1. **ASSESSING POPULATION SIZES, BIOCONTROL POTENTIAL AND MASS PRODUCTION OF THE ROOT BORING MOTH *AGAPETA ZOEGANA* FOR AREWIDE IMPLEMENTATION AND MONITORING OF SPOTTED KNAPWEED BIOCONTROL**

2. **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

3. **COOPERATORS:** Dr. Hariet Hinz (CABI Switzerland), Dr. Urs Schaffner (CABI Switzerland, Dr. Sanford Eigenbrode (University of Idaho), Dr. Heinz Müller-Schärer (University of Fribourg, Switzerland), Brian Marschmann (USDA APHIS PPQ State Director, Idaho), Dr. Rich Hansen (USDA APHIS CPHST, Ft. Collins, Colorado), John (Lewis) Cook (USDI BIA Rocky Mountain Region, Billings, Montana), Dr. John Gaskin (USDA ARS NPARL, Sidney, Montana), Idaho County Weed Superintendents and Idaho-based USFS land managers.
   BCIP CONTACT: Carol Randall, USFS Northern and Intermountain Regions, 2502 E Sherman Ave, Coeur d'Alene, ID 83814, (208) 769-3051, (208) 769-3062, crandall@fs.fed.us

4. **REQUESTED FUNDS: USFS $100,000** (Year 1: $34,000; Year 2: $33,000; and Year 3: $33,000), Project Leveraging: **University of Idaho $124,329**.

5. **EXECUTIVE SUMMARY:** 1) The current status of ecological research suggests that albeit having some impact on spotted knapweed, both, *A. zoegana* and *C. achates* have stronger negative effects on native grasses, thus indirectly benefiting one of most devastating invasive plants in the U.S. 2) This data may misrepresent and underestimate the biocontrol impact of *A. zoegana* and *C. achates* due to use of small herbivory levels in studies. 3) Despite resource-costly distribution efforts for *C. achates* during the past 20 years, ***A. zoegana*** **populations have recently increased to comparable or even greater levels without anthropogenic assistance**. 3) The ecology of *A. zoegana* in the U.S. including its biocontrol impact potential and potential **synergistic effects with *C. achates* are currently poorly understood**. 4) A **mass rearing technique** and respective technology transfer is needed to extend areawide distribution of *A. zoegana.* 5) The work proposed here addresses all above points and develops a technique to mass provide the biocontrol agent.

6. **PROJECT GOALS AND SUPPORTING OBJECTIVES:** The goal of this project is the areawide biological control implementation and assessment for one of the western United States most serious noxious weeds of grassland and forests: 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 will develop distribution and post-release monitoring techniques for *A. zoegana* (BCIP Project Priority No. 1), develop rapid quantitative assessment of root herbivore impact in the field for the knapweed root weevil *Cyphocleonus achates* and *A. zoegana* (BCIP Project Priority No. 2), and identify habitat preferences for both biological control agents, thus economize redistribution resources. We propose to combine quick field sampling methods with analysis of historic insect release records to calculate establishment of spotted knapweed root herbivores along environmental and time

gradients. Finally, we propose to assess overall ecosystem effects of spotted knapweed biological control through analysis of existing and newly collected SIMP (Standard Impact Monitoring Protocol) data. Combined, the proposed work will provide urgently needed data on the root boring moth *A. zoegana*, facilitate its areawide implementation through development of mass rearing technologies, and develop two different approaches to assess overall efficacy of spotted knapweed biological control based on field collected data. This project 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. The University of Idaho weed biocontrol laboratory owns a set of 48 microcosms that allow us to manipulate environmental conditions. With this objective we will establish baseline data on the impact of *C. achates* and *A. zoegana* individually and combined on spotted knapweed plant performance and recruitment under varying climatic conditions over a three year period. The experimental impact data will also assist to corroborate or contradict observed patterns in the field (see Objective 2) (This objective will use **12.5% PE** (project effort)).

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 will use the Nez Perce Tribe Bio-Control Center and EDDMapS database records to revisit release sites for both spotted knapweed root feeding biocontrol agents through time and select additional sites along environmental gradients to assess habitat preferences and estimate distribution and abundance of both insects with regard to human assisted distribution efforts. This objective will provide information on the phenotypic plasticity of each biological control agent with regard to habitat and elevation and provide insight on the dispersal capability and efficacy of *C. achates* and *A. zoegana* with regard to historic and present weed infestation sizes. The objective will direct future redistribution efforts for both biocontrol agents. In a sub-objective, we will develop/improve a quick destructive, quantitative monitoring method for the two root boring insects at field sites (**25% PE**).

Objective 3: Analyze biological control impact using existing and newly collected SIMP (Standard Impact Monitoring Protocol) data on a spatial scale (State of Idaho). The Idaho Biocontrol Task Force (hereafter ID BTF), has collected SIMP data for spotted knapweed for seven years. This data is confounded due to co-occurrence of several biocontrol agents and difficulties to reliably assess root herbivore abundance based on adult counts. We will analyze root herbivore abundance at all existing SIMP field sites using the destructive sampling method developed under Objective 2and include data as covariate in existing SIMP data models. This objective will lead to a modification of the current SIMP protocol for spotted knapweed. This objective will altogether result in a ten year data set, which will provide insight into overall biocontrol efficacy including separation of effects by seed-feeding versus root boring insect biocontrol agents, precipitation and elevation effects. This objective will also provide data on vegetation community responses to spotted knapweed biocontrol (**25% PE**).

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 dependant 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? Objective 4.1 will make use of a second set of 48 smaller microcosms in which we will study the effect of density dependent *A. zoegana* root herbivory on spotted knapweed and surrounding native grasses (**12.5% PE**). For Objective 4.2 we will develop affordable mass rearing protocols to provide land managers with starter releases (**25% PE**). These two sub-objectives will inform land managers at which level (threshold) of root herbivory by *A. zoegana* in the field spotted knapweed will be severely impaired. And it will provide a methodology that can provide insects for redistribution programs. The latter is important because the moth is currently still unavailable and field collections resource intensive and overall cumbersome.

