

Collection and Redistribution Of Biological Control Agents Of Diffuse and Spotted Knapweed

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Diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*C. maculosa*) are introduced, noxious weed species that originated in the Mediterranean region and now infest over 3 million acres in Idaho, Montana, Washington and Oregon. Both species reduce grass forage production, thereby lowering rangeland carrying capacity. They have high fiber content, little nutritive value and low palatability to livestock and wildlife. Furthermore, they form dense stands (See cover photos) that often crowd out other desirable vegetation in an area. Some stands of knapweed have reduced range and pasture carrying capacity to virtually zero.

The knapweeds can be effectively controlled with certain herbicides¹ and by cultivation. However, much of

the land invaded by the weeds is of low economic value, inaccessible or environmentally sensitive, which makes chemical or cultural control impractical. To maintain these weed species below economically damaging levels requires an aggressive vegetation management program that incorporates biological control. With this in mind, a biological control program using host-specific natural enemies, primarily insects, was initiated in the Pacific Northwest and Canada during the 1970's. Since then, a complex of insect species has been introduced and established on spotted and diffuse knapweed in Idaho (Table 1).

¹Chemical control recommendations are included in the current year's PNW Weed Control Handbook.

Table 1. Biological control agents of spotted and diffuse knapweed: Status Report for Idaho.

Natural enemy	Weed(s) ¹	Release ² date(s)	Status	Primary plant stage Attacked	Comments
<i>Urophora affinis</i>	S,D	1975	E,R	Seed head	Seed head gall fly, first species to be released; wide-spread distribution.
<i>U. quadrifasciata</i>	S,D	1981	E,R	Seed head	Seed head gall fly, never officially released in Idaho; migrated from Canada
<i>Metzneria paucipunctella</i>	S	1981	E,R	Seed head	Seed head moth; needs further redistribution.
<i>Sphenoptera jugoslavica</i>	D	1981	E,R	Root	Root-galling beetle; needs further distribution.
<i>Pterolonche inspersa</i>	D,S	1986, 1989-?	U	Root	Root-boring moth; further releases are anticipated.
<i>Agapeta zoegana</i>	S	1989-?	-	Root	Root-boring moth; initial release anticipated for 1989
<i>Pelochrista medullana</i>	D	1989-?	-	Root	Root-boring moth; initial release anticipated for 1989
<i>Cyphocleonus achates</i>	S,D	1989-?	-	Root	Root feeding weevil; release anticipated for 1989
<i>Bangasternus provincialis</i>	D	?	-	Seed head	Seed head weevil; currently being tested for host-specificity.
<i>Aceria centaureae</i>	D	?	-	Rosette	Gall-forming mite; currently being tested for host-specificity.

¹ S = spotted knapweed; D = diffuse knapweed.

² Date first released or found in Idaho; ? indicates actual release date is subject to federal/state clearance and availability.

³ E = established; R = ready for redistribution; U = unknown; - = not present.

Cover Photos: The top photo shows an extensive infestation of spotted knapweed in Kootenai County. The other photo shows a diffuse knapweed problem in Camas County. Notice the absence of grass forage in both pictures.

The first insect released against spotted and diffuse knapweed was the gall fly, *Urophora affinis*. This was later followed by the natural migration of another related species, *U. quadrifasciata*, into Idaho from Ned's Creek, British Columbia, Canada. A root-infesting beetle, *Sphenoptera jugoslavica*, and a seed-feeding moth, *Metzneria paucipunctella*, have also been established on diffuse and spotted knapweed, respectively.

Additional biological control agents, including three root-infesting moths and a root-infesting weevil, are scheduled for release in the near future (Table 1). Other potential natural enemies, including a rosette-infesting mite and a seed head weevil, are currently being investigated in Europe or the U.S., in quarantine. The host plant specificity of the agents is being checked before they are released in the U.S. to ensure their safe introduction. While anticipated release dates for new natural enemies are listed in Table 1, the actual release timing is contingent upon successful host specificity tests and the availability of the natural enemies.

