RANGE

Operational Field Guide

to the propagation and establishment of the bioagent
Larinus obtusus
(Knapweed seedhead weevil)

June 2002

Province of
British Columbia
Ministry
of Forests

Information contained in this Field Guide is comprised of fact and field observations as of June 2002. 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 knapweed biocontrol agent *Larinus obtusus* 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 *L. obtusus,* 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, Food and Fisheries (MAF), Agriculture and Agri-Food Canada (AAFC), Centre for Agriculture and Bioscience International Institute for Biological Control (CAB IIBC) in Switzerland, the British Columbia Cattlemen's Association (BCCA), and several U.S federal and state agencies and universities.

Spotted and diffuse knapweed produce large quantities of seed. Biocontrol activity was initially directed at screening seedhead-feeding insects to reduce seed production, thereby slowing the spread of knapweed. It is recognized that the combined attack of several seedhead feeders is advantageous. *Urophora affinis*, *U. quadrifasciata* and *Metzneria paucipunctella* were introduced between 1970 and 1973 to achieve this purpose. *U. affinis* and *U. quadrifasciata* together have resulted in a decrease in knapweed's seed production from approximately 24,000 to 1,500 seeds per square meter (Harris 1991) while in 1991 Cranston reported the decrease to reach as high as 95%. The knapweed at White Lake, British Columbia is considered to be at, or just beneath, its

replacement level as a result of the attack of *U. affinis*, *U. quadrifasciata* and the root-feeder *Sphenoptera jugoslavica* (released in 1976) (Powell and Meyers 1988). Additional root-feeders were introduced to decrease knapweeds' vigour: *Agapeta zoegana* and *Pelochrista medullana* in 1982; *Pterolonche inspersa* in 1986; and, *Cyphocleonus achates* in 1987. As of 1990, the decrease to 1,500 seeds per square meter had not caused a decrease in knapweed density, and since the root attacking insects appeared to have limited habitat ranges, the screening and importation of additional seed-head feeders took place (Groppe 1990c). *L. minutus*, *Chaetorellia acrolophi* and *Terellia virens*, all introduced in 1991, supplement the impact of *U. affinis* but in different habitat niches. These latter agents are well adapted to the Mediterranean and continental, dry habitats. *Larinus obtusus* was introduced into British Columbia in 1992 to cause the further decrease of knapweed seeds and to fill the niche of moist knapweed habitats (Groppe 1992).

L. obtusus would not adversely affect other seedhead biocontrol agents previously introduced. Firstly, competition will not occur between species preferring separate habitats. For example, in Europe, *L. obtusus* habitat does not overlap habitat for *L. minutus*, *C. acrolophi* or *T. virens* (Groppe 1992). Secondly, attack of the seedheads occurs at different stages of growth so direct competition is often avoided. For example, the galls produced by *U. affinis* attack "have hardened before the weevil attacks the flowerhead and thus cannot be harmed" (Groppe 1992). Thirdly, these insects have developed a variety of feeding strategies for survival. For example, some agents, like *U. affinis* and *T. virens*, tend to colonize dense infestations of knapweed while other agents, like *Chaetorellia* spp. seem specialized in attacking isolated plants (Harris 1998). Also, both *L. minutus* and *T. virens* use the same feeding strategy and oviposit into open flower heads thus avoiding heads which do not open, i.e. heads that have been attacked by receptacle feeders and woody gall formers (Groppe 1992). The extent of these interactions has yet to be investigated in British Columbia.

3. *LARINUS OBTUSUS*

Coleoptera: Curculionidae
Common name: Knapweed seedhead-feeding weevil

BIOLOGY

GENERATIONS PER YEAR: one

ADULT STAGE: Weevils are 5 to 7 mm with a dark-brown colour and patches of white hairs on their backs giving them a mottled appearance (Rees et al 1996). They can emerge from hibernation as early as April as seen in a field cage test in Delemont,

Switzerland. *L. obtusus* adults appear before any flower buds of their host plants are produced. In Rumania, weevils emerged in May and flowering began on May 21. In Yugolslavia, weevils emerged in mid-June and flowering began in the first week of July. Generally in British Columbia, weevils emerge in June but exact timing is site specific. Mating begins at the onset of flowering and continues throughout the weevils' lifespans. The weevils prefer to mate on the upper portions of the plants or on flowers. Mating activity is low in the morning and continually increases in frequency to its greatest level between 11 am and 3 pm (Groppe 1992). A European field test showed that females feeding on knapweed flowers developed fertile eggs while females feeding on knapweed leaves did not develop eggs (Groppe 1992). Females continue to feed on flowers significantly while ovipositing "often cutting off several florets" (Groppe 1992). Females chew holes into the center of a few adjacent marginal florets, cover the area with a secretion and oviposit their eggs into newly opened flowerheads, therefore creating an egg case (Groppe 1992). Another European field test showed that although the ovipositing period lasted five to six weeks after flower feeding began, 50% of the eggs were laid at three weeks after commencement of flower feeding. A 1998 test showed females laid between 21 to 81 eggs with a survival rate of 25 to 80% (Groppe 1992).

EGG STAGE: Eggs are yellowish, round to oval in shape and range slightly in size, for example, up to 1.11 mm in length to 0.80 mm in width at the European test sites (Groppe 1992). Even when weevil populations are dense, the females tend to lay only one or two eggs per flowerhead and more than one adult can develop within a seedhead depending on its size. Laboratory tests showed egg development occurred in 1.5 to 2.5 days at 32^{o}C and 25^{o}C respectively, in 6 to 15.5 days at 20^{o}C and 15^{o}C respectively and not at all at 10^{o}C. Twenty-five degrees Celsius is considered to be optimum for egg development, although, when contained within its egg case, eggs are protected from fluctuating environmental conditions such as temperature (Groppe 1992).

