1. RIGHTING THE SHIP: USING NEWLY BCIP-DEVELOPED HOST SPECIFICITY ASSESSMENT TECHNIQUES TO DEMONSTRATE THE ENVIRONMENTAL SAFETY OF THE HOUNDSTONGUE ROOT WEEVIL WITH THE GOAL TO RE-PETITION THE BIOCONTROL INSECT FOR RELEASE IN THE UNITED STATES

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

5. EXECUTIVE SUMMARY: 1) A 2012 BCIP grant allowed us to develop methodologies to quantitatively and analytically assess early host finding behavior of weed biological control candidates based on olfactorial and visual cues of plant species. 2) We used the seed feeding weevil *Mogulones borraginis* and its target weed *Cynoglossum officinale* as one of the model systems for that project and data collected allows us to submit a cogent petition for the release of the seed weevil in the United States. 3) We used the techniques also to preliminarily study the host finding behavior of a uniquely controversial biocontrol agent, the *C. officinale* root mining weevil *Mogulones crucifer*. 4) This weevil has been released in Canada in 1997 against *C. officinale* and has extremely successfully controlled the weed but is prohibited in the United States because of concerns about its fundamental host range with regard to 5 USFWS T&E listed plant species. 5) In 2010 USDA APHIS PPQ issued 'Pest Alert' for *M. crucifer* in response to findings that the weevil is established in the United States. 6) Our preliminary new data for 10 nontarget plants including 4 of the 5 T&E listed species strongly indicates that the weevil is either repelled by these plant species or behaves indifferently (inability to identify them as host plants), and thus, is much more environmentally safe than previously assumed. 7) The release of the weevil in Canada in 1997 has been decried as one of the most harmful and environmentally irresponsible and consequential actions in biological wed control. 8) Meanwhile, in the absence of effective control means, *C. officinale* has expansively spread throughout the northwestern United States. 9) This project aims to i) document unequivocally the environmental safety of *M. crucifer*, ii) provide data that underscore the importance of early host finding in weed biocontrol agent assessments, iii) re-petition the weevil for release in the United States in order to iv) adjust the public perception of this and other biological weed control programs in the United States.

6. PROJECT GOALS AND SUPPORTING OBJECTIVES: The aim of this project is the application and implementation of novel host specificity assessment techniques focusing on insect host finding behavior to evaluate the environmental safety of *Mogulones crucifer* Pallas, a highly successful biological control agent for one of the northwestern United States' most rapidly spreading noxious weeds of grasslands and forests, *Cynoglossum officinale* L. (houndstongue). The root mining weevil, which was released for the biological control of *C. officinale* in Canada in 1997 is prohibited in the United States over concerns about its broader fundamental host range (Catton et al. 2014, USDA 2010). It however spread southward crossing the Canada-USA border and is now established with large populations northern Washington, Idaho and Montana. The overall goal of this project is to collect a data set demonstrating the environmental safety of *M. crucifer*, which will allow to revoke the existing 'Pest Alert' for the insect (USDA 2010) and to re-petition it for release in the United States in close cooperation with the USWFS. This is accomplished through series of behavioral lab bioassays and field experiments coupled with chemical-ecological and electrophysiological research. This project will develop strongly predictive post-release monitoring techniques for nontargets (BCIP Project Priority No. 1). Target impact monitoring for this system is not required since its impact on *C. officinale*, i.e., its ability to completely and permanently suppress target weed populations has been documented repeatedly in Canada (DeClerck-Floate et al. 2005; Catton et al. 2015; 2016, Rosemarie DeClerck-Floate pers. comm., Susan Turner pers. comm.). Currently, there is no biological control option available for the management of *C. officinale* in the United States. In part because of its toxicity (high quantities of pyrrolizidine alkaloids) and its dispersal mode (epizoochory; seeds attach fur of wildlife and livestock), the plant is largely uncontrolled. As a consequence, infestations have greatly increased in natural areas such as wildernesses and protected grasslands as well as forests throughout western states. As an added product, this project will also develop a semiochemical-based push system that would repel the weevil from areas that harbor protected plant species. While it is our assessment that *M. crucifer* does pose no risk whatsoever to accidentally attack protected nontarget plant species, agencies charged with the conservation and recovery of those plant species may be appreciate of this additional safeguard measure as part of an integrated management strategy for *C. officinale* (BCIP Project Priority No. 3). Data and products of this project will be used to propose to the agency regulating biological weed control in the United States (USDA APHIS PPQ) to modify the sets of criteria used to evaluate environmental safety of candidate species to reflect the importance of host finding behavior. We have raised the issue in an opinion paper (Hinz et al. 2014) and are currently publishing a first data driven manuscript on the topic (Kafle et al. 2016 In review a) but this project will provide compelling empirical data on the topic. This project has four objectives:

