RANGE

Operational Field Guide

to the propagation and establishment of the bioagent
Agapeta zoegana
(Sulphur knapweed moth, yellow-winged knapweed root moth)

March 1998

Province of British Columbia
Ministry of Forests

Information contained in this Field Guide is comprised of fact and field observations as of March 1998. Site specific experiences may vary.

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Forest Practices Branch
Range Section
Noxious Weed Control Program
British Columbia Ministry of Forests

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1. PURPOSE

This document summarizes information for the biocontrol agent *Agapeta zoegana* while it was classified as ‘primary’ and the responsibility of the Forest Practices Branch. The information is a combination of hard facts and field observations. Intended as a ‘field guide’ for those unfamiliar with *Agapeta zoegana*, yet responsible for the continuation of the program, the summary contains pertinent information for field propagation and establishment of the bioagent as well as a historical background of its introduction into British Columbia.

2. INTRODUCTION

The goal of the Ministry of Forests weed control program is to reduce target weed populations to ecologically and economically acceptable levels and to prevent their encroachment into new areas. The biological control portion of the program includes biocontrol agent screening, propagation, release, collection and monitoring.
Implicit in the use of biocontrol methods is the acknowledgment that weed eradication is not a goal. Rather, bioagent species and host weed species exist in predator-prey relationships with the weeds held at acceptable population levels and the insect populations self-sustaining.

The biocontrol program is cooperative among the Ministry of Forests, Ministry of Agriculture and Food, Agriculture and Agri-Food Canada, Centre for Agriculture and Bioscience International Institute for Biological Control (CAB IIBC) in Switzerland, and the BC Cattlemen’s Association, Regional Districts, Montana State University, the US Department of Agriculture (USDA) and the Animal and Plant Health Inspection Service (APHIS).

3. AGAPETA ZOEGANA

Order: Cochylidae
Common name: sulphur knapweed moth, yellow-winged knapweed root moth

BIOLOGY

GENERATIONS PER YEAR: generally one in North America (two to several in Europe)

ADULT STAGE: Adults are 1-2 cm long moths with bright yellow bodies and brown patterns on the wings (Photo 1). Females have a larger body than do males, with a more rounded abdomen. Males have a pair of clasping pincers at the end of the abdomen. Adults emerge from the overwintering generation from mid-June to late-August with emergence timing linked to habitat location and weather patterns. Mating begins almost immediately with eggs being laid throughout the 10- to 14 day life span on lower leaves, especially the rosette. Eggs are oval in shape and somewhat flattened (0.75 mm long and 0.45 mm wide). They are whitish when deposited and turn progressively reddish within 3-4 days.

LARVAL STAGE: Larvae hatch 7-10 days after the eggs are laid and migrate into the root crown. Larvae mine the root tissue just below the outer surface, leaving spiral trails covered by a thin, whitish web. There are six larval instars. Most larval growth occurs throughout the fall before the larvae become dormant for the winter.

PUPAL STAGE: Pupation occurs inside the web near the root crown in late spring/early summer approximately two weeks prior to emergence.

DISPERSAL METHOD: Adults are airborne and disperse by flying. Wind may have an effect on dispersal patterns. Larvae have been found to migrate through the soil to new roots to complete life cycles but as a dispersal method this is not significant.

PHOTO 1: ***Agapeta zoegana*** **(adult)**

RANGE

Native (European) Distribution

A. zoegana is known to occur throughout Europe, except for Greece. The northern distribution limit follows latitude 60°N and the eastern limit is the Ural mountains. Southern and western limits are unknown.

Field observations in Austria, Hungary and Rumania indicate the presence of *A. zoegana* in knapweed stands is primarily determined by site conditions. Within its general distribution area *A. zoegana* is only found in semi-natural undisturbed sites; dry prairies, steppe biotopes and steep, south-facing mountain slopes. It is rare or absent along roadsides, on wasteland and all places where host plants are regularly cut. Grazing by sheep have no adverse effect on populations.

Original sources of the agent were the former Yugoslavia, western Hungary, and eastern Austria.

Potential North American Distribution

Prior to the introduction of *A. zoegana* into North America, a 1988 report from the Commonwealth Institute of Biological Control, Switzerland indicated that typical European collection sites corresponded to the climate of the Canadian spotted knapweed infestation with the exception of our colder winter and shorter summer seasons. It was felt that after a period of acclimation, introductions from Europe could potentially colonize the entire area infested by knapweed at that time.

A. zoegana produced up to three generations per year in its European range depending on climate. However, it was expected to only produce one generation a year in most of the North American spotted knapweed area with two generations possible in limited areas. In 1994 two generations were highly suspected at the Carson site just outside Grand Forks, approximately 500 m from the US border. (See discussion on Growing Degree Days under 'When to Collect').

HABITAT

A. zoegana attacks spotted and diffuse knapweed, showing a preference for spotted knapweed (possibly because spotted knapweed is found on moister sites which *A. zoegana* prefers). It is the root mining larvae that damage the plant. At high numbers or with repeated attack, the larvae can completely destroy knapweed roots or render them susceptible to infection. See Appendix A for detailed information on spotted knapweed (*Centaurea maculosa)* and diffuse knapweed *(Centaurea diffusa).*

A. zoegana has been monitored in knapweed infestations in the Bunchgrass, Ponderosa pine, Interior Douglas-fir and Interior Cedar-Hemlock biogeoclimatic zones, primarily on sites characterized as xeric-hot, xeric-warm, dry-warm and moist-warm. *A. zoegana* appear to prefer the moister associations in the Bunchgrass and Ponderosa pine biogeoclimatic zones and the drier associations in the Interior Douglas-fir and Interior

Cedar-Hemlock zones. Contrary to information on its European habitat, *A. zoegana* is found on disturbed sites, along roadsides and in areas where host plants are mowed.

