *Agapeta zoegana* for Areawide Implementation and Monitoring of Spotted Knapweed Biocontrol

4. **PRINCIPAL INVESTIGATORS**: Mark Schwarzländer, PSES Department, University of Idaho, 875 Perimeter DR MS 2339, Moscow, ID 83844-2339, (208) 885-9319, FAX (208)885-7760, markschw@uidaho.edu; Joseph Milan, USDI Bureau of Land Management, 3948 Development Ave., Boise, ID 83705, (208) 384-3487, FAX (208) 384-3326, jmilan@blm.gov; Paul Brusven, Nez Perce Tribe Bio-Control Center, P.O. Box 365, 22776 Beaver Road, Lapwai, ID 83540, (208) 843-9374, FAX (208) 843-9373, pbrusven@nezperce.org

5. **EXECUTIVE SUMMARY:** 1) Work on the project was delayed due to technical problems finalizing the agreement between the USFS and the University of Idaho. The agreement was finally countersigned on June 27, 2016, prior to which we were not allowed by federal rules to conduct research. 2) Because of the delay we were unable to recruit a graduate student for August 2016 (due to university deadlines) for the research outlined in the agreement. However, a graduate student is currently being recruited and she will join our team for the 2017 field season. 3) We have propagated spotted knapweed and native grass plants for the setup of the microcosms and all plants have been transplanted into microcosm during the 2016 field season. 4) We began to survey select *Agapeta zoegana* field sites for analyses of plant and herbivory traits. These will be used for regression analyses to measure the relative effect of herbivory attack in the field and to identify nondestructive predictive plant size parameters. 5) Analyses of field site data and setup of microcosms will continue through the winter 2016/17. We are currently also propagating insect herbivores for release in the microcosms during the 2017 field season.

6. **PROGRESS BY OBJECTIVE:** The goal of this project is the areawide biological control implementation and assessment for spotted knapweed, *Centaurea stoebe*. This is accomplished through common garden and open field experiments, and modeling activities of existing and newly collected data that emphasize root boring biological control agents of spotted knapweed, specifically the root boring moth *Agapeta zoegana*. This project comprises research in four has four objectives:

   Objective 1: Assessing the impact of the root boring weevil *Cyphocleonus achates* and the root boring moth *Agapeta zoegana* alone and in combination on spotted knapweed performance and recruitment under varying plant competition and climate and climate regimes. We have completed the setup of the 48 microcosm for this objective. We used 2 age classes of spotted knapweed plants (i.e. 1 year-old plants and 3 month-old plants) and field densities of native competitive grass species for the microcosms. *Cyphocleonus achates* and *A. zoegana* will be released in microcosms according to the complete randomized design that we used to setup the experimental plots. We will also apply a drought treatment to half the microcosms beginning in 2017.

Objective 2: Assessments of habitat dependent establishment rates, distribution and abundance of *A. zoegana* and *C. achates* on a spatial scale (State of Idaho). We have visited 4 of the 45 release sites of *A. zoegana* in 2016 to collect plant and herbivory data. We use the date form those 4 field sites for regression analysis to identify predictive non-destructive plant parameters that will reduce the time/workload to assess all 45 field sites. Because herbivory attack rates at all 4 sites was very low, we will continue comprehensive sampling until we have data for a few field sites with higher attack rates. It is our intention to visit a total of 50-60 field sites per year and repeat visits every year of this project to assess between year variability of herbivory attack and correlate it to environmental parameters, especially climate (Tab. 1).

Objective 3: Analyze biological control impact using existing and newly collected SIMP (Standard Impact Monitoring Protocol) data on a spatial scale (State of Idaho). We obtained SIMP data collected in years past in the State of Idaho for spotted knapweed biological control and have begun to control data spread sheets for errors and consistency. We reorganize data for statistical digestion and make decisions for which field sites we have consistent time series data. In addition, we have try to resolve how to treat data with regard to confounding effects of root and seed herbivory present at field sites. This will be one of the main research objectives of the incoming graduate student in collaboration with our statistician Dr. William Price (university of Idaho).

Objective 4: In this objective we will address two specific practical implementation questions with regard to *A. zoegana*: 1) what intensity of root herbivory needs to be reached (density dependent herbivory) to negatively affect spotted knapweed growth or reproduction or kill plants? And 2) what is the most cost effective way to mass produce large numbers of the moths? For this objective, we have setup the second set of microcosms and we have begun to acquire *Agapeta zoegana* with our partner the Nez Perce Tribe Biocontrol Center. We will not consider artificial diet for mass rearing efforts as it is generally accepted that artificial diets are not cost efficient. The mass rearing efforts will be conducted using potted plants and field cage setups in a cropland comparable setting. These will be setup during the 2017 field season.

Table 1: Field site locations of preliminary sites visited in summer 2016 to assess field abundance of *Agapeta zoegana* and *Cyphocleonus achates* and list of plant variables and herbivory assessed for regression analyses (n = 30 for all field sites).

Site	Name	Coordinates	Sampling date
1	Powell Ranger Station (North)	N46.522706, W114.684425	07/26/2016
2	Powell Ranger Station (South)	N46.501997, W114.669801	07/26/2016
3	Farragut State Park	N47.952200, W116.605700	07/28/2016
4	Athol (Brunner Rd.)	N47.903295, W116.768390	07/31/2016

Site	Mean no. / plant		Mean length (cm)		Mean diameter (mm)		% roots with previous attack	Mean no. *Cyphocleonus*			Mean no. *Agapeta*		Dry weight (g)	
	Stems	Buds	Longest stem	Root	Root crown	Root (2 cm below crown)		Larvae	Pupae	Neonates	Larvae	Pupae	Above-ground	Root
4	1.36	13.93	53.91	16.51	5.81	4.55	0	0	0	0	0	0	3.421	0.921
3	2.20	11.93	40.05	14.76	6.70	5.13	0	0	0	0	0	0.033	2.507	1.023
2	2.90	26.83	58.69	17.49	8.72	6.61	6.67	0	0	0	0	0.100	6.897	2.276
1	1.46	11.36	54.74	14.18	5.94	4.54	0	0	0	0	0	0.033	2.737	0.810

Fig. 1: Setup of one of two 48-plot microcosm experiments at the University of Idaho Parker Research Farm in Moscow, ID (top). Treatments of drought, climate variation are randomly assigned to microcosms. Each microcosm contains 8 large and 8 small randomly assigned *C. stoebe* plants and a mix of native grass species (bottom).