Objective 1: Assessing the host finding behavior of the root mining weevil *Mogulones crucifer* with regard to the remaining (5th) untested USFWS threatened and endangered (T&E) listed Boraginaceae species (*Oreocarya crassipes*), additional critical confamilial North American plant species in the genera *Cryptantha* and *Oreocarya* and select close Eurasian relatives of *C. officinale*, using 1) behavioral bioassays, 2) gas chromatography mass spectrometry (GC-MS) of foliage and floral volatile organic compounds (VOCs), and 3) Gas chromatography electro-antennodetection coupled with flame ionization detection (GC-EAD/FID). Our laboratory has developed and successfully implemented the instrumental approach outlined above as part of a previously awarded BCIP proposal and already conducted preliminary studies for *M. crucifer* between 2013-2015. The approach allows to reliably and nondestructively assess behavioral responses (attraction, indifference or repellence) of *M. crucifer* to headspace volatile blends. This objective assesses the ability of *M. crucifer* to perceive a plant as a host (This objective will use 25% PE (Project Effort).

Objective 2: Assessing the evolutionary stability of host finding behavior in *M. crucifer* **with regard to feeding history.** We were able to show strong host finding specificity in *M. crucifer* using 10 North American species including 4 of the 5 confamilial T&E plant species (Kafle 2016). There exists however, very little information on the stability of behavioral host selection of specialist herbivore insects, especially with regard to their feeding history. This information is important to assess the likelihood of a host switch (for example if the host weed populations get depleted) and to help convincing the USFWS about the safety of this insect. To demonstrate the stability (and genetic basis) of the observed host-fidelity in *M. crucifer*, we test behavioral responses of female *M. crucifer* half-sibling lines reared for several generations (parental – F4) on *C. officinale*, a close European relative of *C. officinale* and a North American nontarget, which are within the fundamental host range of the weevil. All insects will be stores individually for advanced next generation genetic analysis in a collaborative project with a Univ. of Idaho Ph.D. student (Amanda Stahlke), to explain the strength of the *C. officinale*–*M. crucifer* host fidelity and underlying mechanisms (25% PE).

Objective 3: Identification of volatile(s) causing repellence in *M. crucifer* and development of a push system. We found consistently that floral and foliage volatiles of all tested *Hackelia* species (incl. the T&E listed H. venusta) caused repellence in *M. crucifer*. With this objective we want to identify responsible volatile(s) and develop these into an IPM push system. We have volatile organic compound blends (VOCs) for all *Hackelia* species stored but not yet identified bioactive VOCs (i.e., those that trigger an antennal response in *M. crucifer*). We will use techniques outlined in Objective 1 to identify bioactive VOCs and measure behavioral responses to the identified VOCs. This process may be more difficult than anticipated (e.g. if bioactive VOCs are present in very small concentrations) but our lab is equipped to overcome those potential pitfalls. Over the duration of this project we will isolate VOCs causing repellence in *M. crucifer* and test a push system in the field (25% PE).

Objective 4: The final objective of this project will address an important question pertaining to the successful re-petitioning of *M. crucifer* for release in the United States: **Can laboratory bioassay findings be transferred to the open field?** We will conduct series of field tests with select nontargets including T&E species to confirm that observed indifference or repellence in the laboratory translates to no-attack of plant species in the field. Specifically, we will test the odor plume admixture (OPA) hypothesis, which assumes that 'spill over attack' is due to weevils confused by a plume of host plant and nontarget volatiles (Kafle et al. In review a). The data obtained with this objective will help to convince the USFWS, about the environmental safety of *M. crucifer*. Open-field experiments will be conducted on tribal lands in areas where *M. crucifer* is naturalized (Blackfeet Nation, the Confederated Tribes of the Colville Reservation, or the Kalispell Tribe of Indians) (25% PE).

The four objectives will result in a comprehensive data set, which will be used to re-petition the insect in close negotiations with the USFWS and USDA APHIS PPQ. We have communicated extensively with USDA APHIS PPQ Pest Permit Branch in Riverdale, MD and USDA APHIS PPQ State Plant Health Directors in western states and PPQ personnel agrees with us that if we are able to convince the USFWS about the environmental safety of the weevil, the agency would favorably consider and fast-track a petition for release (Also see Products and Outcomes below).