Under an insulating snow cover, *A. zoegana* is tolerant of cold winter temperatures, however it requires a long growing season to develop (perhaps a factor of cumulative Growing Degree Days) and therefore does not appear to do well at elevations above 1000 m.

4. HISTORY OF INTRODUCTION

In 1982, *A. zoegana* was approved for release in North America for the control of diffuse and spotted knapweed. From 1982 to 1985, *A. zoegana* was released at a limited number of field sites in British Columbia, with little success. Small shipments of agents where introduced to field sites under a cage or in the open. It was hoped that the population would establish and increase to allow for collection and redistribution. A few males were caught in pheromone traps but few if any larvae were found in roots.

In May of 1985, a Ministry of Forests Propagation Facility (MOFPF) was established at the Agriculture and Agri-Food Canada Research Station in Kamloops, to overcome the difficulties of direct field releases. Insects from Europe were either very low in number or were received as eggs or larvae which were hard to handle in the field. In addition, future collections from Europe were felt to be limited due to loss of knapweed habitat.

Agents were placed in two 3x3 m field cages that were planted with a mixed stand of diffuse and spotted knapweed. Considered to be an efficient and inexpensive method for initial establishment and for maximizing yield, the number of plots was expanded in subsequent years to a potential of 220, with several species of agents on a variety of weed species. Concern over concentrating the propagation of knapweed root-attacking agents at one location lead to the establishment of a smaller propagation site at Selkirk College, Castlegar in 1986. This facility remained in operation until 1992.

After the initial establishment year of 1985, information on *A. zoegana* behaviour and life cycle was collected. Each year adult moths were collected and redistributed into new plots at the facility or to field sites and methods of collecting, transporting and releasing were tested.

Field releases were monitored to determine establishment, distribution, spread and suitability of sites for recollection at the branch, region and district levels. Nelson Forest Region began to establish their own collection and redistribution program. The tables below will give an appreciation of the expansion of the *A. zoegana* program from 1985 to 1997.

After the 1993 season, propagation of *A. zoegana* was terminated at the Kamloops Research Station. A single tent remains for the capture of adventive *A. zoegana* found at the facility and for display purposes since the moths are the most colourful and easiest to see of all the biocontrol species.

By 1994 all collections were from established field sites. At this time, Nelson Forest Region had an established collection and redistribution program of A. zoegana and were able to supply requests from other regions. Silviculture Practices Branch continued to collect and redistribute mainly within the Kamloops Forest Region due to proximity to the MOFPF and because Nelson Forest Region was managing the east side of the province for the Branch program.

Plots were reestablished at the Kamloops facility in 1995. There was concern that *A. zoegana* was not as advanced in the biocontrol program as desired since no new large yielding sites had been found in the Kamloops Region. The plots were dismantled in 1997 since more sites were found to yield insects, particularly the Old Hedley site discovered in 1996.

During 1993 and 1994, a limited number of vegetation transects were established to monitor the effects of biocontrol agents on target weed species.

SUMMARY OF COLLECTIONS AND RELEASES

The following tables summarize collection and redistribution data for the Propagation Facility and by Forest Region and District. Differences in the numbers reared/field collected and released stems from: insects retained at the propagation facility to maintain tent populations (particularly early in the program); insects perishing; shipped single releases that have been split in two when released in the field; and missing data.

Collection and release summary of *Agapeta zoegana* in B.C.

	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Reared & Collected	1,974	6,034	1,166	5,042	11,560	9,528	13,808	19,896	25,689	27,470	11,940
Released	1,116	5,426	1,147	4,433	9,736	7,457	13,268	22,637	25,387	27,040	12,040

Propagation Facility

Insects Reared in MOF Propagation Facility Tents

	1987	1988	1989	1990	1991	1992	1993	1997
Insects Received for Propagation Tents (# of tents)								76 (3)
Reared at MOFPF (# insects)	1,974	6,034	469[1]	3,601	5,754	1,890	3,916	

[1]The winter of 1988/89 caused severe damage to the plants in the propagation plots and subsequently the agents on them. All moths reared in 1989 were kept at the propagation facility to replenish the tented populations.

Kamloops Forest Region

Field collections

#Insects

Site	1990	1991	1992	1993	1994	1995	1996	1997
Barriere			100					
Brass Kettle	486		7					
Cinnamon Ridge			550		100		11	
Cinnamon Ridge, Munitions & Adams River						853		
Clearwater Airport	52							
Douglas Lake Road						1,762	761	253
Douglas Lake Road & Monte Lake							150	
Falkland						442	34	205
Fountain Valley					382			
Gladwin Trailer Park					48			
Little Shuswap						100		
Louis Creek								367
McLure								44
Mission Flats							2	10
Munitions							9	145
Old Hedley Road							11,526	3,260
Pavillion Lake		4,805	997	2,017	1,539	1,259		
Research Branch trial plots		108						
Seton Lake			4,195	6,206	3,819	295		
Spences Bridge								208
Station Road, Westwold							103	
Sunnybrae Road						28		
Unknown							4	
TOTAL	538	4,913	5,849	8,223	5,888	4,719	12,600	4,492

Field releases by district

#Insects (#Releases)

DISTRICT	1982	1985	1986	1987	1988
Clearwater				194 (93)	
Kamloops		10+ (2)[1]	80 (2)	159 (3)	2,236 (26)
Lillooet				157 (2)	405 (7=1[1])
Merritt				37 (1)	
Penticton		(1)[1]		50 (1)	
Salmon Arm	(1)[1]		155 (2)	84 (2)	522 (10)
Vernon				141 (3)	1,190 (9)
TOTAL	(1)	10 (3)	235 (4)	822 (15)	4,353 (53)