Knapweed Control Expectations

Survival of a large or small number of transferred natural enemies does not mean that immediate weed control will occur. The newly established natural enemies will serve as colonizers, and several years will be required for their descendants to reach population levels that can effectively stress the weeds. The knapweed biological control programs are still in their infancy. Only four insects are established, and three of them are seed consumers. These insects by themselves will not eradicate or even control knapweeds. Merely reducing seed production will not kill the plants although the feeding activity of the natural enemies may weaken the plants. Other natural enemies, working in concert with the species already established, are needed to exert more stress on the knapweed plants. Such stress ultimately may cause plant death or reduce the competitive ability of the weeds to a point where desirable plant species can out-compete them.

An important consideration relative to new invasions of knapweeds into previously uninfested areas is that eradication by herbicides, hand grubbing, cultivation or other techniques is preferable. While releasing natural enemies to help reduced seed spread, etc., might be worthwhile, quick eradication of a small infestation would be better in the long run. The natural enemies established to date will not, by themselves, totally control or eradicate the weeds or prevent their spread.

The goal of biological control is not to eradicate the weeds but to reduce their competitive ability so that

knapweed-infested sites can be recolonized by desirable plant species. Biological control should, therefore, be part of a larger vegetation management effort that includes the use of herbicides, fertilizers, grass reseeding and grazing management techniques. Research is in progress to identify how these control measures can be integrated.

Life Cycle of Spotted and Diffuse Knapweed

Spotted knapweed is a short-lived perennial. Diffuse knapweed is a winter annual, occasionally acting as a biennial. Seeds of both species germinate during summer or fall, forming a seedling with a rosette of leaves. In their early stages of growth, both spotted and diffuse knapweed have deeply cut grey-green leaves with short, thin, grayish hairs and ridged stems. In the following spring, flower stalks develop and seeds are produced in August. Since spotted knapweed is a perennial, new rosettes are also formed during the summer and spring from established root systems.

Spotted knapweed grows 12 to 60 inches tall, is more erect and has more limited branching than the bushy diffuse knapweed which grows 4 to 40 inches in height. Diffuse knapweed will often break off at maturity and tumble with the wind. Dead spotted knapweed stems generally remain erect during the winter unless crushed by heavy snow-pack.

Both weed species are capable of producing flower buds until frost, but normally they start to senesce when soil water is depleted. Peak bud production for both spotted and diffuse knapweed generally occurs from late June to mid-July, depending on seasonal temperature and rainfall patterns.

The flower heads of spotted knapweed are larger than those of diffuse knapweed. The bracts of spotted knapweed seed heads have dark, comb-like tips that give the head a spotted appearance. The seed heads open soon after the seed is mature. Some of the seeds have a short pappus (tufts of hairs) on top that enables them to be carried by the wind, much like a parachute, thereby allowing them to spread short distances from the infested areas. The flowers of spotted knapweed are usually pink to purple, but occasionally white.

The uniformly colored bracts of diffuse knapweed seed heads are comblike and armed with small spines that give the plant its disagreeable, spiny character. Some bracts have dark spots much like those of spotted knapweed, but these plants can be distinguished from spotted knapweed by the presence of a long spine on the tip of each bract. Diffuse knapweed flowers are primarily white and occasionally pink to lavender. Diffuse knapweed seeds do not shatter as readily as those of spotted knapweed. This enables the seeds to be spread with the tumbling of the diffuse knapweed plant in late summer and fall.

Life Cycle of *U. affinis* and *U. quadrifasciata*

Larvae of the flies, *U. affinis* and *U. quadrifasciata*, cause the formation of galls in the seed heads of diffuse and spotted knapweed. The adult flies are small, generally less than $\frac{3}{16}$ of an inch in length. Adults of both species have dark bands on their wings, but the bands of *U. quadrifasciata* are much bolder and more complete than those of *U. affinis* (Fig. 1). Female flies can easily be distinguished from males by their long ovipositors, which they use for egg-laying.

