



Operational Field Guide

to the propagation and establishment of the bioagent *Cyphocleonus achates* (Knapweed root weevil)

March 1998



Province of British Columbia Ministry of Forests

The contents of this Field Guide may not be cited in whole or in part without the advance written approval of the Director, Forest Practices Branch, Ministry of Forests, Victoria, British Columbia

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

Operational Field Guide

to the propagation and establishment of the bioagent Cyphocleonus achates (Knapweed root weevil)

March 1998



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

TABLE OF CONTENTS

1.	PURPOSE1
2.	NTRODUCTION1
3.	CYPHOCLEONUS ACHATES1
	BIOLOGY1
	RANGE
	Native (European) Distribution
	НАВІТАТЗ
4.	HISTORY OF INTRODUCTION4
	SUMMARY OF COLLECTIONS AND RELEASES5
	Propagation Facility6Kamloops Forest Region6Field collections6Field releases by district7Nelson Forest Region7Field collections7Field collections7Field releases by district8Other Forest Regions8Field releases by region8Other Release Destinations9
5.	REDISTRIBUTION9
	FIELD COLLECTION9
	What and where to collect
	SHIPPING12
	FIELD RELEASE13
	Potential release sites
6.	MONITORING15
	AGENTS16
	PLANTS17
	RESULTS17

APPENDICES

Appendix A – HOST WEEDS	
Appendix B – LITERATURE CITED	
Appendix C – RELEASE AND MONITORING FORMS	

LIST OF PHOTOGRAPHS AND PICTURES

Photo 1 – Cyphocleonus achates adult (Knapweed root weevil)	2
Photo 2 – Comparison of plant and root size of unattacked vs. attacked plants	
Photo 3 – Cinnamon Ridge collection site (Kamloops Forest Region)	5
Photo 4 – Spotted knapweed (Centaurea maculosa Lam.)	19
Photo 5 – Diffuse knapweed (Centaurea diffusa Lam.)	20
Picture 1 – Characterisitics for sexing Cyphocleonus achates (Knapweed root	
weevil)	11

1. PURPOSE

This document summarizes information for the biocontrol agent *Cyphocleonus achates* while it was classified as 'primary' and the responsibility of the Forest Practices Branch. The information is a combination of hard facts, observations and best guesses. Intended as a 'field guide' for those unfamiliar with *Cyphocleonus achates*, 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. CYPHOCLEONUS ACHATES

Coleoptera: Curculionidae Common name: Knapweed root weevil

BIOLOGY

GENERATIONS PER YEAR: one

ADULT STAGE: Adults are mottled grey-brown weevils, 0.5 to 1.75 cm long. Adults emerge from the overwintering generation in mid July to early September and feed on knapweed leaves, preferring those of young plants. When the new weevils emerge their bodies are soft and have a dark, reddish colour. Over a few days the cuticle becomes hardened and their mature coloration develops. Females have a very round abdomen. Males have flat abdomens. A European study found that mating began within 1-2 weeks if adults were early emerging and immediately if they were late emerging. Nelson Region has noted mating never begins before August, even if adults emerge in early July. A single female will mate several times during her 10 week

lifespan. European studies indicate egg laying takes place from late July to mid September. Egg laying has been observed to commence the third week of August in Nelson Region, continuing until frost. Egg laying is highly specialized. Females excavate a tunnel through the soil and into the root crown using their heads and chew an attachment site. A single egg is laid and secured. The egg is then encased with soil particles and/or plant debris held together with a secretion. The soil tunnel is then filled in. A successful oviposition takes on average 26 minutes. Average fecundity rates are estimated to be 45 eggs/female.

LARVAL STAGE: Larvae hatch 10-12 days after eggs are laid. They immediately start tunneling and develop in the root crown. There are four larval instars, with the second instar overwintering. Third and fourth instar larvae cause a gall-like enlargement in the root. Galls have been measured at 2-4 cm long and around 1cm in width.

PUPAL STAGE: Pupation occurs in spring to early summer within the galled root. The pupal period lasts about two weeks. The new adult weevil chews a hole in the root to emerge.

DISPERSAL METHOD: The adult weevil is flightless. Walking is the only method of dispersal.