Combined, the four objectives will result in a comprehensive data set, which will provide pertinent distribution, abundance and impact data for the greatly understudied and underutilized root boring moth *A. zoegana.* The project will provide comparable data for the root boring weevil *C. achates*, which is actively redistributed throughout the western U.S. and reared on artificial diet at significant resource costs. This project will provide important data on the compatibility and potential synergisms of both root feeding biological control agents of spotted knapweed, a topic for which there exist currently no data in North America. Finally, this project will reconcile contradicting data on the ability of the current suite of biological control agents to control spotted knapweed over time and the consequences of this ability for neighboring plant communities. The results of this project will result in methodology development and technology transfer products regarding post-release impact monitoring and redistribution of spotted knapweed root feeding biocontrol agents (see Products and Outcomes below).

7. **PROJECT JUSTIFICATION/URGENCY:** Spotted knapweed *Centaurea stoebe* L. ssp. *micranthos* (Gugler) is an herbaceous Eurasian perennial Asteraceae that has been introduced to North America with contaminated hay shipments from Asia and Europe around the 1890s. It has since become one of the most widespread and most problematic invasive plants in North America. Spotted knapweed currently occurs in 46 U.S. states and 6 Canadian provinces. It is declared as noxious in 14 states including the entire western U.S and 4 Canadian provinces (Winston et al. 2010). Spotted knapweed invasions are often characterized by dense stands that competitively exclude native species completely (Callaway et al. 1999). Despite more than 50 years of chemical control efforts, spotted knapweed spread by the mid 1980s onto more than 2.5 million hectares (Chicoine et al. 1985) and onto more than 4 million hectares in the 1990s (Müller-Schärer and Schroeder 1993). Alongside *Bromus tectorum* L., cheat grass, and *Euphorbia esula* L., leafy spurge, spotted knapweed is considered one of the most economically destructive exotic invaders in the northwestern U.S. and southwestern Canada (Callaway et al. 1999). Spotted knapweed was also recently mentioned as one of the key noxious weeds impairing endangered sage grouse habitat in Nevada (J.J. Goicoechea, pers. comm.).

Since the 1970s a total of 13 arthropod species have been introduced to North America for the classical biological control of spotted knapweed. Some of the biological control agents have become widespread and abundant such as the seed feeding fruit flies *Urophora affinis* (Frauenfeld), *U. quadrifasciata* (Meigen), the seed feeding weevils *Larinus minutus* Gyllenhal, *L. obtusus* Gyllenhal, and the root feeing weevil *Cyphocleonus achates* (Fåhraeus). The effects of these biocontrol agents have been investigated in numerous studies during the past 15 years with varying and often contradicting outcomes (Knochel and Seastedt 2010). In the early 2000s, spotted knapweed exhibited regional population level declines with greater than 60% plant density reductions in some U.S. states. While some authors credited biological control for these declines (Corn et al. 2006, 2007, Jacobs et al. 2006, Knochel et al. 2010, Story et al. 2006, Story et al. 2008), the majority of studies attributed it exclusively to drought (Pearson and Callaway 2006, Pearson and Fletcher 2008, Ortega and Pearson 2011) or dismissed the influence of biocontrol on the phenomenon outright (Ortega et al. 2012). Studies demonstrated that there is little indication for competitive interactions between seed feeding and root mining biological control insects of spotted knapweed (Stephens and Myers 2014, Van Hezewijk and Bourchier 2012) but showed instead that seed and root herbivores have generally additive impacts on the invasive weed (Knochel et al. 2010, Van Hezewijk and Bourchier 2012). These findings are in concurrence with the cumulative stress hypothesis, which states that plants can no longer tolerate or compensate for herbivory if it exceeds a threshold load, regardless of whether it is caused by individual or a suite of herbivore species (Knochel et al. 2010, Knochel and Seastedt 2010). On the other hand, there are numerous common garden and greenhouse studies that have shown that spotted knapweed tolerates or even over-compensates for root mining herbivory by *C. achates* (Ortega et al. 2012) and by the root mining moth *Agapeta zoegana* (L.) (Newingham et al. 2007, Steinger and Müller-Schärer 1992). More importantly, studies associated both root boring biological control agents with indirect negative effects on native grass species (Callaway et al. 1999, Ortega et al. 2012, Ridenour and Callaway 2003, Thelen et al. 2005). In a recent study, Knochel and Seastedt (2010) attempted to reconcile the contradictive data, proposing that compensation or impairment of spotted knapweed populations depends on the intensity of biological control herbivory and resource availability. Indeed, there is a remarkable lack of data on realized spotted knapweed root herbivory levels in the field (but see Randall 2015 for an exception) and some of the studies associating root herbivores with negative effects on native grasses (e.g. Ridenour and Callaway 2003, Ortega et al. 2012) used questionably low levels of root herbivory.

Recently, data has been reported that the assumed rare root mining moth *A. zoegana* (Winston et al. 2014) has dramatically increased in distribution and abundance in the States of Montana and Idaho and the Province of British Columbia (Randall 2015, Schwarzländer unpubl. data, Joseph Milan, pers. comm., Val Miller, pers. comm.). *Agapeta zoegana* or the 'sulfur knapweed moth' was first reported as established in the U.S. in Montana in 1991 (Story et al. 1991) but the abundance was generally characterized as low and the biocontrol agent since not considered a priority for redistribution (Winston et al. 2014). The moth has not been the target of larger redistribution efforts (Fig. 1), in part due to its limited availability and in part due to its cryptic life history. The scarcity of knowledge on *A. zoegana* in North America is, however, unacceptable especially given the fact that the moth can now be found at field sites throughout Idaho coexisting with - and as abundant as the actively distributed *C. achates* (Randall 2015, Schwarzländer unpubl. data). The very little information on *A. zoegana* in North America can be summarized as follows: The moth first established successfully in the U.S. in Montana in 1991 (Story et al. 1991), has a strong preference for larger and bolting plants (Story et al. 2000, Smith and Story 2002), did not show any additive impact with grass competition during a field study in Montana (Story et al. 2000), and in a greenhouse study conducted in Europe showed no competitive interactions with *C. achates* (Steinger and Müller-Schärer 1992). We could not find any accounts on abundance and distribution in the field, attack patterns, interactions with other root herbivores or impact. In stark contrast to this paucity of information, intensity of attack has been consistently greater for *A. zoegana* during the past 4 years at three field sites in Idaho at which the moth co-occurs with *C. achates.* Attack rates at these sites ranged from 19 – 25% of spotted knapweed roots sampled