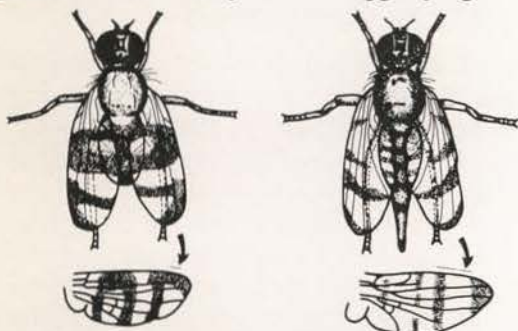


Fig. 1. Adult male *Urophora quadrifasciata* and female *U. affinis*.

The life histories of the two flies are similar. They have one or two generations per year and both overwinter as mature larvae within the seed heads. Pupation of the overwintering generation begins around the first of May and continues until mid-July. Adults begin to emerge the last week of May or the first week of June. Peak adult emergence of the overwintering generation usually occurs the first or second week of July, coinciding with peak bud production of the knapweed (especially spotted knapweed) plants. Eggs of both species are laid within the small, developing flower buds. First-generation larvae may be found from mid-June to early August. Most larvae from the first generation of *U. affinis* will overwinter, but a small portion of them will continue to develop, pupate and emerge as adults to begin a second generation. Second-generation larvae may first be found in early September. They too will overwinter as mature larvae and continue their development again the following spring.

The seasonal development of *U. quadrifasciata* is similar except that most first generation larvae will complete their development and produce a second generation. Only a small portion of the first generation will remain as mature larvae that will eventually overwinter and complete their development the following year. In summary, *U. affinis* has primarily one generation per year while *U. quadrifasciata* has primarily two generations per year.

The galls formed by *U. affinis* are hard and distinct (Fig. 2). When a larva hatches within the flower bud, it moves down to the receptacle (base of the flower) where it begins to feed. This causes the production of abnormal plant tissue growth that eventually covers the larva. The highly nutritive internal gall tissues are energy-sinks that essentially steal nutrients from other



Fig. 2. Hard gall of *U. affinis* in spotted knapweed seed head. Gall has been opened to expose the larva (maggot) within.

plant parts, stressing the plants in addition to directly destroying seeds within the infested seed heads.

The galls of *U. quadrifasciata* are not as apparent as those of *U. affinis*. They are often difficult to see and the larvae appear "naked" (Fig. 3). When a larva hatches, it enters a floret and moves down into the developing ovary. The larva then causes increased cell growth of the ovary that it feeds upon. This less massive type of gall apparently does not lead to as significant an energy drain on the rest of the plant as the gall of *U. affinis*. The impact of this species is limited to the destruction of the ovary it feeds within and the abortion of some of the surrounding ovaries.



Fig. 3. Exposed larva of *U. quadrifasciata*. This species does not have a hard gall.

U. quadrifasciata disperses better than *U. affinis*, probably because it has primarily two generations per year. When second-generation adults emerge in August or September, they must locate knapweed buds for egg laying. Since many spotted knapweed infestations have completed bud production by August, *U. quadrifasciata*



Fig. 4. Adult of the seedhead moth, *Metzneria paucipunctella*, on spotted knapweed.

must disperse to find available buds, usually on wetter sites such as roadsides and stream beds, or at higher elevations. Since diffuse knapweed can produce buds over a protracted period of time, compared to a typical spotted knapweed infestation, second-generation flies may continue to infest available buds within a site, or disperse to other sites. Thus, *U. quadrifasciata* serves an important role in the infestation of knapweed buds that would have escaped attack by first-generation *U. affinis*.

Both fly species may be found in the same seed heads. They are generally compatible with each other, and in combination they can lead to lower levels of seed production. Once *U. affinis* becomes established at a spotted or diffuse knapweed site, however, it usually will become the dominant fly species. Spotted knapweed seed heads typically contain 5 *Urophora* spp. galls each, but may contain up to 25 galls. The smaller diffuse knapweed seed heads usually do not contain more than 5 galls each.



Fig. 5. Mature larva of *M. paucipunctella* within spotted knapweed seed head. The larva has also fed on some of the *U. affinis* galls located behind it.

