Biological Control of Russian Knapweed and Other Weeds Invasive to Lands Managed by the BLM - Final Report 2003 to 2008

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Tansy ragwort infestation Lincoln Co. MT – Photo (MSU)

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Summary - This report covers research conducted by J. Littlefield at Montana State University during the calendar years of 2003 to the end of 2008. It provides a review of research presented in earlier reports plus activities conducted in 2008. More details of several projects are provided in the attachments provided . Target weeds for this project included: hawkweeds, whitetop, field bindweed, Russian knapweed, and rush skeletonweed. The scope of work may be categorized under three broad objectives: 1) Identification of new potential agents and host specificity testing; 2) Clearance of agents through the regulatory channels and the eventual field release of these agents; and 3) Monitoring of biocontrol agents and their target plants, including pre-release monitoring of weed populations.

A petition for the field release the gall wasp, *Aulacidea subterminalis*, for the control of orange hawkweed has been drafted and will be submitted to the USDA-APHIS and TAG. Additional agents for hawkweed are being investigated at CABI Bioscience. This work is partially supported by MSU and with BLM funds (test plant collection). The host specicifty testing of the gall mite, *Aceria drabae*, for the control of whitetop was collected was completed, although additional testing of different mite biotypes continues, as well as he testing of additional agents at CABI Europe in Switzerland. A field survey of the eriophyid mite, *Aceria malherbae*, indicated that the gall mite is widespread at field bindweed sites throughout much of eastern Montana. This indicates a rapid expansion of the mite within the state. For Russian knapweed, the flower gall flies, *Urophora kasachstanica* and *U. xanthippe*, have been approved by TAG for release in Montana but their release is questionable due to concerns by the US Fish & Wildlife Service. Approval for the release of a stem galling wasp, *Aulacidea acroptilonica* was granted in 2008 and a release permit for the gall fly, *Jaapiella* ivannikovi is expected in 2009.. Both the wasp and gall fly being reared at the MSU quarantine. The root-feeding moth, *Bradyrrhoa gilveolella*, for the control of rush skeletonweed, was reared and larvae were consigned to the BLM in Idaho.

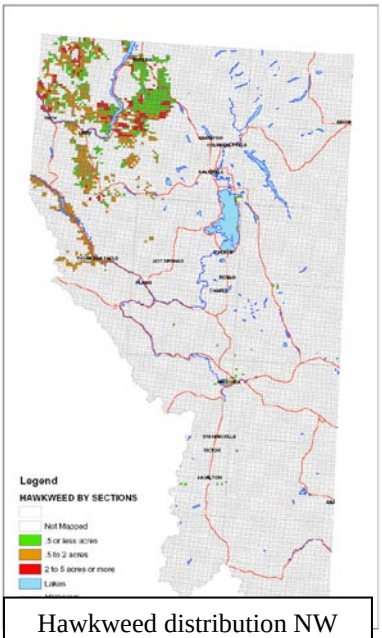
Invasive Hawkweeds – *Hieracium* species

Cooperators:
Linda Wilson – University of Idaho (formerly)
Gitta Grosskopf - CABI Europe – Switzerland

Introduction

Invasive hawkweeds - Both meadow hawkweed (*Hieracium caespitosum*) and orange hawkweed (*H. aurantiacum*) are undergoing rapid range expansion in the Pacific Northwest, in particular in northwestern Montana. This rapid expansion and quick vegetative dominance of a site has outpaced the ability of managers to track, locate and treat these weed infestations by conventional means. As a management tool to help control these weeds, a biological control program has been initiated by the Biocontrol of Hawkweed Consortium.

Biological control of invasive meadow and orange hawkweed in the United States has been investigated over the past seven years. Host specificity tests of several hawkweed insects for use in New Zealand have been conducted by Swiss researchers at CABI Europe - Switzerland. Five insects were identified and tested for use in New Zealand. Additional host specificity testing has been conducted to determine their potential in North America. Of these five insects, two have been dropped from consideration, two are still being tested and the last insect, the gall wasp *Aulacidea subterminalis*, has complete its testing and appears to be sufficiently host specific for field release. Several other insects have been considered by CABI, and of these two additional gall wasp, *Aulacidea hieracii* and *Aulacidea pilosella*, may be promising.



Hawkweed distribution NW Montana - 2005

Objectives

The objectives of this project were to: 1) Determine host specificity of several biocontrol agents; and 2) Investigate rearing techniques and establish rearing colonies of promising agents.

Results

Objective 1) Determine host specificity of several biocontrol agents.

Plant Collection – Collections of plant material were made primarily by Linda Wilson (formerly University of Idaho)(partially funded by this BLM agreement through MSU), although several

species were collected by MSU personnel. These plants were either grown in the greenhouse for seed or sent directly to CABI and/or MSU for testing. These included: *H. albertinum, H. albiflorum, H. argutum, H. bolanderi, H. fendleri, H. gracile, H. gronovii, H. longipilum, H. longiberbe, H. parryi, H. venosum, Crepis atribarba*, *Microseris nutans,* and *Lygodesmia juncea*. In addition CABI provided seeds of *H. carneum, H. gronovii*, and *H. scabrum* to MSU. The ability to obtain and grow these native test plants has been the major limiting factor in our testing of biocontrol agents for hawkweeds. Many of these native plants have been difficult to grow, especially in Europe, and often they have not produced viable seeds.

During the collection of plant material (by MSU) two native insects were reared. A stem boring cynipid wasp was discovered from *H. scouleri* (ID) and a flower head moth from *H. gracile* (MT). Neither of these two insects has yet been identified to species.

Table 1. The following plant species were collected and were provided to MSU and CABI Bioscience for testing:

Species	Origin	Tribe	Seed/Plants
CATEGORY 1: Genetic types of invasive *Hieracium* species			
H. aurantiacum	I	Hieraciinae	P
H. pilosella (WA)	I	Hieraciinae	P
H. caespitosum	I	Hieraciinae	P
H. floribundum	I	Hieraciinae	P
H. glomeratum	I	Hieraciinae	P
H. pilosella	I	Hieraciinae	P
H. piloselloides	I	Hieraciinae	P
CATEGORY 2: Species in the same genus as *Hieracium*.			
Species in the subgenus *Hieracium*			
H. canadense	N	Hieraciinae	P
Species in subgenus *Stenotheca*			
H. albertinum	N	Hieraciinae	P
H. albiflorum	N	Hieraciinae	P
H. bolanderi	N	Hieraciinae	P
H. carneum	N	Hieraciinae	P
H. fendleri	N	Hieraciinae	P
H. gracile	N	Hieraciinae	P
H. greenei	N	Hieraciinae	P
H. gronovii	N	Hieraciinae	P
H. horridum	N	Hieraciinae	P
H. longiberbe	N	Hieraciinae	P
H. parryi	N	Hieraciinae	P
H. scabrum	N	Hieraciinae	P
H. scouleri var. *cynoglossoides*	N	Hieraciinae	P

CATEGORY 3: Genera in the Asteraceae family.

3. Species in different subtribes of the same tribe (Lactuceae) as *Hieracium*

Crepis atribarba	N	Crepidinae	P
Crepis intermedia	N	Crepidinae	P
Agoseris grandiflora	N	Microseridinae	P
Krigia biflora	N	Microseridinae	P
Microseris nutans	N	Microseridinae	P
Lygodesmia juncea	N	Stephanomeriinae	P
Stephanomeria tenuifolia	N	Stephanomeriinae	P

Objective B. Determine the host specificity of *Aulacidea subterminalis*.

Methods - Galls of the wasp, *Aulacidea subterminalis*, were received in the spring of 2001 through 2006 (Littlefield 2009). These were from a rearing colony maintained by G. Grosskopf, CABI-Europe, Delémont, Switzerland. Galls were kept in moist vermiculite in cold storage (approximately 8^{o} C) until plants were in the correct phenological stage for infestation, i.e. producing stolons or vegetative shoots. Orange hawkweed (*Hieracium aurantiacum*) plants from Idaho and Montana were used as a control. In addition, the gall wasp's native host, mouse-ear hawkweed (*H. pilosella*), also was used as a control in the test. Galls were removed from cold storage throughout the summer, and were kept at room temperature until adult emergence. Three adult females were then transferred per replicate test plant. Wasps were originally (2001) contained on the plant using a 9-cm diameter plastic cage. The cage was vented at the top using a 100 mesh screen and cages were cut to the size of the plant. In subsequent years cages were replaced with netting since it was determined that the plastic used for caging was slightly phytotoxic to hawkweeds. After three days of exposure, adults were removed. Plants were inspected after approximately four months to determine the presence of galls.

Figure 1. Host specificity testing at MSU.

Results - North American Tests - During the North American host specificity test, galls were only induced on four exotic species: *H. pilosella* (its native host), *H. aurantiacum, H. flagellare*, and *H. floridundum*. No native or other test plants were infested. All host plants (with the exception of *H. floribundum*) are closely related based upon a proposed phylogeny by Gaskin and Wilson (2007) of North American *Hieracium* species. Gall induction on *H. floribundum* in the Montana tests appears atypical since this species was not infested in tests conducted by CABI. DNA testing of *H. floribundum* plants used in the MSU tests indicated that plants shared a greater genetic affinity to the subgroup containing *H. caespitosum, H. floribundum* and *H. glomeratum* than the subgroup containing *H. aurantiacum, H. flagellare* and *H. pilosella* (Gaskin per.comm.). Detailed information regarding the testing of *A. subterminalis* can be found in Littlefield et al. 2008 (see attachment).

Based upon test results from MSU, as well as testing of *A. subterminalis* for importation into New Zealand, this wasp appears to be sufficiently host specific for introduction into North America. Host utilization by *A. subterminalis* is confined to a few closely related hawkweed species all of which are Old World species. Native North American hawkweeds are precluded from attack in that they do not produce stolons in which the gall wasp induces galls and completes its development. All native North American hawkweeds are phylogenticallydistant and distinct from the exotic, and often weedy, hawkweeds of the Pilloella group. A petition for its field release has been drafted for submission to APHIS/TAG (see attachment).

CABI Testing– MSU (through the Montana Noxious Weed Trust Fund) is supporting CABI Bioscience Switzerland Centre in their biocontrol activities on invasive hawkweeds. CABI is currently testing two hoverflies *Cheilosia urbana* and *C. psilophthalma*, mass rearing the Hieracium gall wasp, *Aulacidea subterminalis* for MSU, and have initiated host range investigations with *Aulacidea pilosellae*, a small cynipid inducing galls of the mid-rib of leaves, flower head stalks, and stolons. Additional information can be found in Grosskopf et al. 2008. A petition for the release of *Cheilosia urbana* is currently being drafted by CABI and MSU.

Several fungi (*Entyloma hieracii* and *Rhizoctonia* sp) have recently been collected in Europe by Dr. A. Caesar, USDA-ARS, Sidney, MT. Preliminary tests to determine the potential use of these fungi are currently underway in the plant pathogen quarantine at MSU.

Objective 3) Investigate rearing techniques and establish rearing colonies of promising agents.

The biological control agent for invasive hawkweeds closest to being released is the gall wasp *Aulacidea subterminalis*. This gall wasp is sufficiently host specific, produces only females which gives the species a reproductive advantage, and appears to impact the plant in laboratory tests (Klöppel 2003). Although the majority of wasps reared from material received from CABI have been used for host specificity studies, a small rearing colony has been maintained at the quarantine lab at MSU of the past two years. In addition, studies have been initiated to determine gall development and the suitability of the various hawkweeds as hosts of the wasp. These studies will hopefully allow us to increase the number of wasps available for future field releases.