(Randall 2015). Similarly, we found 55% of roots attacked during an opportunistic survey of a pasture in Browning, MT, with the majority of attacked roots containing more than one *A. zoegana* larvae. At that same field site, several thousand *C. achates* were previously purchased and released. For a widespread invasive plant such as spotted knapweed classical biological control may be the only management tool that realistically offers at least the potential for sustainable control (Bernd Blossey pers. comm.). In line with the cumulative stress hypothesis for spotted knapweed (Knochel and Seatstedt 2010), there is an immediate urgency to assess the increased abundance, distribution and attack intensity of *A. zoegana* at a spatial scale, its interactions and potential synergisms with *C. achates,* and to derive respective implementation strategies from the results of these investigations.

Proposed action: The recently discovered large populations and more widespread distribution of *A. zoegana* may potentially offer different outcomes in spotted knapweed biocontrol programs or accelerations of spotted knapweed weed density declines. Since the biocontrol community was literally unaware of this cryptic distribution expansion and since the field impact of *A. zoegana* and interactions with other biological control agents are unknown, we can currently not deduce technology transfer guidelines or implementation strategies for this biocontrol agent. In British Columbia, the areawide decline of spotted knapweed has been attributed to increases of the density of root herbivores in general and *A. zoegana* specifically (Val Miller, pers. comm.). The overall goal of the work proposed here is to assess *A. zoegana* population sizes along environmental gradients on a spatial scale (State of Idaho), study their impact on spotted knapweed and potential synergisms with other spotted knapweed biocontrol agents, specifically *C. achates* as these two root herbivores co-occur in spotted knapweed populations and even within the same roots (Randall 2015, Schwarzländer, unpubl. data).

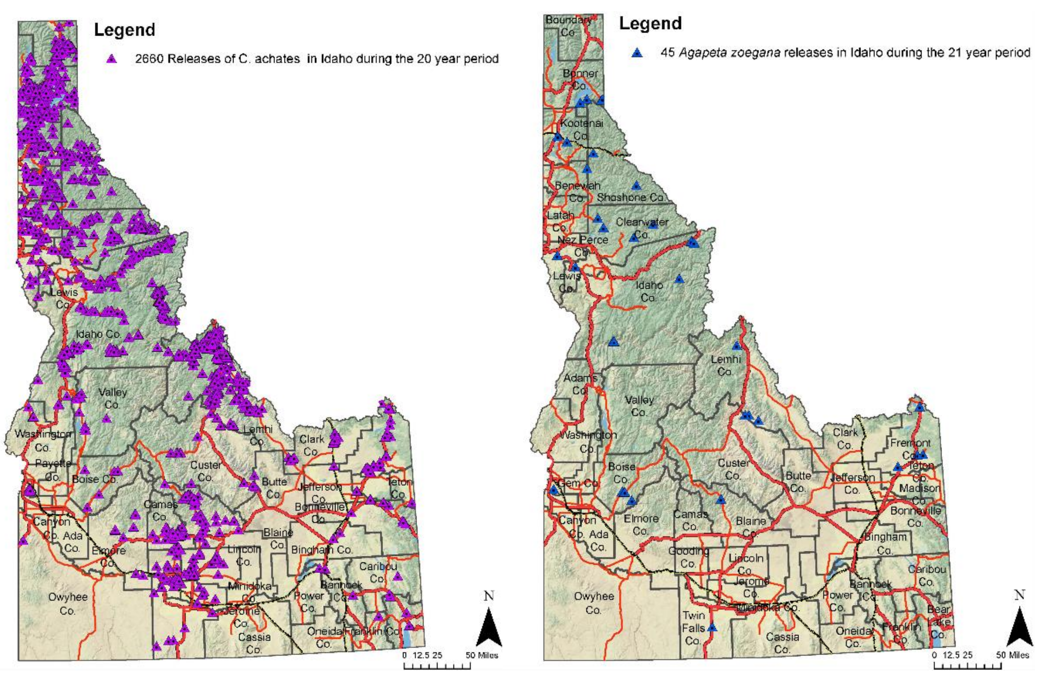

Fig. 1 Documented *Cyphocleonus achates* releases in the State of Idaho (left) between 1994 and 2014 (n = 2660) and *Agapeta zoegana* releases (right) in Idaho between 1993 and 2014 (n = 45). Data provided by the Nez Perce Bio-Control Center; Accessed 10/15/2015.

8. **APPROACH: Plant and insect materials and common gardens.** We have collected seeds throughout the years from local spotted knapweed populations and maintain collections at our laboratory. We have germinated several 100 seeds from a local Moscow, ID population in Spetember 2015 and these seedlings will be transplanted into microcosms by the end of November 2015. *Cyphocleonus achates* adults and *A. zoegana* adults for the common garden experiments will be kindly provided by the Nez Perce Bio-Control Center. The weed biocontrol lab at the University of Idaho owns two sets of microcosms. These are one block of 48 round 3.25m² microcosms (to be used for Objective 1) with a depth of 1.5m. Soil in microcosms has been undisturbed since 2003 and last experimentation was conducted in 2011. We recently applied herbicide to remove all vegetation in microcosms in preparation to plant spotted knapweed and native grasses. A second block consists of 48 1.3m² microcosms, also 1,5m deep with undisturbed soil since 2003 (to be used for Objective 4.1). We will use Idaho fescue *Festuca idahoensis* Elmer, and bluebunch wheat grass *Pseudoroegneria spicata* (Pursh) A. Löve as native grass competitors since both are dominating and sympatric occurring species in Idaho short grass prairie. Microcosms have advantages over regular field plots which include control over soil nutrient conditions, soil moisture and isolation from neighboring vegetation effects.