Life Cycle of *M. paucipunctella*

Larvae of this moth feed within the seed head of spotted knapweed. Adult moths (Fig. 4) emerge in June and early July. Females lay their yellowish eggs on the bracts at the base of the mature flower buds. Eggs hatch in about 10 days and the larvae enter the flower buds as they open. More than one larva may enter an individual bud, but only one survives because larvae are cannibalistic. Young larvae feed on the developing florets; older larvae (Fig. 5) feed on the developing seeds. Mature larvae overwinter within the seed head, and pupation occurs in May and early June.

Each moth larva destroys several knapweed seeds and may also feed upon some of the *U. affinis* and *U. quadrifasciata* larvae within the same seed head (note damage to the *U. affinis* gall in Fig. 5). Fortunately, the number of fly larvae destroyed is small and those that are consumed already have had their impact on seed production, so *M. paucipunctella* is considered to be compatible with the gall flies.



Fig. 6. Adult of the root-boring beetle, *Sphenoptera jugoslavica*, on diffuse knapweed.

Life Cycle of *S. jugoslavica*

Larvae of this beetle infest the roots of diffuse knapweed. In southern Idaho, adult beetles (Fig. 6) emerge from early July through early August. The brown, adult beetles feed on the knapweed foliage, causing some minor damage. After mating, they lay their eggs on the leaf petioles near the base of the plant. Eggs hatch in 2 to 4 weeks, depending on temperatures during that period. The larvae chew into the leaf petioles, make their way down the petiole into the root crowns and eventually cause the production of swollen galls in the upper portion of the tap-roots (Fig. 7). Usually only one living larva will be found in a plant. The larva overwinters inside the gall and continues to feed and develop in the spring. Pupation occurs in June and up to mid-July. There is no peak time of adult emergence, however, male beetles emerge a week or so before females.



Fig. 7. Mature larva of *S. jugoslavica* in taproot of diffuse knapweed.

The feeding of the larvae within the roots greatly reduces rosette growth and delays bolting for one or more seasons. If plants do bolt, they are not as vigorous as uninfested plants. The overall impact of the beetle infestation is to weaken the rosette. This may predispose it to other mortality factors, reduce its competitive ability and eventually lead to a reduction in weed density and seed production.

Collection and Redistribution of Natural Enemies

Seed Head-Infesting Species — Collection and redistribution schedules for the flies and the moth are summarized in Table 2. These insects can be relocated by three different methods, depending on the timing of the redistribution and considerations regarding the spread of knapweed seed. The easiest way to redistribute these natural enemies is to collect knapweed stems that have natural enemy-infested seed heads, tie them in bundles and place them at the chosen release sites during spring and early summer. The natural enemies will develop within the old seed heads and emerge when the temperatures allow them to complete development. Be sure to inspect natural enemy collection sites before collecting seed heads to confirm that fly and moth larvae are present.

Table 2. Collection and redistribution schedule for *Urophora* spp., *Metzneria* and *Sphenoptera*.

Agent	Appropriate transfer stage	Collect	Transfer
<i>Urophora affinis</i> or <i>U. quadrifasciata</i>	larvae/pupae	Sept. to late May	late May through early June
	adults	late June to late July	late June to late July
<i>Sphenoptera jugoslavica</i>	adults	early to late July	early to late July
<i>Metzneria paucipunctella</i>	larvae/pupae	Sept. to late May	late May through early July

Refer to the text for specific information pertaining to collection and redistribution methods.

The major problem with this approach is that knapweed seed can also be dispersed with the infested seed heads. This is not likely to be a problem if the release sites are already heavily infested by the weeds, but it may spread seed if bundles are handled carelessly during transport. An alternative to this approach is to collect seed heads during the fall or early spring and place them in a release cage (Fig. 8). The adult flies and moths will emerge and escape from the cage in the spring when warmer temperatures allow them to complete their development. The cage helps prevent the spread of seeds that may have remained in the seed heads. Seed heads collected in the fall or winter can be stored in a release cage that is placed in a protected area, such as a barn, to provide ambient temperatures. This is necessary to prepare the insects for normal development in the spring. The cages can be moved to the weed-infested sites any time in early spring when small buds are available.