6. PROJECT JUSTIFICATION/URGENCY: *Cynoglossum officinale* L. (houndstongue) is a monocarpic short-lived perennial native to Europe and western Asia (de Jong et al. 1990). It was first introduced to North America in the 1800s (Brand 1921; Upadhyaya et al. 1988) and has since invaded eight Canadian provinces and all but 2 states in the USA (USDA NRCS 2016). It invades grass- and rangelands at ease and has become an overwhelming management problem in open forests, forest clearings and forests with grazing permits (Dickerson and Fay 1982; Upadhyaya et al. 1988). Originally considered a fringe weed in British Columbia in the 1980s, rapid invasion of the northwestern United States combined with the inability to effectively manage *C. officinale* in wildlands, remote or inaccessible areas has led to the rise of this weed to the forefront of land manager concerns in Idaho, Colorado, Montana and Wyoming. Recently, *C. officinale* has also become a growing problem in North- and South Dakota. The uncontrolled increase of the weed can be attributed to larger quantities of burred nutlets that attach to fur of mammals and are distributed from transportation corridors throughout wildernesses, grasslands and forests (Svensson and Wigren 1990; De Clerck-Floate 1997). Organic ranches where *C. officinale* is distributed by grizzlies foraging for wild carrots (Malou Anderson-Ramirez, pers. comm.) or ranchers that attempt to preserve ecosystem health (Lanie White, pers. comm.) are equally affected by the weed. *C. officinale* infestations pose serious problems. Ecologically, the plant competes with native forbs especially for moisture (Upadhyaya et al. 1988). Economically, *C. officinale* reduces forage production in rangelands and pastures (Jacobs & Sing 2007). In addition, the plant contains high concentrations of pyrrolizidine alkaloids (van Dam et al. 1995), which when consumed freshly or dry as hay are toxic to livestock and cause often fatal kidney disease (Baker et al. 1991; Baker et al. 1989; Knight et al. 1984; Stegelmeier et al., 1996).

Areawide sustained long term control of *C. officinale* using herbicides or mechanical means or both is economically infeasibly and pragmatically unachievable (DeClerck-Floate & Schwarz-länder 2002a; b). A program to develop classical biological control for *C. officinale* was initiated in 1987 (Hinz et al. 2007; Schwarzländer 1999). *Mogulones crucifer* (=*M. cruciger* Herbst, =**Ceutorhynchus cruciger** Herbst) is a root mining weevil native to Eurasia. The weevil typically has one generation per year in its native range but can have a partial second generation (Schwarzländer 1997). The experimental host range of *M. crucifer* has been studied between 1988 and 1996. In brief, the weevil has a broader fundamental host range but in choice tests prefers its sole known Eurasian field host (Jordan et al. 1993; Schwarzländer 1996). Based on these results, a petition for field release of *M. crucifer* in the United States and Canada was submitted in 1996 to the Technical Advisory Group (TAG). Subsequently, the Canadian Biocontrol Review Committee approved the release of *M. crucifer* in Canada in 1997 following a favorable recommendation by TAG but approval for release in the United States was not granted because of the concern of the United States Fish and Wildlife Service (USFWS) over the environmental safety of the weevil with regard to native threatened and endangered listed (T&E) species (DeClerck-Floate & Schwarzländer 2002b). Additional no-choice and choice larval development test conducted between 2001-2004 showed that several native confamilial plants including 3 T&E species supported partial development of *M. crucifer* (Andreas 2004). Since its original release in 1997 in British Columbia, weevil populations have increased greatly and dispersed widely (De Clerck-Floate et al. 2005). *M. crucifer* has crossed the Canada-USA border and is spreading south and westward at approximately 12km (7.5miles) per year and its abundance is increasing rapidly (Winston 2011). Recent post-release monitoring studies in Canada showed however, that nontarget attack by *M. crucifer* on a native confamilial species sympatrically occurring with *C. officinale*, *Hackelia micrantha* (Eastw.) J.L. Gentry, can be explained by spillover (Catton et al. 2014; 2015). The authors also conclude that population level non-target impacts are highly unlikely to occur from this spillover as *M. crucifer* is unable to maintain populations on the nontarget (Catton et al. 2015; 2016). As is true for other biological weed control agents, the ecological host range of *M. crucifer* is narrower than its fundamental host range (De Clerck-Floate & Schwarzländer 2002b). The assessment of the host selection behavior, which is mediated by various sensory cues provides important data on the relative attractiveness of these ecologically at-risk nontarget species and thus, is useful in predicting the ecological host range (Wheeler & Schaffner 2013). For *M. crucifer* there is preliminary data that indicate that the host selection by this weevil is mediated by the VOCs emitted by its host plant *C. officinale* (Andreas et al. 2008a; b; Kafle 2016). The objective of this project is to assess nontarget attack risks of *M. crucifer* that take into account the early stages of the host selection behavior and its underlying phytochemical basis. In doing so, this project will elucidate the role that host finding may have in predicting the ecological host range of *M. crucifer*.