LARVAL STAGE: Egg hatch is dependent on temperature (see above). Once hatched, larvae move down into the flowerhead, feeding as they go and consuming seeds and pappus hairs. Larvae took 17 days to develop through three instars to pupation in an outdoor field trial in Europe (Groppe 1992). Third instar larvae create holes in flowerheads and cover them with pappus hairs, seeds, etc., stuck together to form upright cocoons approximately 5 to 8 by 8 to 9 mm in size (Groppe 1992).

PUPAL STAGE: Pupation occurs within the cocoon created by the larvae. Outdoor European field trials showed pupal development took about nine days (Groppe 1992).

F1 ADULTS: In Europe, emergence of F1 adults occurs five weeks after the onset of flowering. They join the hibernated weevils (parents) to create a total and greater peak

than the peak seen three to four weeks previously consisting of only hibernating weevils (Groppe 1992). F1 adults feed on foliage before moving to hibernation places in the soil and litter around the root area. It is known that these weevils “can hibernate twice and live for a second summer” but it is not known if they can oviposit a second summer (Groppe 1992).

DISPERSAL METHOD: The adult weevil walks and flies to reach its mate and host plants.

Photo 1 *Larinus obtusus* adult

RANGE

Native (European) Distribution

Larinus obtusus has been reported throughout the central and western range of spotted knapweed (*Centaurea maculosa*) and diffuse knapweed (*C. diffusa*). Its preference is for the moister habitats of these plants (Groppe 1992).

Two subspecies of *L. obtusus*, *L. obtusus* ssp. *obtusus* and *L. obtusus* ssp. *australis*, exist across a wide range in Europe (Figure 1). Insect populations involved in screening tests came from Rumania and Yugoslavica, which, according to the map, would be *L. obtusus* ssp. *obtusus* (Groppe 1992)

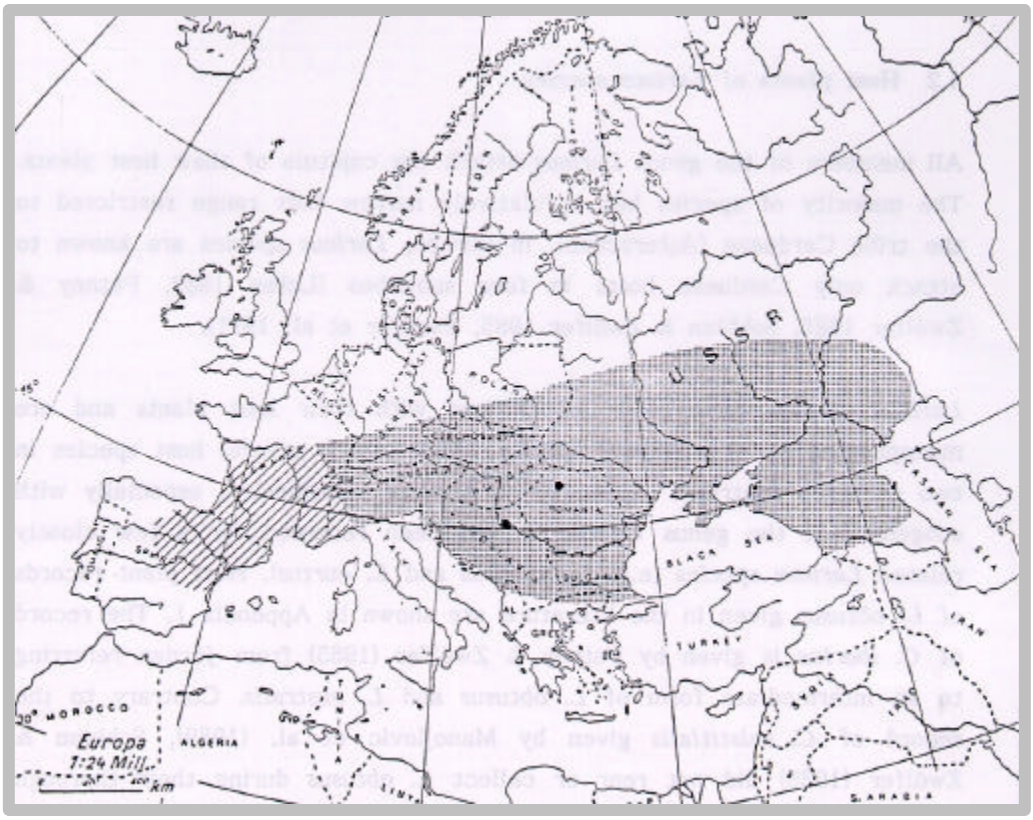

Figure 1: Native European distribution of *L. obtusus* ssp. *obtusus* (dots) and *L. obtusus* ssp. *australis* (thatched) and the locations of the two test populations (Groppe 1992)

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

Predicted North American Distribution

L. obtusus was expected to cover the same general range in North America as *L. minutus* (Figure 2). However, *L. obtusus* and *L. minutus* do not co-occur in Europe (Groppe 1992).