Photo 1: Cyphocleonus achates (adult)

RANGE

Native (European) Distribution

Cyphocleonus achates has a wide native geographic range. It is found in eastern and southern Europe and Asia Minor, including the former Czechoslovakia, Austria, Hungary, Romania, Bulgaria, Greece, Turkey, Syria, and the former USSR. The original agents imported into North America came from Austria, Greece, Hungary, and Romania.

Potential North American Distribution

Prior to the introduction of *C. achates* into North America, European reports indicated that *C. achates* was expected to establish throughout the warmer and drier parts of the range of diffuse and spotted knapweed in North America.

HABITAT

C. achates attacks spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*Centaurea diffusa*), showing a preference for spotted knapweed. Larvae mine and gall the central vascular tissue of the roots. Newly hatched larvae mine into the root cortex. Feeding by older larvae causes considerable damage to the root. Adult weevils feed on the foliage but do not seem to have a significant impact on older host plants. However, in laboratory observations, young knapweed rosettes were completely defoliated and if the central bud was attacked the plant was killed.

The growth of small knapweed plants is stunted and the shoots of attacked plants are significantly shorter and produce fewer flowers than those of healthy plants. Seed production is reduced due to feeding within the root crown impeding nutrient flow into the stem.



Photo 2: Comparison of plant and root size of unattacked (left) vs attacked (right) plants

Ministry of Forests, Forest Practices Branch (Range Section)

Because spotted knapweed plants produce a larger root than diffuse knapweed, the attack rate is often much higher on spotted knapweed. More larvae are able to survive in the same plant. Multiple or repeated *C. achates* attack has been noted to cause reduced knapweed growth and increased mortality.

C. achates 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 and dry-warm. The weevils seem to prefer well drained, sandy textured soils that lack dense vegetation other than knapweed. Sites which accumulate heat are preferred, such as south facing slopes or flat, open areas. One observation noted the weevils established well on hot sites with gravelly soils that were adjacent to water bodies.

See Appendix A for detailed information on Spotted Knapweed (*Centaurea maculosa*) and Diffuse Knapweed (*Centaurea diffusa*).

4. HISTORY OF INTRODUCTION

C. achates was approved for release in North America in 1987. The Ministry of Forests Propagation Facility (MOFPF) at Kamloops received a total of 49 adults in September of 1987. After a confinement period to ensure breeding, 35 of the adult weevils were placed into propagation cages. (Seven pairs were sent to an uncaged release site near Osoyoos.) An additional 26 weevils were released at Selkirk College in the Nelson Forest Region that same year.

The propagation tents were maintained from 1987 to 1991 after which time they were terminated. Due to a high attack and mortality rate of host weeds it was difficult to maintain plants in the plots. Containing the weevils was also a problem. They either chewed through the mesh or possibly burrowed under the tents.

Two field collection sites were established in the Kamloops area at Cinnamon Ridge in 1988. These sites have been used since 1991 for collection and redistribution of *C. achates*.



Photo 3: Cinnamon Ridge collection site, Kamloops Forest Region

Plots were re-established at the Kamloops facility in 1995. There was concern that *C. achates* was not as advanced in the biocontrol program as desired since no new large yielding collection sites had been found in the Kamloops Region. The plots were dismantled in 1997 since existing field collection sites were still yielding large numbers of weevils and many more sites were found to have established.

Selkirk College propagation facility also received agents in 1987. A total of 26 adults were released into two plots. In 1988, one more plot was established, as well, 9 weevils were field released. By 1989 it became apparent that the weevils could not be contained, so tenting the plots was terminated. *C. achates* were field collected from the propagation area from 1989 to 1994. The site yielded 2998 weevils for redistribution between 1988 and 1992 at which time extensive plant mortality decreased the value of the site for collection.

The monitoring of field releases in the province has established several field sites for the collection of *C. achates*.

SUMMARY OF COLLECTIONS AND RELEASES

The following tables summarize collection and redistribution data. Any differences between the numbers reared/field collected and the numbers released can be attributed to: 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.

	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Reared & Collected	79	306	269	636	1,087	3,028	2,051	11,255	11,906	28,528	18,487
Released	75	286	227	360	676	2,066	2,052	10,908	11,770	28,475	18,618

Collection and release summary of Cyphocleonus achates in B.C.