Proposed action: The behavioral host finding data collected for *M. crucifer* during the past three years and field data published by Catton et al. (2014; 2015; 2016), have greatly altered the scientific view on the environmental safety of *M. crucifer*, which is considered as extraordinarily successful in Canada but was considered an environmental threat in the United States. We propose to conduct series of host finding bioassays, underlying electrophysiological and phytochemical ecological experiments, and field experiments with critical native Boraginaceae species to document the environmental safety of the root weevil. With documented indifference (2 T&E species) and repellence (2 T&E species, Fig. 1), we believe that it is not only possible but likely that this research (including the remaining 5th T&E species) will produce data sufficiently convincing to re-petition *M. crucifer* for release in the United States. We will from the very onset of this project engage and interact with the USFWS. This would not only lead to sustained control of *C. officinale* in the USA but would also allow reversing some of the bias held by conservation biologists towards biological weed control.

7. **APPROACH: Plant and insect materials and common gardens.** After 19 years of effort we have for the first time successfully acquired seeds of the 5th T&E listed species Oreocarya crassipes (I.M. Johnst.). This species has never been subjected to any kind of testing. In addition we obtained seeds for 4 never tested native **Crypantha** species and **Adersonglossum virginianum** (L.) J.I. Cohen (syn: **Cynoglossum virginianum** L.:), and some Eurasian close relatives of *C. officinale* in the genera *Cynoglossum, Rindera*, and *Solenanthus*. We also continue to maintain specimen of the remaining 4 T&E species and of the former native congeners of *C. officinale*. We will

propagate all plant species in pots and use plants for olfactorial bioassays and headspace foliage and floral VOC collections. We also will collect VOV headspace of T&E species at field sites in Washington State, Oregon, California and Texas. We maintain a lab colony of *M. crucifer* at the Univ. of Idaho for testing but have also identified field sites in northern Idaho to augment or colony if necessary. One of the advantages of a rearing colony is the availability of naïve females although we have shown that there is no difference in behavioral responses between naïve and experienced *M. crucifer* (Kafle et al. In review a). Field experiments will be conducted at sites with naturally occurring *M. crucifer* populations on tribal lands in Washington State at the Confederated Tribes of the Colville Reservation or the Kalispell Tribe of Indians or in Northern Montana at the Blackfeet Nation.