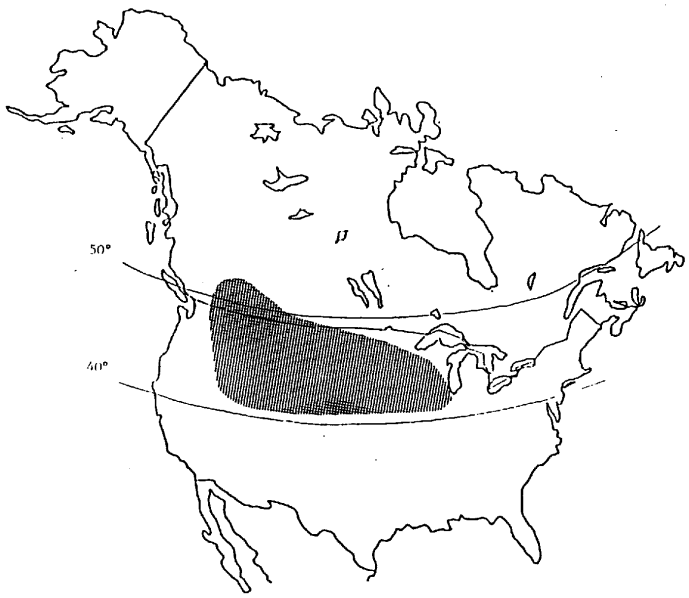

Figure 2: Predicted distribution of *Larinus obtusus* in North America (Groppe 1992)

Ovipositing *L. obtusus* females prefer large flowerheads. Since spotted knapweed flowerheads are larger than those of diffuse knapweed and *L. obtusus* prefers the moister areas of knapweed ranges, *L. obtusus* would do best attacking spotted knapweed in its moist habitat sites (Groppe 1992).

Knapweed in North America occurs up to altitudes of 3,000 m, two times the elevation known for European sites (Groppe 1992).

HABITAT

L. obtusus will attack both spotted and diffuse knapweeds. In B.C., the weevils have successfully established in the moist and warm range of spotted knapweed's habitat. Typically, the knapweed plants found in this range are tall and lush as seen at Squaam Bay and Hiuihill Creek, both in the Interior Douglas-Fir moist warm two zone (IDFmw2).

Photo 2: Spotted knapweed at Squaam Bay

Photo 3: Spotted knapweed at Hiuihill Creek

In the Nelson Region, the Interior Cedar-Hemlock dry warm (ICHdw) biogeoclimatic zone is preferred by *L. obtusus* over other zones, even the Interior Cedar-Hemlock moist warm two (ICHmw2). Supporting evidence involves releases made in the year 2000. Of 25 releases made in the ICHdw zone, 22 (88%) were positive when monitored in 2001. However, only 14 of 21 sites (67%) in the ICHmw2 zone were positive when monitored in 2001. Additionally, weevils at the ICHdw sites dispersed further (almost all more than 500 m) than those at the ICHmw2 sites. Further supporting evidence involves three releases made in 1994. When monitored in 2001, the single release in the ICHdw had experienced a population explosion and a large dispersal pattern (Photos 4 and 5). Conversely, the two sites located in the IDF undifferentiated (IDFun) zone had not experienced a large increase and dispersal patterns totalled a mere 100 m.

Photo 4: Kootenay Canal 1994 release

Photo 5: Kootenay Canal 1994 release close-up

Unfortunately, the Kamloops Forest Region does not have any listed ICHdw zones. Early Kamloops Forest Region releases (made in 1993 and 1994) in the Ponderosa Pine very dry hot (PPxh2) and a variety of very dry IDF (IDFx) sites did not establish with *L. obtusus*. However, subsequent releases (1999 and later) made in IDF very hot dry (IDFxh1 and IDFxh2) sites that were in close proximity to significant water bodies (large creeks, rivers and lakes) have established. The moderating effect of these water bodies appear to create micro habitats similar to the moist, warm spotted knapweed range. The 2000 release seen in Photo 6 is located next to Weyman Creek. Additionally, *L. obtusus* has been released into the IDF moist warm two (IDFmw2) and the ICH moist warm two and three (ICHmw2 and ICHmw3) zones in the Kamloops Forest Region since 1999. These biogeoclimatice zones have also been conducive to *L. obtusus* survival which further supports the observation that the weevils prefer the moist and warm range of spotted knapweed's habitat. These zones, however, are limited in this region and available sites will soon be exhausted.

Photo 6: Weyman Creek release

Although the 1999 *L. obtusus* release in the Vancouver Forest Region was damaged, it is expected that subsequent releases made in the zones of the IDF wet warm (IDFww) (Photo 7), the Coastal Douglas-Fir moist maritime (CDFmm), and the Coastal Western Hemlock moist submaritime (CWHms1) (Photo 8), dry submaritime (CWHds1) (Photo 9 and 10) and very dry maritime (CWHxm1) will establish and expand. *L. obtusus* should do well in almost all spotted knapweed sites in the Vancouver Forest Region.

Photo 7: Spuzzum release

Photo 8: North of Sailor Bar release

Photo 9: Yale release

Photo 10: Emory Creek release

The highest elevation in the Kamloops Forest Region for *L. obtusus* establishment is 865 m (Baldy Mt. Road in Clearwater Forest District) while in the Nelson Forest Region, sites between 600 m and 1000 m yield the highest number of weevils.

Photo 11: Baldy Mountain Road release

To date, the most northern established site of *L. obtusus* is found approximately 8 km south of Blue River in the ICHmw3 at 693 m. Also in this zone, not as far north but at a higher elevation (729 m) is a site near Wells Gray Park. The plants are sparse and the soils are cobbled, yet, the moisture and temperature are conducive to *L. obtusus* survival.

Photo 12: Most northern established site of *L. obtusus*

Photo 13: Site near Wells Gray Park

Slope does not appear to be a factor as establishment has occurred on a large range of slopes. Aspect may be a minor factor as establishment has occurred on sites with aspects ranging from 28^{o} to 280^{o} but no sites have been reported positive in the Kamloops Forest Region with a northern exposure. However, this may be a site specific feature. In the Nelson Forest Region, establishment has occurred on all aspects of sites, even north and north-eastern facing sites. These latter sites were found in Kootenay Lake Forest District where the biogeoclimatic zone is the most favoured by *L. obtusus*, i.e. ICHdw.