Another redistribution method is to collect adult flies with an insect net in early summer. The flies can then be placed in a container and transported to the release site. The flies prefer buds $\frac{1}{4}$ inch or less in diameter,



Fig. 8. Release cage for containing knapweed seed heads and associated seed during bioagent release.

so the best time to do this is in early summer when small buds are available. Adult flies can be released in late summer, but such releases will be effective only if small buds are present. Because adult moths are difficult to collect, this method of redistribution is not recommended. If adult moths are collected, however, they can be released a little later than the flies since the adult moths will lay their eggs on larger buds (Table 2).

Release of large numbers of flies is not necessary to ensure establishment and eventual colonization. Usually as few as 25 to 50 adults are enough. Large numbers of flies are readily available, however, so we encourage releasing at least 500 flies to ensure a faster buildup of their populations.

We do not know what numbers of adult *M. paucipunctella* are necessary for effective establishment, but we have been successful with releases of fewer than 100 moths at a site. Again, if moths are readily available, release several hundred per site. This will facilitate a quicker population buildup. As is true with the beetles, the moth's sex ratio can vary depending on when they are collected. Early collections may be biased toward males.

Root-infesting Species — *S. jugoslavica*, established on diffuse knapweed, is the only root-infesting species currently established on the knapweeds. Other species are scheduled for future release (Table 1). While this discussion will deal specifically with *S. jugoslavica*, similar techniques may be appropriate when the other species are ready to be redistributed. Refer to Table 2 for a summary of the appropriate collection and redistribution schedule.

Root-infesting insects can be redistributed in two ways. The easiest method is to collect adults. The alternative is to collect roots infested with larvae or pupae. We recommend that only adult insects be redistributed since moving infested roots is difficult and probably less successful. Adult beetles are best collected using a sweep net during the early evening hours.

Large numbers of beetles are not necessary for successful establishment at a new site. Releases of 25 to

50 adult beetles are often adequate. Remember, however, that early-season beetle collections may contain more males than females due to their differential emergence periods. Collect enough beetles to compensate for this problem.

Whenever transporting any insects or insect-infested plant materials, avoid exposing them to extreme temperatures. Insects collected when the weather is warm should be packed in an ice chest if possible. If you use ice to keep the insects cool, protect them from water that might melt during transport. Do not put wet plant material into plastic bags without ventilation. You may drown the insects or cause excessive decay of the plant material. Keep insects or infested plant material out of direct sunlight, particularly the dashboards and seats of vehicles.

Selection of Release Sites

Each of these natural enemies can survive in a variety of areas that support spotted and diffuse knapweed. When trying to develop local "field nurseries" from which natural enemies can later be collected for further redistribution, the best sites are large, open stands of weeds that are free from disturbances such as livestock and pesticide use. This type of site also is desirable but not so crucial for routine redistributions where the insects are introduced into an area without concern for future collections and redistributions.

Sources of Natural Enemies for Redistribution

Idaho has many sources of knapweed natural enemies. *U. affinis* and *U. quadrifasciata* are well distributed throughout the state, particularly on spotted knapweed. They can be found in the northern panhandle, in western Idaho as far south as Riggins and in eastern Idaho as far south as Salmon. Equally good sources of flies are diffuse knapweed infestations in southcentral Idaho, especially Camas (Fairfield area) and Blaine (Ketchum-Hailey area) counties.

M. paucipunctella populations are increasing in northern Idaho (Kootenai, Bonner and Boundary counties), in central Idaho (Idaho County) and in eastern Idaho (Lemhi County). It should be available in numbers sufficient for statewide redistribution by 1989. Care should be taken not to over-exploit these populations until then to allow their populations to stabilize.

S. jugoslavica is currently established near Fairfield in Camas County, near Hailey in Blaine County, near Shoshone in Lincoln County and in Gooding County. It is available in limited numbers for redistribution.