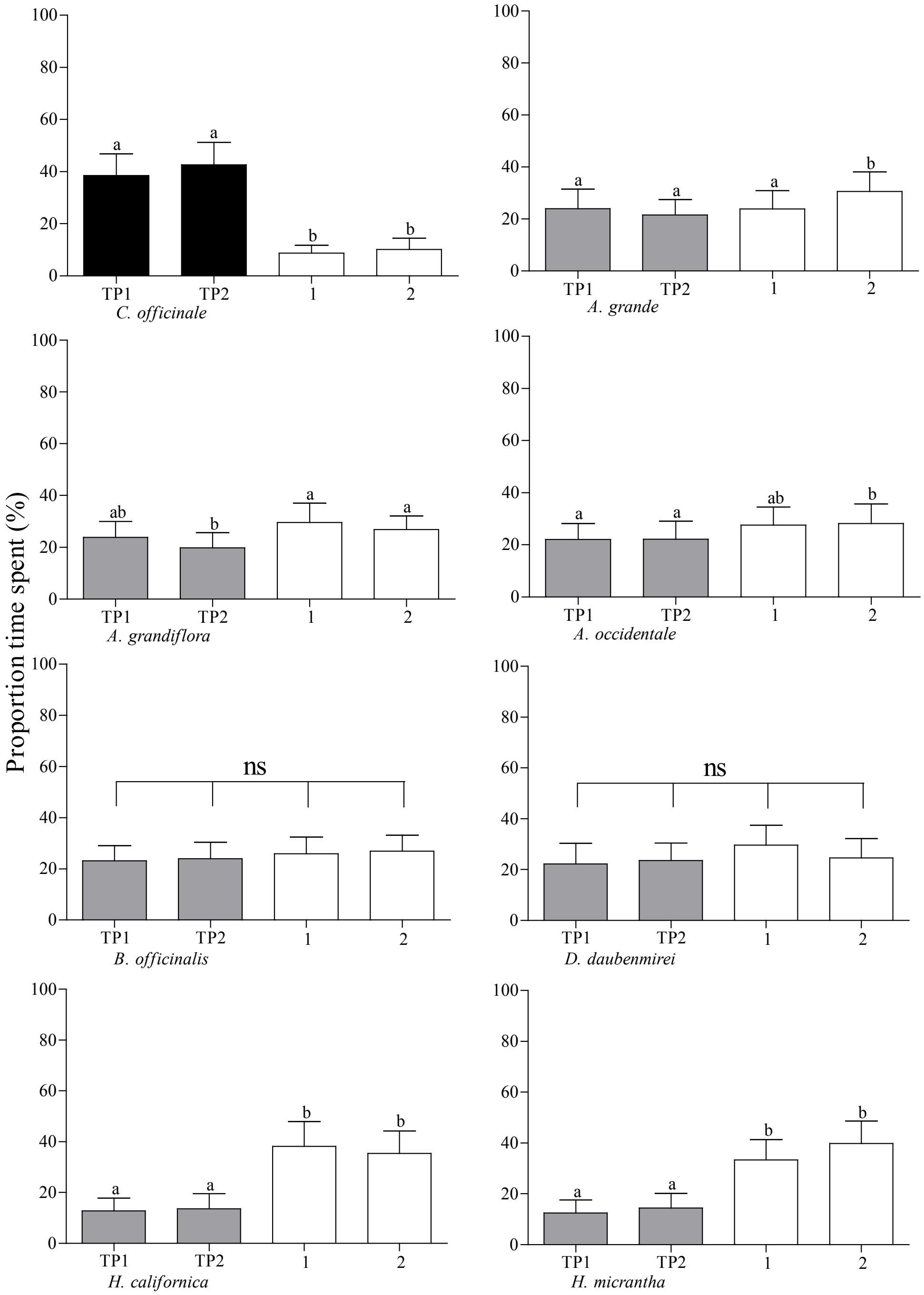

Fig. 1 Proportion of time spent by female *Mogulones crucifer* in each of four quadrants of a four-armed olfactometer arena using volatile headspace of one plant species in two quadrants (TP1 and TP2) and purified air in two quadrants (1 and 2). Differing letters on top of bars denote significant differences (n=20) (Categorical log linear model followed by single degree of freedom contrast analysis, $p<0.05$). Black bars, *Cynoglossum officinale*; Grey bars, test plant species; White bars, purified air. Tests were conducted with 10 confamilial nontarget species, including 4 T&E species. Attraction was only found in *C. officinale*, indifference or tendency to repellence in 7 plant species, and repellence in 3 *Hackelia* species. Other variables tested included the Initial Choice (IC) and Final Choice (FC) of females at the end of the 20 min trials and yielded very similar results (Kafle 2016).

Objective 1: Behavioral responses of *Mogulones crucifer* to VOCs of rare, threatened and endangered North American and Eurasian confamilial plant species of *C. officinale* and their underlying electrophysiological phytochemical bases. The techniques used for these tests have been developed during the last four years as part of a previous BCIP award to investigate the host finding behavior of the *C. officinale* seed weevil Mogulones borraginis (F.). The techniques were also used in 2014-2016 for tests with *M. crucifer* (Park et al. In review; Kafle et al. In review a; b). In brief, we conduct behavioral bioassays with greenhouse propagated and field collected headspace VOCs blends using a 4-armed circular olfactomter arena and measure the initial choice (IC), final choice (FC) and proportion of time spent in each of 4 quadrants during 20-minute bioassays with individual *M. crucifer* females (Vet et. al. 1983). Data will be analyzed using $\chi 2$ tests followed by logistic regression, log linear categorical models, and generalized linear mixed models. We will conduct gas chromatography mass spec-trom-etry (GC-MS) of foliage and floral VOC blends of respective plant species to identify VOC composition relative concentrations for species and individuals among species (greenhouse propagated vs field collected). We will conduct gas chromatography electro-antennodetection experiments coupled with flame ionization detection (GC-EAD/FID) to identify bioactive volatile organic compounds (those that cause a reaction in insect antennae) in select plant species. Based on GC-EAD/FID data, principal component analyses (PCA) will be conducted to separate plant species the weevil has no ability of identifying as potential hosts (Kafle 2016). We will conduct two separate sets of experiments based on foliar and floral VOCs and we expect albeit largely differing VOC compo-sitions, similar behavioral results (Kafle et al. In review a). For a more detailed and technical description meth-odological approaches used, we would happily forward write-ups to the panel at any time **(25% Project Effort, PE).**

Objective 2: Assessment of the stability of *M. crcucifer* - *C. officinale* **host fidelity over generations depending on feeding history.** We have demonstrated host specific behavioral responses for *M. crucifer* using a set of 10 confamilial plant species including 4 of the 5 T&E and we are able to pinpoint that one VOC unique to *C. offic-inale,* methyl isolvalerate, may largely responsible for this host fidelity. We will test the strength of the observed host fidelity with regard to the feeding experience of *M. crucifer*. This objective will also be used to study the genetic basis for the observed host-fidelity using next generation genetic techniques (collaborative project with Univ. of Idaho Ph.D. student Amanda Stahlke). We will test behavioral responses of naïve *M. crucifer* females using a subset of plant species as described in Objective 1. We will then rear half-sibling lines of those females on *C. officinale*, a sympatric Eurasian confamilial not known to be a field host of *M. crucifer* but easily supporting its development (*Borago officnalis* L.). We will also rear half-sibling lines on a North American nontarget species that supports development of the weevil *Hackelia micrantha* (Eastw.) J.L. Gentrya. We will rear half-sibling lines for 4-5 generations (Parental to F4 generation) and test behavioral responses and biological traits (fecundity and fertility) of *M. crucifer* for each generation and each host plant. All weevils will be individually stored following behavioral bioassays for genetic analyses. We hypothesize that regardless of the host plant species, Parental – F4 female *M. crucifer* will maintain their specific host fidelity. We will rear all weevils on greenhouse propagated plants of each of the three plant species under identical environmental conditions. This is a laborious objective. However, we believe that the quality of data obtained will justify the amount of work outlined. The heritable stability of behavioral host fidelity has to our knowledge been rarely tested in a classical biological weed control program. We expect the findings to be particularly insightful and convincing **(25% PE)**.