Objective 1: Individual and combined impact of *A. zoegana* and *C. achates* on spotted knapweed populations under varying environmental conditions. This will be a factorial design with *A. zoegana*, *C. achates*, both root herbivores and no root herbivory as one treatment factor. Microcosms will contain field densities of bluebunch wheatgrass, Idaho fescue and spotted knapweed, all of which are currently propagated. For spotted knapweed, we will use plants of two different age/size classes to account for and measure preference of either root herbivore for a given plant size. The competition levels will be low grass competition (half of that found in spotted knapweed invaded natural areas), normal grass competition (equal to that at invaded natural areas) and normal grass competition at changed climate (using heat lamps to mimic an average climate temperature scenario for north central Idaho for 2040 (obtained from the University of Idaho's REACCH PNA program www.reacchpna.org). The experiment will be conducted over 3 ½ years. Soil will not be disturbed. Major response variables measured will be spotted knapweed mortality, seed head production per stem and unit area and spotted knapweed recruitment. Native grass mortality and plant size parameters will also be measured. Realized spotted knapweed root herbivory (no. larval stages per root) will be assessed through random harvest of select plants on an annual basis. We will use ANOVA and Repeated Measure ANOVA techniques to analyze data. This objective will provide data on effects of root herbivory by *A. zoegana*, *C. achates* and both insects on spotted knapweed performance and native grasses in a realistic setting. In addition, we will - after consultation with our statistician (Dr. William Price) - potentially add treatments to the experimental setup in given years. For example we may add a drought event in a set of microcosms in a given year (**12.5% PE**).

Objective 2: Assessments of habitat dependent establishment, distribution and abundance of *A. zoegana* and *C. achates* based on historic release data. For this objective, we will closely work with USFS and BLM field personnel and Weed Superintendents throughout Idaho counties. We will draw on release information for both root herbivores retrieved from the Nez Perce Bio-Control Center database and existing spotted knapweed SIMP monitoring sites throughout the state. We will categorize release sites using environmental filters such as original infestation size, elevation, soil type, habitat, release date, release size (no. of insects) and visit a set number of sites each year for destructive spotted knapweed plant sampling based on the SIMP monitoring protocol (http://www.agri.state.id.us/Categories/PlantsInsects/NoxiousWeeds/Bio_Control.php. In brief, all plants in ten 0.125m² frames along a 20m transect will be collected to assess current biological control root herbivore abundance rates. Plants will be transported back to the University of Idaho via bonded carrier or processed in the field. Above- and below ground size and fresh weight will be measured, seed head no. counted and roots will be dissected for insect instars. All sites will be visited during elevation-adjusted times when larvae/pupae of both root herbivores can be found in spotted knapweed roots. We will use this data to correlate biocontrol establishment rates with habitat characteristics and release date. In the case of *A. zoegana* this will be admittedly be difficult given the relative small number of releases made during the past 20 years. We do, however, expect to find *A. zoegana* established at many sites at which it has never been released. In total we aim to visit 120 field sites (40 per year) for this objective or more depending on assistance from local land managers (**25% PE**).

Objective 3: Analysis of existing and newly collected SIMP (Standard Impact Monitoring Protocol) data on a spatial scale (State of Idaho). The Idaho Biocontrol Task Force (chaired by Joseph Milan) has collected SIMP monitoring data for spotted knapweed over the past seven years, on average for 30 field sites throughout the state. We have unique expertise modelling SIMP data for the effects of biocontrol herbivory and contrasting it from other environmental factors such as precipitation or temperature (Price 2014, Weed and Schwarzländer 2014) and will work closely for this objective with Dr. Aaron Weed (now USDI NPS) and our statistician Dr. William Price. While the SIMP data on spotted knapweed is more comprehensive than that analyzed for Dalmatian toadflax (Weed and Schwarzländer 2014) and Canada thistle (Price 2014, Weed Price and Schwarzländer, in preparation), it is also more challenging for two reasons: 1) seed feeding herbivory effects and those of root feeding herbivores are confounded at many SIMP sites and 2) herbivory data for root herbivores is missing or in instances potentially underrepresenting the realized root herbivory levels. In addition, potential effects of *A. zoegana* are completely missing in the existing SIMP data set. This is because presence and numbers of the moth were not assessed as part of the protocol since it was assumed that the moth is rare and not present at SIMP monitoring sites. Under this objective we will visit all current SIMP sites during 2 years and collect destructive transect plant samples (see Objective 2) in the vicinity of the permanent SIMP monitoring transects to assess the realized root herbivory at all sites. We will use this data as covariates in the SOIMP effect modelling efforts.

We will also modify the SIMP monitoring protocol to include *A. zoegana* and to collect data that provide more reliable data for root herbivore abundnace. This will be accomplished through different adult monitoring approaches and subsequent regression and correlation analysis of those adult counting assessments and realized root herbivory intensity using field sites as replicates.

Combined, the existing and newly gathered information will result in a ten year monitoring data set, which to our knowledge is one of largest biocontrol monitoring data sets collected. It will provide insight into overall biocontrol efficacy including separation of effects of seed feeding versus root boring insect biocontrol agents. It will also provide pertinent data on the effects of precipitation (drought) on spotted knapweed performance and surrounding vegetation communities and the interactions of biocontrol and precipitation (**25% PE**).

Objective 4: *Agapeta zoegana* larval herbivory damage threshold and propagation techniques. This objective has two applied sub objectives: In Objective 4.1 we will assess the threshold or density at which *A. zoegana* larval feeding will severely damage spotted knapweed reproduction and survival. We will use a second set of 48 1.3m² microcosms to set up a full factorial design with two factors, *A. zoegana* herbivory at 4 logarithmic levels and changing climate (using heat lamps) at 3 cumulative day degree levels. As in Objective 1, microcosms will be populated with spotted knapweed plants and native grass competition comparable to densities found in the field. Response variables will include spotted knapweed survival, reproductive output per stem and per unit area and recruitment. We will also measure native grass performance and realized root herbivory. Data will be analyzed with ANOVA and Repeated Measure ANOVA techniques. This experiment will be conducted over a 3 ½ year period. This experiment will provide essential data on the biocontrol potential of the root boring moth and help prioritize spotted knapweed biocontrol implementation, redistribution and resource allocation efforts (**12.5% PE**).