DISTRICT	1990	1991	1992	1993	1994
Clearwater	100 (1)	500 (5)	100	500 (5)	
Kamloops	1,986 (21)	3,295 (30)	475	3,350 (33)	4,387 (44)
Lillooet		600 (4)	403	500 (5)	
Merritt		850 (10)		1,000 (10)	
Penticton	98 (1)	1,200 (12)[1]	100	600 (6)	500 (5)
Salmon Arm		700 (8)		500 (5)	1,245 (11)
Vernon	495 (5)	1,100 (10)	761	800 (8)	855 (8)
TOTAL	2,679 (28)	8,245 (79)	1,839	7,250 (72)	6,987 (68)

DISTRICT	1995	1996	1997
Clearwater	500 (5)	1,087 (11)	281 (3)
Kamloops	3,800 (32)	3,107 (30)	1,315 (13)
Lillooet	570 (5)	1,900 (19)	821 (8)
Merritt	1,600 (16)	3,000 (30)	1,034 (10)
Penticton	1,170 (11)	4,500 (45)	426 (5)
Salmon Arm	1,040 (10)	700 (7)	530 (4)
Vernon	1,700 (17)	1,650 (16)	
TOTAL	10,380 (96)	15,944 (158)	4,407 (42)

[1]Number was not recorded.

Nelson Forest Region

Field collections

#Insects

Site	1989	1990	1991	1992	1993	1994	1995	1996	1997
Carson					146	12,463			
Deer Park						200	450		
Genelle								19	150
Glass House									300
Hall Siding								100	
Heinz Field						100	1,000	356	
Hills							1,750		
Kid Creek								100	
McDonald Creek							1,380		
Passmore						400	11,815	13,465	3,700
Salmo								100	800
Sanca Creek				599	618	1,750	950	100	
Selkirk College	697	903	893	1,190	905	2,250	3,675	575	
7 Mile Dam									100
Sheep Trap								155	850
Slocan Park									1,480
TOTAL	697	903	893	1,789	1,669	17,163	21,020	14,870	7,380

Field releases by district

#Insects (#Releases)

DISTRICT	1982	1986	1987	1988	1989
Arrow		98 (1)	275 (5)	282 (8+1[1])	157 (4)
Boundary	(1)[1]	79 (1)		78 (3)	183 (5)
Kootenay Lake				189 (5)	127 (4)
TOTAL	(1)	177 (2)	275 (5)	549 (17)	467 (13)

DISTRICT	1990	1991	1992	1993	1994
Arrow	619 (2)	604 (2)	2,074 (20)	1,870 (19)	6,550 (60)
Boudnary	46 (1)			510 (4)	3,200 (28)
Cranbrook		400 (2)		707 (5)	600 (4)
Golden					300 (3)
Invermere		300 (2)	98 (1)		300 (3)
Kootenay Lake	105 (1)	637 (2)	1,660 (16)	2,324 (23)	3,400 (33)
Revelstoke					200 (2)
TOTAL	770 (4)	1,941 (8)	3,832 (36)	5,111 (51)	14,550 (133)

DISTRICT	1995	1996	1997
Arrow	1,750 (58)	5,620 (43)	1,400 (13)
Boundary	4,600 (40)	2,600 (24)	3,300 (29)
Cranbrook			1,100 (6)
Golden		200 (2)	
Inveremere		200 (1)	
Kootenay Lake	3,210 (23)	1,550 (13)	1,380 (13)
Revelstoke		200 (2)	200 (1)
TOTAL	14,960 (121)	10,370 (85)	7,380 (62)

[1]Number released was not recorded

Other Forest Regions

Field releases by region

#Insects (#Releases)

REGION	1987	1988	1990	1991	1993	1994	1995	1996
Cariboo								
100 Mile House		119 (2)			100 (1)			
Williams Lake			400	400	307 (2)	500 (3)		
Prince Rupert							100 (1)	
Vancouver								
Chilliwack					100 (1)	100 (1)	210 (2)	500 (5)
Port Alberni	24 (2)				100 (1)			
TOTAL	24 (2)	119 (2)	400	400	607 (5)	600 (4)	310 (?)	500 (5)

Other Release Destinations

Distribution numbers recorded in this table are only the releases given directly to municipalities and Native Bands by this office. This covers only a minute percentage of the releases given to these groups by MOF. The MOF Regions and Districts maintain relations with these and other groups and share a portion of their releases with them following Regions' and Districts' obligation to release the agents on Crown land.

#Insects (#Releases)

DESTINATION	1988	1989	1992	1994	1995	1996	1997
Alberta							
Waterton Park				300 (3)			
City of Kamloops							153 (2)
Montana	250						
Native Bands						100 (1)	100 (1)
Ontario							
Guelph					200		
Other	513	39	100				
TOTAL	763	39	100	300(3)	200	100 (1)	253 (3)

5. REDISTRIBUTION

Redistribution of agents is a critical part of the biocontrol program. To ensure distribution throughout *A. zoegana's* potential provincial range, personnel must be able to recollect from field releases and make releases into new sites.

FIELD COLLECTION

What and where to collect

Field collecting of *A. zoegana* involves walking through a stand of knapweed looking for bright yellow moths. Newly emerged moths are a deep yellow but quickly fade to pale yellow as they age and lose wing scales. Moths emerge from the root crowns and can often be seen on stems near the plant bases. They also cling to the undersides of leaves, particularly when it is hot.

Lush knapweed plants can be a better bet for finding the moths. However, in the ICHdw, Nelson Region staff have found it easier to collect on drier micro-sites where knapweed plants are still green, have a smaller stature, and are less dense. Mature moths do not feed significantly and therefore do not require green leaf material.

The following are suggested Collection Site Criteria*:

- Sites should be between 0.5 and 1.5 ha in size.
- The average estimated knapweed density should be greater than 5 plants/metre^2.
- Soils should be free of dense moss cover.
- Topographies of successful collection sites have varied: flat, bowl shaped, forest openings, south facing slopes. All have been able to accumulate heat units.
- Insects established for at least 3 to 4 years (dependent on size and density of weed infestation, site characteristics, weather conditions, number of agents in initial release, predators/ parasites etc.).
- Frequency of attack greater than 50%.
- Average larvae density greater than 100% (total #larvae/total #plants sampled) X 100
- Preferably located on Crown Land with easy access within 100 km of regional or district offices.
- Sites should be easily traversed for collection.