Objective 3: Identification of volatile organic compounds responsible for *M. crucifer* repellence and devel-opment of a semiochemical based push system. Our current data strongly indicates *M. crucifer* repellence to foliage headspace VOC blends of 3 North American *Hackelia* species tested including the T&E species *Hackelia* venusta (Piper) H. St. John. Similarly, we found repellence in Mogulones borraginis towards floral headspace VOCs of 2 *Hackelia* species (Park et al. in Review). We want to identify VOCs in foliar and/or floral *Hackelia* speciesVOC blends that trigger repellence behavior in *M. crucifer*. To do so, we use techniques described in Kafle (2016) and under Objective 1 of this proposal. We have conducted gas chromatography mass spectrometry (GC-MS) for foliage and floral volatiles of several individuals of 3 *Hackelia* species but will add more field collected samples. We will conduct gas chromatography electro-antennodetection tests coupled with flame ionization detection (GC-EAD/FID) using *Hackelia* spp VOC blends to identify bioactive VOCs assuming that these com-pounds may play a role in the observed repellence. We will then acquire pure compounds, as we have done for *C. officinale*, and test behavioral responses of *M. crucifer* to these compounds. We are aware that repellence may not necessarily be caused by a specific compound but a blend of VOCs and/or that GC-MS may not detect bioactive

VOCs that are present in very small concentrations. In the latter case, while laborious, we possess the instrumentation and methodologies to identify small concentration bioactive VOCs. Identified repelling VOCs will be tested in common garden experiments for their efficacy with exposed and manipulated (visible but odorless) *C. officinale* plants. We would make this push system available as additional safeguard **(25% PE)**.

Objective 4: Transferability of behavioral host finding responses from the laboratory to the field. Open field tests will be designed to test observed indifference and repellence patterns of *M. crucifer* with regard to select native nontarget species. In these sunray-style plot designs, *M. crucifer* will be released at a central point and movement and potential attack of nontargets at various distances will be measured in the presence and absence of the host plant *C. officinale*. We hypothesize that nontarget attack will categorically not occur in the absence of *C. officinale*. Other plot designs will be used to test at what minimum distance 'spill over attack' (i.e., attack of a nontarget plant in the proximity of *C. officinale*) will occur. Specifically, we will test how an odor plume admixture (OPA) (Kafle et al. in Review) may cause 'spill over' attack. For this objective we intend to use at least 2 of the 5 T&E Boraginaceae species that can easily be propagated (*Amsinckia grandiflora* (A. Gray) Kleeb. ex Green and Plagiobothrys strictus (Greene) I.M. Johnston) in order to provide more compelling data on the environmental safety of *M. crucifer*. Set design experiments will be conducted in common gardens, in a large greenhouse at the University of Idaho, and in areas where *M. crucifer* is naturalized on tribal lands in northern Montana and/or northern Washington State (i.e., the Blackfeet Nation, Confederated Tribes of the Colville, or Kalispell Tribe of Indians Reservations) **(25% PE)**.

8. EXPECTED PRODUCTS AND OUTCOMES: The data generated in this project will be used to prepare a new petition to the Technical Advisory Group (TAG) for the release of *Mogulones crucifer* in the United States. The data will be summarized in a M.S. and a Ph.D. thesis of two current graduate students at the University of Idaho (Jessica Fung and Karuna Nepal) and be published in 5 refereed journal articles on 1) the behavioral responses of *M. crucifer* to closely related Eurasian and North American confamilials, 2) VOCs causing repellence in *M. crucifer* and their use in IPM push systems, 3) the strength of early host finding host fidelity in *M. crucifer*, 4) odor plume admixture as a mechanism explaining transitory nontarget attack in biological weed control, and 5) a review/opinion article on the *Cynoglossum officinale* biological control program with regard to the relative importance of the fundamental and ecological host ranges of biocontrol agents. We also will write a manual in the USFS FHTET biocontrol manual series entitled **'Biology and Biocontrol of Houndstongue.'**

APPENDIX I – Workflow/load and timeline

Workflow/load: To accomplish the work proposed in the four objectives outlined above, the University of Idaho will split responsibilities between a current Ph.D. graduate student (Jessica Fung) and a M.S. student (Karuna Nepal). The BLM (Joey Milan) has pledged to provide additional support in addition to the funds sought with this proposal to enable successful graduation of both students and accomplishment of all work items outlined above. The University of Idaho will dedicate 0.4 FTE of Research Support Scientist (Brad Harmon) effort and 0.20 FTE of Principal Investigator effort (Schwarzländer) to this project. A Ph.D. student in the University of Idaho's Evolutionary Biology and Computational Biology Program (Amanda Stahlke) will teamwork with Jessica Fung on Objective 2. In addition, the graduate students will be assisted by Environmental Science Undergraduate 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, this is a large human resource investment that should be sufficient to complete the objective set forth above.