Under Objective 4.2 we will develop effective mass propagation techniques for *A. zoegana.* The University of Idaho has experience in the propagation of other difficult to rear root moths such as *Bradyrrhoa gilveolella* (Treitschke), which we reared at more than a thousand individuals a year. We do not assume that *A. zoegana* will be as difficult to propagate for a number of reasons but we will spend considerable amounts of time developing an affordable resource effective propagation method. We intend to take two different approaches to propagate *A. zoegana*, one using larger 5 gallon pots and another using larger tubs typically used for livestock watering stations. In either case, containers will be filled with standardized potting soil and we will regularly fertilize spotted knapweed plants grown in containers, since it is known that fertilization will increase insect production and plant tolerance to herbivory. Both types of containers will be covered with gauze prior to the emergence period of insects. We will throughout the course of method development keep a ledger on materials and labor to provide cost-per-insect information for each method. In addition, this objective will provide data on the life history of the moth. Indications are that the moth is univoltine in North America while it completes 2 – 3 generations in Europe (Corn et al. 2009). As we did for the propagation of *Bradyrrhoa gilveolella*, we will closely work with - and transfer all propagation technologies to the Nez Perce Tribe Bio-Control Center to maximize implementation (**25% PE**).

9. EXPECTED PRODUCTS AND OUTCOMES: The data generated in this project will be published in **4 refereed journal articles** on 1) the combined effect of the two root herbivores, 2) the density dependent root herbivory under changing climates, 3) modelling the 10 years of SIMP data and 4) the habitat specific establishment and abundance of the root herbivores based on past releases. This project will provide a thorough assessment of the potential of the recently increase populations of *A. zoegana* to contribute to the successful biologically based management of spotted knapweed. In addition, we will develop a **cost-effective mass propagation method** for *A. zoegana* to aid redistribution and implementation efforts. We will use all data to update the USFS **FHTET 'Biology and Biocontrol of Knapweeds'** manual at the end of this project.

APPENDIX I – Workflow/load and timeline

Workflow/load: To accomplish the objectives proposed, the University of Idaho will seek a Ph.D. graduate student who will be mainly responsible for all work. We will, however dedicate 0.4 FTE of Research Support Scientist effort and 0.25 FTE of Principal Investigator effort to this project. In addition, the graduate student will be assisted by 4 Environmental Science Seniors who will work on sub-objectives as part of their senior research projects. Finally, the graduate student will be supported during the summer months of each study year by a full time student worker. Combined, the manpower will be sufficient to complete the objective set forth above.

Timeline:

2016											
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Objective 1 - Microcosm experiment											
			Objective 2 – Release site sampling					BM1			
				Objective 3 – SIMP				BM2			
				Objective 4.1 - Microcosm experiment							
				Objective 4.2 – Rearing							
2017											
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Objective 1 - Microcosm experiment											
			Objective 2 – Release site sampling								
				Objective 3 – SIMP							
				Objective 4.1 - Microcosm experiment							
				Objective 4.2 - Rearing							
2018											
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Objective 1 - Microcosm experiment											BM3
			Objective 2 – Release site sampling					BM4			
				Objective 3 – SIMP				BM5			
				Objective 4.1 - Microcosm experiment					BM6		
				Objective 4.2 – Rearing					BM7, 8, 9		

Anticipated project period is **03/01/2016-12/31/2018**. Benchmarks (BM) as follows: **BM1**, Review/fine tuning of methodological approach following analysis of first field season data; **BM2,** Review/fine tuning of methodological approach following analysis of first field season data; **BM3**, Submission of article on combined root herbivory and changing climate; **BM4**, Submission of article on establishment and abundance of root herbivores based on historic releases; **BM5,** Submission of article modeling SIMP data (effect of herbivory and other environmental factors on spotted knapweed and surrounding vegetation); **BM6,** Submission of article on *A.* zoegana threshold intensity attack; **BM7**; Submission of article on cost effective mass rearing; **BM8,** Revision of knapweed biocontrol manual; **BM9,** Final project report to USFS FHTET.

APPENDIX II - References

Callaway, R. M., T. H. deLuca, and W. M. Belliveau. 1999. Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. Ecology. 80: 1196-1201.

Chicoine, T. K., P. K. Fay, and G. A. Nielsen. 1985. Predicting weed migration from soil and climate maps. Weed Science. 34: 57-61.

Corn, J. G., J. M. Story, and L. J. White. 2006. Impacts of the biological control agent *Cyphocleonus achates* on spotted knapweed, *Centaurea maculosa*, in experimental plots. Biological Control. 37: 75-81.

Corn, J. G., J. M. Story, and L. J. White. 2007. Effect of summer drought relief on the impact of the root weevil *Cyphocleonus achates* on spotted knapweed. Environmental Entomology. 36: 858-863.

Jacobs, J. S., S. E. Sing, and J. M. Martin. 2006. Influence of herbivory and competition on invasive weed fitness: Observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environmental Entomology. 35: 1590-1596.

Knochel, D. G., N. D. Monson, and T. R. Seastedt. 2010. Additive effects of aboveground and belowground herbivores on the dominance of spotted knapweed (*Centaurea stoebe*). Oecologia. 164: 701-712.

Knochel, D. G., and T. R. Seastedt. 2010. Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. Ecological Applications. 20: 1903-1912.

Müller-Schärer, H., and D. Schroeder. 1993. The biological control of *Centaurea* spp. in North America: do insets solve the problem? Pesticide Science. 37: 343-353.

Newingham, B. A., R. M. Callaway, and H. BassiriRad. 2007. Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory. Oecologia. 153: 913-920.

Ortega, Y. K., and D. E. Pearson. 2011. Long-term effects of weed control with Picloram along a gradient of spotted knapweed invasion. Rangeland Ecology and Management. 64: 67-77.

Ortega, Y. K., D. E. Pearson, L. P. Waller, N. J. Sturdevant, and J. L. Maron. 2012. Population-level compensation impedes biological control of an invasive forb and indirect release of a native grass. Ecology. 93: 783-792.