*These criteria also need to be kept in mind when establishing future collection sites (current release sites) to ensure a future population of *A. zoegana* in the field. See discussion under Field Release.

PHOTO 2: Old Hedley Road collection site, Kamloops Forest Region

PHOTO 3: Passmore collection site, Nelson Forest Region

How to collect

Vacuum aspirators have been found to be an efficient method for collecting the moths. Mouth operated aspirators were eliminated as a method because of irritation to the throat caused by wing scales. Sweep nets were eliminated due to insect damage.

An aspirator is actually a hand held vacuum cleaner modified with a series of rubber stoppers, plastic tubing and 1 litre bulk food container.

Some weed material or absorbent paper is put into each container to provide the insects with something to cling to. Any plant material that is used needs to be on the dry side and seed free. Lush green leaves will transpire, resulting in condensation in the container and drown moths. Toilet tissue has been used with success in the Nelson Region although there is some concern about scale loss during shipping when using paper products. A quality, 2-ply brand is needed to avoid crushing and compacting. Enough toilet tissue is used to loosely fill the container (3 to 4 connected sheets).

Moths are aspirated head first to minimize damage to wings. A suggested technique for collecting is to invert the container when aspirating because the moths generally fly to the top and the next step of exchanging lids is easier. This technique works better when using plant material in the container and Nelson Region has never found the need to invert. Rather, they slow the moths down by placing the container in a cooler for a few minutes or just tapping the container bottom once or twice to dislodge moths and quickly change lids. Escaped moths are minimal and a few extra (3 to 5) are aspirated just in case.

When 50 (or a few more) moths are collected, the lid used during aspiration is quickly exchanged for a lid with a mesh opening. (A hole is cut in a lid and a small-mesh screen is glued over the opening). Mesh screen on the storage lid is critical to allow ventilation and to prevent a build up of condensation that can damage the insects' wings. Containers are kept cool and out of direct sunlight in a portable cooler with ice packs wrapped first in plastic bags and then in paper towels to absorb any condensation.

Efficiency of collection increases with experience. Each collection site is unique and a strategy for collecting (how to actually walk through the site) can only be developed from experience - trial and error so to speak. Under good collecting conditions a very experienced person can collect between 300 and 350 moths/hour. However, realistically 100 moths per hour is normal. Two or three people working together increases efficiency.

When to collect

Time of Year

Peak collection periods for the Nelson Region have occurred from one week to about one month in advance of the Kamloops Region. From past records peak collection time in Nelson Region is from the first week of July to the third week of August. The Kamloops Region peak seems to be between the second to third week of July to the third week of August. These dates have shifted by up to two weeks later in cool wet years. Very dry, hot weather conditions have also been noted to affect emergence. Larvae have been found dead in desiccated roots and the number of moths collected declined. Also see discussion under Additional Considerations below.

It is hypothesized that heat accumulation is the most important factor that determines emergence of *A. zoegana*. Reports from Europe hint at this as well.

It has been observed in the Nelson Region that a warm, dry autumn promotes an early peak emergence the following summer. Yearly weather patterns must be considered when predicting the emergence of *A. zoegana*.

Collecting during peak emergence not only makes sense for efficiency reasons but has implications for sex ratio as well. According to Shelford 1994, the sex ratio is slightly in the females favour during peak emergence and females have a higher fecundity at this time. Being able to collect desirable sex ratios and highly fecunded females has positive implications for redistribution.

Time of Day

Wind, temperature and light may hinder collection. Cool conditions during the morning and early (6:30pm - 8:30pm) evening, have been found to be the best times in the Kamloops Region. Moths have been noted to 'hide' from intense heat and often rest on the underside of leaves making detection harder. If morning and evening temperatures are too low, the moths become lethargic and perhaps resting out of site, difficult to find.

Nelson Region has found success between 10:30am to 3pm unless temperatures climb over 34^{o}C. Collection during hot afternoon temperatures is hard on personnel and the moths are difficult to catch since they are highly active and move quickly to avoid capture. Ideal collection days are noted to be warm with a high overcast. The moths rest on the top of plants but are not flighty.

Collection in the late evening before sunset can be difficult. The angle of the sun creates long shadows and the yellow hew of the sunlight during this period obscures visibility of the moths. Following sunset, light conditions are better for catching moths, but generally there is only about half an hour of collection time before it is too dark to see the insects.

Additional considerations

Intervals of cool weather and rain have been noted to 'interrupt' emergence and hence collection. A return to clear skies and hot temperatures, caused a second 'flush' of moths to occur and new brightly coloured specimens were found in large numbers.

Intervals of prolonged high temperatures and drought conditions have been speculated to cause larvae to enter aestivation (a suspended state similar to hibernation). A site in Kamloops Region was monitored during the spring with abundant larvae found. However, fewer than expected moths were collected at the site after the adverse weather conditions.

No conclusions on the longevity of a collection site can be made at this time. Some *A. zoegana* sites have yielded large numbers of moths in the first year of collection with moderate numbers for several years following. One site failed to yield agents even though healthy larvae were found in the roots prior to the collection season. Other sites have had consistently high yields two or more years running.

Consideration must be given to leaving a viable population at the site following the collection season. During the days/weeks spent collecting at a site, females are continually mating and ovipositing over their 12-14 day lifespan, however to ensure a continued population there needs to be some moths left at the conclusion of the collecting season. When numbers begin to drop at a site a decision to terminate collection for the season needs to be made.