Soils found at sites where *L. obtusus* established in the Kamloops Forest Region were clay/loam or silty layers over well-drained coarse materials. Oxeye daisy and moss have been seen at many established sites in the Kamloops Forest Region. Compacted gravel till to silt loam soils are found in the Nelson Forest Region sites.

As with other biocontrol agents that reside in the soil throughout the winter, good snow cover is assumed to be beneficial to protect *L. obtusus* weevils from drastically varying temperatures.

4. HISTORY OF INTRODUCTION

Screening of *L. obtusus* occurred between 1988 and 1991. *L. obtusus* was approved for release in 1992 and the first releases into British Columbia were made in 1993.

Of the 2,905 weevils received from CABI in July 1993, 100 were sexed to ensure an equal ratio of males to females and placed in a tent at the Ministry of Forests Propagation Facility (MOFPF). When monitored in the tent and in two field releases later that year**,** no weevils, nor evidence of their existence was found.

In spring of 1994, no adult weevils were found at the MOFPF. An additional 200 were added to the same tent in July, 1994. Again, these were sexed for an equal ratio of males to females. Adults were found on August 22 and September 26, 1994. These weevils were expected to overwinter in the soil and plant litter and emerge in the spring of 1995. Weevils were also found at all four 1993 releases in the Kootenay Lake Forest District.

In early June, 1995 weevils began to emerge from the tent at the MOFPF, but the population was small. *Urophora* flies, which were abundant in the plot, were aspirated from the tent to reduce any possible interspecific competition. The residual population of weevils was left to reproduce in the tent, therefore, no weevils were redistributed to the field in 1995.

Also in 1995, four field sites in the Arrow Forest District and three sites in the Kootenay Lake Forest District were monitored for establishment. Varying results were found but when these sites were re-monitored in 1996, all proved to be positively established. This variation was also seen in 1997 when three of five (60%) sites were found to be established.

The Kamloops Forest Region field sites were monitored over several years. All early releases (made in 1993 and 1994) were put into zones of PPxh2, IDFxh2, IDFxh204, and IDFxw01. None were found to be positive.

In 1998, one field release in the Cariboo Forest Region, eight field releases in the Kamloops Forest Region and five field releases in the Nelson Forest Region were monitored. Zero, four (50%) and five (100%) of the field releases were found to be established in each of the Forest Regions, respectively.

In 1999, the single *L. obtusus* tent was dismantled at the MOFPF. Possibly three of the nine sites in the Kamloops Forest Region that were monitored were established with *Larinus* weevils. However, *L. minutus* had been released close to these sites and with the help of Dr. Rob Bourchier (AAFC), the sites were found to be established with *L. minutus*. In the Nelson Forest Region, releases of *L. obtusus* and *L. minutus* were not placed in close proximity to one another so confidence was high that all of the six field releases monitored and found established were indeed *L. obtusus*. When the Kootenay Canal site in the Nelson Forest Region began to yield *L. obtusus* weevils for collection in 1999, redistribution of this agent occurred in the Nelson, Kamloops and Vancouver Forest Regions. Sites chosen in the Kamloops and Vancouver Forest Regions had environmental characteristics as similar as possible to the successful sites found in the Nelson Forest Region.

In 2000 and 2001, the *L. obtusus* population at Kootenay Canal continued to increase in number and has been the sole supply of this agent for all regions in British Columbia.

When monitored in 2000 and 2001, 25 of 30 (83%) of the 1999 and 2000 releases in the Kamloops Forest Region were positively established.

To date, 82 of the total 96 (pre-2001) sites in the Nelson Forest Region have been found positively established (85%).

The single 1999 *L. obtusus* release in the Vancouver Forest Region was found mowed in 2001, subsequently no weevils were found. Confidence is high, however, that subsequent 2000 and 2001 releases made in this region will establish in the IDFww, the CDFmm, the CWHms1, the CWHds1 and the CWHxm1 zones.

SUMMARY OF COLLECTIONS AND RELEASES

The following tables summarize collection and redistribution data.

Collection and release summary of *Larinus obtusus* in B.C.

	1993	1994	1995	1996	1997	1998	1999	2000	2001
Reared & Collected			0	0	0	0	18,400	38,400	40,760
Released	2,905	3,200							

Propagation Facility

Insects Reared in MOF Propagation Facility Tents

	1993	1994	1995	1996	1997	1998	1999[a]
Insects Received for Propagation Tents (# tents)	100	200					
Reared at MOFPF							

[a] The *L. obtusus* plot was excavated March 2000 after determining the lack of success at the propagation facility was due to improper habitat conditions.

Cariboo Forest Region

Field releases by district

#Insects(#Releases)

DISTRICT	1993	1994
Chilcotin		
Horesfly		
Quesnel		
Williams Lake		300 (1)
100 Mile House	200 (1)	
TOTAL	200 (1)	300 (1)

Kamloops Forest Region

Field releases by district

#Insects(#Releases)

DISTRICT	1993	1994	1999	2000
Clearwater			800 (2)	6400 (11)
Kamloops		900 (3)	1600 (3)	1400 (3)
Lillooet				
Merritt	200 (1)	600 (2)		
Penticton				
Salmon Arm			800 (2)	2400 (3)
Vernon	1565 (3)			2600 (5)
TOTAL	1765 (4)	1500 (5)	3200 (7)	12,800 (22)

Nelson Forest Region

Field collections

#Insects

SITE	1999	2000	2001
Kootenay Canal	18,400	37,400	40,760
Kootenay Canal East		1000	
TOTAL	18,400	38,400	40,760