Pearson, D. E., and R. M. Callaway. 2006. Biological control agents elevate hantavirus by subsidizing deer mouse populations. Ecology Letters. 9: 443-450.

Pearson, D. E., and R. J. Fletcher. 2008. Mitigating exotic impacts: Restoring deer mouse populations elevated by an exotic food subsidy. Ecological Applications. 18: 321-334.

Price, J. R. 2014. Post-release assessment of classical biological control of Canada thistle (*Cirsium arvense*) in the western United States. Moscow: University of Idaho. 130 p.

Randall, C. 2015. Spotted knapweed (*Centaurea stoebe*) root sampling for knapweed root weevils (*Cyphocleonus achates*) and knapweed root moths (*Agapeta zoegana*). CFO-TR-15-002. 17 pp.

Ridenour, W. L., and R. M. Callaway. 2003. Root herbivores, pathogenic fungi, and competition between *Centaurea maculosa* and *Festuca idahoensis*. Plant Ecology. 169: 161-170.

Steinger, T., and H. Müller-Schärer. 1992. Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. Oecologia. 91: 141-149.

Stephens, A. E. A., and J. H. Myers. 2014. Testing biological control agent compatibility: *Cyphocleonus achates* and *Larinus minutus* on diffuse knapweed. Biological Control. 70: 48-53.

Story, J. M., N. W. Callan, J. G. Corn, and L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control. 38: 227-232.

Story, J. M., L. Smith, J. G. Corn, and L. J. White. 2008. Influence of seed head-attacking biological control agents on spotted knapweed reproductive potential in western Montana over a 30-year period. Environmental Entomology. 37: 510-519.

Thelen, G. C., J. M. Vivanco, B. Newingham, W. Good, H. P. Bais, P. Landres, A. Caesar, and R. M. Callaway. 2005. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. Ecology Letters. 8: 209-217.

Van Hezewijk, B. H., and R. S. Bourchier. 2012. Impact of *Cyphocleonus achates* on diffuse knapweed and its interaction with *Larinus minutus*. Biological Control. 62: 113-119.

Winston, R, C.B. Randall, R. DeClerck-Floate, A. McClay, M. Schwarzländer. Field guide for the biological control in the Northwest. USDA Forest Service FHTET-2014-08. 333 p. Morgantown, WV.

APPENDIX III - Budget estimates

Year 1	University of Idaho Matching Funds	Funds provided by USFS FHP FHTET
SALARY AND FRINGE (Ph.D. student) 12 months graduate Research Assistantship (@ $2,250/month) + 2.5% fringe/benefit		$27,000.00 $675.00
SALARY AND FRINGE (PI Schwarzländer) 1.5% FTE (base $85,155) ($1,277) and 31.6% fringe/benefits ($404)		$1,277.00 $404.00
OPERATIONAL EXPENSES Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation		$924.00
TRAVEL Reimbursement for vehicle mileage for travel to field sites to collect SIMP root data @ $0.555/mile for 4,000 miles ($2,220)		$2,220.00
EQUIPMENT Laptop computer and/or digital media ($1,500)		$1,500.00
INDIRECT COST 50% Unrecovered indirect costs (@ 45.3% of TDC) as not allowed under this agreement $7,701); 0.25 FTE salary Research Support Scientist (Harmon) ($12,454); 0.25 FTE salary Principal Investigator (Schwarzländer) ($21,288)	$7,701.00 $12,454.00 $21,288.00	
SUBTOTALS	**$41,443.00**	**$34,000.00**

Budget Justification Year 1:
Salaries: 12 months graduate Research Assistantship (RA) @ $2,250/month and 2.5% fringe/benefits ($27,000 and $675, respectively);
Salaries: 1.5% mandatory charge PI salary (base: $85,155) and 31.6% fringe/benefits ($1,277 and $404, respectively);
Operational Expenses: Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation ($924);
Travel: Partial reimbursement for repeated field trips travel to field sites throughout ID @ $0.555 per mile for approximately 4,000 miles ($2,220);
Equipment: Laptop computer/iPad/tablet or digital media ($1,500);

University of Idaho Cost Sharing: Partially (50%) waived Indirect Costs @ 45.3% ($7,701); and partially matched salary of Research Support Scientist (Harmon) @ 0.25 FTE (base $49,816) ($12,454) and Principal Investigator Schwarzlaender @ 0.25 FTE (base $85,155) ($21,288) for a total of ($41,443).

Year 2	**University of Idaho Matching Funds**	**Funds provided by USFS FHP FHTET**
SALARY AND FRINGE (Ph.D. student) 12 months graduate Research Assistantship (@ $2,250/month) + 2.5% fringe/benefit		$27,000.00 $675.00
SALARY AND FRINGE (PI Schwarzländer) 1.5% FTE (base $85,155) ($1,277) and 31.6% fringe/benefits ($404)		$1,277.00 $404.00
OPERATIONAL EXPENSES Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation		$924.00
TRAVEL Reimbursement for vehicle mileage for travel to field sites to collect SIMP root data @ $0.555/mile for 4,000 miles ($2,220)		$2,220.00
EQUIPMENT Digital media/Laptop repair/replacement ($500)		$500.00
INDIRECT COST 50% Unrecovered indirect costs (@ 45.3% of TDC) as not allowed under this agreement $7,701); 0.25 FTE salary Research Support Scientist (Harmon) ($12,454); 0.25 FTE salary Principal Investigator (Schwarzländer) ($21,288)	$7,701.00 $12,454.00 $21,288.00	
SUBTOTALS	**$41,443.00**	**$33,000.00**

Budget Justification Year 2:

Salaries: 12 months graduate Research Assistantship (RA) @ $2,250/month and 2.5% fringe/benefits ($27,000 and $675, respectively);

Salaries: 1.5% mandatory charge PI salary (base: $85,155) and 31.6% fringe/benefits ($1,277 and $404, respectively);

Operational Expenses: Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation ($924);
Travel: Partial reimbursement for repeated field trips travel to field sites throughout ID @ $0.555 per mile for approximately 4,000 miles ($2,220);
Equipment: Laptop computer/iPad/tablet or digital media ($500);
University of Idaho Cost Sharing: Partially (50%) waived Indirect Costs @ 45.3% ($7,701); and partially matched salary of Research Support Scientist (Harmon) @ 0.25 FTE (base $49,816) ($12,454) and Principal Investigator Schwarzlaender @ 0.25 FTE (base $85,155) ($21,288) for a total of ($41,443).