Initial attempts to rear the wasp in the laboratory were not overly successful. One problem was our selection of a host plant. We initially reared the wasp on *H. flagellare* thinking it was mouse ear hawkweed, the native host of the wasp. Although galls are induced on this species, we believe it to be a less suitable host. We also have had better gall production when we keep female wasps on the plants for longer durations of up to 5-7 days. We suspect that the wasps select stolons in a specific stage of development for oviposition and that by placing females on plants for longer durations we optimize our chance that suitable stolons will be present. Another problem has been poor emergence of adults after cold treatment. In general we have had good wasp emergence from field grown plants whereas emergence from laboratory plants have been less. This past year we were able to keep galls (field collected autumn 2004) in cold storage for one and a half years and still have viable adults emerge (spring 2006).

To aid us in the rearing of the wasp, we have initiated gall development studies on two hawkweed hosts – mouse ears and orange. Plants with developing stolons were exposed to female wasps for a period of 3-5 days. The number of stolons and their diameter and length were recorded every week for twelve weeks. The presence of galls (Figure 2) was also noted. Galls were remove later in the autumn and placed within cups containing vermiculite. These were placed in cold storage and removed the following spring to allow for adult emergence.

Figure 2. Galls on mouse eaar hawkweed

In comparing the length of infested verses uninfested stolons, it appears that female wasps tend to select shorter stolons for oviposition (Figure 3), although orange hawkweed stolons were slightly longer. Stolons on which galls were eventually inducted were approximately 11% shorter that those which did not produce galls (8.4 cm vs 9.5 cm at exposure). Although infested stolons continue to elongate their final length was still approximately 11 % less than uninfested stolons. Variation in the data (Figure 3) can be attributed to plant mortality and different observers (i.e. measurers).

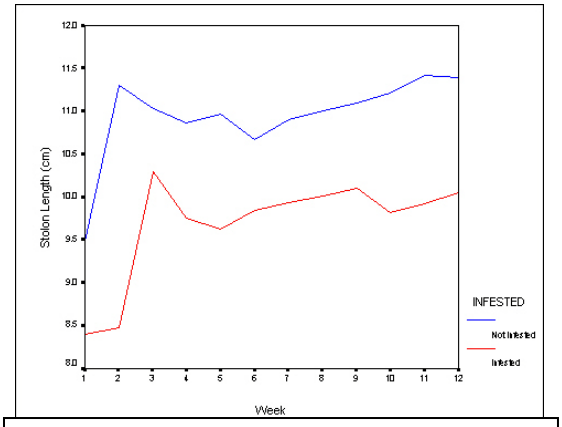


Figure 3. Length of infested and non-infested

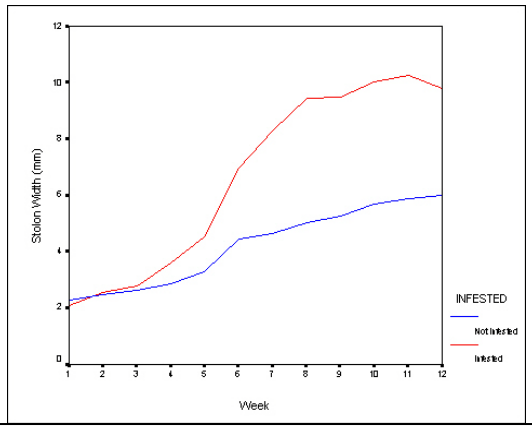


Figure 4. Tip diameter of infested and non-infested stolons.

Gall development was visibility apparent approximately 6 weeks after exposure to female wasps, although measurements suggest initial gall development as early as four or five weeks after exposure (Figure 4). Gall diameter continues to increase until week ten after which it tapers off

In comparing the two hosts, orange and mouse ear, although mouse ear hawkweed is the natural host of the wasp galls on orange hawkweed were significantly greater in diameter ($F=8.659$ $P=0.004$) (Figure 5). Gall development appears more rapid for the first 4-6 weeks after exposure before tapering off for two weeks. Galls reached their maximum development ten weeks after exposure. Gall are multi-chambered (Figure 6), therefore diameter is dependent upon the number of wasp larvae present. Although we had adult emergence from these galls after cold treatment, live larvae were still present at time of gall dissection. These galls were returned to cold storage to see if larvae will complete development with additional exposure to cold temperatures. Because of limited gall material in 2007 and 2008 these tests will be repeated in 2009.

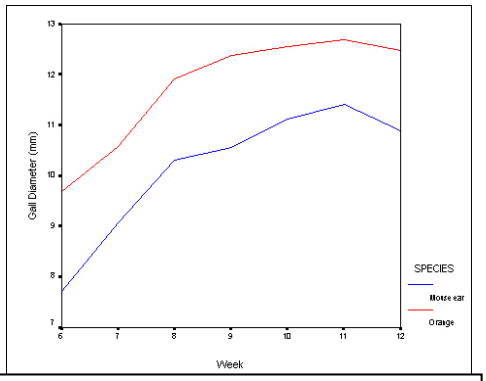


Figure 5. Tip diameter of infested and non-infested stolons by hawkweed species.

Figure 6. Multi-chamber gall of Aulacidea subterminalis – CABI photo.

Testing of *Aulacidea subterminalis* has been completed at MSU and a petition for release has been drafted for submission to APHIS/TAG. Greenhouse rearing of this wasp will be conducted in 2009 to augment populations of future field release. Gall development and impact studies as well as greenhouse rearing of the wasp will continue. CABI will continue work with *Cheilosia psilophthalma* and *Aulacidea hieracii*. We will also be assisting in the development of potential plant pathogens for the control invasive hawkweed species at the micro-containment laboratory at MSU.

Whitetop - *Lepidium (Cardaria) draba*

Cooperators:

Hariet Hinz - CABI Bioscience Switzerland Centre
J. Kashefi USDA-ARS-EBCL, Greece
Massimo Christofaro, BBCA, Rome, Italy

Introduction

Lepidium (*Cardaria) draba* (Brassicaceae) (heart-podded hoary cress, also known as whitetop) is one of two hoary cress species introduced into North America. This species is native to southwestern Asia, but is naturalized throughout Europe. It was first collected in 1864 in New York and through subsequent introductions is now wide spread throughout Canada and the US. Forcella and Harvey (1980) indicated that in the northwestern states of the US, only one county was infested by *C. draba* in 1900 and by 1980 over 66 counties had infestations. One additional species of hoary cress has been introduced into North America and have also become weedy. L*epidium appelianum* (= *Cardaria pubescens*), globe-podded hoary cress, is also wide spread but is less of a problem in western Canada and the US.

Hoary cresses are perennial weeds that reproduce from seeds and a spreading root system. The root system consists of vertical and lateral roots from which rosettes and shoots arise, and is very similar to that of Canada thistle. Vertical roots may penetrate as deep as five feet (Selleck 1965), thus making control through cultivation difficult. Plants may produce up to 850 flowers with a total seed out put of 1,200 to 4,800 seeds. Nearly 84% of the seeds germinate the first year. A single plant grown without competition can produced as many as 455 shoots in one year.

Hoary cresses are difficult to control using chemical or cultural control practices. To date no biological control agents have been introduced into North America. Currently *Aceria drabae* and a root/ stem galling weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae) are being considered as possible biological control agents by Montana State University and the USDA-ARS European Biological Control Lab in France. *Aceria drabae* has been found in Eastern Europe and Eurasia. Mites induce leafy galls on the stems or inflorescences (Figure 7), which either stunts the plant or reduces seed formation. Although the mite has been reported on a number of mustard species, field observations (Lipa 1978) indicate that the mite has a narrow host range. Host specificity tests on the mite are quite advance. *Ceutorhynchus assimilis* is also under consideration but has not been collected due to changes in key personnel at the EBCL.

Figure 7. Mite Infested (left), uninfested plant (right)

Five additional species are all being investigated at the CABI Europe in Delémont Switzerland. Tests with the gall-forming weevil *C. cardariae* are the most advanced. Approximately 80 species (half of which are indigenous to North America) been tested under no-choice and choice

conditions thus far. Although several plant species supported gall development in no-choice tests; in choice tests this weevil appears to be specific. Additional plant species are needed to complete the testing of this agent. The stem-mining weevil *C. merkli* does not appear to be quite as specific and due to low infestation of hoary cress, this species has been temporarily dropped from testing but may be revisited in the future. Testing of the seed-feeder *C. turbatus* continues. Eggs and mature larvae were only found on the closely related *Lepidium campestre* and *L. draba*. Testing is not as advanced as other agents due to problems with synchronizing seed pod and weevil development, and weevil mortality due to too many larvae within a seed pod. *Psylliodes wrasei* has been
tested on 45 plant species of which 16 species (7 North American species) supported larval development. However, multiple choice field cage tests were be made in 2007 to determine if this beetle is worth continuing to test. The beetle is common and can damage the plant. Another root feeding weevil, *Melanobaris semistriata* has been located in the Caucasus. Limited larval transfer tests have been conducted on this species and thus far the results look promising.

Cooperative Participation

In an effort to curb the spread of this noxious weed, several state, federal and international agencies, universities and organizations have joined together to explore biological control options and study the ecology and systematics of this important pest plant. The Hoary Cress Consortium, formed in 2001, includes representatives and researchers from a variety of international organizations, federal, state and county agencies, and university institutions. A similar consortium group has also been formed for perennial pepperweed but funding for biocontrol work has been limited.

Six insect species and one mite are currently being investigated as potential biological control agents for hoary cresses in North America (Table 1).

Table 1. Potential biological control agents currently under investigation.

A. ***Species name***	**Feeding niche of larvae**	**Investigating organization**
Aceria draba	Stem/flower galls	Montana State University
Ceutorhynchus assimilis (= *C. pleurostigma*)	Root and stem galls	USDA-ARS France & Sidney, MT, MSU
Ceutorhynchus cardariae	Shoot and petiole galls	CABI
Ceutorhynchus turbatus	Seeds	CABI
Melanobaris semistriata	Root feeder	
Psylliodes wrasei	Developing shoots	CABI

Objectives

The objectives of this project were to: 1) continue the host specificity studies for the mite, *Aceria drabae* and initiate host specificity tests of the weevil, *Ceutorhynchus assimilis*; and 2) to determine if more suitable or effective mite biotypes exist for North American whitetop varieties.

Results

Objective 1) Continue the host specificity studies for the mite, *Aceria drabae* and initiate host specificity tests of the weevil, *Ceutorhynchus assimilis*.

Test list – A proposed host specificity test plant list for potential biological control agents for hoarycress, perennial pepperweed and dyer'swoad has been drafted by Dr. Linda Wilson and me and has been submitted to TAG for review (see attachment). This list was designed to allow testing for several weedy mustards in the western US. The list is comprised of 104 species separated into seven test categories. Although this is a lengthy list, the intent was that not all species would be tested due to the nature of the specific biocontrol agent and some redundancy in some of the plant genera. For example in host specificity testing at CABI the gall weevil *Ceutorhynchus cardariae* has been observed to develop on a species of *Stanleya*, therefore we included several species of *Stanleya* for testing. These species would not be need for other agents, such as the mite. TAG has reviewed the list and some reviewers have made recommendations as to additional plants. These recommendations are being reviewed by researchers. In general the reviews of the list were favorable. In addition a number of recent research papers on the phylogeny the mustards and Lepidium have been published and has allowed us insights as to the relationships of the native test plants to the target host.

Plant and seed collection has been conducted by Littlefield, Dr. John Gaskin (USDA-ARS, Sidney, MT), and Dr. Richard Scott (funding through Fremont County Weed & Pest, WY). I have collected many of miscellaneous plants of the list from a variety of sources. John Gaskin has collected many of the important *Lepidum* species and other native species. Dr. Richard Scott (Central Wyoming College) has also collected a number of native mustards in Wyoming. These seed have been provided to both CABI and MSU. Although most of the test species on the list have been collected we are still lacking less than ten percent of the plants needed. For the hoarycress project, Dr. Mark Schwartzlander, University of Idaho has put together a collection of common whitetop haplotypes and is currently growing them.