SHIPPING

Collected insects are shipped to new release sites in the 1 litre bulk food containers used during collection. Traditionally, a minimum of two containers (100 insects) were used for each release site unless larger releases were designated. To ensure population establishment and a faster increase in population numbers, releases of a minimum of four containers (200) insects are recommended. The containers are packaged into carefully sealed boxes to avoid insect escape during shipment. The boxes are then filled with ice packs to keep the moths cool and reduce their activity, which could result in damage during transport. Ice packs are wrapped first in plastic bags to contain the majority of condensation and then in paper towel to prevent further condensation from building up on the inside of the containers and damaging the insects' wings or causing them to drown. The boxes are kept out of direct sunlight. The agents are shipped quickly via courier or bus (or plane) to release locations due to the fragility of the insects and their short life span.

FIELD RELEASE

Potential release sites

A potential release site needs to meet certain criteria to ensure success and longevity. It must meet Ministry needs from a program and logistic standpoint, i.e. travel distance, land tenure, accessibility. It must also be conducive to agent survival and micro-site conditions seem to be more important for success of *A. zoegana* releases than do the specific biogeoclimatic zones.

Below are suggested criteria based on observations of past sites which have been successful:

Criteria

- Sites should be between 0.5 and 1.5 ha in size. Releases on sites larger than 1.5 ha can be made, keeping in mind the moths may disperse over an area that makes them difficult to collect in the future (they tend to concentrate in pockets on larger sites, however, the time from release to collectable densities can be lengthy). Releases on sites less than 0.5 ha may also establish, yet, after 3 to 4 years, the population of plants may have dwindled from attack to the extent that the number of plants yielding moths may be too few to consider the site efficient for collection.
- The average estimated knapweed density should be greater than 5 plants/metre2.
- Topographies of successful sites have varied: flat, bowl shaped, forest openings, south or southwest facing slopes. All have been able to accumulate heat units.

Considerations

- Elevations above 800 m have lower establishment success rates.
- Fine, uniformly textured soil appears to have a negative effect on establishment. (An exception is the Carson site.)
- Moss has been noted on sites with poor establishment but this is not consistent.
- Strong prevailing winds may disperse moths too widely.

- Dust from nearby roads may hinder establishment.
- Sites receiving cold air drainage may be poor choices, especially if they are relatively flat, allowing cold air to pond.
- Site should be easily traversed for collection.

The following are suggested steps to take when making a site selection:

1. Plan release site locations prior to requesting agents. Release sites should be pre-selected the fall or spring prior to release of agents. This avoids ‘drop and dash’ releases and promotes overall weed management planning. An unofficial rule is to space current year releases at least 1 km apart.
2. Determine tenure and stability of land management. Preferably a site will be located on Crown Land with MOF mandated as the steward and have a cooperative tenure holder. Other suitable locations may be land under the jurisdiction of other agencies with the goals of controlling weeds and establishing/maintaining working relationships. Release sites might be located in or close to relevant municipalities with the goal of future cost effective collection sites. An example is a municipal water reservoir which is long term and most activity, particularly herbicide spraying, is prohibited.
3. Make sure the site will not be disturbed after release. Crown control of the site is preferred with future management known. (Grazing was not considered a detrimental factor in European reports - nothing has been noted in BC reports). Discuss future development plans for the site wherever it is located.
4. Check previous release records and maps to ensure no prior release of the agent has been made at a potential site. An unofficial rule is that a distance of 1 km constitutes a separate release.
5. Monitor plants at potential site to ensure the agent is not already present through natural dispersal.
6. Check the immediate vicinity of the proposed release site for ant hills and wasp nests to minimize predation.
7. Mark selected release site with a stake so that it may be relocated to monitor insect progress and weed population decline.

Insect release

Before any insects can be released there is preparatory work that needs to be completed at the site. Make sure that all paperwork, photo’s, site maps, measurements, etc. are completed before opening any lids, otherwise people will be treading on insects.

Generally each release has consisted of 100 adult moths unless a larger release is designated for a site. Occasionally there are 4 or 5 more to account for any escapes in collecting. In a research study by Powell, Sturko and Wikeem (no date) it was found that an increase in the number of insects released at a site resulted in an increase in the "peak emergence rate". When agents have been released at all critical sites, consideration can be given to adding further agents to sites where establishment has not been confirmed or a larger population of the agents is required.

Agents are released primarily by Ministry of Forest Regional and District personnel and occasionally by staff from the Ministry of Agriculture, Fisheries and Food. Other people who may receive insects include Native Bands on reserve and private lands, private citizens, Ministry of Transportation and Highways and University staff.

The following are suggested steps to take when making an insect release:

1. <u>Mark the release site with a semi-permanent stake</u> to assist relocation efforts for follow-up agent establishment and weed impact monitoring.
2. <u>Fill out the 'Biological Control Release Record'</u> (see Appendix C) that is shipped with the moths **accurately** and **completely**. Information on the forms is fundamental to further analysis of the program. One completed copy of the Release Record is kept in the District office and one is returned to the MOF Regional office. The forms are then collated to create a provincial database traditionally administered by the Ministry of Agriculture, Fisheries and Food.
3. <u>Create accurate site maps complete with permanent tie points</u>. This is essential for future monitoring of the release site.
4. <u>Take Photographs.</u> They have proven to be a useful tool to both relocate the release site and to provide an ocular comparison of the site over time. A suggested method and form (EM-9) is outlined in the Habitat Monitoring Manual.
5. <u>Gently release the moths</u> (once the paper work is completed) at one location by the stake. They will disperse themselves from this initial release point. It is more difficult for insects to propagate if they are spread over a large area.

Additional considerations

Initial releases for the season should be made in similar latitudes or further south than collection sites to ensure temperatures are conducive to agent establishment. As northern release sites warm, they can receive insects. Once northerly temperatures begin to drop, releases should be directed south to provide for the longest possible establishment season.

If more than one collection site is available, it is preferable to redistribute moths into habitats similar to those they are acclimatized to.