Year 3	**University of Idaho Matching Funds**	**Funds provided by USFS FHP FHTET**
SALARY AND FRINGE (Ph.D. student) 12 months graduate Research Assistantship (@ $2,250/month) + 2.5% fringe/benefit		$27,000.00 $675.00
SALARY AND FRINGE (PI Schwarzländer) 1.5% FTE (base $85,155) ($1,277) and 31.6% fringe/benefits ($404)		$1,277.00 $404.00
OPERATIONAL EXPENSES Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation		$924.00
TRAVEL Reimbursement for vehicle mileage for travel to field sites to collect SIMP root data @ $0.555/mile for 4,000 miles ($2,220)		$2,220.00
EQUIPMENT Laptop computer and/or digital media ($500)		$500.00
INDIRECT COST 50% Unrecovered indirect costs (@ 45.3% of TDC) as not allowed under this agreement $7,701); 0.25 FTE salary Research Support Scientist (Harmon) ($12,454); 0.25 FTE salary Principal Investigator (Schwarzländer) ($21,288)	$7,701.00 $12,454.00 $21,288.00	
SUBTOTALS	**$41,443.00**	**$33,000.00**

Budget Justification Year 3:

Salaries: 12 months graduate Research Assistantship (RA) @ $2,250/month and 2.5% fringe/benefits ($27,000 and $675, respectively);

Salaries: 1.5% mandatory charge PI salary (base: $85,155) and 31.6% fringe/benefits ($1,277 and $404, respectively);

Operational Expenses: Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation ($924);

Travel: Partial reimbursement for repeated field trips travel to field sites throughout ID @ $0.555 per mile for approximately 4,000 miles ($2,220);

Equipment: Laptop computer/iPad/tablet or digital media ($500);

University of Idaho Cost Sharing: Partially (50%) waived Indirect Costs @ 45.3% ($7,701); and partially matched salary of Research Support Scientist (Harmon) @ 0.25 FTE (base $49,816) ($12,454) and Principal Investigator Schwarzlaender @ 0.25 FTE (base $85,155) ($21,288) for a total of ($41,443).

MARK SCHWARZLÄNDER

Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, Idaho, 83844-2339 Phone: (208) 885-9319, Fax: (208) 885-7760, E mail: markschw@uidaho.edu

Professional Preparation

University of Kiel, Germany, Biology M. Sc., 1993
University of Kiel, Germany, Biology, Ph. D., 1999

Appointments

2014 - present: Professor of Entomology, University of Idaho
2007 - 2014: Associate Professor, Co-Director of CRISSP, University of Idaho
2000 - 2007: Assistant Professor of Entomology, University of Idaho
1997 - 2000: Research Scientist, CABI Europe – Switzerland

Expertise

Biological weed control, insect herbivory, insect ecology, insect behavior, plant population biology, plant ecology, interdisciplinary studies.

Selected Recent Publications (total 45; #graduate students)

Hinz, H.L., **Schwarzländer, M**., Gassmann, A. and Bourchier, R.S. Successes we would not have had: a retrospective comparison of predicted versus realized host range of weed biological control agents in North America. Invasive Plant Science and Management. *In Press.*

#Weed, A.S. and **Schwarzländer, M.** 2014. Density-dependence, precipitation, and biological control agent herbivory influence landscape-scale dynamics of the invasive Eurasian plant *Linaria dalmatica.* Journal of Applied Ecology. 51: 825–834.

Gaskin, J.F., **Schwarzländer, M.**, Hinz, H.L., Williams, L. III, Gerber, E., Rector, B.G. and Zhang, D.Y. 2013. Genetic identity and diversity of perennial pepperweed (*Lepidium latifolium*) in its native and invaded ranges. Invasive Plant Science and Management. 6(2):268-280.

Gaskin, J.F., **Schwarzländer, M**., Kinter, C.L., Smith, J.F. and Novak, S.J. 2013. Propagule pressure, population structure and geographic origins of an apomictic plant invasion on three invaded continents; a comparison of introduction dynamics around the globe. American Journal of Botany. 100(9): 1871-1882.

Goulet, E.J., Thaler, J., Ditommaso, A., **Schwarzländer, M.** and Shields, E.J. 2013. Impact of *Mecinus janthinus* (Coleoptera: Curculionidae) on the growth and reproduction of *Linaria dalmatica* (Scrophulariaceae). The Great Lakes Entomologist 46(1-2): 90-98.

Gaskin, J.F., **Schwarzländer, M.**, Williams, L. III, Gerber, E. and Hinz, H.L. 2012. Minimal genetic diversity in the facultatively outcrossing perennial pepperweed (*Lepidium latifolium*) invasion. Biological Invasions 14:1797-1807.

Hinz, H.L., **Schwarzländer, M**., #McKenney, J.L., #Cripps, M.G., Harmon, B.L. and Price, W.J. 2012. Biogeographical comparison of *Lepidium draba* between its native, expanded and introduced range. Biological Invasions 14:1999-2016.

#Szűcs, M., Eigenbrode, S.D., **Schwarzländer, M.** and Schaffner, U. 2012. Hybrid vigor in the biological control agent, *Longitarsus jacobaeae*. Evolutionary Applications 5(8): 489-497.