Field releases by district

#Insects(#Releases)

DISTRICT	1993	1994	1999	2000	2001
Arrow	290 (1)	1200 (3)	8600 (25)	12,000 (28)	10,400 (16)
Boundary					
Columbia					
Cranbrook			600 (2)	1000 (2)	1200 (1)
Invermere					
Kootenay Lake	550 (3)		5600 (12)	12,200 (24)	22,960 (33)
TOTAL	840 (4)	1200 (3)	14,800 (39)	25,200 (54)	34,560 (50)

Vancouver Forest Regions

Field releases by district

#Insects(#Releases)

DISTRICT	1999	2000	2001
Campbell River			500 (1)
Chilliwack	300[a] (1)		5200 (7)
Mid Coast			
Port McNeill			
Queen Charlotte Islands			
South Island			500 (1)
Squamish		400 (1)	
Sunshine Coast			
TOTAL	300 (1)	400 (1)	6200 (9)

[a] 400 *L. obtusus* weevils were shipped, 100 died in transport.

5. REDISTRIBUTION

Redistribution of agents is a critical part of the biocontrol program. To ensure distribution throughout *L. obtusus*' 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 collection of *L. obtusus* involves sweeping or visually locating adult weevils in a knapweed infestation and hand picking them from plants. Weather conditions have some effect on locating the weevils. Prior to bloom, the weevils climb up the plants when the temperature rises above approximately 25ºC. After bloom starts, they are up on the plants feeding and mating for most of the day. A seemingly more important factor in collection than daily temperature variations is the position in the weevils' cycle. Prior to flower formation, the weevils are located on the stems, rosette leaves and florets. Following flower formation, and particularly when ovipositing begins, the weevils are found on the flowerheads.

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/m^2.
- Topographies of successful sites have varied. All have been able to accumulate heat units. A moist warm climate is needed.
- Clay/loam or silt over well-drained coarse material is the common soil texture found at many sites in the Kamloops Forest Region with established *L. obtusus* populations. Compacted gravel till to silt loam soils are found in Nelson Forest Region sites.
- 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 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 *L. obtusus* in the field. See discussion under Field Release.

How to collect

L. obtusus weevils are swept from knapweed plants and separated from other debris by either aspirating the weevils out of the sweep net or putting the net contents in a

refrigerator to cool the insects down and then separating out the weevils, or a combination of both methods. Aspirating the weevils from the net while in the field works best as seeds, plant parts, other insects, etc. can be left at the site and the weevils are not jeopardized by storing them with spiders and other predators. It is easier for an experienced person to obtain a 'clean' container when it is hot since the weevils are more active and more readily climb out of the debris found in the bottom of the net. Depending on care taken, experience and luck, the collection may be free of any contaminants listed above, but, it is good practice to still clean the collection after placing the weevils in a refrigerator. It has been observed that approximately two to three weeks after the numbers of weevils greatly increase (~ mid-July), the weevils begin to tenaciously cling to the plants and are difficult to collect with sweep nets. This action may coincide with ovipositing. At this time, it is more efficient, and less harmful to the insects and their host plants, to hand pick with bare hands or aspirate the weevils as they cling to the flowerheads. Aspirating directly off the plants is not as effective when the weevils are really clinging. Plants need to be approached slowly when using these methods. 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. Once this 'clinging' activity has begun, however, collections should subside to ensure the collection site continues its population and the females transported to new locations still have eggs to be oviposited to start a new colony.

The weevils are placed into containers with a mesh opening in the lid. Mesh screen on the storage lids 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 containers (this works best with large numbers of weevils per plant); tipping the plant over your hand; or plucking the weevils off the plant from below to avoid the weevil dropping to the ground. When it is hot and the weevils are active, all methods that require constantly opening the lids of the containers are not effective as the weevils will fly back out. It is best in this case to combine hand picking and aspirating. 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 as it takes experience to develop an 'eye' for the weevils on the plants.

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). Do not use plant material that is too lush as it produces a lot of condensation inside the containers that may drown the insects. Remove open flowerheads before placing knapweed into containers so females will not be encouraged to oviposit and no eggs will be lost. 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.

Indoors, the numbers of weevils are confirmed. *L. obtusus* can be sexed, looking for characteristics similar to those used for sexing *Cyphocleonus achates*, yet, a strong hand lens or a microscope is needed so generally this is not done. If the weevils are to be kept for any length of time before releasing, they should be stored in quantities of 100/container. The weevils will need to be transferred to clean containers every two days and supplied fresh knapweed for foraging. When readied for field delivery, containers may be combined to make quantities of 200/container.

L. obtusus may also be collected by harvesting seedheads in late August, just prior to the emergence of the next generation of adults. Enough stem should be left attached to the seedheads so several may be tied together in small bundles which are then tied to individual plants at the release site. Do not pull up plants when collecting if the intent is to maintain the infestation as a collection site. In order to obtain an estimate of the survival rate of the weevils, open the heads of several plants and calculate an average number of weevils per plant, percentage of seedheads infested or number of weevils per seedhead if harvesting spotted knapweed. The larger head can support more than one developing weevil. This method is more time consuming and there is a high potential to spread seeds from site to site.

When to collect

Time of Year

The collection period is between late June to late July with peak collection generally occurring in the second and third weeks of July. Peak collection may be up to two weeks earlier in the Nelson Forest Region than in the Kamloops Forest Region, depending on the weather. *L. obtusus* can be found into the beginning of August, however, it is felt that collection needs to be terminated earlier than this (at the end of July) to allow for establishment on release sites (and to avoid collection of any early F1 emerging adults). Eggs require time to hatch, pupae must develop and adults must be able to emerge and feed for a short time before entering the leaf litter prior to frost.