Phenology of the knapweed may be a consideration when timing releases. In a 1988 report studying oviposition, rosettes were found to be more frequently attacked than flowering plants. The lower leaves of the flowering plants had dried before oviposition commenced and the roots were less suitable for larval development. Rosettes were preferred over flowering plants for oviposition.

If agent establishment at a release site is uncertain or the knapweed infestation is particularly large, re-release of agents may need to be considered. Before re-releasing at a site the Region/District plan needs to be reviewed, i.e. Can agents be spared for re-release at a site that may or may not be conducive to the agents' survival when they could be placed at a new site?

6. MONITORING

Monitoring of field sites can be carried out to determine:

1. whether the agent has established at the release site
2. the density of agents per plant or area
3. how far the agent has spread from the release point
4. the agent's preferred habitat and current range
5. areas that are unsuitable to the agent
6. any effects the agent has had on the weed population
7. potential collection sites
8. if collecting from the site has had any effects on plant or agent populations
9. agent life cycle information i.e. emergence dates, effects of weather.

Depending on the type of information being sought the monitoring technique will vary. Reconnaissance methods can be used to assess parameters such as site suitability, presence or absence of agents, dates of emergence etc. A suggested monitoring form ('Release Site Monitoring Form') detailing information to collect at each site can be found in the Appendix C. Many details listed on this form can be found on the original release form. Some will not change over time and need not be duplicated if the information is already recorded, while other details, particularly the plant's dispersal description, may change.

A more rigorous method is needed for quantifiable information on insect and plant populations. It is suggested that this type of monitoring be planned at selected sites as dictated by the constraints of program planning, time and budget.

A suggested transect method is as follows:

1. <u>Find the release stake</u> or from the description on the release form, the closest position to the release stake. Mark a starting point.
2. <u>Determine 4 directions</u> from the starting point for running the transects. If cardinal directions cannot be used, determine 4 non-cardinal right angles. Pace out 25 m in each direction.
3. <u>Pace out three parallel transects</u> at least 2 metres apart: two 33 m long and one 34 m long if the site does not allow for this transect design.
4. <u>Pace out two 50 m parallel lines</u> lying side by side or staggered (as can be accommodated by the site). The intent is to monitor 100 plants randomly if the site does not allow for either of the above transect designs.
5. <u>Choose the closest plant to the toe of the boot</u> and dig it up for inspection at one metre intervals along each transect.

A form for this method (Biocontrol Agent Monitoring Form) is found in Appendix C.

AGENTS

Sites can be monitored for the presence of adult moths in July and early August.

Roots are monitored for larvae in the fall and early spring but not too early or inexperienced eyes may not detect the small larvae. Mid-May to early-June when the larvae have grown and are easy to detect seems to be the preferred time period. It is possible to check for pupae in early summer before adult emergence has occurred.

After digging up a knapweed plant the outer surfaces of the roots are gently scraped with a knife to remove dirt or rinsed with water to remove dirt. Evidence of tunnelling from feeding, white webbing or the presence of larvae or pupae is sought. Live larvae are usually found in the vicinity of silk-lined tunnels which tend to be concentrated on the outer surface of the root, just under the bark. When scraping with a knife, the webbing can easily be distinguished from the bark by the difference in texture. The presence of living larvae indicates new attack. Old attacks will not have any larvae present and the mines are usually larger.

Useful parameters to note include larvae density, frequency of attack, average larvae density:

$$\text{larvae density} = \frac{\text{Total \# larvae}}{\text{Per plant}}$$

$$\text{frequency of attack} = \frac{\text{Total \# of plants attacked}}{\text{Total \# of plants sampled}} \times 100$$

$$\text{average larvae density} = \frac{\text{Total \# of Larvae}}{\text{Total \# plants sampled}} \times 100$$

PLANTS

A method needs to be developed for measuring responses of the host weed population. Useful parameters to monitor would be: density, height, production, seed production, cover, frequency and root response.

RESULTS

Monitoring has found that *A. zoegana* can establish in the Bunchgrass, Ponderosa pine, Interior Douglas-fir, Interior Cedar-Hemlock, Coastal Douglas-fir and Coastal Western Hemlock biogeoclimatic zones. Specific site parameters are more difficult to summarize and analyze. However some generalities have been noted previously in the suggested criteria for site collection and release.

With the differences in latitude, future monitoring information may be best analyzed on a Region or District level.

Dispersal information is best presented in map form.

APPENDICES

Appendix A - HOST WEEDS

Spotted Knapweed *Centaurea maculosa* Lam.

The plant

- A short-lived perennial, introduced with forage or grain seeds from south-eastern Europe. First Canadian collection made at Victoria, B.C. in 1893. Spreads by seed. Mature plants are 0.3-1.5 m tall, with long fibrous tap roots. Stems are somewhat hairy when young and highly branched. The basal and lower stem leaves are pinnately lobed; the upper leaves have smooth margins. Young leaves have a fine coating of hairs. Flowers are 1-1.5 cm long, pink to purple (occasionally white); bracts of the flower head are black tipped, giving the head a spotted appearance. Flowering occurs from July through to September. Prolific seeds are black or brown, 3 mm long, and topped with bristles up to half the length of the seed. The plant contains volatile oils with a distinctive smell and an extremely bitter taste.

Habitat

- Found throughout British Columbia, but primarily at lower to mid-elevations of the southern interior including the entire Kootenays, Okanagan, Thompson-Nicola, Cariboo-Chilcotin, Fraser Valley, Vancouver Island and several Gulf Islands. It occurs in isolated pockets elsewhere in the province (Prince Rupert Forest Region and Peace River area). Estimated to cover about 60,000 ha of semiarid rangeland in BC (Muir 1986) and has potential to occupy 1.1 million ha.