Skills:

- Grant writing experience (109 cooperative agreements and 9 national competitive grants for a total funding spending authority of $3,967,833)
- Major advisor of graduate students in biological weed control (completed: 2 Ph.D. and 9 M.S. students; currently advising: 2 Ph.D. and 2 M.S. students)
- Extension specialist training professional land managers and tribal nations in biological weed control implementation/invasive plant identification (conducted more than 100 technology transfer field days across USA and Canada)

JOSEPH D. MILAN

Bureau of Land Management, Boise District, 3948 Development Ave., Boise, Idaho, 83705, Phone: (208) 384-3487, Fax: (208) 384-3493, email: jmilan@blm.gov

Professional Preparation

College of Idaho, Caldwell, Idaho, Biology, B.S., 2001
University of Idaho, Moscow, Idaho, Entomology, M.S., 2005

Selected Posters and Publications

Milan, J., M. Schwarzlaender, A. Weed, J. Price. 2014. Post-Release Monitoring: A Regional Approach to Assess Impact Across the Invaded Ranges of Multiple Target Species. 14th International Symposium on Biological Control of Weeds, Skukuza, Kruger National Park, South Africa. March 2-7, 2014.

Milan, J., A. Weed, M. Schwarzlaender, P. Brusven, C. Randall. 2013. Is a Regional Interagency, Multi-Year, Multi-System Post-Release Impact Assessment Program Possible? In: Proceedings of the 13th International Symposium on Biological Control of Weeds. Ed. By Wu, Y., T. Johnson, S. Sing, S. Raghu, G. Wheeler, P. Pratt, K. Warner, T. Center, J. Goolsby, and R. Reardon. 461.

Sing, S., D.Weaver, S. Ward, J. Milan, C.L. Jorgensen, R. Progar, A. Gassmann and I. Tosevski. 2011. Hybrid weeds! Agent biotypes!: Montana's ever-evolving toadflax biocontrol soap opera. 13th International Symposium on Biological Control of Weeds, Waikoloa, HI. September 11 – 16, 2011. (poster)

Progar, R., Markin, G., Milan, J., Barbouletos, T., and Rinella, M. 2011. Population Dynamics and Impacts of the Red-headed Stem Borer on Leafy Spurge (*Euphorbia esula*). Invasive Plant Science and Management, 4, 183-188.

Progar, R., Markin, G., Milan, J., Barbouletos, T. and Rinella, M. 2010. Inundative Release of *Aphthona* spp. Flea Beetles (Coleoptera: Chrysomelidae) as a Biological "Herbicide" on Leafy Spurge in Riparian Areas. Journal of Environmental Entomology, 103, 242-248.

Milan, J.D., Harmon, B.L., Prather, T.S., and Schwarzlaender, M. 2006. Winter mortality of *Aceria chondrillae*, a biological control agent released to control rush skeletonweed (*Chondrilla juncea*) in the western United States. Journal of Applied Entomology, 130, 473-479.

Selected Presentations

Milan, J. Establishing new biocontrol agents, how to use them, and more biocontrol agents on the horizon. 2015. Nevada Weed Management Association Conference. Sparks, Nevada, October 28-29, 2015.

Milan, J., M. Schwarzlaender, A. Weed, J. Price. 2014. Post-Release Monitoring: A Regional Approach to Assess Impact Across the Invaded Ranges of Multiple Target Species. 14th International Symposium on Biological Control of Weeds, Skukuza, Kruger National Park, South Africa. March 2-7, 2014.

Milan, J. Biological Control of Noxious Weeds. 2012. Northwest Intertribal Agricultural Council. Fort Hall, Idaho, August 15-16, 2012.

Milan, J., A. Weed, M. Schwarzlaender. 2011. Is a regional interagency, multi-year, multi-system post-release impact assessment program possible? A case study of Dalmatian toadflax." XIII International Symposium on Biological Control of Weeds (ISBCW 2011), Waikoloa, Hawaii, September 11-16, 2011.

Milan, J. Idaho's Biological Control Program. 2010. Northern Rockies Invasive Plant Conference. Coeur D'Alene, Idaho, October 24-29, 2010.

PAUL A. BRUSVEN

Nez Perce Bio-Control Center. Nez Perce Tribe, PO Box 365, Lapwai, Idaho, 83540 Phone: (208) 843-9374, Fax: (208) 843-9373, E mail: pbrusven@nezperce.org

Professional Preparation

University of Idaho, Moscow, Idaho, Ag. Mechanization B.S., 1990

Appointments

2003-date: Nez Perce Bio-control Center Coordinator
1994-2003: Soil Conservationist for the Nez Perce Tribe, Land Services Program
1991-1994: Water Resources Planner/Specialist for the Nez Perce Tribe, Water Resources Division
1990-1991: Soil Agronomist for the Nez Perce Tribe, Wildlife/Soils Program
1985-1990: Part time farm machinery operator, Moscow, Idaho

Expertise

- Conservation planning and report writing
- Budget development and management
- Ability to motivate people to accomplish specific goals and objectives
- Wetland delineation training, certificate obtained
- Introduction to GIS/GPS with Arc View training, certificate obtained
- Aerial photography/remote sensing training, certificate obtained
- ASTM, Environmental Site Assessments for Commercial Real Estate training, certificate obtained
- Computer literate; Microsoft Word, Excel, Arc View (GIS software), and others
- Soil/water sampling and planning
- Agriculture farm equipment operation and calibration

Experience

- Served as Coordinator for the Nez Perce Bio-control Center supervising 2-5 employees, securing funding, managing budgets, rearing biological organisms, providing education workshops, and worked cooperatively with a variety of organizations including private, county, state, federal, tribal and universities .
- Worked closely with the Bureau of Indian Affairs, Realty Program in agriculture lease development
- Worked on soil/water/ invasive weeds related issues for the Nez Perce Tribe both on/off reservation
- Familiar with federal farm programs and implemented many on tribal lands and treaty areas
- Grant/proposal writing to obtain funding for bio-control of weeds/soil/water quality restoration work
- Developed/Implemented conservation plans to protect Nez Perce Tribal and private lands
- Developed budgets and managed them for specific land conservation/improvement projects
- Collected and stored land data in computer software such as the Geographical Information System
- Gave oral presentations to educate tribal and non-tribal landowners/operators
- Worked daily with the general public regarding federal, state and tribal agricultural programs
- Coordinated with inter-tribal and outside governmental agency on land management projects/activities

Other Qualifications

- Member, Idaho Biocontrol Task Force, Idaho Weed Control Assoc., Idaho Weed Coordinating Committee
- Leadership Idaho Agriculture, Class of XXI Graduate
- Member, Soil & Water Conservation Society