Host specificity tests: Aceria drabae – Host specificity testing of the mite *Aceria drabae* (Figure 8), has been conducted over a period of ten years. Testing during this period was intermittent due to primarily shipping problems. The problem of mite mortality during shipment was not uncommon. In 2003 two shipments of *Aceria drabae* sent to Bozeman died during transit despite no delays in shipment, and again in 2007 and with several shipments made in 2008. This is an itermittant problem since we recieved healthy mites from Greece in 2004 and 2006. Other mite researchers have experienced the same problem. We suspect that mites overheat during transit and the addition of large amounts of blue ice seems to help.

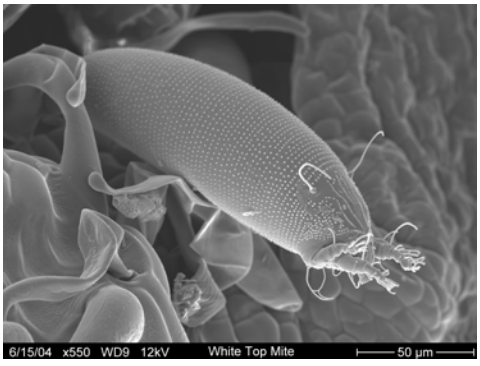


Figure 8. Aceria drabae

A shipment was received in May of 2008 from Javid Kashefi (USDA-ARS) from Greece. These were used to complete host specificity tests. Testing was conducted on 253 plant replications consisting of 24 different plant species (Table 2). Plants were inspected for mites two weeks after inoculation and again at 30 days. Mites and mite galls were only observed on the target host whitetop (*Lepidum draba*). Approximately 71% of plants were infested with mites and 57% had obivious galling.

In previous tests at MSU, *Aceria drabae* has only been observed to infest whitetop. Over 80 different plant species and varieties (Table 3) have been tested, although some species had a low number of replications. The mite was very host specific and only infested its intended host *Lepidium draba*. The closely related hairy hoarycress, *Lepidium appelianum*, was not infested. Previous testing indicted that this species or at least this particular haplotype is not a suitable host. The mite appears to be sufficiently host specific and a TAG petition is currently being drafted.

Objective 2) Determine if more suitable or effective biotypes exist for North American whitetop varieties.

The gall mite *Aceria drabae* is known from a variety of locations across Eastern Europe (e.g. Romania, Greece, Poland, Turkey and western Russia). Since gall mites tend to be very host specific, different biotypes of the mite may be present that are more adapted to whitetop genotypes present in North America, or to climatic conditions found in Montana.

Mite populations have been surveyed for and found in Hungary and Romania (by CABI Bioscience) and at least five sites by USDA-ARS, EBCL and BBCA, Rome Italy: Thessaloniki, Greece; Kayseri, Turkey; Erzurum, Turkey; Krasnodar, Russia; and Almaty, Kazakhstan. Although mites were collected by CABI from Hungary and Romania in 2005, they could not be shipped to MSU due to problems renewing APHIS permits. One shipment was received from Romania in 2006. Due to unknown reasons nearly all the mites were received dead or dying. Although some mites were transferred to plants, we were not able to start a colony form this collection. Mites were placed in ETOH for future DNA analysis. Again in 2008 shipments were received from Bulgaria, Turkey and Kazakstan and all the mites died while in transit. Tests will be repeated in 2009.

<u>*Ceutorhynchus assimilis* –</u> A root/ stem galling weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae) is also being considered as a possible biological control agent by the USDA-ARS European Biological Control Lab in France. DNA analyses in combination with preliminary host range tests revealed that *C. assimilis*, recorded from the literature as oligophagous, consists of at least two different host races, one of which appears to be specific to *L. draba* (Fumanal et al. 2004). Studies of this weevil will continue in Montana.

Due to several retirements at the USDA-ARS European Biological Control Laboratory the gall weevil, *Ceutorhynchus assimilis* was not collected in 2005. A small collection of the weevil was made in 2008 by B. Fumanal. Only a few adults were received at the MSU quarantine lab. These were placed on plants and were able to produce a small number of galls. An additional collection

of the weevil has been made in France by M. Bon of the USDA-ARS-EBCL during the spring of 2009. These will be shipped to Bozeman once adults have emerge..

Future Work

The host specificity testing of *Aceria drabae* was completed in 2008, although additional plants species may still be tested if necessary. Biotypes of the mites and their potential impact will be investigated to determine if superior populations exist. Screening of additional agents will continue at CABI. Testing of another promising agent, *Ceutorhynchus cardariae*, will hopefully be completed in 2008 or early 2009. CABI will continue host specificity studies of potential biocontrol agents.

Table 2. Host specificity testing of *Aceria drabae* at MSU – 2008

Species	Common Name	Native/ Introd[2]	# Tested	Mites 2 Weeks	Mites 30 Days	Galls present
Arabidopsis thaliana	Mouseear Cress	Intro.	17	-	-	-
Arabis glabra	Tower-Mustard	Nat.	16	-	-	-
Arabis holboellii	Holboell's Rockcress	Nat.	8	-	-	-
Berteroa incana	Hoary False Madwort	Intro.	8	-	-	-
Caulanthus crassicaulis var. *crassicaulis*	Thickstem Wild Cabbage	Nat.	17	-	-	-
Cleome serrulata	Rocky Mountain Beeplant	Nat.	1	-	-	-
Erodium cicutarium	Red Stem Stork's Bill	Intro.	6	-	-	-
Erysimum inconspicuum	Shy Wallflower	Nat.	3	-	-	-
Lepidium crenatum	Alkalai Pepperweed	Nat.	16	-	-	-
Lepidium davisii	Davis' Pepperweed	Nat.	11	-	-	-
Lepidium draba	Whitetop	Intro.	21	+	+ (15 plants)	+ (12 plants)
Lepidium flavum		Nat.	7	-	-	-
Lepidium lasiocarpum var. *lasiocarpum*	Shaggyfruit Pepperweed	Nat.	10	-	-	-
*Lepidium montanum (?)*var. *angustifolium*	Mountain Pepperweed	Nat.	9	-	-	-
Lepidium montanum var.*montanum*	Mountain Pepperweed	Nat.	8	-	-	-
Lepidium papilliferum	Idaho Pepperweed	Nat.	15	-	-	-
Lepidium obongum		Nat.	16	-	-	-
Lepidium sordidum	Sordid Pepperweed	Nat.	4	-	-	-
Lesquerella hemisphysaria	Intermountain Bladderpod	Nat.	16	-	-	-
Lesquerella ludoviciana	Keeled Bladderpod	Nat.	16	-	-	-
Pelargonium zonale	Zonal Geranium	Intro.	5	-	-	-
Schoenocrambe linifolia	Salmon River Plainsmustard	Nat.	7	-	-	-
Thelypodium laciniatum	Cutleaf Thelypody	Nat.	16	-	-	-
Thlaspi idahoensis	Idaho Pennycress	Nat.	7	-	-	-

Table 3. Status of host specificity testing of *Aceria drabae*.

Species	Common Name	Test Cat[1]	Native/Introd. /Eur[2]	Phenology[3]	Infested[4]
Alyssum alyssoides	Pale Madwort	3	Intro.	A, B	-
Arabidopsis thaliana	Mouseear Cress	3	Intro.	A	-
Arabis aculeolata	Walso Rockcress	3	Nat.	P	-
Arabis blepharophylla	Rose Rockcress	3	Nat		-
Arabia breweri	Brewer's Rockcress	3	Nat		-
Arabis caucasia		3	Intro		-
Arabis dispar	Pinyon Rockcress	3	Nat		-
Arabis glabra	Tower-Mustard	3	Nat.	B, P	-
Arabis holboellii	Holboell's Rockcress	3	Nat.	B, P	-
Arabis koehleri	Koehler's Rockcress	3	Nat		-
Armoracia rusticana	Horseradish	6	Intro.	P	-
Barbarea verna		7	Intro		-
Berteroa incana	Hoary False Madwort	7	Intro.	A, B, P	-
Brassica juncea	India Mustard	3	Intro.	A, P	-
Brassica napus	Rape	3, 6	Intro.	A, B	-
Brassica nigra	Black Mustard	3, 6	Intro.	A	-
Brassica oleracea capitata	Cabbage	3, 6	Intro.	P	-
Brassica oleracea gimmifera	Brussel Sprouts	3, 6	Intro.	P	-
Brassica oleracea italica	Broccoli	3, 6	Intro.	P	-
Brassica rapa	Field Mustard	3	Intro.	A, B	-
Camelina microcarpa		3	Intro.		-
Camelina sativa	Gold-Of-Pleasure	3	Intro.	A, B	-
Capsella bursa-pastoris	Shepherd's Purse	3	Intro.	A	-
Cardamine cordifolia var. *lyallii*	Large Mountain Bittercress	4b	Nat	P	-
Cardamine pachystigma	Serpentine Bittercress	4b	Nat	P	-
Caulanthus anceps (= *Guillenia lemmonii*)	Lemmon's Mustard	3	Nat.	A,B	-
Caulanthus crassicaulis var. *crassicaulis*	Thickstem Wild Cabbage	3	Nat.	B, P	-
Caulanthus inflatus	Desert Candle	3	Nat.	A	-
Cleome serrulata	Rocky Mountain Beeplant	5	Nat.	A	-
Conringia orientalis					-
Crambe tatarica	Crambe	7	Intro.	B	
Draba albertina	Slender Whitlow-Grass	3	Nat	P	
Draba aurea			Nat		-
Draba densifolia	Dense-Leaf Whitlow-Grass	3	Nat.	P	-
Draba grayana			Nat		-
Draba oligosperma	Few-Seed Whitlow-Grass	3	Nat	P	-
Erodium cicutarium	Red Stem Stork's Bill	6	Intro.	A, B	-
Erysimum capitatum var.	Sanddune Wallflower	3	Nat.	B, P	-

capitatum					
Erysimum inconspicuum	Shy Wallflower	3	Nat.	B, P	-
Hesperis matronalis	Dames Rocket	3	Intro.	B, P	-
Iberis umbellata	Globe Candytuft	3	Intro.	A	-
Isatis glauca		2	Eur.	B,P	-
Lepidium appelianum	Hairy Whitetop	1	Intro.	P	-
Lepidium campestre	Cream-anther Field pepperwort	2	Intro.	A	-
Lepidium davisii	Davis' Pepperweed	2	Nat.	P	-
Lepidium densiflorum var. *densiflorum*	Common Pepperweed	2	Nat.	A, B	-
Lepidium draba	Whitetop	1	Intro.	P	+
Lepidium huberi	Huber's Pepperweed	2	Nat.	P	-
Lepidium lasiocarpum var. *lasiocarpum*	Shaggyfruit Pepperweed	2	Nat.	A, B	-
Lepidium latifolium	Broadleaved Pepperweed	1	Intro.	P	-
Lepidium montanum var.*montanum or nevadense*	Mountain Pepperweed	2	Nat.	B, P	-
Lepidium nanum	Dwarf Pepperweed	2	Nat.	P	-
Lepidium oblongum	Veiny Pepperwort	2	Nat.	A	-
Lepidium papilliferum	Idaho Pepperweed	4a	Nat.	B, P	-
Lepidium perfoliatum	Clasping Pepperweed	1	Intro.	A	-
Lepidium sativum	Garden pepperwort	2	Intro.	A	-
Lepidium sordidum	Sordid Pepperweed	2	Nat.	A, B	-
Lepidium virginicum var. *medium*	Poorman's Pepperweed	2	Nat.	A, B, P	-
Lesquerella arizonica		4b	Nat		-
Lesquerella occidentalis	Western bladderpod	4b	Nat		-
Lesquerella fedleri	Fendler's Bladderpod	4b	Nat.	P	-
Lesquerella hemisphysaria	Intermountain Bladderpod	4b	Nat.	P	-
Lesquerella ludoviciana	Keeled Bladderpod	4b	Nat.	P	-
Lesquerella montana	Mountain.Bladderpod	4b	Nat		-
Lobularia maritima	Sweet Alyssum	3	Intro.	A, B	-
Nasturtium officinale	Watercress	3	Nat.	P	-
Pelargonium zonale	Zonal Geranium	6	Intro.	A	-
Physaria chambresii var. *membranacea*	Chamber's Twinpod	3	Nat.	P	-
Physaria floribunda		3	Nat		-
Raphanus sativus	Cultivated Radish	3, 6	Intro.	A, B	-
Rorippa sinuata	Spreading Yellowcress	3	Nat.	P	-
Schoenocrambe linifolia	Salmon River Plainsmustard	4b	Nat.	P	-
Sinapis alba	White Mustard	3	Intro.	A	-
Smelowskia ovalis	Alpine False Candytuft	3	Nat.	P	-
Stanleya albescens	White Prince's-Plume	3	Nat.	P	-
Stanleya pinnata var.	Desert Princesplume	3	Nat.	P	-

pinnata					
Streptanthus farnsworthianus	Evalyn's Jewelflower	3	Nat.	A	-
Thlaspi arvense	Field Pennycress	3, 6	Intro.	A	-
Thlaspi idahoensis	Idaho Pennycress	3	Nat.	A, P	-
Thlaspi montanum var. siskiyouense	Siskiyou Pennycress	3	Nat.	P	-
Thelypodium laciniatum	Cutleaf Thelypody	3	Nat.	B	-
Tropaeolum majus	Nasturtium	6	Intro.	A, P	-