Propagation Facility

Insects Reared in MOF Propagation Facility Tents

	1987	1989	1990	1991	1995	1997
Insects Received for Propagation Tents (# of tents)	35(1)	71(1)	86(1)		240(5)	(4)
Reared at MOFPF (# Insects)	35	306	22	0	420	

Kamloops Forest Region

Field collections

#Insects								
SITE	1989	1991	1992	1993	1994	1995	1996	1997
Aberdeen							1,844	621
Aberdeen & Sugarloaf							209	
Aberdeen & Sugarloaf & Cinnamon Ridge						3,300		
Cinnamon Ridge	1	56	1,181	1,388	3,923	1,400	8,817	3,244
Deadman								560
Sugarloaf Rd					34		583	696
Okanagan Falls								303
#9 Rd. Oliver							835	334
Okanagan Falls and Oliver								322
Research Branch trial				139				
TOTAL	1	56	1,181	1,527	3,957	4,700	12,288	6,018

DISTRICT	1987	1988	1989	1991	1992	1993	1994	1995	1996	1997
Clearwater		30 (2)					199		500 (5)	500
							(2)			(5)
Kamloops		54 (1)		336	812	535	2,745	2,580	4,599	2,600
				(3)	(8)	(5)	(28)	(25)	(46)	(26)
Lillooet					400	200	500	600	800 (8)	400
					(4)	(2)	(5)	(6)		(4)
Merritt		30(1)			277	209	1,498	1,100	2,260	1,100
					(3)	(3)	(15)	(11)	(24)	(11)
Penticton	14 (1)				100	394	1,700	1,000	2,235	786
					(1)	(4)	(17)	(10)	(23)	(6/7)
Salmon Arm							402	100	400 (4)	
							(4)	(1)		
Vernon				200	209	300	964	700	2,240	400
				(2)	(2)	(3)	(9)	(7)	(23)	(4)
TOTAL	49 (2)	240	86 (1)	536	1,798	1,638	8,008	6,320	14,773	5,786
		(5+)		(5)	(19)	(17)	(80)	(65)	(149)	(56)

Field releases by district #Insects(#Releases)

Nelson Forest Region

Field collections

#Insects	#Insects								
SITE	1989	1990	1991	1992	1993	1994	1995	1996	1997
Genelle								396	18
Kootenay Canal			41		108	866	640	11	
Lone Pine								119	507
Nine Mile					604	5,442	4,950	7,758	2,596
Salmo						959	1,600	5,300	8,226
Selkirk	246	636	601	996	159	31			
Sheep Trap							10	2,656	1,222
TOTAL	246	636	642	996	871	7,298	7,200	16,240	12,569

Field releases by district #Insects (#Releases)

#Insects (#Rele	eases)						
DISTRICT	1987	1988	1989	0990	1991	1992	1993
Arrow	26 (1)	20(2)	15 (2)	102 (2)	82 (1)		
Boundary			16 (2)		100 (1)		77 (1)
Cranbrook							100 (1)
Golden							
Invermere							
Kootenay Lake			82 (7)	258 (4)			78 (1)
Revelstoke							
TOTAL	26 (1)	20 (2)	113 (11)	360 (6)	182 (2)	No releases	255 (3)

DISTRICT	1994	1995	1996	1997
Arrow	860 (8)	2,030 (20)	6,484 (74)	3,774 (33)
Boundary	1,152 (11)	2,100 (17)	2,000 (20)	5,014 (45)
Cranbrook	100 (1)		160 (1)	1,180 (11)
Golden			100 (1)	
Invermere		300 (3)		
Kootenay Lake	629 (6)	710 (7)	3,623 (40)	2,312 (22)
Revelstoke			200 (2)	300 (2)
TOTAL	2,741 (26)	5,140 (47)	12,567 (138)	12,580 (113)

Other Forest Regions

Field releases by region

#Insects(#Releases)

REGION	1991	1992	1993	1994	1995	1996	1997
Cariboo							
Quesnel				100 (1)			
Williams Lake			109 (2)	100 (1)			
100 Mile House	65 (1)		50 (1)	100 (1)			
Prince Rupert					100 (1)		
Vancouver							
Campbell River						360 (4)	
Chilliwack				100 (1)		300 (3)	
Duncan						100 (1)	
Port Alberni		100 (1)				100 (1)	
Sunshine Coast							228 (2)
TOTAL	65 (1)	100 (1)	159 (3)	400 (4)	100 (1)	860 (9)	228 (2)

Other Release Destinations

Distribution numbers recorded in this table are restricted to the releases given <u>directly</u> to municipalities and native Bands by this office. Total field distribution numbers will be higher since MOF regions and districts maintain relations with these and other groups and share a portion of their releases with them.