Growing conditions

- Grows in a wide range of environmental conditions, though mostly in grasslands and open forests of the Bunchgrass, Ponderosa Pine, Interior Douglas-fir and Interior Cedar-Hemlock, as well as the Coastal Western Hemlock and Coastal Douglas-fir biogeoclimatic zones. Local infestations occur in the Montane Spruce and Englemann Spruce-Subalpine Fir zones. A rapid colonizer of disturbed soils, it can also displace native vegetation in undisturbed sites.

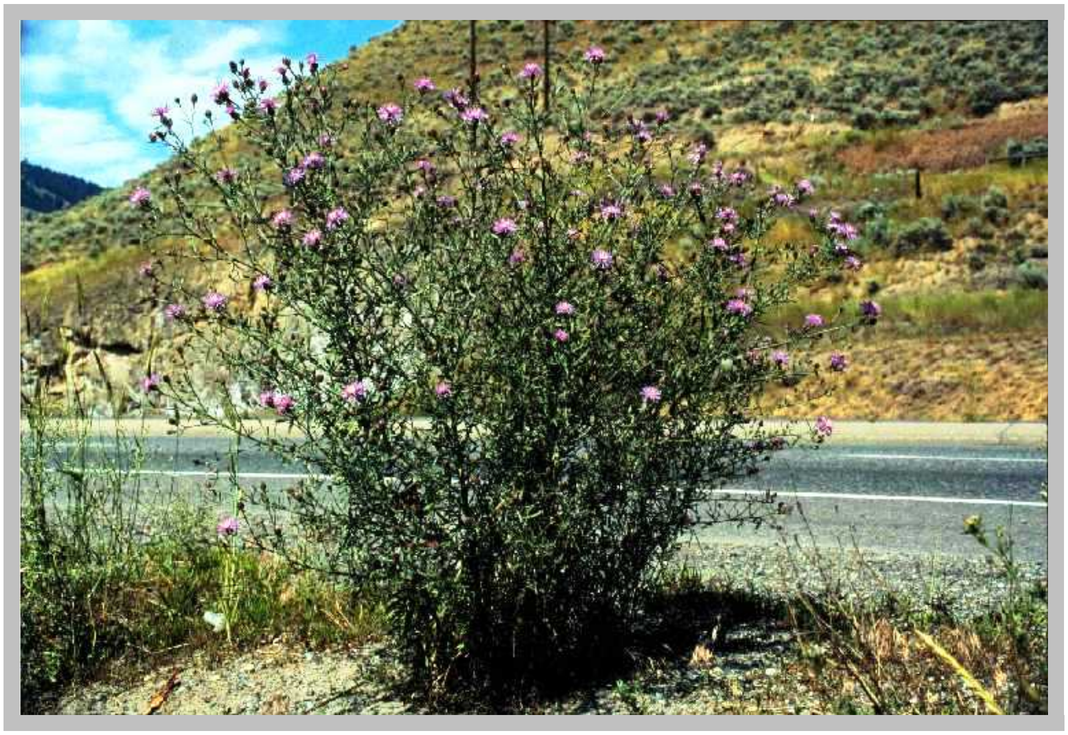

PHOTO 4: Spotted knapweed (*Centaurea maculosa*)

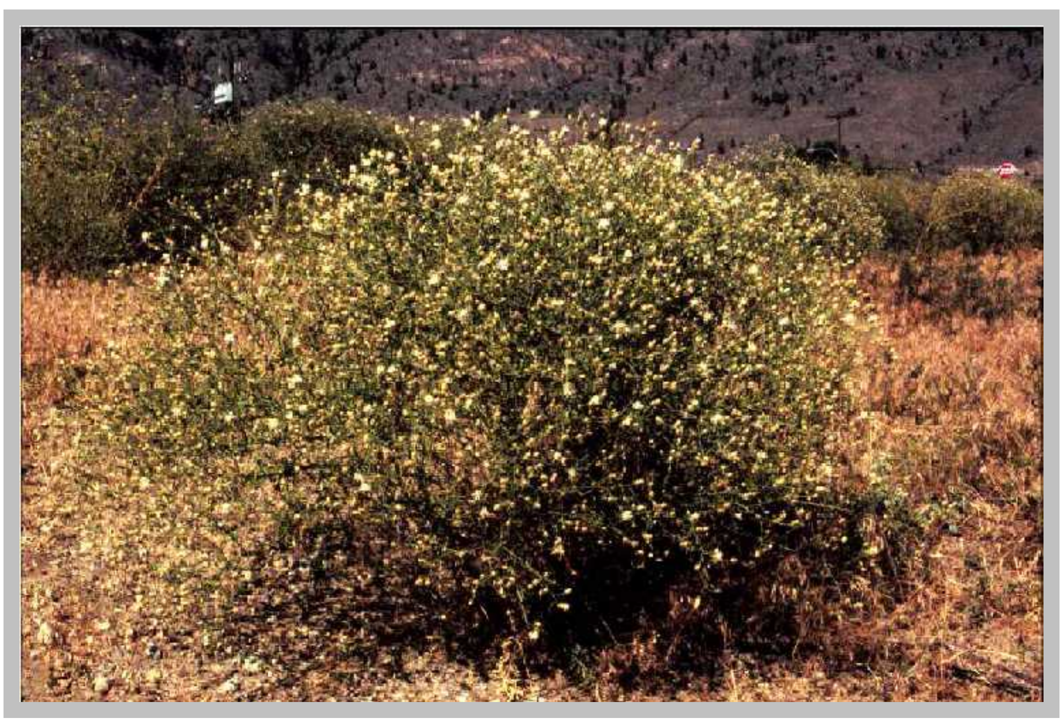

PHOTO 5: Diffuse knapweed (*Centaurea diffusa*)

Diffuse Knapweed *Centaurea diffusa* Lam.