Proposed timeline:

2017											
JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
		Objective 1 - Behavioral bioassays, chemicals and ecological, electrophysiological experiments									
		Objective 2 - Strength and evolutionary stability of host fidelity							BM2		
			Objective 3 - Repellance push system						BM3		
			Objective 4 - Common garden and open field experiments								

2018											
JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Objective 1 - Behavioral bioassays, chemicals and ecological, electrophysiological experiments											
		Objective 2 - Strength and evolutionary stability of host fidelity							BM4		
			Objective 3 - Repellance push system						BM5		
			Objective 4 - Common garden and open field experiments								

2019											
JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Objective 1 - Behavioral bioassays, chemicals and ecological, electrophysiological experiments											BM1
		Objective 2 - Strength and evolutionary stability of host fidelity							BM6		
			Objective 3 - Repellance push system						BM7		
			Objective 4 - Common garden and open field experiments							BM8	

2020											
JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Objective 1 - Behavioral bioassays, chemicals and ecological, electrophysiological experiments											BM9
		Objective 2 - Strength and evolutionary stability of host fidelity							BM10		
			Objective 3 - Repellance push system						BM11		
			Objective 4 - Common garden and open field experiments							BM 12, 13, 14, 15	

Anticipated project period is **04/01/2017-12/31/2020**. Benchmarks (BM) as follows: **BM1**, Summary of behavioral response data with new set of critical plant species; **BM2**, Testing parental generation and successful rearing of F1 lines on hosts/non hosts; **BM3**, Analysis of *Hackelia* volatile blends; **BM4**, Testing F1 lines, rearing F2 lines; **BM5**, Identification of bioactive compounds; **BM6**, Testing F2 lines, rearing F3 lines; **BM7**, Testing push system; **BM8**, Summary of common garden and field experiments; **BM9**, Final project report to USFS FHTET, Submission of article on behavioral responses and underlying chemical processes; **BM10**, Submission of articles on strengths of host fidelity and genetic basis for host finding host specificity; **BM11**, Submission of article on a repellence system confusing/repelling *M. crucifer*; **BM12**, Submission of article on transferability of host finding results to field, **BM13**, Submission of houndstongue biocontrol project saga, **BM14**, Submission of petition to TAG; **BM15**, preparation of houndstongue FHTET biocontrol manual.

APPENDIX II - References

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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 (@ $2,333/ month) + 2.0% fringe/benefit		$28,000.00 $560.00
SALARY AND FRINGE (PI Schwarzländer) 1.5% FTE (base $86,860) ($1,303) and 31.1% fringe/benefits ($405)		$1,303.00 $405.00
OPERATIONAL EXPENSES Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation		$572.00
TRAVEL Reimbursement for vehicle mileage for travel to hound-stongue field and SIMP sites @ $0.54/mile for 4,000 miles ($2,160)		$2,160.00
EQUIPMENT Laptop computer and/or digital media ($1,000)		$1,000.00
COST SHARING Salary PI Schwarzlaender $5,715 + $1,778 (31.1%) fringe/ benefits, salary Research Support Scientist Harmon $3,353 plus $1,372 (40.9%) fringe/benefits, F&A on salaries and fringe/bene-fits $5,758, UI waived F&A $16,024	$5715.00 $1,778.00 $3,353.00 $1,372.00 $5,758.00 $16,024.00	
SUBTOTALS	**$34,000.00**	**$34,000.00**

Budget Justification Year 1:
Salaries: 12 months graduate Research Assistantship (RA) @ $2,333/month and 2.0% fringe/benefits ($28,000 and $560, respectively);
Salaries: 1.5% PI salary (base: $86,860) and 31.1% fringe/benefits ($1,303 and $405, respectively);
Operational Expenses: Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation ($572);
Travel: Partial reimbursement for repeated field trips travel to field sites throughout ID @ $0.54 per mile for approximately 4,000 miles ($2,160);
Equipment: Laptop computer/iPad/tablet or digital media ($1,000);
University of Idaho Cost Sharing: Waived Indirect Costs @ 47.13%* ($16,024); and partially matched salary of Research Support Scientist Harmon @ $3,353 salary and $1,372 fringe and Principal Investigator Schwarzlaender @ $5,715 salary and $1,778 fringe ($12,218). Plus Indirect Costs on shared salaries ($5,758) for a total of $34,000.
*Please note 47.13% is a blend of the 46% UI federally negotiated rate from 4/2017 to 6/2017 and the 47.5% federally negotiated rate from 7/1/2017 to 3/2017.