1 CATEGORY 1: Genetic types of *Cardaria/ Lepidum latifolium* (varieties, races, genotypes, etc.); CATEGORY 2: North American species in the same genus as *Cardaria/Lepidium*, including economically and environmentally important plants; CATEGORY 3: North American species in other genera in the Brassicaceae family, divided by tribe, including economically and environmentally important plants; CATEGORY 4: Threatened and endangered species in the Brassicaceae family, divided by genus and tribe; CATEGORY 5: North American species in other families in the Capparales order that have some phylogenetic, morphological, or biochemical relationship to the target weed, including economically and environmentally important plants; CATEGORY 6: North American species in other orders that have some morphological or biochemical relationship to the target weed, including economically and environmentally important plants; and CATEGORY 7: Any plant on which the biological control agent or its close relatives (within the same genus) have been previously recorded to feed and/or reproduce.

2 Origin: N= Native; Intro.= introduced into US; Eur.=European only

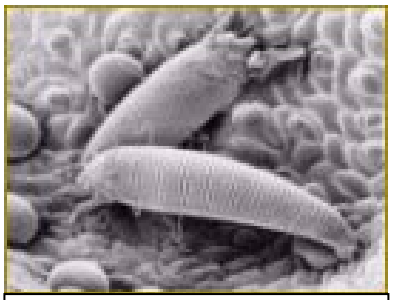
3 Life stage: A= annual; B= biennial; P=perennial

4 - = Not infested; + Infested

Field Bindweed – *Convolvulus arvensis*

Introduction

Field bindweed, *Convolvulus arvensis* L. (Convolvulaceae) is one of the most aggressive, perennial weeds of grain producing areas of the northern Great Plains of North America. Although field bindweed is a persistent weed of croplands, it also occurs in pastures, gardens, roadsides, right-a-ways and vacant land. Field bindweed has been identified as a target species for biological control by APHIS. To control this weed biologically, the gall mite, *Aceria malherbae* Nuzzaci (Acari: Eriophyidae) (one of only two agents approved by APHIS for field release) has been released in the United States. Although the mite has been successfully established at three locations in Montana, the establishment and spread of the mite at other sites is not currently known**.** Successful biological control would provide an alternative management strategy for field bindweed.



Aceria malherbae mites

Objectives

Previous releases sites of *Aceria malherbae* will be inspected for successful establishment of the mite.

Approach

Sites of *Aceria malherbae* were inspected from mid-August to mid-September. Percent mite infestation was determined at each site (see below). If mite galls were observed, visual inspections of adjacent field bindweed infestations were made parallel and perpendicular to the prevailing winds until mites or field bindweed can no longer be located. A more general survey along major roads was also conducted in Eastern Montana.

Microhabitats at selected *Aceria malherbae* releases in were characterized as to site/ environmental conditions, plant productivity, soil conditions, and current or past land utilization. Sites were characterized as to elevation, aspect, average precipitation, and mean temperatures (if available). Field bindweed density, plant composition, percent cover and biomass were estimated for each sampling unit. Ten 1/4 m^2 quadrates were sampled along a 50 m transect extending through the center of the release site. From these quadrates, field bindweed density (i.e. number of stems) and number of infested stems were determined, and all plant material were clipped, bagged, and dried to determine biomass (g). Plants were separated as to field bindweed, grasses and forbs to determine species composition. Percent cover at each sampling unit was visually estimated. Physical properties and nutrient quality of the soil were also determined. Soil compaction was measured using bulk density estimates. Soil from upper horizon (A) was sampled using a manual soil corer. Two representative samples were collected from each site and

combined and sub-sampled for soil analysis: % organic matter, sand, silt and clay, soluble salts, pH, and various minerals/micronutrients. All laboratory work, with the exception of the soil analyses, was conducted on the MSU campus.

Results

Field Releases & Monitoring

In July 2003, a release of Aceria malherbae was made on BLM property (former Altman Ranch) which borders the Clark Fork of the Yellowstone south of Laurel, MT. Mites were released using gall material that was chopped into fragments and then sprinkled onto bindweed plants. Subsequent inspections of the site indicated that the mites established on plants in 2003 and increased in population in 2004. Additional mites were released at the site in 2004. Unfortunately most of the release site has since been sprayed and cultivated, thereby eliminating most of the mites. Follow-up spraying in 2006 eliminated the remaining mite population at the original release. We were able to locate several small patches of bindweed near the Clark Fork River which were infested with the mite. These were located approximately a quarter mile from the initial release.

In 2004 we located an addition bindweed mite site on Bureau of Reclamation land near Huntley, MT. We were initially going to make a release of the mite at this location, but as we were inspecting the site we noted infested plants. Further inspection indicated that the mite was well at the site and had dispersed for several miles. We speculated that the mite was placed here by the Weed Supervisor of Yellowstone County in the late – 1990's. Mites were sent to Yellowstone Co. for release but no site location was provided at the time.

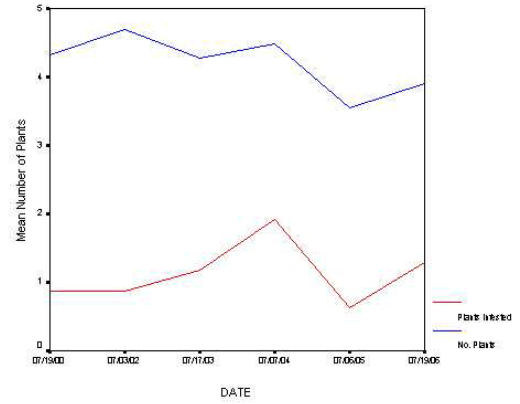


Figure 9. Field bindweed density at Roy, MT.

As with the infestation at a long term monitoring site located at Roy, MT, the infestation at Huntley was also patchy in distribution. We surveyed the site to determine if cover of grasses, field bindweed and other forbs contributed to this patchy distribution. We hypothesized that either the density of field bindweed or the lack of cover increased plant infestation by the mite. Seventy five ¼ m quadrates were established at the site – 25 in areas of light, moderate and heavy infestation. Percent infestation and percent cover of grasses, forbs, field bindweed and bare ground were estimated for each quadrate. Step wise regression analysis was performed on this data. No significant correlations were observed in the data except for percent cover by grasses. As grass cover increased infestation of bindweed plants by the mite decreased. The reasons for this relationship are unclear.

In August 2006 we planned to release the gall mite at the NRCS Bridger Plant Materials Center, outside of Bridger Montana, but found that *A. malherbae* to be already present. Mites were also located near Edgar. It is not certain as to the source of these mites. The BLM Laurel release is the only documented release of the mite in the area (over 25 miles from Bridger), although mite may have also come from illicit or undocumented releases.
2006 appeared to be a good mite year at two additional release sites: one on Bureau of Reclamation land near Huntley, MT and the other near Roy, MT. Although the bindweed density at the Bureau of Reclamation site was very low, an adjacent site was found containing large numbers of mites. This area was mowed earlier in the summer and much of the bindweed that resprouted was infested, although the density of the mite was somewhat patchy. There may be a few patches of BLM land nearby that may contain the mite. At Roy, mite and bindweed populations continue to be monitored. Bindweed density (Figure 9) appears to have remained stable at this site, although there are variations in density from year to year. For example the bindweed density and the presence of the mite was reduced compared to previous years but in 2006 plant and mite density rebounded. The mite was also located for the first time at a release made at the CM Russell Wildlife Refuge, within a few miles of land administered by the BLM.

Geographic Location In Which The Project Took Place - The geographic range of the survey was expanded to include the following Montanan counties: Big Horn, Carbon, Custer, Daniels, Dawson, Fergus, Flathead, Gallatin, Garfield, Lewis & Clark, McCone, Park, Phillips, Prairie, Richland, Roosevelt, Rosebud, Stillwater, Sweetgrass, Treasure, Valley, Wibaux, and Yellowstone (Table 3). Due to budget and time restrictions surveys were not conducted in Hill, Judith Basin, and Teton Counties. Except for Teton County, these releases were made in 2006 and therefore were of lower priority.

Previous releases of the mite occurred in: Fergus, Flathead, Gallatin, Hill, Judith Basin, Lewis & Clark, McCone, Park, Phillips, Richland, Roosevelt, Teton, and Yellowstone. Infested field bindweed sites were located at all of these counties (Hill, Judith Basin and Teton not sampled), although not all sites had the mite present.

The remaining counties: Big Horn, Carbon, Custer, Daniels, Dawson, Garfield, Golden Valley, Musselshell, Petroleum, Prairie, Rosebud, Stillwater, Sweetgrass, Treasure, Valley, Wheatland, and Wibaux represent counties where the mite was not known to previously occur.

Mite populations tended to be quite variable. Most field bindweed sites in eastern Montana were infested with mites. In general only a few plants were infested at each site, but occasionally heavy pockets of the mites were observed, e.g. sites near Vida (McCone Co.) or Opheim (Valley Co.). Many of the sites visited in Yellowstone Co. had significant infestations of *Aceria malherbae*. In contrast, sites located in the western portions of the state (e.g. Flathead, Gallatin, Lewis & Clark and Park Counties) had low levels of infestations despite the mites being established for a period of ten years at some sites (i.e. Flathead and Gallatin Counties). At these sites only an occasional plant was infested, with only one or two leaf galls observed. These sites occur at relatively higher elevations and receive more rainfall.

The majority of the sites sampled were along roadsides or vacant land, where disturbance (other than mowing) is minimal. Mites were also located in highly disturbed sites such as hay land, pastures and cultivated/ fallow fields. Plant cover at infested sites was characterized by moderate grass cover or open ground. Bindweed cover ranged from 6-
25% and the presence of other forbs was low (less than 5%). Infested sites were generally more open and drier habitats. Soil conditions at high verses low mite infestations did not vary greatly. Soil texture was thought to be a key characteristic in the ability of the mite to overwinter, but the soil texture ranged from sandy loams to heavy clay soils; and reflected more the location of the particular site.

Conclusions

Aceria malherbae is well established within the state of Montana. Although many counties were not sampled, the mite appears to be widespread in eastern portions of the state. It is thought that the mite has a wider distribution than what is indicated by the survey. The mite may have also dispersed into surrounding states such as North Dakota and Wyoming, or the Canadian province of Saskatchewan. The sources of the mite are not known. Although releases were made in the eastern part of the state (e.g. McCone, Roosevelt, and Richland Counties) many of these sites were subsequently destroyed due to cultivation, did not apparently establish or until recently did not appear to have spread. The wide spread distribution of *Aceria malherbae* may indicate that the mite may be wind dispersed to a greater extent than was previously thought (perhaps originating from Yellowstone Co.). Despite the mite being widespread, its current impact on field bindweed appears to be low in many places. Yellowstone County appears to have the highest density of *Aceria malherbae* compared with other Montanan counties. Perhaps with time, the mite may reach comparative levels.