The plant

- A biennial to short-lived perennial, spreading primarily by seed. Stems are 60-90 cm in height with many branches. Leaves are 5-20 cm long, hairy, and highly divided. Leaves alternate from the stem, with basal leaves forming a rosette. Flower heads are numerous, urn shaped, and covered with small, narrow bracts ending in sharp, rigid spines. Flowers are white or occasionally pink or purple. Prolific seeds are black to dark brown, 3 mm long, and lack a developed fringe of hairs (pappus). Diffuse knapweed contains volatile oils with a distinctive smell and extremely bitter taste.

Habitat

- Widely distributed throughout British Columbia, though primarily in the Kootenays, Thompson-Nicola, Okanagan, Kettle River, and Fraser Canyon areas of the southern interior. Occurs in pockets and at lower abundance in the Cariboo-Chilcotin.

Growing conditions

- Occurs over a wide range of ecological types, though it tends to dominate dry valley bottoms in the Bunchgrass zone and transition areas of Ponderosa Pine, and Interior Douglas-fir biogeoclimatic zones. A rapid colonizer of disturbed soils, it can also invade and displace native vegetation in undisturbed areas.

Appendix B - LITERATURE CITED

Muller, H., D. Schroeder and A. Gassman. 1988. Investigations on *Agapeta zoegana* Haw. (Lep. Cochylidae), a possible biocontrol agent of spotted knapweed, Centaurea maculosa Lam. (Compositae) in Canada. European Station Commonwealth Institute of Biological Control, Delemont, Switzerland.

Powell G., A. Sturko, B. Wikeem and P. Harris. 1994. Field Guide to the Biological Control of Weeds in British Columbia. Land Management Handbook Number 27. Province of British Columbia, Ministry of Forests.

Powell G., A. Sturko and B. Wikeem. Fecundity, survival and growth of *Agapeta zoegana* L. (Lepidoptera: Cochylidae), propagated for the biological control of knapweeds. Currently submitted to the Journal of Biological Control.

Shelford, C. 1994. Body measurements and emergence patterns of *Agapeta zoegana*, a biological control agent of spotted knapweed (Centaurea maculosa). Min.For., Range Br., (Mimeo) 23p.

APPENDIX C – RELEASE AND MONITORING FORMS

BIOLOGICAL CONTROL RELEASE RECORD

SITE NUMBER: D __ __ /___ ___ ___ ___/___ ___/___ ___ ___

BIOAGENT: ________________/____________ **WEED SPECIES:** ___ ___ ___ ___ / ___ ___ ___

SOURCE: _______________________ **STAGE:** ADULT PUPA LARVA EGG OTHER

COLLECTION: __ __ / __ __ / __ __ (YY/MM/DD) **RELEASE:** __ __ / __ __ / __ __ (YY/MM/DD) **TIME:** __ __ : __ __

#RELEASED ________________ **JURISDICTION** ________________ **RELEASED BY:** _______________

DISTRICT: ___________________________________ **RANGE UNIT NAME:** ____________________

PRIVATE LAND **Owner:** ____________________________ **Phone:** ( ) ___ ___ ___ - ___ ___ ___ ___

ADDRESS: ____

LOCATION:____

____

BCGS MAP: __ __ __ __ __ __ __ **UTM :** |__|__| |__|__|__|__|__|__| |__|__|__|__|__|__|__| ________

ZONE EASTING NORTHING DATUM

WEED DENSITY: <1 plant/m^2 2-5 plants/m^2 6-10 plant/m^2 >10 plant/m^2

SIZE OF INFESTATION: < 100 m^2 101-400 m^2 401-2500 m^2 2501-5000 m^2 5001-10000 m^2 >1 ha.

WEED DISTRIBUTION: Continuous Stand: Scattered Patches: Distribution Code (1-9):_____

SLOPE %: _______ **ASPECT ^{0} :** _______ **ELEVATION m :** ______ **BIOGEO UNIT:** _ _ _ _/_ _ _

zone/subzone /variant/site series

MONITORING for ESTABLISHMENT

Date Monitored
y/m/d

__ __ / __ __ / __ __	Yes	No	Photo
__ __ / __ __ / __ __	Yes	No	Photo

COMMENTS: ___________________________

____

____

____

SKETCH MAP (Indicate North)

RELEASE SITE MONITORING

DATE:	**AGENT:**
SITE NUMBER:	**SITE NAME:**
MAP NO.:	

WEED DENSITY:

<1 plant/m^2	____	2-5 plants/m^2	____
6-10 plants/m^2	____	>10 plants/m^2	____

SIZE OF INFESTATION:

<100m^2	____	100-400m^2	____
400-2500m^2	____	2500-5000m^2	____
5000-10000m^2	____	>1 ha	____

WEED DISTRIBUTION:

Continuous Stand	____	Scattered Patches	____

ACCESS TO SITE:

Easy	____	Difficult	____
Describe if necessary.			

SITE TOPOGRAPHY:

Flat	____	Bowl Shaped	____
Forest Openings	____	Close to River/Lake	____
Terraced	____	Hillside	____
Other (describe)	____		

TRAVERSABILITY OF SITE:

Easy	____	Difficult	____
Describe if necessary			

SOIL DESCRIPTION:

Moss covered	____	Gravel	____
Clay	____	Silt	____
Compact	____	Loose	____
Sandy	____	Other (describe):	____

SLOPE (%): ____ ASPECT (^{o}): ____ ELEVATION (m): ____

BIOGEOCLIMATIC CLASSIFICATION: _____

DISTANCE FROM KAMLOOPS (km): ____

LAND OWNER:

RECOMMENDATION:

COMMENTS:

BIOCONTROL AGENT MONITORING FORM

SITE NUMBER: ______________________ DATE: (YR/M/D) ____________

AGENT: ______________________ LOCATION: ____________

RELEASE DATE: ______________________ TARGET PLANT: ____________

METERS		NORTH	SOUTH	EAST	WEST
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
MAX DIST.					

PERCENT ATTACK: ____________

COMMENTS: ____