The dependence of emergence and hence collection on the accumulation of enough heat units has not been observed or investigated. The literature does, however, describe the dependence of larvae hatching at an optimum temperature of 25^{o}C (Groppe 1992).

It is unknown whether male *L. obtusus* emerge before the females from the soil/leaf litter in the spring to create an uneven sex ratio of newly emerged weevils. It is possible to identify male and female *L. obtusus* weevils but it requires either a strong hand lens or a microscope and a method of slowing the weevils down, such as putting them in a cooler or refrigerator, to investigate the shape of their abdomens. This is not practical

for field work. This fact, combined with the time period needed for the female's ovaries to mature, leads to the practice of leaving the first observed weevils in the field until the population increases and then collecting without determining the sex of the weevils.

Time of Day

L. obtusus weevil activity has been found to be dependent on weather conditions, the time of day and is closely linked to the flowering period of local host knapweeds (Groppe 1992). Sunny/partially cloudy days have been found to be better for collecting than cooler days. In 1999, collection numbers in the Nelson Forest Region with partially cloudy days were on average 517 weevils/hour; sunny days were on average 404 weevils/hour; and cloudy/rainy days were on average 225 weevils/hour. Temperature is also important as shown by higher numbers collected at 30^{o}C (in 1999, 650 weevils/hour) than collected at 20^{o}C (in 1999, 400 weevils/hour). On hot afternoons weevils have been seen to fly around. Keeping these factors in mind, collection can generally take place between 10 am and 5 p.m. Additionally, prior to flowering the weevils can be found all over the knapweed plants. Yet, when flowering commences, the weevils tend to congregate on the top third of the plant and even cluster in groups of two to five on the flowerheads (maximum number found in a cluster was 15 in 1999).

It is recommended not to sweep for the weevils in or following rain. Caution should be taken when hand-picking the weevils following a rain shower. The weevils can either drown in small amounts of water or their elytra (wing covers) can be damaged when they get stuck to the canvas net or the wall of the containers.

Additional considerations

The CAB IIBC September 30, 1994 Quarterly Report on Weeds notes that continual yearly collection of weevils from a site will cause a significant decline in their numbers. Refraining from collecting for a year or more may be necessary to allow the population to recover. Depending on operational goals, it may be worthwhile to rotate collection sites from year to year to sustain their insect populations, but keeping in mind the potential for the knapweed infestation to decline below the level that provides for a good collection site.

SHIPPING

Collected insects are shipped to new release sites in 1 litre bulk food containers. Traditionally, one container (100 insects) was 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 200 insects are recommended. In the Nelson Forest Region releases with varying numbers were made in the year 2000. When monitored in 2001, the sites having had 200, 400 and 600 weevils released the previous year had 50%, 77% and 85% of the sites established, respectively. A single

release made in the year 2000 with 1,200 weevils was positively established in 2001. The remaining 50%, 33% and 15% of the sites having had 200, 400, and 600 weevils released on them may still prove to be established. The number of weevils at these sites may be below a current detectable level. Therefore, for fast detection and increase in population numbers, the most efficient number of weevils to use for a release would be between 400 and 600. However, some releases made with 200 weevils have proven positive the following year and yielded a small number for collection in their second year. The decision for optimum numbers to release will be dependent on the number of weevils available to collect and on the type of habitat available at the recipient site. Ideal habitat sites will ensure a greater chance for establishment and faster increases in weevil populations.

The shipping containers are well ventilated and contain sufficient knapweed to feed the weevils during transport. Be certain to remove any open flowerheads before placing the knapweed in containers so females will not be encouraged to oviposit and no eggs will be lost. 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. The cold packs 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 that have been successful.

Criteria

- Release sites should be large enough to support a viable insect population with potential for natural dispersal.
- Soils may be clay/loam to fine and uniformly textured, but preferably well-drained.
- Sites with other vegetation present, even moss, have been reported as suitable for *L. obtusus*.
- The average estimated knapweed density should be greater than 5 plants per m^2. (This is only a guideline, a moist, warm habitat seems to be more critical than plant spacing.)

- Sites should not be shaded.
- Topographies of successful sites have varied. All have been able to accumulate heat units. A warm, moist climate is needed.

Considerations

- *L. obtusus* is virtually indistinguishable in the field from *L. minutus* (another knapweed seed-feeding weevil). *L. obtusus* can be distinguished from its close relative, *L. minutus* by the shape and size of their rostrums (snouts). "*L. minutus* has a shorter snout that widens at the end so in general more of a triangle tip whereas the *L. obtusus* has a longer more finger-like snout with no flaring at the end" (Bourchier pers comm 1998). However, this rostrum feature is virtually impossible to distinguish in the field and even poses difficulty for trained entomologists in the laboratory. Even though these two agents do not co-exist in their European homeland due to different habitat requirements, care should be taken when making releases so as not to overlap these two species or place them too close together so their sites merge. The future goal is for these two agents to cover the total breadth of their habitats and therefore reside in close proximity, but until they do, it is advantageous to have them separated in order to determine their survival success and host weed control.
- *L. obtusus* experiences mortality from parasitism, pathogens and predators (mainly birds when they seek seeds in the capitula) (Groppe 1992). Large bird populations, therefore, such as those found around marshes, should be avoided when making releases of *L. obtusus* weevils.
- Releases at any elevation should be attempted. The highest recorded elevation where establishment has been successful is 865 m in the Kamloops Forest Region and 1,090 m in the Nelson Forest Region.
- Combinations of site features should be kept in mind when deciding on release sites. When liberating weevils at sites that are in their extreme range of one feature (e.g. IDFxh) consider choosing sites where other features are moderate (e.g., average elevation and a southerly aspect).
- 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. 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.
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 that 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. 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 bird colonies, ant hills and wasp nests to minimize predation.
7. Mark selected release sites with a stake and take GPS coordinates 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.