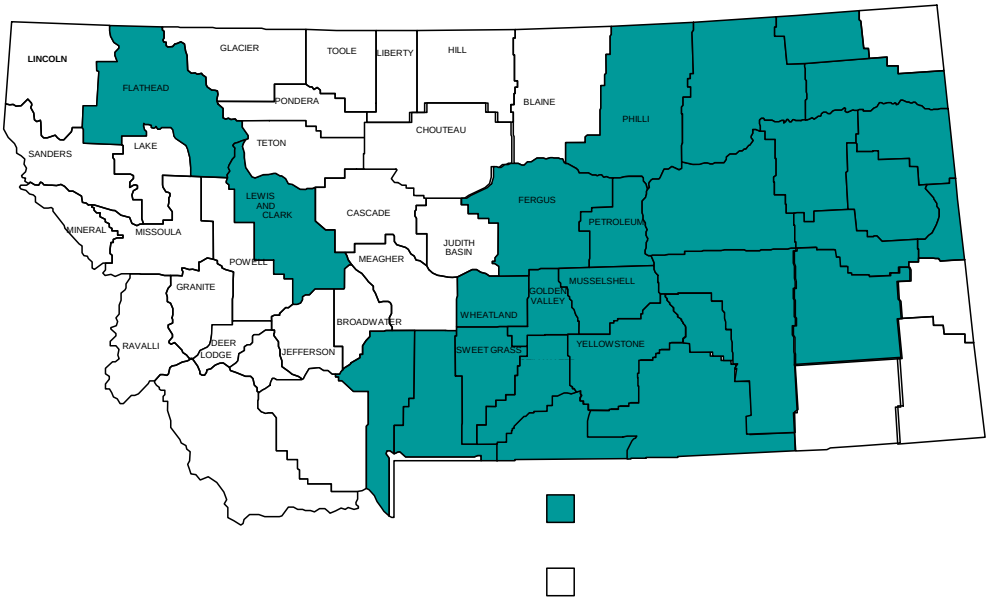
Table 3. *Aceria malherbae* sample locations - Montana 2007 and 2008

County	Location	Presence	Mite Level	Elevation
Big Horn	Hardin	Y	1	2855
Carbon	Bridger	Y	3	3745
Carbon	Edgar	Y	1	3487
Custer	Diamond Ring Ranch	Y	3	2367
Daniels	Scobey	Y	1	2475
Dawson	East of Prairie Co. line - rest stop	Y	1	2252
Dawson	West of Wibaux Co. line	Y	1	2614
Fergus	191 & Birdwell Rd	Y	1	3079
Fergus	Bohemian Corner	Y	1	3246
Fergus	North of Roy	Y	1	3117
Fergus	North of Roy	Y	1	3129
Fergus	North of Roy	Y	1	3115
Fergus	North of Roy	Y	1	3073
Fergus	North of Roy	N	-	3100
Fergus	North of Roy	Y	1	3191
Fergus	North of Roy -	Y	2	3138

	Release 1994/95			
Fergus	Roy	N	-	3479
Flathead	Kalispell	Y	1	2979
Gallatin	Belgrade	Y?	1	4468
Gallatin	Bozeman	N	-	4777
Gallatin	Bozeman	N	-	4777
Gallatin	Bozeman - MSU	Y	1	4840
Gallatin	Bozeman - MSU	N		4849
Gallatin	Manhattan	N	-	4260
Gallatin	Manhattan	N	-	4270
Gallatin	Springhill	N	-	4744
Gallatin	Springhill	N	-	4643
Gallatin	Springhill	N	-	4623
Garfield	Rest Stop - Garfield/ McCone Line	Y	1	2468
Golden Valley	Ryegate	Y	1	3710
Lewis & Clark	East Helena	Y	1	4261
McCone	North of Vida - Near Jct 13 & 201	Y	2	2418
Musselshell	S. Roundup	Y	1	3500
Park	East of Livingston - Mission Rd	N	-	4359
Park	East of Livingston - Yellowstone River	N	-	4422
Park	East of Springdale Fishing Access	Y	1	4303
Park	Livingston - Release	Y	1	4579
Park	Springdale Exit - I 90	Y	1	4162
Petroleum	Flatwillow	Y	1	
Phillips	CMR - Release 2001	Y	1	2277
Phillips	CMR - Release 2006	Y	2	2270
Phillips	CMR - Release 2006	Y	1	2274
Prairie	Terry	Y	1	2304
Prairie	Terry	Y	1	2284
Richland	Crane - Seven Sisters WMA	Y	1	1990
Richland	Savage - Elk Island WMA Rd	Y	1	1992
Richland	West of Lambert - Release	Y	1	2434
Roosevelt	North of Wolf Point - Rt 13	Y	2	2416
Rosebud	East of Rosebud	Y	3	2530
Rosebud	Hathaway	Y	1	2487
Rosebud	West of Forsyth	Y	1	2697
Stillwater	Columbus	Y	3	3606
Stillwater	East of Columbus - Molt Rd	Y	1	3805

Stillwater	Park City	Y	1	3400
Stillwater	West of Columbus - Springtime Rd	Y	1	3707
Stillwater	West of Park City	Y	1	3432
Sweetgrass	Big Timber	N	-	4036
Sweetgrass	Greycliff - Pelican Fishing Access	Y	1	3896
Sweetgrass	Reed Point	Y	2	3760
Treasure	West of Hysham	Y	1	2895
Valley	East of Opheim	Y	3	3250
Wheatland	Harlowton	Y	1	4317
Wibaux	East of Dawson Co. line	Y	1	2840
Wibaux	West of ND state line	Y	1	2826
Yellowstone	Billings - River Front Park	Y	2	3176
Yellowstone	Billings - Zoo Ave.	Y	2	3268
Yellowstone	Custer	Y	1	2740
Yellowstone	East of Billings - I90 & 94	Y	2	3134
Yellowstone	East of Laurel	Y	1	3280
Yellowstone	Huntley - East of Museum	Y	3	3008
Yellowstone	Huntley - Museum	Y	2	3033
Yellowstone	Huntley - Release (?)	Y	1	3039
Yellowstone	Huntley - River	Y	1	3065
Yellowstone	Pompey's Piller	Y	1	2897
Yellowstone	Pompey's Piller - Visitor Center	N	-	2905
Yellowstone	South of Huntley - Hogan Rd	Y	1	3160
Yellowstone	South of Huntley - Shadow Canyon	Y	1	3130
Yellowstone	South of Laurel - BLM release 2003	Y	1	3274
Yellowstone	Worden	Y	3	3050
Yellowstone	West of Huntley	Y	1	3133
Yellowstone	West of Custer	Y	3	2968

Aceria malherbae Survey – 2007 &



Russian Knapweed – *Acroptilon repens*

Cooperators & Co-investigators:

U. Schaffner CABI-Europe, Switzerland
T. Collier, University of Wyoming, Laramie
Rich Hansen, USDA-APHIS Fort Collins, CO
US Fish & Wildlife Service, CMR Refuge
Dana Berner USDA-ARS, Fort Detrick, MD

Introduction

Research and surveys by various Russian, USDA, and more recently CABI scientists have identified numerous potential biocontrol organisms attacking Russian knapweed. In the past years J. Littlefield - MSU (through support of the Noxious Weed Trust Fund) and U. Schaffner – CABI Europe (supported by Wyoming) have conducted host specificity testing of six agents: two *Urophora* gall-flies (MSU), an *Aceria* foliage mite (MSU), a *Napomyza* root fly (CABI), a stem-galling wasp (Aulacidea) (CABI), a tip gall midge (*Jaapiella*) (see cover photo.) (CABI), and a root-boring moth (*Cochylimorpha*) (CABI),. Petitions have been submitted to and approved by TAG for the release of the *Urophora* flies, gall wasp, and gall midge (currently being review). We expect a release permit for the gall wasp to be issued in early 2008. Release of the gall flies is currently being held up by the Fish & Wildlife Service due to concerns as to non-target ovipositional probing by the flies (no eggs were laid). The gall midge is very host specific and should be approved. The root moth, although damaging, has been difficult to work with. Several other agents have been rejected due to non-target attack on other plant species. Other agents have been investigated include a flower infesting mite, defoliating and stem boring beetles. The mite appears to be the more host specific of these agents. Surveys for new agents have occurred in Turkey, Uzbekistan, Kazakhstan, western China, and is finishing up in Iran.

Objectives

The objectives of this project were to: 1) Release the flies, *Urophora kasachstanica* and *U. xanthippe*, when they become available; 2) Rear the gall wasp *Aulacidea acroptilonica* and the gall fly *Jaapiella ivannikovi*; 3) Assist in the preparation of petition of potential biological control agents, and 4) Continue to monitor the rate of spread of Russian knapweed at study sites and survey for native natural enemies.

Results

Objective 1. Release the flies, *Urophora kasachstanica* and *U. xanthippe*, when they become available

Two species of gall flies have been identified as potential biocontrol agents of Russian knapweed: *Urophora kasachstanica* and *Urophora xanthippe* (referred to in the literature as *U. maura*). Both flies induce a lignified gall in

the flower head which reduces seed production. *Urophora kasachstanica* has only been collected on Russian knapweed, whereas *Urophora xanthippe* has been recorded to infest Russian knapweed, as well as, a possible record on *Centaurea virgata* spp. *squarrosa* (squarrose knapweed).

Host specificity tests were completed for the gall flies. A petition for the field release of the two flies (a copy will be provided upon request) was submitted in April 2003 to the USDA-APHIS and TAG for review. On December 30, 2003 TAG approved the petition for release and an environmental assessment was drafted and sent to the USDA-APHIS-PPQ for review in November 2004 along with a PPQ 526 permit requesting the release of these flies in Montana. In 2007 this EA was rejected by the Fish & Wildlife Service due to concerns as to non-target ovipositional probing by the flies (no eggs were laid). It is not certain if this issue can be resolved, although additional information is being collected.

Objective 2) Rear the gall wasp *Aulacidea acroptilonica* and the gall fly *Jaapiella ivannikovi*.

Several shipments of gall wasp *Aulacidea acroptilonica* were received at the MSU quarantine lab for rearing. Due to high levels of parasitism, populations of the wasp will need augmentation prior to making extensive field releases.

Two populations of the gall wasp *Aulacidea acroptilonica* was received in April 2006 from U. Schaffner, CABI Bioscience. Approximately 202 galls of a population from Uzbekistan and 761 galls from Turkey were received. Galls from Uzbekistan population were significantly larger than those from Turkey (Figure 10). Galls were placed in cold storage and were periodically removed for adult emergence starting on May 1st. Adults began to emerge from the galls approximately 6 days after removal from cold storage. Wasp emergence was poor and was delayed for those galls that were kept in cold storage for the longest period of time (to June 6th). Average emergence for the wasps from Uzbekistan was 9.0 days (range 6-11 days) and 12.5 days for the Turkish wasps (range 6-21 days). Adult emergence was low, only 20 adults emerged from the Uzbek galls (18 ♀: 2 ♂) and 132 adults from the Turkish galls (111 ♀: 21 ♂). Wasps were relatively heavily parasitized by several parasitoids wasps. Approximately 91% of the individual wasps that emerged from the Uzbek galls were parasitoids compared with 70% from the Turkish galls. Wasps were caged on suitable sized Russian knapweed plants between 5 days and until their deaths. Of the 77 plants/stems exposed to the gall wasp only two galls were induced (both of the Uzbek wasps). Russian knapweed plants were of good health and size (4.26 $\pm$ 1.53 mm diameter at base and 13.3 $\pm$ 16.9 cm in height). Upon dissection of the stems later in the summer, it appeared that wasp either probed or laid some eggs in about a third of the stems but for some reason eggs were either not laid or did not hatch. I suspect that a combination of environmental conditions due to the cages used or unfavorable temperature may have resulted in poor gall production.