#Insects (#Releases)

	1991	1995	1996
Native bands			400 (4)
City of Kamloops			959 (9)
Highland Valley Copper Mine			200 (2)
MOE Ecological Feserves			80 (1)
Alberta – Native Bands			400 (4)
Ontario – Guelph		200 (2)	
USA	100(1)		
TOTAL	100 (1)	200 (2)	2,039 (20)

5. REDISTRIBUTION

Redistribution of agents is a critical part of the biocontrol program. To ensure distribution throughout *C. achates*' potential provincial range, personnel must be able to recollect from field releases and make releases into new sites. Since the weevils do not fly, increasing their rate of spread is of particular importance.

FIELD COLLECTION

What and where to collect

Field collection of *C. achates* involves visually locating adult weevils in a knapweed infestation and hand picking them from plants or off the ground. Weather conditions determine location of the weevil. During hot weather, the weevils can be found on the upper portions of the stem and on the flowerheads. During cool and wet weather, they bury themselves in the leaf litter and loose soil around the base of the plant. Newly emerged weevils can be found sitting on flowerheads where they crawl presumably to enhance hardening of their cuticle by the sun.

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/metre2.
- Topographies of successful sites have varied. All have been able to accumulate heat units. A hot climate is needed.
- Elevations above 800 m have lower establishment success rates (best success in Nelson Region is below 700 m). At higher elevations the typical growing season is probably too short to allow establishment. There has been some success on

limited microsites at higher elevations, however, these populations are expected to die out after a cold, wet year.

- Soils should be free of active soil disturbance and coarse textured. Fine, uniformly textured soil appears to have a negative effect on establishment.
- Sites receiving cold air drainage may be poor choices, especially if they are relatively flat, allowing cold air to pond.
- Preferably on Crown land with easy access within 100 km 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 *C. achates* in the field. See discussion under Field Release.

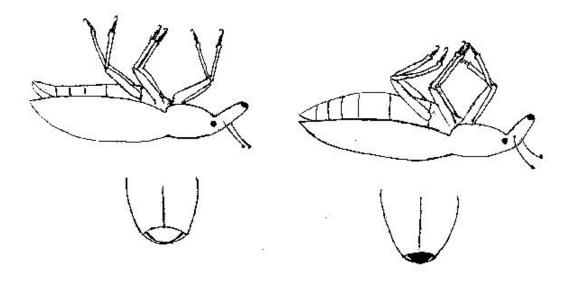
How to collect

The weevils are hand picked with bare hands and placed into containers with a mesh opening in the lid (Nelson Region uses ice cream pails). Mesh screen on the storage lid is critical to allow ventilation and to prevent a build up of condensation that can drown the insects. Depending on experience the task can be accomplished several ways: tipping the plant over the collection container (this works best with large numbers of weevils per plant); tipping the plant over your hand; or plucking the weevil off the plant from below to avoid the weevil dropping to the ground. Avoid pulling a clinging weevil off a plant. They need to be gently persuaded to avoid injury. What ever the method used, beginner collectors may find it easier to crouch down at the plant level.

Knapweed is placed in the containers to provide feed and a place for the weevils to cling (it also helps avoid weevils crawling over each other, fighting and injuring one another). Start with some plant material in the bottom making sure it does not reach the top, creating escape ladders. As more weevils are caught, add plant material and be ready to use the lid to prevent escapes. 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.

Plants need to be approached slowly. If the weevils notice movement or the plant is shaken, they drop to the ground and lie motionless on the soil where they are well camouflaged. During warm temperatures the weevils can be found in the upper plant canopy, often at the very top if it is particularly hot. Collecting during cool temperatures will entail looking for the weevils near plant bases, within or under basal leaves and often close to the root crown. Do not pull up plants when collecting if the intent is to maintain the infestation as a collection site.