Releases of 200, 400 and 600 should be considered. See the discussion under Shipping for more details.

Primarily Ministry of Forests regional and district personnel and occasionally staff from MAFF release agents. 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. Mark the release site with a semi-permanent stake and take GPS coordinates to assist relocation efforts for follow-up agent establishment and weed impact monitoring.
2. Fill out the 'Biological Control Release Record' (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**.**

3. Create accurate site maps complete with permanent tie points. This is essential for future monitoring of the release site.
4. Take photographs. 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. Gently release the weevils (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 again to provide for the longest possible establishment season. Preferably, the plants will be at the same phenological stage at the release site and the collection site.

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 the agent 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; and
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. However, once the type of information is identified, the monitoring techniques should be consistent for all sites to be able to compare the resulting information. 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. Find the release stake or from the description on the release form, the closest position to the release stake. Mark a starting point.
2. Determine four directions 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. Pace out three parallel transects at least 2 metres apart: two 33 m long and one 34 m long if the site does not allow for the previous transect design.
4. Pace out two 50 m parallel lines 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. Make two sweeps of the plants per metre with a sweep net (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 consistently, for example, every five metres, count and record the number of weevils.

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

AGENTS

Sites can be monitored for the presence of adults from as early as May, but generally in June, for weevils emerging from hibernation, then through July, August and early September for the F1 generation. There may be an overlap in the two generations sometime in late July or early August. Weevils can be observed on the plants in the spring, either on the stems, rosettes or florets prior to flower formation and later on the

flowerheads. Temperatures can range from 20°C to 30°C+. If it is quite cool or having just rained, the weevils may not be on the plants, yet when it is warm they move up onto the tops of the plants and can be easily seen (Photo 14). Characteristic 'shot-holes', the result of adults emerging from the seedheads, can be observed in late July and into the fall or even the following spring. Upon opening seedheads, the empty cavity created by larvae feeding on seeds is evident (Photo 15).

Photo 14: *Larinus obtusus* residing on the top of a spotted knapweed plant

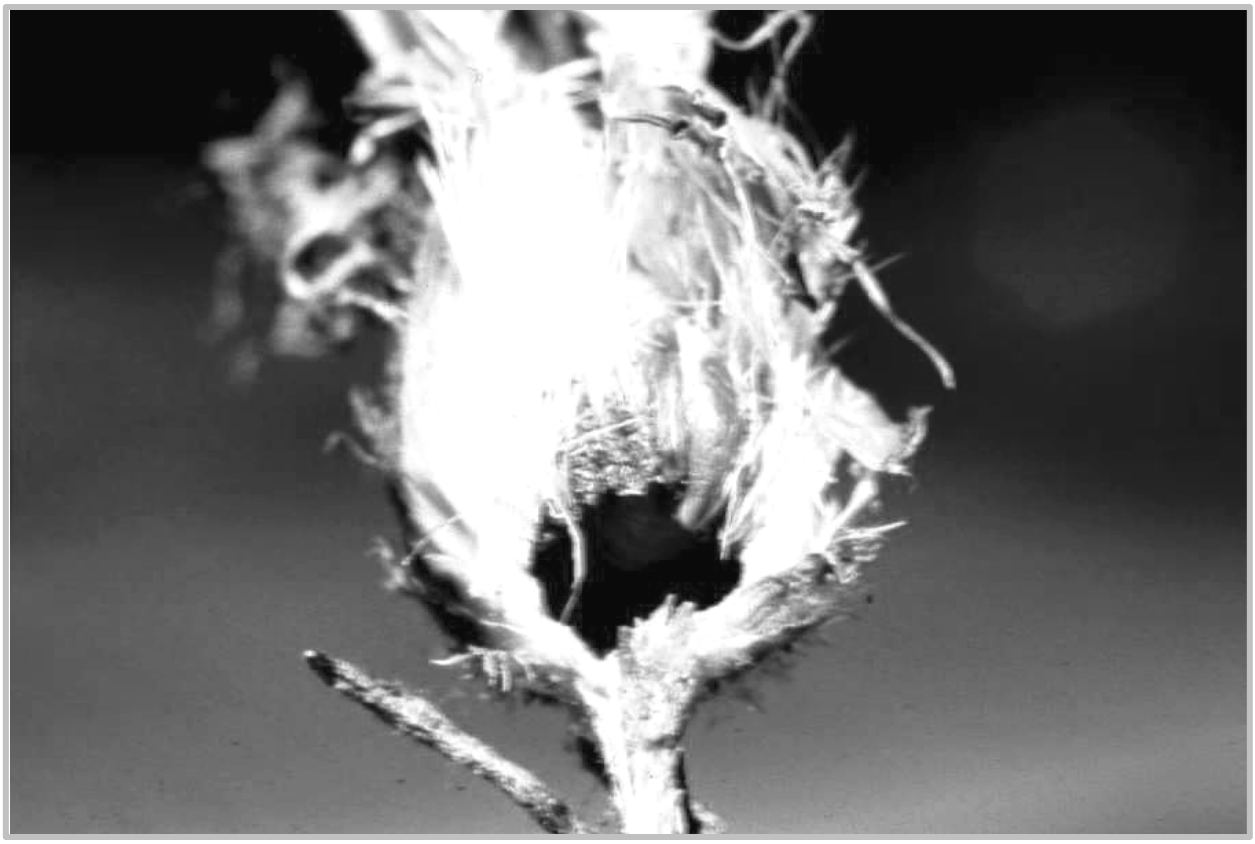

Photo 15: Cavity in spotted knapweed seedhead created by *Larinus obtusus*

Older releases of *L. obtusus* may disperse further depending on several factors, for example, health of the original weevils liberated, habitat conditions, site disturbance and weather over the years. Monitoring in 2001 in the Nelson Forest Region showed 1993 and 1994 sites had dispersed on average 1,575 m while 1999 sites had dispersed 230 m. These dispersal patterns may have been a result of population explosions between 2 to 5 years as suggested by the co-op student Corey Kuromi in 2001 or there may have been gradual increases of 100 to 200 m/year.