Figure 10. Aulacidea acroptilonica galls (Turkish pop. on left, Uzbek pop. right)

Two populations of the gall wasp *Aulacidea acroptilonica* were received in April 2007 from U. Schaffner, CABI Europe. Approximately 20 galls of a population from Uzbekistan and 50 galls from Turkey were received. Galls were placed in cold storage and were removed for adult emergence starting on May 8th. Adults began to emerge from the galls approximately 9 days after removal from cold storage. Adult emergence was low, only 18 adults emerged from the Uzbek galls (17 ♀: 1 ♂) and 42 adults from the Turkish galls (28 ♀: 14 ♂). Wasps were relatively heavily parasitized by several parasitoids wasps. Of the 46 plants/stems exposed to the gall wasp only 13 galls were induced (8 Turkish and 5 Uzbek). Russian knapweed plants were of good health and size (4.1 ± 1.1 mm diameter at base and 7.9 ± 5.4 cm in height). Galls were harvested in late summer and placed in cold storage for rearing in the spring of 2008. No adults emerged from these galls in 2008.

Aulacidea acroptilonica female

In 2008 we received additional gall material from CABI. Approxmately 1200 galls were received. Adults were reared form a third of the galls and were used to inoculate plants in the quarantine greenhouses. Over 215 galls were reared. These will be used for field releases in 2009. The remainder of the gall material was kept in cold storage until a release permit was granted by APHIS. Unfortunately we kept the gall too long in storage and adult emergence was very poor. The few adults that emerged were used for additional greenhouse rearing. A shipment of *Aulacidea* was received in April 2009 and adults will be used for eventual field releases.

A shipment of the gall fly *Jaapiella ivannikovi* was received from CABI in late August 2007. Sixty four adults (40 ♀: 24 ♂) emerged from the gall material. These adults were placed on Russian knapweed plants. Two generations of flies were reared before the colony collapse in November. It is uncertain as to the reason for this collapse. I suspect that poor mating in the previous generation resulted in the production of mostly males.

Another shipment the gall midge arrived in October 2008 and a laboratory colony has been started. The number of gall reared has steadily increased from approximately 45 galls for the first generation of midges to close to 800 galls for the seventh generation.
These will be used for making field releases in Wyoming and Montana, once a release permit is granted.

Objective 3) Assist in the preparation of petition of potential biological control agents.

The environmental assessment for the field release of the two *Urophora* flies (*U. kashastanica* and *U xanthippe*) on Russian knapweed was not completed by APHIS. The US Fish and Wildlife did not concur that the flies would have no significant impact on threatened and endangered species during Section 7 consultation. Occasional probing of several Cirsium flower heads by Urophora females (no egg deposition) was observed in no-choice tests in the lab. In open field tests female Urophora were only observed on Russian knapweed. Based upon the no-choice tests the Fish & Wildlife Service concluded that this probing may adversely impact seed production of

T&E Cirsiums. Although this decision was made in spring of 2007 it was not until October that it was relayed to me.

Petitions for the release of two agents screened at CABI Bioscience were drafted Drs. Collier (U. of Wyoming), Schaffner (CABI) and myself. The petition for the release of the gall wasp, *Aulacidea acroptilonica* has been reviewed by TAG with a recommendation of field release. An EA was drafted by APHIS and a release permit was granted in July 2008. A second petition for the release of the tip gall midge, *Jaapiella ivannikovi* was sent to TAG early in 2007 and approved by TAG in 2008. APHIS drafted an EA for *Jaapiella*. We expect a release permit to be granted in June of 2009. This agent is sufficiently host specific to Russian knapweed and therefore we do not expect problems with its approval. See attachments for copies of the TAG petitions for *Aulacidea acroptilonica* and *Jaapiella ivannikovi*.

Objective 4. Continue to monitor the rate of spread of Russian knapweed at study sites and survey for native natural enemies.

Field sites showing promise as potential release sites were monitored as to plant density, infestation size, clonal growth, flower and seed development and associated vegetation. This monitoring proved base-line data as to the spread and ecology of Russian knapweed.

Clones - Twenty point infestations (referred to as clones) were selected in 2000 at three sites at the Charles M. Russell Wildlife Refuge, Phillips County, Montana. Clones were selected based upon their distinctness from other point infestations and their size. We selected various size clones to ascertain if plant density increased with size or age of the clone. Clones ranged in size from 30 m^2 to over 930 m^2 in area at the time of selection.

Clones were samples four times during the summer: June (late May to early June); July (late June to early July); August (late July to early August); and September (mid September to mid October). The geographic center of each clone was determined and used as the origin. In June and September, transects were established north, south, east and west from the center of the clone. Measurements were made along each transect to the outer plant (plants were 1 meter either side of the transect) to determine the radius of the infestation. In June, quarter $meter^2$ quadrates were established along the north and west transect at the center of the clone, at the edge of the clone (outer – recorded 5 plants in from the furthest plant), and mid-way between. Stem counts, stem height (cm), and number of flowers (buds, full flower & post flowers) were recorded for each quadrate. In September the biomass of each quadrate was taken. These samples were dried and separated by Russian knapweed, other forbs and grasses. These were weighed to the nearest 0.1 g.

Summary of Results

Stem Density - There were no significant differences between stem density of the center of the clone and the middle portion. Stem density, as expected, was less at the periphery of the clone. In June, plant density ranged from 10 plants per 0.25 m^2 at the center to 3 stems at the periphery (Fig. 11). In general stem density did not change significantly over the duration of the study or within years. In some years, such as 2000, stem density decreased over the season partially due

to stems being grazed or broken off by wildlife, primarily elk. This seemed more common within the clone where stem density was greater, and less at the periphery. In other years this feeding seemed to stimulate the production of additional shoots, especially early in the season. Late in the season, August through September, additional shoots emerged within the plots. This was especially evident after a moderate to heavy rain. This can also be illustrated in the expansion of the clones (see below).

Stem Height - No significant differences were observed for stem height of plants from the center to the edge of the clone, although plants at the periphery of infestations were slightly shorter. In June stem height ranged between 30 cm to 40 cm (Fig. 12). Height increased during July but decreased slightly in August, and September probably due to stems being snapped off due to the movement of animals through the plots.

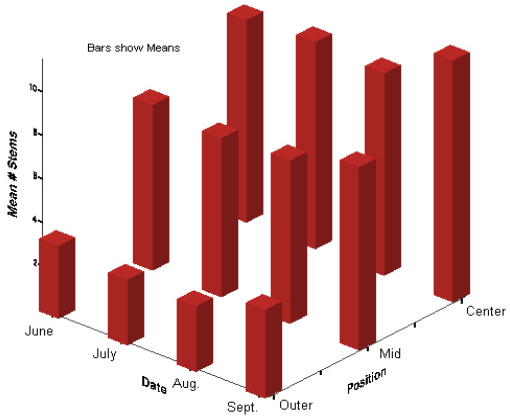


Figure 13. Flower/bud numbers of Russian knapweed within the clone

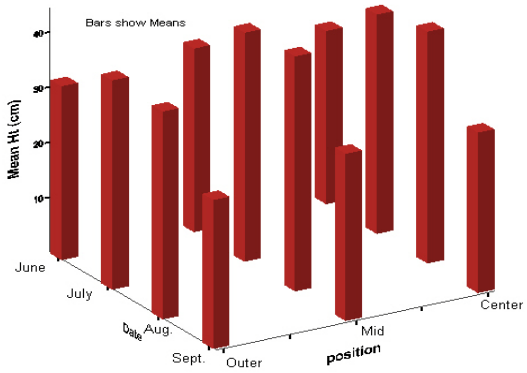


Figure 12. Russian knapweed stem height within the clone.

Flower Production – In May and early June Russian knapweed plants are still in the vegetative stage of development. Few flower buds were observed at this time (Fig. 13). By July, peak flowering has occurred, and depending upon adequate rainfall flowering will continue until fall, especially on newly emerged shoots. By the later part of July, flowering was approximately half finished. A number of buds never flowered or flower heads were lost during the summer due to stem breakage or by grazing by wildlife (especially early in the summer). The number of heads per plant ranged from approximately 25 per stem along the margins to 20 per stem at the center of the infestation. Plants at the periphery, although approximately the same height or a bit less, produce slightly greater numbers of flowers compared to the middle and center portion of the clone.

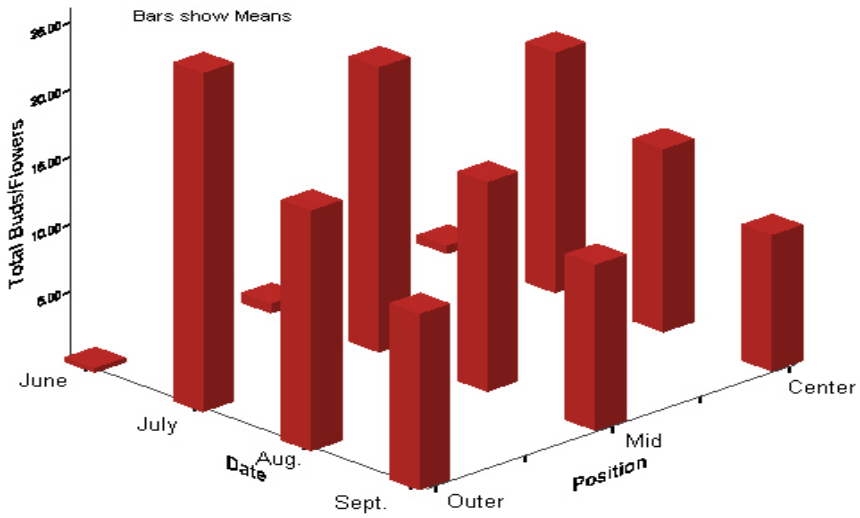


Figure 13. Russian knapweed flower production within the clone

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Spread of Clones – To determine the seasonal spread of clones, four transects (north, south, east and west) were measured in early June and again in September. The data for all transects and clones were combined for the various years and seasons (Fig. 14). Overall there was a general expansion of the clones between 2000 spring and 2003 fall. On average the clones grew approximately 1.5 m outwards along the radius from spring 2000 to fall 2003, or and average of 37 cm per year. For comparison, in Turkey Russian knapweed clones grew only 25 cm from spring to spring (Schaffner per. comm.). In 2002 and 2003 we observed a slight decrease in the size of the clone in the following spring, perhaps due to overwintering mortality of the outer plants or perhaps they were just late in emerging. Most of the expansion that we observed during the season was probably result of mid-summer precipitation stimulating the emergence of new stems. This was very evident in 2001, 2002 and to a lesser extent in 2003. When we sampled in September, flowering plants, plants with buds, and a few rosettes were evident.

Figure 14. The lateral spread of Russian knapweed clones in 2000 to 2003

Figure 15. Biomass of Russian knapweed, forbs and grasses within clones.