These insects are also available from out-of-state sources, but federal and/or state permits are required

to move them across state or country borders (i.e., Montana or Canada). Contact the Idaho State Department of Agriculture if you anticipate collecting or purchasing bioagents from another state or Canada. If you transport plant material with the natural enemies into Idaho from outside the state or the U.S., destroy the plant material after the insects have been released. Do not release the noxious weeds with the insects.

We recommend that in-state sources of bioagents be used as much as possible. Since these insects are already adapted to Idaho conditions, they are more likely to establish at the new sites. Use of in-state insects lessens the likelihood of introducing parasites of the natural enemies or new biotypes of the weeds (from plant material that might accompany the bioagents). Finally, permits are not required for the movement of in-state material. This cuts down on paperwork.

Monitoring Natural Enemy Populations

Before any large-scale efforts in relocating natural enemies to new knapweed-infested areas, survey the target knapweed populations to see if the agents are already present. The general, statewide distributions of the insects do not represent a complete picture of the insects' current distribution. All the insects released to date are capable of substantial dispersal on their own, though they do vary in dispersal tendencies. *U. quadrifasciata*, for example, has been found everywhere knapweeds are found in Idaho, and most of these populations are a result of natural dispersal.

Seed-Infesting Species — Timing of the survey is important. Adult gall flies that emerge from the overwintering generation may be found from mid-June to late July (Table 2). Second-generation adult flies may be seen later in the summer and fall, but numbers are usually low. An insect sweep net is useful for surveying the adult gall flies. One of the best ways to survey for gall flies is to open and inspect seed heads for galls and larvae. The fly galls and maggots within the seed heads are easy to see and identify (Figs. 2, 3). This is best done in the fall, winter and early spring (Table 2). Fly galls and associated larvae may be small and difficult to find in summer. Larvae and pupae of the spotted knapweed seed head moth, *M. paucipunctella*, are quite obvious (Fig. 5) within the seed head and can be found from fall until spring as well. Adult moths are difficult to find. The small yellow eggs of the moth are located on the bracts at the outside base of the bud near the stem, but they are often difficult to see.

Root-Infesting Species — Larvae and pupae of *S. jugoslavica* are easily seen within the upper roots of plants dug or pulled from the ground (Fig. 7). The root is usually swollen where the beetle is located and the presence of the beetle can be verified by cutting the root open. Adult beetles can be surveyed by using a sweep-

net. The early evening period is best for survey and collection purposes.

Records of survey results and redistribution efforts are extremely useful for the computer mapping process being developed at the University of Idaho. These maps describe distributions of both the knapweed and biological control agents. This information is important in planning future weed management activities. Survey and redistribution report forms can be obtained from your Extension agricultural agent or the State Department of Agriculture and returned to those same offices. If survey report forms are not available, record the date, location, weed species, insect species and density (or at least presence or absence), the source of your bioagents, the observer's name and any other notes that help describe the situation. This information can be sent to the same offices mentioned above.

Future Redistribution Efforts

Until recently, most knapweed bioagent redistributions were conducted by University of Idaho personnel. Since the appropriate methodology has been developed, federal, state and county personnel have become involved as well. A new program, being developed by the Plant Pest Quarantine and Protection unit of the U. S. Department of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS-PPQ), involves the redistribution of knapweed natural enemies throughout the western region. USDA-APHIS, in cooperation with the University of Idaho, the Idaho State Department of Agriculture, various federal agencies such as the USDA-BLM (Bureau of Land Management) and USDA-FS (Forest Service) and the counties, has

further organized survey and redistribution efforts for the gall flies. University of Idaho personnel, in cooperation with USDA-APHIS, have and will continue to relocate the spotted knapweed seed head moth throughout the state.

Most redistribution of the root-infesting beetles, *S. jugoslavica*, in southcentral Idaho has been undertaken by the University of Idaho in cooperation with the multi-county, multi-agency Wood River RC&D project. USDA-APHIS recently (1987) became involved with the redistribution of this natural enemy.

Anyone interested in participating in the natural enemy survey and redistribution efforts is encouraged to do so.

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