In the lab, the numbers of weevils are confirmed, sexing is checked and they are put into containers in mating pairs. Early in the season surplus males are kept in separate containers until enough females are collected to make pairs. Due to the size of the weevils, the amount of knapweed they eat, and the frass they create, their numbers are kept at approximately 10 to 15 pairs per container. Containers are cleaned daily with warm water and replenished with fresh, preferably lush, knapweed that has plenty of leaf matter but also some stalk for the weevils to cling to. Pairs are kept only until 100 insects are collected. Just before shipping they are placed in two bulk food containers of 25 pairs each.



Picture 1: Characteristics for sexing *Cyphocleonus achates*

Once caught, the weevils are sexed and separated into male and female containers. This can take place in the field or back at the lab. Adult weevils can be sexed by turning them on their backs. Females have very round abdomens whereas the male abdomen is flat. While struggling to right themselves, the male abdomen curves upwards and becomes concave. The cloaca opens and a fawn-coloured, curved protrusion can be observed. The female cloaca does not open as large as the male, and it is triangular in shape and darker than the males. When a day's collection is complete, the total number of males and females is tallied and written on the containers.

Note: Nelson Region has gone to a random sexing of a portion of the collected weevils (1 bucket) from a site. Collection numbers have been so high at some sites that individual sexing is not practical. The weevils are maintained in ice cream pails and then counted 50 to a bulk food container prior to shipping for release.

When to collect

Time of Year

The collection period is between early July and mid October with peak collection generally occurring from mid August to mid September. *C. achates* can be found

Ministry of Forests, Forest Practices Branch (Range Section)

emerging well into October, however, it is felt that collection needs to be terminated earlier than this (mid September) to allow for establishment on release sites. Eggs require time to hatch and newly emerged larvae must be able to tunnel into roots before frost.

There has been a big variation in peak collection time between sites and on the same site from year to year. Emergence and hence collection seems to be dependent on weather, specifically the accumulation of enough heat units. No investigations have been undertaken to verify this, however, annual reports mention several times the effect of cold weather on emergence.

It has been noted that early emerging weevils are predominantly male (2:1). As the season progresses the ratio becomes more balanced and during the last few weeks females dominate. In recent years, the 50:50 male to female shipping ratio has been maintained until the proportion of females dominates in the field. At this time the ratio of males to females found in the field is the ratio used for shipping. It was also observed that early emerging males did not mate with available females until later in their life cycle. Egg laying was also observed to be less in the first weeks of emergence (agreeing with the noted M:F ratio and behaviour).

Time of Day

Sunny days have been found to be better for collecting than cooler days. Collecting on cloudy days is considered a less efficient use of time. Collecting is easiest during the afternoon (warmest part of the day) but can continue all day long. The weevils are on the upper parts of the plant during hot temperatures and are easier to find. In the morning and on cooler or rainy days the weevils are more difficult to locate but can be found at the base of plants and under dead leaf debris around plant bases.

SHIPPING

Collected insects are shipped to new release sites in 1 litre bulk food containers. 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 well ventilated and contain sufficient lush knapweed to feed the weevils during transport. Containers are packaged into carefully sealed boxes to avoid insect escape during shipment. Cold packs are wrapped first in plastic bags to contain the majority of condensation and then in newspaper or paper toweling to prevent further condensation from building up inside the containers and damaging or drowning the insects. These are used to keep the weevils cool and reduce their activity if they are traveling any distance. The agents are shipped quickly via courier or bus to release locations.

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 establishment.

Below are suggested release site criteria and considerations. They are based on observations of past sites which have been successful.

Criteria

- Release sites should be large enough to support a viable insect population with potential for natural dispersal.
- The average estimated knapweed density should be greater than 5 plants per metre2. (This is only a guideline. Soil conditions and lack of litter/soil cover seem to be more critical than plant spacings).
- Soils should be undisturbed (no active disturbance), coarse textured and have a minimum of litter. Fine, uniformly textured soil appears to have a negative effect on establishment.
- Topographies of successful sites have varied. All have been able to accumulate heat units. A hot climate is needed.

Considerations

- Elevations above 800m have lower establishment success rates.
- Sites receiving cold air drainage may be poor choices, especially if they are relatively flat, allowing cold air to pond.

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

- 1. <u>Plan release site locations prior to requesting agents</u>. Release sites should be preselected the fall or spring prior to release of agents. This avoids 'drop and dash' releases and promotes overall weed management planning.
- 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. <u>Make sure the site will not be disturbed after release</u>. Crown control of the site is preferred with future management known. Discuss future development plans for the site wherever it is located.