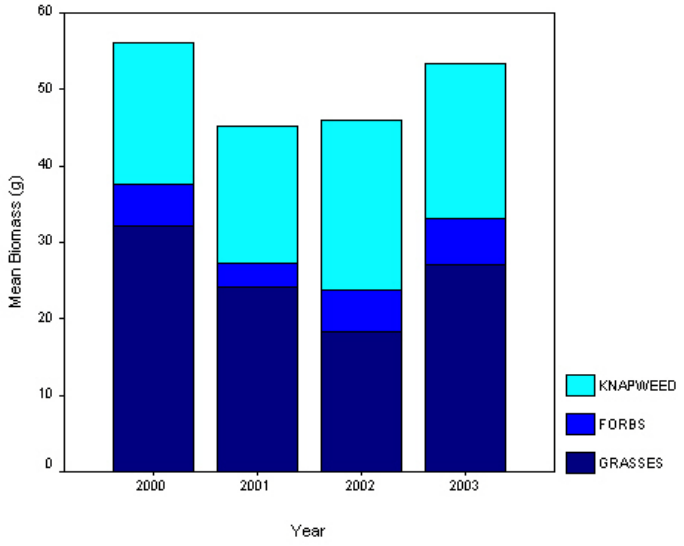

Figure 16. Changes in biomass of Russian knapweed, forbs and grasses from 2000 to 2003

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Comparison of Russian knapweed clones from Montana with those in Turkey - The clonal study in Montana was conducted in a similar manner to a study conducted in Turkey by U. Schaffner and associated at CABI Bioscience. These studies will allow for comparison of Russian knapweed infestations in its native range with those of where the weed has been introduce. In general Russian knapweed was more invasive in Montana as determined by several attributes (Table4). Plant density was nearly three times as great within the infestations in Montana compared to those found in Turkey. Plant densities were very similar at the periphery of infestations. As expected biomass of Russian knapweed was greater at Montana sites, but the biomass of other forbs and grasses wee much higher than at site found in Turkey. This may be due to the sites in Turkey being more disturbed (by cultivation, grazing, construction, etc.). Lateral spread of the infestations was greater in Montana, approximately 37 cm per year compared to 25 cm in Turkey. Also seed production was much greater in Montana. In Turkey very little seed was produced due to the presence of insects infesting flower heads. Most flower heads in Turkey had less than one seed per head.

Table 4. Comparison of Russian knapweed clones from Montana with those in Turkey.

Attributes	Montana Relative to Turkey
Stem Density	>>
Plant Height	>
Number of Flowers	>
Seed Production	>>
Lateral Spread	>

Potential release sites are still being monitored at the CMR Wildlife Refuge. The rate of clonal growth has been measured for the past six years on individual clones of Russian knapweed. In the past two years naturally occurring insects and plant pathogens have been surveyed at these sites. Very few insects have been found in association with Russian knapweed. The most damaging appears to be several grasshopper species, primarily *Melanaplus sanguinipes*. Two plant pathogens have been collected for comparison with overseas pathogens being considered for biological control. Although widespread and common, the rust *Puccinia acroptili*, has been reported for the first time in the literature from Montana (CMR Wildlife Refuge) and Wyoming (Fremont Co. near Bass Lake) (Bruckart et al. 2006). A second pathogen, a leaf spot fungus *Cercosporella acroptili* (Figure 12) was also located at the CMR National Wildlife Refuge. This fungus appears identical to an isolate from Turkey (FDWSRU 98-001) based on morphology and DNA ITS 1 and 2 sequences (GenBank #779164) (Eskandari et al. 2006). This fungus can cause large numbers of leaves to prematurely die, although this damage appears to have little impact on the plant.

Figure 12. *Cercosporella* leaf spot on Russian knapweed..

Rush Skeletonweed – *Chondrilla juncea*

Cooperators:

George Markin, USFS
J. Kashefi , USDA European Biological Control Lab – Greece
Joey Milan, BLM Boise, Idaho
P. Brusvan & M. Hanks, Nez Perce Biocontrol Center
Boise & Challis National Forests
Heather Prody – Montana State University

Introduction

Rush skeletonweed, *Chondrilla juncea* L. (Asteraceae), was first identified in the United States in 1807. *Chondrilla juncea* now infests over 6.2 million acres of rangeland in the Pacific Northwest (Cogan 2002). An estimated 41,000 ha per year, in the West, is being infested by *C. juncea* (Littlefield, et al 2000). Common infestations occur on rangeland, pasturelands, croplands, residential properties, transportation right-of-ways, and other areas that have frequent soil disturbances (McCafferry, Piper, Callihan, Coombs 2002).

In an attempt to control the spread of *C. juncea* a biological control program was implemented during the early 1970's to introduce host-specific natural enemies in the Pacific Northwest. Currently, *Cystiphora schmidti* (gall midge), *Eriophytes chindrillae* (gall mite), and *Puncia chondrillina* (rust fungus) have been established for the biological control of C. *juncea* in Idaho, Oregon, Washington, and California. These agents do not provide adequate control throughout the range of *C. juncea* in North America (Littlefield, et al 2002); therefore continuation of the study for appropriate bio-control agents is currently being conducted at Montana State University, Bozeman, MT. The purpose for the studies at the University are focused on developing a list of key soil and climate factors that can be used for identifying and selecting appropriate release sites for the establishment of *Bradyrrhoa gilveolella* in Idaho.

The root feeding moth *Bradyrrhoa gilveolella* (Treitschke) (Lepidoptera, Pyralidae) has been studied as a potential bio-control agent. *B. gilveolella* was approved for release in the United States in 2002. The host specificity of *B. gilveolella* was conducted under laboratory conditions in Montpellier, France, Thessaloniki, Greece (Carssche, Wapshere 1975) and Montana State University Bozeman, United States. The reasoning for the release of *B. gilveolella* in Idaho included: 1)comparable climate conditions between Idaho and the native range of the insect; 2) larva feed on the roots of *C. juncea* decreasing nutrient flow to the plant and killing smaller plants; 3) no other root feeding organism has been introduced as a bio-control agent on *C. juncea* in North America; 4) feeding damage from the larva combined with other bio-control agents will decrease plant vigor; 5) the moth has two generations a year will continue to damage the plant throughout the growing season; and 6) the moth appears to be host specific to *C.juncea (*Littlefield, et al 2002).

Objectives

The rush skeletonweed biological control project has two phases:

Project I. Distribution and monitoring of the root feeding moth *Bradyrrhoa gilveolella*.

The objectives of this project were: 1) Make collections of the moth from European sites, and screen the moth in quarantine**;** 2) Rear the moth for release; 3) Release the moth and monitor its success.; and 4) Habitat

Project II. Surveying, Rearing, and Screening of New Biocontrol Agents in Europe, Eurasia, and MSU

Project I. Distribution and monitoring of a root-boring biological control agent.

Bradyrrhoa gilveolella (Treitschke) (Lepidoptera, Pyralidae) a root-feeding moth, found in Europe, was introduced to North America as a potential biological control agent for Rush Skeleton weed. Initial release of *B. gilveolella* in southern Idaho was based on the following factors: 1) Comparable climate conditions between Idaho and the native range of the insect, 2) Larvae feed on the roots of *C. juncea* decreasing nutrient flow to the plant and killing smaller plants, 3) No other root feeding organisms have been introduced as biocontrol agents on *C. juncea* in North America, 4) Feeding damage from the larvae combined with other bio-control agents will decrease plant vigor, 5) Because the moth has a potential of two generations a year, the larvae can continue to damage the plant throughout the growing season, and 6) The moth appears to be host specific to *C. juncea* (Littlefield et al. 2002). *Bradyrrhoa gilveolella* was approved for release in the United States by the USDA-APHIS in 2002. Initial release of the moth was conducted using infected plants in November 2002. The success of the moth as a biological control agent relies on the ability to effectively establish and redistribute the moth.

In autumn 2002 the root-feeding moth *Bradyrrhoa gilveolella* (Lepidoptera, Pyralidae) was approved by the USDA-APHIS for release in the United States. Initial releases have been made in southern Idaho in November 2002 and during the summers of 2003, 2004 and 2006. The moth has been able to survive the winters of Idaho and emerge the following year. Although recovery of empty pupal cases and feeding tubes has been made, the establishment of the moth at these releases has not been confirmed. In 2006 only a few moths were able to be reared from material collected from Europe. These were used for studies of the moth's biology, one field release, and for the establishment of an insectary at the Nez Perce Biological Control Center. Key to the success of this biocontrol agent is our ability to effectively rear and establish it.

Objective 1) Make collections of the moth from European sites, and screen the moth in quarantine.

Collections of *Bradyrrhoa gilveolella* were made by G. Markin, USFS and J. Kashefi, USDA-ARS from two locations in Greece: Lake Prespa in northwestern Greece and near Asprovalta along the northeastern coast. Past collections of the root moth have been made from Lake Prespa, whereas the site near Asprovalta is a newly discovered site. Both sites have a sandy soil type, although the coastal site is a much hotter site in comparison.

Shipments of infested root material were received at the MSU quarantine laboratory on 10- May 2006 and a smaller second shipment from Lake Prespa was received on 6-July 2006.

Larval development at the Asprovalta site was better synchronized in comparison to the Lake Prespa site. Larvae collected at the coastal site were in the late instar of development and just starting to pupate, whereas the larvae from Lake Prespa were more variable in development, from mid-instar larvae to pupae. We observed a high level of disease associated with the

Bradyrrhoa larvae

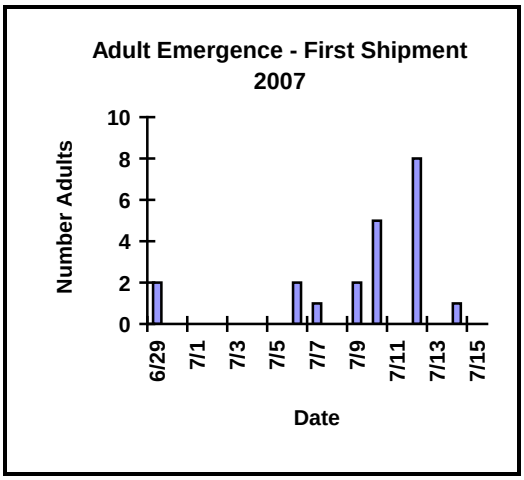
Asprovalta *Bradyrrhoa*, primarily secondary bacteria and fungi (*Bauvaria bassiana*). Of 86 larvae received only 7 (8%) emerged as adults, 2 were parasitized and the remaining died of disease or other causes. We speculated that the larvae were stressed since this collection was made approximately a week before being received in quarantine. From the Lake Prespa collection we recovered 166 larvae, of which 22% were dead upon arrival. Live larvae were placed on a rearing diet or transferred to live plants. Nine adults eventually emerged (7% emergence) but over a period of eight weeks. The late June collection of *Bradyrrhoa* contained only a few larvae, from which we reared 7 adults.

In 2007_two collections of rush skeletonweed roots infested with *Bradyrrhoa* were made near Lake Prespa in northwestern Greece and were screened through the MSU quarantine lab. The first shipment was received June 18th and the second shipment on July 20th. Twenty-one adult *Bradyrrhoa* emerged from this first shipment (180 roots) and 27 adult *Bradyrrhoa* from the second (200 roots). Adults emerged over a period of approximately 15 days. Adults were placed in oviposition tubes and eggs were collected after they were deposited. Only a few eggs were obtained from the first shipment and these were used for laboratory rearing whereas greater number of eggs were harvested from the second shipment and were used for both rearing and field release.

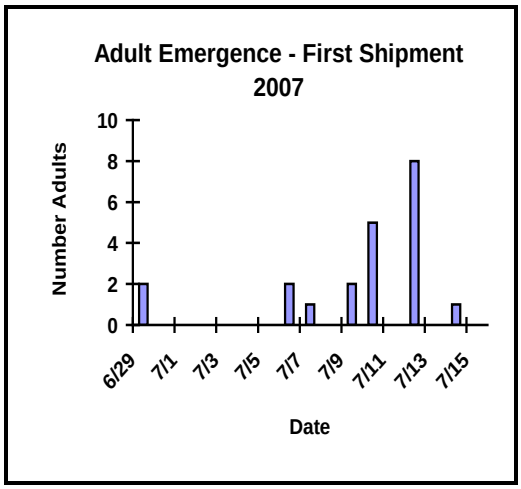
Bradyrrhoa shipment from Greece

Adult ovipoistion tube

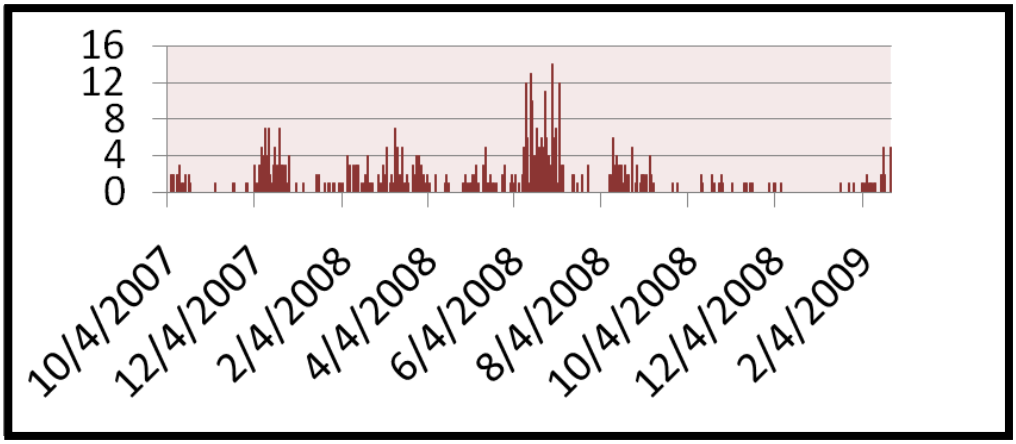
Currently we are maintaining a colony of *Bradyrrhoa* within the quarantine laboratory at MSU. We have had two periods of adult emergence, a small peak in October and a second peak in early December. Adult longevity and egg productions appear to be less for this winter population compared to those that emerged in the summer. We suspect that temperature and lighting may play a role. We plan to continue our rearing throughout the winter.