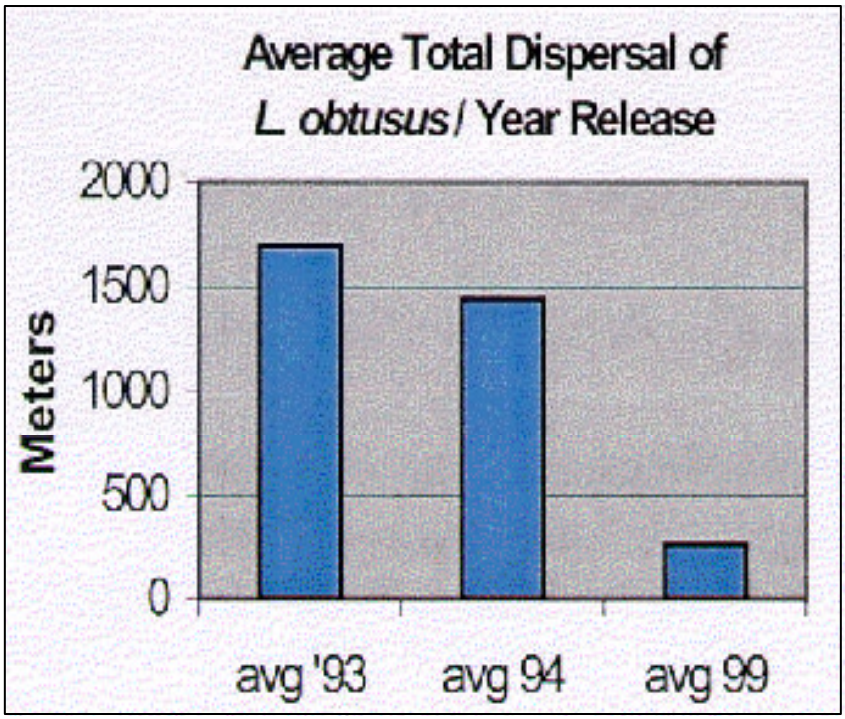

Figure 3: Average total dispersal of *L. obtusus* in 1993, 1994 and 1999 (Kuromi 2001)

If *L. obtusus* weevils do not appear established at a site, monitor adjacent patches of knapweed to determine if they have moved to more desirable habitat conditions than those at the original release location.

PLANTS

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

RESULTS

When attacked, most seeds within the flowerhead are consumed and others are used to form the cocoon. Attack rates at Rumanian and Yugoslavian field sites were 37% and 76%, respectively (Groppe 1992). The weevils do feed on knapweed leaves but the result has not been studied enough to distinguish the damage from other biocontrol agents. Studying the flowerheads for damage due to females feeding and ovipositing can indicate the existence of *Larinus* weevils.

To date, monitoring has found that *L. obtusus* can establish in the moist warm range of spotted knapweed found within the Interior Douglas-fir and Interior Cedar-Hemlock biogeoclimatic zones with a preference for the latter. Further zones containing the specific knapweed requirements (generally found along the coast) are expected to support *L. obtusus* populations. 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.

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, British Columbia, in 1893. Spreads by seed. Mature plants are 0.3 to1.5 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 to1.5 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 semi-arid rangeland in British Columbia (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 16: Spotted knapweed (*Centaurea maculosa*)

Diffuse Knapweed (*Centaurea diffusa*) Lam.

The plant

- A biennial to short-lived perennial, spreading primarily by seed. Stems are 60 to 90 cm in height with many branches. Leaves are 5 to 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 17: Diffuse knapweed (*Centaurea diffusa*)

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Appendix C - MONITORING FORMS

BIOLOGICAL CONTROL RELEASE RECORD

SITE NUMBER: **D** ___ ___/___ ___ ___ ___ /___ ___ /___ ___ ___

District /Agent Code /YY /Release #

BIOAGENT: _____ /_____ **WEED SPECIES:** ___ ___ ___ ___ / ___ ___

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

COLLECTION:__ __ / __ __ / __ __ **RELEASE:**__ __ / __ __ / __ __ **TIME:**__ __: __ __

Y M D **Y M D**

 # 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 plants/m^2 ☐ < 10 plants/m^2 ☐

SIZE OF INFESTATION: < 100 m^2 ☐ 101-400 m^2 ☐ 401-2500 m^2 ☐ 2501-5000 m^2 ☐

5001-10000 m^2 ☐ > 1ha. ☐

WEED DISTRIBUTION: Continuous Stand ☐ Scattered Patches: ☐

SLOPE %:___ **ASPECT^0:**___ **ELEVATION m:**___ **BIOGEO UNIT:** __ __ __/__ __ __/__ __ __

zone/subzone - variant/site series

MONITORING for ESTABLISHMENT

Date Established					Photo:	
Y	M	D	Yes	No	Yes	No
__	__	__	☐	☐	☐	☐
__	__	__	☐	☐	☐	☐
__	__	__	☐	☐	☐	☐

SKETCH MAP (Indicate North)

COMMENTS:

RELEASE SITE MONITORING FORM

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: _____