- 4. <u>Check previous release records and maps to ensure no prior release of the agent</u> <u>has been made at a potential site</u>. An unofficial rule is that a distance of 1 km constitutes a separate release.
- 5. <u>Monitor plants at potential site</u> to ensure the agent is not already present through natural dispersal.
- 6. <u>Check the immediate vicinity of the proposed release site for ant hills and wasp</u> <u>nests</u> to minimize predation.
- 7. <u>Mark selected release sites with a stake</u> so that it may be relocated to monitor insect progress and weed population decline.

Insect release

Before any weevils 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 consists of approximately 100 adult weevils (50 males and 50 females), transported in two bulk food containers of 25 pairs each. Nelson Region uses 50 females as minimum criteria for a release and in years with fewer males have made 50 female:30 male releases to 'stretch' the male numbers. Early shipments contained equal quantities of males and females. As the proportion of females collected increases, the proportion of females to males shipped may also increase, reflecting the same ratio as found in the field.

Agents are released primarily by Ministry of Forest regional and district personnel and occasionally staff from the Ministry of Agriculture, 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 weevils **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 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 weevils</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 reports from Europe commented on the compatibility of *C. achates* weevils and *Agapeta zoegana* moths. (See Summary of *A. zoegana* for information on the agent). It was felt there would be minimal if any displacement of the moths by the weevil. The moths were observed to attack relatively small rosettes whereas *C. achates* females selected larger rosettes and spent a longer time ovipositing on these plants. The larvae of *A. zoegana* fed mainly on the outer regions of the root while *C. achates* attacked the inner cortex. Larger plants (with larger roots) were able to support both agents.

Nelson Region has had success with releasing *C. achates* into sites that already have established *A. zoegana* populations. It is felt that *A. zoegana* 'opens' up the knapweed canopy and renders the site more suitable to *C. achates* establishment (warm air and sun reaches the soil to increase temperature).

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 again to provide for the longest possible establishment season.

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

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 sitesTo determine if collecting from the site has had any effects on plant or agent populations.
- 8. if collecting from the site has had any effects on plant or agent populations t.To determine potential collection sites.
- 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 (A)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 four 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 the above 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 a uniform number of plants randomly, if the site does not allow for either of the above transect designs
- 5. <u>Make two sweeps of the plants per metre with a sweep net</u> (a sweep is defined as a single pendulum swing in one direction). This works out to be approximately one sweep per step. Check the sweep net every five metres, count and record the number of weevils.

A form for this method (Biocontrol Agent Monitoring Form) is found in Appendix C. The average number of agents may be calculated per metre and recorded or the count for every five metres may be included on the form. Either way, the percent attack will be equivalent.

AGENTS

Sites can be monitored for the presence of adults from late June to September. Weevils can be observed on warm days in the upper plant canopy. The inside of knapweed roots are monitored for presence of larva or pupae May to early June. Roots are usually enlarged if there are *C. achates* larvae or pupae inside. Knapweed plants are dug up and the roots cut open.

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

larvae density	= <u>Total # larvae</u>	
	Per plant	100
frequency of attack	= <u>Total # of plants attacked</u> Total # of plants sampled	x 100
	Total # of plants sampled	
average larvae density	= <u>Total # of Larvae</u>	x 100
	Total # plants sampled x 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 *C. achates* can establish in the Bunchgrass, Ponderosa pine, Interior Douglas-fir, Interior Cedar-Hemlock and Coastal Douglas-fir 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 seed grain (apparently alfalfa) from southeastern 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. 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.

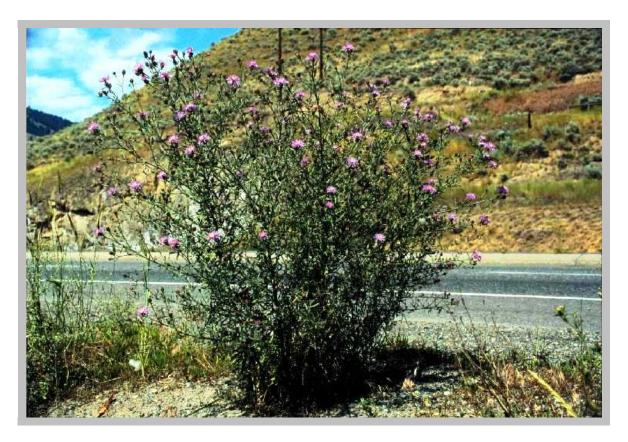


Photo 4: Spotted knapweed (Centaurea maculosa)

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.