Bradyrrhoa adult emergence first shipment



Bradyrrhoa adult emergence second shipment



Bradyrrhoa rearing – MSU 2007 to 2009

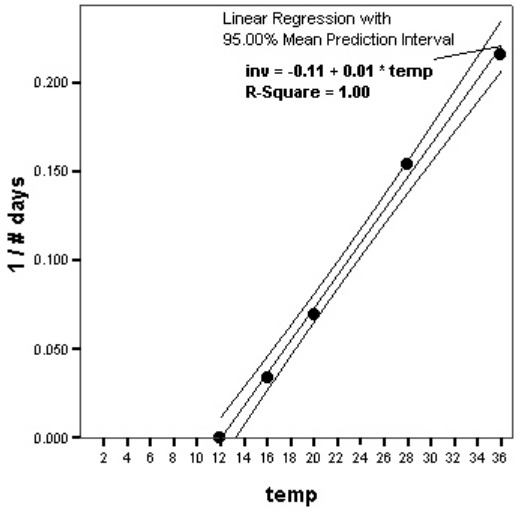
Thermal Development Studies - The objective of the study is to assess the effects of constant temperatures for three developmental stages of *Bradyrrhoa gilveolella:* egg to I instar larva,

larvae to pupae, and pupae to adult. Specifically aimed to determine the minimum and maximum developmental thresholds for determining degree-day requirements of each developmental stage. Each stage was assessed with two repetitions at temperatures of 4ºC (only one repetition conducted), 12ºC, 20ºC, 28ºC, and 36ºC using one hundred eggs per temperature. Observations were taken daily for hatching eggs. Once the larvae hatched they were moved to plants within the same growth chambers. Observations were conducted based on the size of the developing larvae; >1 cm would not be checked for 30 more days, < 1 cm check weekly, 1 cm < 1.5 cm check daily for pupal development. Pupae were removed and placed in individual containers remaining in the chambers. Pupae were assessed daily for emergence of adults. The eggs to I instar larvae development section produced a nearly complete data set resulting an approximation of the minimum developmental temperature for the eggs. The approximate minimum developmental threshold temperature for eggs is 18ºC. More temperatures need to be assessed in 2005 to obtain the maximum developmental temperature. The first instar to pupae, and pupae to adult section of the study did not produce complete data sets. The procedures have been reevaluated and changes have been made to successfully obtain complete data sets.

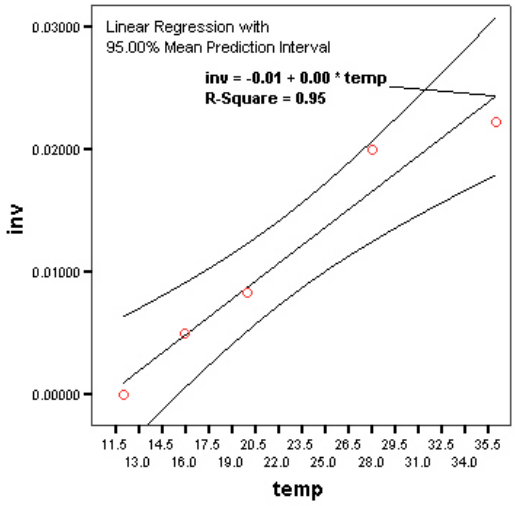
Table 5 Summary of Bradyrrhoa shipments and rearing.

Year	# Shipments	# Adults	# Eggs	Fate
2002	**3**	**12**	**120**	**1st Field Release (ID)**
2003	**2**	**57**	**3400**	**1200 Field Release (ID)** **1340 USFS** **550 Nez Perce** **310 Rearing (MSU)**
2004 **(Rearing)**	**4**	**36** **47**	**1721** **2989**	**1480 Field Release (ID)** **410 USFS** **260 Nez Perce BC** **280 Canada** **2280 Rearing/Research (MSU)**
2005 **(Rearing)**	**2**	**15** **18**	**700**	**200 Nez Perce BC** **500 Rearing/Research (MSU)**
2006	**2**	**25**	**500**	**350 USFS** **150 Rearing (MSU)**

2007 (Rearing)	**2**	**48 182**	**1217 2156**	**500 Field Release (ID) 2104 Nez Perce BC 150 USFS 620+ Rearing (MSU)**
2008 (Rearing)	**1**	**0 371**	**0 10,788**	**- 20 A Field Release (ID) 2020 20A Nez Perce BC 3,945 USFS 1,000 BLM 3,823Rearing (MSU)**



Bradyrrhoa Egg development



Bradyrrhoa larval development

Objective 3) Release the moth and monitor its success.

Releases & Monitoring - Two releases of *Bradyrrhoa* were made in 2007. One release was made on BLM property in Boise County near Lucky Peak Reservoir. The release was made in early August by inoculating plants with larvae. Several larvae were recovered during a visit in mid-September. A second release was also made in late October along the Salmon River north of Riggins. A previous release(s) of *Bradyrrhoa* was made at this site by G. Markin (USFS). As with the Lucky Peak release, plants were inoculated with newly hatched larvae.

Bradyrrhoa lab colony - MSU quarantine

Bradyrrhoa release site Little Gallagher Creek, Idaho

Previous *Bradyrrhoa* releases made from 2002 to 2006 were visited and plants sampled for presence of larvae or feeding tubes. These sites were in the vicinity of Cambridge (2003 & 2004), Idaho City (2003), Banks (2003), Danskin Creek (2006) and Little Gallagher Creek (2002, 2003, 2004 & 2006). Although we have determined in the past that *Bradyrrhoa* completed their development at several sites (i.e. Cambridge, Idaho City and Little Gallagher Creek) after plants were inoculated with larvae, no recovery of larvae has been made until 2007. One mid-size larva was recovered at Little Gallagher Creek (Fig. 8) in September. This indicates that a small population of the moth may exist at this site. In previous years we have recovered plants with empty feeding tubes or dead pupae from this site but until now we have not observed live larvae. Although a cage release of adult moths was made at this site in 2006, no larvae or feeding tubes were observed in 2007 within the release cage. At the other release sites the moth has not been recovered. At the Cambridge site some webbing and feeding on the roots has been observed but we have attributed this, most likely, to the feeding of another moth species.

Objective 2: Habitat associations of B. gilveolella infestations at sites in Bulgaria and Greece with those in Idaho

Habitat Analysis – Past attempts to establish *Bradyrrhoa* in both Australia and Argentina have failed. We hypothesize that the moth may be restricted to certain habitats. To enhance its chances for establishment, key environmental factors associated with rush skeletonweed patches infested with *Bradyrrhoa gilveolella* in Europe were compared with those of potential release sites located in south central Idaho.

Twenty three rush skeletonweed sites in northern Greece, southern Bulgaria, and south central Idaho, were characterized as to site/ environmental conditions, plant productivity, soil conditions and chemistry, and current or past land utilization.

Quantitative variables were compared using t-tests, and qualitative variables by contingency table analysis. Principal component analysis (PCA) (using PC-ORD 4.0) was used to examine the habitat associations and the presence of *Bradyrrhoa.* Multiple response permutation procedures (MRPP) were use to compare differences between sites with and without the moth.

Results were somewhat inconclusive. Both biotic and site conditions varied among sites. For example Rush skeletonweed density was greater in Idaho and European sites with *Bradyrrhoa*. The presence of other forbs was greater at European sites, but the grass cover was greater in Idaho but less at sites with *Bradyrrhoa* in Europe. The level of other biocontrol agents such as the rust, mite or midge was greater in Idaho and nearly nonexistent at European sites; but feeding by other insects was greater in Europe, with the exception of that by grasshoppers which were common in Idaho. Mean temperatures are cooler in Idaho in part due to higher elevations and latitudes but summer temperatures (not shown) are compatible to Lake Prespa, Greece (the main collection site for the moth). Soil chemistry differs, in part, between European sites and Idaho. In Idaho, calcium and nitrates tended to be lower but higher in potassium. Several Bulgarian sites had higher levels of heavy metals due to their location near smelters. Although the soil at several *Bradyrrhoa* sites was very sandy, the moth was also found at sites with loamier type soils. The moth was not present at sites with heavier soils.

Sites with and without the moth could not be differentiated with MRPP tests. In PCA analysis, sites in Europe where *Bradyrrhoa* was present were loosely grouped. The site at Lake Prespa, Greece is somewhat unique being located on a sandy isthmus between two lakes. Other sites with *Bradyrrhoa* were grouped more in association with other European sites and some Idaho sites (in which *Bradyrrhoa* has been released). PCA analysis is somewhat limited due to the low number of sites with the moth. In addition other European sites where the moth is absent may be suitable sites which moths have yet to locate and colonize. Further sampling will be conducted of sites infested with *Bradyrrhoa* and other potential release sites in North America (e.g. northern Idaho, eastern Washington) to increase the robustness of the analysis and possible predictions.

Project II. Surveying, Rearing, and Screening of New Biocontrol Agents in Europe, Eurasia, and MSU

An import permit for the importation of *Schinia cognate* into the U.S. was submitted to APHIS but was not issued in time for the insect to be imported this year. We concentrated our activities in obtaining various plants for host specificity testing. A host test plant list had been developed Birdsall, Markin and Littlefield for testing *Bradyrrhoa*. This list has been modified slightly for testing additional rush skeletonweed agents. Currently there is 55 plant species and varieties that we would like to test. We now have 44 of these species or varieties being grown in our containment greenhouses or as seed. Of the remaining species we have been able to located potential collection sites for nearly all. One problem that

Schinia cognata adult. Photo by Tamás Baranyi

we will have testing some of these plants is that their flowering period is not well synchronized with the activity of the moth. Flowers of field collected plants have been frozen and will be used to determine if the moth larvae will utilize these flowers as food. To speed up the testing, overseas testing of introduced weed or crop species will be conducted by overseas cooperator, whereas the majority of native North American plants will be tested at the MSU containment facility.

In this objective we planned to import promising insects into the MSU quarantine for rearing and additional study of their biology and host specificity. We planned to initially work with the bud feeding moth, *Schinia cognata* from Bulgaria and Greece, as well as obtain test plants for the testing of other agents in Europe or Russia. A shipment of pupal *Schinia cognata* (a flower infesting noctuid) was received from Greece for host specificity testing. Only two of approximately 40 pupae emerged and both adults promptly died. These adults appeared to be deformed. The death of these adults and pupae were perhaps due to rearing or shipping conditions. A second shipment could not be arranged due to low numbers of the moth present at field sites in Greece. Due to very dry conditions at many locations in Greece, rush skeletonweed plants produced very few flowers late in the summer thus limiting the population of *Schinia* at collection sites.

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