YEAR 2	University of Idaho Matching Funds	Funds provided by USDA FHP FHTET
SALARY AND FRINGE (Ph.D. student) 12 months graduate Research Assistantship (@ $2,333/month) + 2.0% fringe/benefit		$28,000.00 $560.00
SALARY AND FRINGE (PI Schwarzländer) 1.5% FTE (base $86,860) ($1,303) and 31.1% fringe/benefits ($405)		$1,303.00 $405.00
OPERATIONAL EXPENSES Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation		$572.00
TRAVEL Reimbursement for vehicle mileage for travel to houndstongue field and SIMP sites @ $0.54/mile for 2,148 miles ($1,160)		$2,160.00
EQUIPMENT Laptop computer and/or digital media ($1,000)		$1,000.00
COST SHARING Salary PI Schwarzlaender $5,872 + $1,826 (31.1%) fringe/benefits, salary Research Support Scientist Harmon $3,353 plus $1,372 (40.9%) fringe/benefits, F&A on salaries and fringe/benefits $5,579, UI waived F&A $15,675	$5715.00 $1,778.00 $3,353.00 $1,372.00 $5,758.00 $16,024.00	
SUBTOTALS	**$33,000.00**	**$33,000.00**

Budget Justification Year 2:
Salaries: 12 months graduate Research Assistantship (RA) @ $2,333/month and 2.0% fringe/benefits ($28,000 and $560, respectively);
Salaries: 1.5% PI salary (base: $86,860) and 31.1% fringe/benefits ($1,303 and $405, respectively);
Operational Expenses: Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation ($572);
Travel: Partial reimbursement for repeated field trips travel to field sites throughout ID @ $0.54 per mile for approximately 2,148 miles ($1,160);
Equipment: Laptop computer/iPad/tablet or digital media ($1,000);
University of Idaho Cost Sharing: Waived Indirect Costs @ 47.5% ($15,675); and partially matched salary of Research Support Scientist Harmon @ $3,353 salary and $1,372 fringe and Principal Investigator Schwarzlaender @ $5,355 salary and $1,666 fringe ($11,746). Plus waived Indirect Costs on shared salaries ($5,579) for a total of $33,000.

YEAR 3	University of Idaho Matching Funds	Funds provided by USDA FHP FHTET
SALARY AND FRINGE (Ph.D. student) 12 months graduate Research Assistantship (@ $2,333/month) + 2.0% fringe/benefit		$28,000.00 $560.00
SALARY AND FRINGE (PI Schwarzländer) 1.5% FTE (base $86,860) ($1,303) and 31.1% fringe/benefits ($405)		$1,303.00 $405.00
OPERATIONAL EXPENSES Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation		$572.00
TRAVEL Reimbursement for vehicle mileage for travel to houndstongue field and SIMP sites @ $0.54/mile for 2,148 miles ($1,160)		$2,160.00
EQUIPMENT Laptop computer and/or digital media ($1,000)		$1,000.00
COST SHARING Salary PI Schwarzlaender $5,872 + $1,826 (31.1%) fringe/ benefits, salary Research Support Scientist Harmon $3,353 plus $1,372 (40.9%) fringe/benefits, F&A on salaries and fringe/benefits $5,579, UI waived F&A $15,675	$5715.00 $1,778.00 $3,353.00 $1,372.00 $5,758.00 $16,024.00	
SUBTOTALS	**$33,000.00**	**$33,000.00**

Budget Justification Year 3:
Salaries: 12 months graduate Research Assistantship (RA) @ $2,333/month and 2.0% fringe/benefits ($28,000 and $560, respectively);
Salaries: 1.5% PI salary (base: $86,860) and 31.1% fringe/benefits ($1,303 and $405, respectively);
Operational Expenses: Field supplies and laboratory consumables, soil media and partial support for bench fees for space in greenhouse for plant propagation ($572);
Travel: Partial reimbursement for repeated field trips travel to field sites throughout ID @ $0.54 per mile for approximately 2,148 miles ($1,160);
Equipment: Laptop computer/iPad/tablet or digital media ($1,000);
University of Idaho Cost Sharing: Waived Indirect Costs @ 47.5% ($15,675); and partially matched salary of Research Support Scientist Harmon @ $3,353 salary and $1,372 fringe and Principal Investigator Schwarzlaender @ $5,355 salary and $1,666 fringe ($11,746). Plus waived Indirect Costs on shared salaries ($5,579) for a total of $33,000.