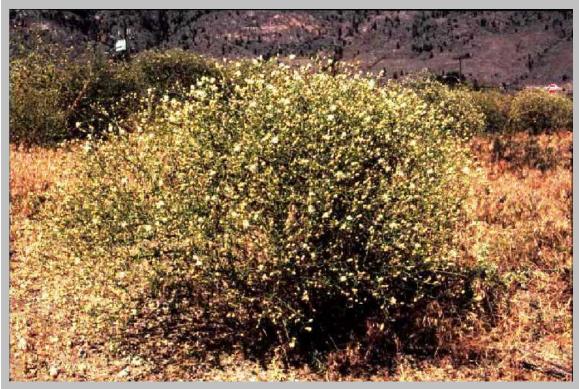


Photo 5: Diffuse knapweed (*Centaurea diffusa*)

Ministry of Forests, Forest Practices Branch (Range Section)

Appendix B - LITERATURE CITED

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.

Stinson, C.S.A. 1987. Investigations on *Cyphocleonus achates* (Fahr.) (Col. Curculionidae), a possible biological agent of spotted knapweed (*Centaurea maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) (Compositae) in North America. CAB International Institute of Biological Control, European Station.

Appendix C – RELEASE AND MONITORING FORMS

ALSO SER		/ 1			
B	IOLOGICAL	CONTROL REI	LEASE R	ECORD	
A COLUMN	SITE NUM	BER: D /		//_	
BIOAGENT:/	W	VEED SPECIES:		_/	
SOURCE:	STAGE: AD	ULT PUPA	LARVA	EGG OTHE	R
COLLECTION: / / /	RELEA	ASE:///		TIME::	
YY/MM/DD #RELEASED J	URISDICTION	YY/MM/DD	FLEASED	RV∙	
DISTRICT:					
PRIVATE LAND Owner:					
ADDRESS:					
BCGS MAP:	UTM: _ _		_	_	
WEED DENSITY: <1 plant/m ²		EASTING ts/m ² 6-10 pla	ant/m ²	NORTHING >10 pl	DATUM ant/m ²
SIZE OF INFESTATION:		101-400 m ²			
	2501-5000 m ²	5001-10000 r	m^2	>1 ha.	
WEED DISTRIBUTION: Continuou SLOPE %: ASPECT ⁰ :				ribution Code (1- OGEO UNIT:	
MONITORING for ESTABLISHME	ENT				
Date MonitoredYMDYesNo	Photo				
/ /					
//					
Comments					

RELEASE SITE MONITORING

DATE: AGENT:

SITE NUMBER: SITE NAME:					
MAP NO.:					
WEED DENSITY: 1 plant/m ² 6-10 plants/m ²		2-5 plants/m ²			
$\begin{array}{c} \text{SIZE OF INFESTATION:} \\ < 100 \text{m}^2 \\ 400 - 2500 \text{ m}^2 \\ 5000\text{-}10000 \text{m}^2 \end{array}$		$ \begin{array}{c} 100 - 400 \text{ m}^2 \\ 2500 - 5000 \text{ m}^2 \\ >1 \text{ ha} \end{array} $			
WEED DISTRIBUTION: Scattered Patches		Continuous Stand			
ACCESS TO SITE: Easy Describe if necessary:		Difficult			
SITE TOPOGRAPHY: Flat Forest opening Terraced Other (describe)		Bowl shaped Close to river/lake Hillside			
TRAVERSABILITY OF SITE: Easy Describe if necessary:		Difficult			
SOIL DESCRIPTION: Moss covered Clay Compact Sandy		Gravel Silt Loose Other (describe)			
SLOPE (%):	ASPECT (⁰):	ELEVATION (m):			
BIOGEOCLIMATIC CLASSIFICA DISTANCE FROM KAMLOOPS (
LAND OWNER:					
RECOMMENDATION:					
COMMENTS:					

BIOCONTROL AGENT MONITORING FORM

SITE NUMBER:				DATE: (YR/M	[/D)	
AGENT:			LOCATION:	LOCATION:		
RELEASE DATE:				TARGET PLA	NT:	
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: