Rhinocyllus conicus Froel

INVASIVE SPECIES ATTACKED: Nodding thistle (*Carduus nutans* L.), bull thistle (*Cirsium vulgare* (Savi) Ten.), Canada thistle (*C. arvense* (L.) Scop.), marsh plume thistle (*C. palustre* (L.) Scop.) and plumeless thistle (*C. acanthoides* L.)

TYPE OF AGENT: Seed feeding beetle (weevil)

COLLECTABILITY: Mass

ORIGIN: France

DESCRIPTION AND LIFE CYCLE

Adult:

Adults are 3 - 7 mm long, oblong, black weevils (Powell et al. 1994; Harris 2005). They have patches of brown and light grey hairs distributed over their backs. Their rostrum (nose) is short, making it distinctly different from Larinus planus which occurs on many of the same plants (Harris 2005). The summer generation adults emerging in August have the same hair tufts as the spring generation, but the hair appears patchy yellow at first, which gives them the appearance of being covered with pollen (Coombs et al. 1996).

Fig. 1. *R. conicus* adult (credit Powell et al. 1994)

Adults emerge from overwintering locations in the spring depending on the latitude, for example, in mid to late April in Virginia U.S.A. while in Alberta they appeared in early June (Gassmann and Louda 2001). Emerged adults feed on foliage and disperse by flying, typically in the spring to seek out host plants for feeding, then breed and oviposit. Mating and egg-laying begins in early summer (Powell et al. 1994). Oviposition is concentrated at the beginning of the flowering season in Canada. The length of the oviposition period was found to vary from year to year in Montana (Gassmann and Louda 2001). Eggs are laid during the entire single flower phase of nodding thistle, but only during three waves of the plumeless thistle bloom phase. Each female lays an average of 200 eggs on involucral bracts of flower heads singularly or in multiples of two to five eggs (Harris 2005; Shorthouse and Lalonde 1984; Gassmann and Kok 2002). Over 50 eggs may be laid on a single terminal nodding thistle head (Harris 2005). Eggs can be easily found on the outside of the bud bracts in the third week in July in B.C. Females cover the egg with a cap of masticated plant particles (Shorthouse and Lalonde 1984). The cap appears as a wart, protects the egg from predation and supports the newly hatched larvae to penetrate the bract tissue (Gassman and Kok 2002; Shorthouse and Lalonde 1984). Over time, the cap turns light brown and is highly visible. Early oviposited eggs may be accidentally laid into enclosed terminal leaves, in these instances, the resulting larvae will not survive. The time to develop from an egg to a first generation adult varies in different locations (Gassmann and Louda 2001).

Fig. 2. *R. conicus* eggs oviposited on nodding thistle bracts.

The summer generation adults remain in their cell chambers for several weeks before they emerge, changing from cream or reddish tan to almost black. If they are in the seed head portion of the plant they will chew through the upper part of the flower bud. Those in the stem chew through the stem wall near the base of the bud and exit through the opening (Rees 1977). They remain on the plants for a short time before hibernating, however those that emerge during >16 hour days will go on to produce a second generation. Individual weevils can survive up to 15 months (Powell et al. 1994).

Egg:

Eggs incubate for 6 - 9 days (Powell et al. 1994). Laboratory tests showed a minimum threshold temperature of 11°C is required for egg hatching (Gassmann and Louda 2001).

Larva:

Larvae remain creamy white, "C" shaped, and with pale yellow-brown heads during all instar stages (Rees 1977). Laboratory tests showed a threshold temperature of 16-20ºC for larval development, depending on the stage (Gassmann and Louda 2001). If eggs have been laid onto leaves surrounding a terminal flower bud but no head develops, the larvae will not survive (Harris 2005). Smith and Kok (1985) state that newly hatched larvae can die from starvation if they do not receive the appropriate nutrition as they mine the bracts and the outside of the receptacle. This material may have less nutritional value or may contain a higher level of secondary chemical compounds than the receptacle tissue and the larvae may have depleted energy reserves when they eventually reach the receptable. The larvae tunnel into the receptacle and move either to central or upper receptacles or, rarely, to the peduncle. The cells near the tunnels turn brown (Shorthouse and Lalonde 1984). When the central receptacle is occupied, the larvae tunnel back and forth and feed initially on several types of cells then exclusively on callus cells in the tunnels and later in a chamber of callus cells that result from the feeding. When the upper receptacle beneath the developing floret is occupied, the larvae's feeding causes the formation of a chamber from the rapid proliferation of callus cells in this area. The larvae feed on these callus cells until they are consumed and as the larvae grow larger, they will feed on unaffected receptacle cells. In the rare instance the larvae inhabit the peduncle, they feed on the pith cells (Shorthouse and Lalonde 1984). In B.C., swollen upper stems have been observed which can indicate infestation by *R. conicus*. Multiple larvae can develop successfully within a single head, however, within heavily infested heads high mortality can occur, suggesting intraspecific competition (Gassmann and Kok 2002). In Montana, the mortality climbed from 23.3% to 82.5% when the number of larvae went from 15.6 to 21.3 per head (Gassmann and Louda 2001).

Fig. 3. ***R. conicus*** **larva in nodding thistle seedhead (credit Powell et al. 1994)**

Fig. 4. Nodding thistle seedhead with multiple ***R. conicus*** **larvae and chambers**

In Canada, larvae have been found to aggregate and the degree of aggregation varies from year to year. The number of larvae found infesting heads has been correlated with the size of the head (Gassmann and Louda 2001). Up to 65 larvae can develop in nodding thistle heads that have a 30 mm radius (Harris 2005). Infested heads can often be identified by protruding tufts of hair pushed out by the larvae (Gassmann and Kok 2002). Fourth instar larvae form an egg-shaped chamber with hard, thick, black walls made of plant frass and fecal matter (Shorthouse and Lalonde 1984). Multiple chambers fuse together and become a large mass (Rees 1977). Larvae complete feeding in 45-55 days after oviposition (Harris 2005).

Pupa:

Pupae are creamy white during the pupation period which takes place inside the chamber and lasts 8 - 14 days. New immature adults linger in the chamber for several weeks before chewing an exit hole to escape and feed (Rees 1977).

Overwintering stage:

Adult weevils emerge six weeks after oviposition then search for sites to overwinter in the soil, leaf litter or woody debris (Laing and Heels 1978).

Life Cycle Summary

Biocontrol Agent ↓	Activity of Interest	Jan to Apr	May		Jun		Jul		Aug		Sep		Oct to Dec
			1 – 15	16-31	1 – 15	16-30	1 – 15	16-31	1 – 15	16-31	1 – 15	16-30	
Rhinocyllus conicus	**Lifecycle**	overwintering adult	adult			larva		pupa	adult				overwintering adult
	Monitor			adult		egg/larva		pupa		adult			
	collect							larva/pupa		adult			

EFFECTIVENESS ON NODDING THISTLE HOST PLANT

R. conicus is an important agent intended for control of nodding/musk (*Carduus nutans*) and plumeless (*C. acanthoides*) thistles which reproduce strictly by seed. Predictions for use of *R.conicus* as a biocontrol agent were positive as this weevil has a propensity to lay large numbers of eggs and to disperse them (spreading the risk) and would exert significant pressure on its host plant once it was removed from the restraints placed on it by its co-evolved competitors and parasitoids in Europe. However, in Nebraska, they saw the adult weevils had a higher tendency to aggregate together than in Europe (Gassmann and Louda 2001).

Fig. 5. Mixed nodding and bull thistle *R. conicus* site in North Thompson area (Ponderosa pine zone)

Adults feed on foliage and leave signature rounded feed holes over the entire leaf surface. *R. conicus* have been found to deposit their eggs according to the size of the flower head. The trend was identified that showed more eggs were laid on larger heads. This supports the notion that larger heads have a greater capacity to support greater numbers of larvae but also reflects the relationship between total supply of eggs and flower heads, or that larger heads present more surface area for eggs to be deposited (Zwolfer and Preiss 1983).

R. conicus preferentially feed on the terminal buds and seldom choose the lateral buds of secondary flowers and feed in the stem just below the point of attachment of the flower (Rees 1977). A study in Gallatin Valley, Montana showed the *R. conicus* reduced the seed in terminal flower heads of nodding thistle but missed the later secondary heads (Gassmann and Louda 2001).

The larvae create larval cells and when there are many, the larval cells will occur in a continuous string, the longest of which recorded in the 1974 investigation in Montana was 47 cm. When many larvae feed in the stems, or when they congregate in that area, the flower above dies. The flower appears stunted since the area below is constricted from the dead tissue. The flower does not produce seed and the larvae experience high mortality in the dead tissue (Rees 1977). Where nodding thistle flower heads are highly attacked so they only consist of the involucral bracts, some pappus hairs, and the blackened larval chambers made of feces and frass, the heads will tend to be shaped like inverted hearts, unlike unattacked heads that are cylindrical-shaped. Additionally, the pappus hairs and floret tubes of attacked heads are shorter and have no purple petals unlike the longer pappus hairs, floret tubes and purple petals of unattacked heads (Shorthouse and Lalonde 1984).

C. nutans produces more stems and more flowers when damaged, but not killed, by herbicide or mechanical treatments, trampling, etc. *R. conicus* feed within the inflorescence which prevents seed development without initiating regrowth of the plant (Phillipps 1982). This feeding not only consumes seeds but also affects the viability of unconsumed seeds (Rees 1977). Monitoring in Saskatchewan showed a distinct decline in the nodding thistle plants and a decline in the number and size of flower heads. The production of callus cells as a result of the *R. conicus* larvae feeding mentioned above is gall-like and presumably decreases the plant's resources, potentially resulting in the decline in number and size of flowers in Saskatchewan (Shorthouse and Lalonde 1984). Each larva contributes by reducing an average of 16 seeds in the head it occupies and suppresses the development of seed in other heads. At two sites in Saskatchewan, the seed production was decreased by 99% (Harris 2005). U.S.A. studies have demonstrated *R. conicus* to be responsible for seed destruction and lowered viability of its host. Specifically in Virginia, a measured mean reduction in nodding thistle density of 95% was recorded after only six years (1969-1975) (Laing and Heels 1978; Rees 1977). In New Zealand six larvae inside receptacles 1.3 cm in size decreased seed production by 85%. When more than 20 larvae were found in receptacles, viable seed was rarely produced (Phillipps 1982).

Proper land management greatly enhances the control of thistle by the weevils, especially the prevention of overgrazing (Gassmann and Kok 2002). However, in Canada, forage losses to nodding thistle are considered insignificant. In the provincial prairies, nodding thistle is now found on disturbed lands around cities, on light soils in sheep pastures and where mechanical or chemical treatments of the thistle has subsequently reduced the number of weevils present (Harris 2005).

British Columbia History

Difficulties to control nodding thistle led to investment into Canada's contribution into the screening research that ran from 1961 to 1968 (Gassmann and Louda 2001). The main source of weevils introduced into Canada and the U.S.A. to control nodding thistle (and plumeless thistle) is from the southern French Rhine valley where *R. conicus* uses *C. nutans* almost exclusively as its breeding host (Zwolfer and Preiss 1983). *R. conicus* was first introduced into Canada in 1968 near Belleville, Ontario and Regina, Saskatchewan to control both nodding and plumeless (*C. acanthoides* L.) (Laing and Heels 1978). *R. conicus* was released into B.C. for control of nodding thistle (*C. nutans*) in 1979 west of Williams Lake. *Trichosirocalus horridus* (rosette/root crown feeding weevil) was also introduced for nodding thistle that same year. *Urophora solstitialis* (seed-feeding fly) was introduced in 1991 but was never recovered and *Puccinia carduorum* (rust) and *Larinus planus* (seed-feeding weevil) arrived adventively in the province. The weevil also feeds on the non-native thistles bull (*Cirsium arvense*), Canada (*C. vulgare*) and, more recently, marsh (*C. palustre*). Transfer onto subsequent host thistles has been explored in the literature.

Nodding (*Carduus nutans*) and Plumeless (*C. acanthoides*) thistles:
These plants are considered under biological control in B.C. *R. conicus* co-exist in nodding and plumeless thistle stands with *Larinus planus* and *Trichosirocalus horridus*.

Bull (*Cirsium vulgare*) and Canada (*C. arvense*) thistles:
The weevils appear to move freely onto these less preferred thistle species when nodding thistle becomes displaced. *R. conicus* larvae have been found in the same bull thistle seed heads with *U. stylata* larvae. However, most of the bull thistle flower buds are produced after the *R. conicus'* oviposition period. The weevils co-exist with *Larinus planus* and *Urophora cardui* in Canada thistle stands. Canada thistle can withstand attack by aborting attacked buds and replacing them, as well as delaying senescence in the male flowers until after the larvae have finished feeding (Harris 2005).

Fig. 6. *R. conicus* dispersal site on nodding thistle between Merritt and Kamloops (Interior Douglas-fir zone)

Marsh/marsh plume Thistle (*C. palustre*):
The Robson Valley houses the most extensive population of marsh thistle in the province. Significant ecological values depicted by several nearby federal and provincial parks, farming, and cattle range interests are at risk in this and surrounding areas. As marsh thistle poses a significant threat to B.C.'s interests, it was determined that investigation into the use of R. *conicus* was warranted. Historic screening research showed that *R. conicus* would attack *C. palustre* (Gassmann and Louda 2001). Between 1997 and 2000, attempts to establish *R. conicus* on marsh thistle with weevils collected from nodding thistle near Princeton in B.C.'s southern interior were made but none established sustainably. It was suggested that the weevil failed to adapt to climatic conditions in the Robson Valley which are significantly different than Princeton, or the weevil was not synchronized with marsh thistle, or a combination of both. *R. conicus* collected in Alberta were thereafter released in the Robson Valley in 2002, 2004 and 2005. The initial survival of *R. conicus* indicated there was potential for attack, however, long term population establishment failed on all sites. Gassmann and Louda (2001) suggest that "preference and synchronization, based on physical conditions and host plant quality, could influence host plant use by *R. conicus* in the field in North America."

Concurrently, funding was provided to CAB International for overseas surveys for additional potential Canada thistle (as this species is not yet considered under biological control) and marsh thistle biological control agents in 2004 and 2005 but was discontinued following the conclusion that all pertinent global areas had been surveyed and no new potential agents were found. No further screening was to take place (R. Bourchier pers. comm. 2006).

To determine if *R. conicus*' lack of survival was due to the weevil failing to develop viable life stages on marsh thistle in field conditions, a trial was conducted between 2010 and 2014 in the Robson Valley. Results of that trial are summarized and showed no failing of any life cycle stage. Multiple studies in the U.S.A have shown several patterns of *R. conicus* use of their thistles, one of which was "exponential growth on native thistles in Nebraska showed a long (i.e. more than 20 years) lag after introduction" (Gassmann and Louda 2001). With time, acclimation for persistent establishment in this area may occur.

Fig. 7. *R. conicus* dispersal site on Plumeless thistle near Kamloops (Interior Douglas-fir zone)

HABITAT AND DISTRIBUTION

Native:

R. conicus' native origin occurs in North Africa, South and Central Europe, the Caucasus Mountains, Kazakhstan and Asia Minor. Populations of R. conicus from the southern French Rhine valley have been the main source of weevils for control of nodding (and plumeless) thistle in Canada and the U.S.A. (Zwolfer and Preiss 1983).

The preferred habitat of thistles in Europe is in dry and warm areas below elevations of 600 m (Gassmann and Louda 2001). R. conicus establishes wherever the host thistles grow in well drained soils and favours dense stands over widely spaced or scattered patches (Powell et al. 1994; Harris 2007).

North America:

"Initial utilization of the native *Cirsium* species as hosts has been greatest on the early flowering species, and lowest on the high elevation (4150 m) species" (Gassmann and Louda 2001).

Although *R. conicus* can experience high larval mortality within nodding thistle heads, reportedly due to intraspecific competition, larval mortality in *Cirsium* thistle heads have been reported much higher in the Gallatin Valley in Montana a few years after the initial releases (Gassmann and Louda 2001).

Known Preferences in British Columbia on Thistle Species:

R. conicus has been found to have self-dispersed by March 2015 onto infestations of nodding (19 sites), plumeless (9 sites), bull (17 sites), and Canada (30 sites) thistles and recently onto marsh plume thistle (1 site) (Table 1) (BCMFLNRO 2015). The relative abundance of these dispersed sites amongst the species may not reflect a preference by the weevil but rather is likely a combination of relative abundance of the plant species in B.C. (nodding thistle has drastically declined in B.C. since the weevil's initial introduction in 1979), the accessibility of plant sites by the weevil and the attention given by invasive plant managers to recording these sightings.

Biogeoclimatic Ecosystem Classification Zones:

R. conicus has been released into a variety of biogeoclimatic (BEC) zones in the province. For more information, go to www.for.gov.bc.ca/hra/plants/application.htm. Subsequent monitoring of these sites has been intermittent. However, the natural dispersal of this agent into the different BEC zones has been documented at 76 sites by March 2015 (BCMFLNRO 2015). The habitat of the dispersal sites may be more indicative of the weevil's preferences than the survival, or lack thereof, at release sites. *R. conicus* has been found in the Bunchgrass (2 sites); Coastal Douglas-fir (22 sites); Coastal western hemlock (14 sites); Interior cedar-hemlock (3 sites); Interior Douglas-fir (26 sites); Montane spruce (1 site) and Ponderosa pine (8 sites) biogeoclimatic zones.

Site Criteria:

Site criteria have been summarized from the literature and from existing B.C. sites (BCMFLNRO 2015). In B.C., *R. conicus* has been released onto 97 sites (26 of which have been recorded as established) or self-dispersed to 76 sites on six invasive thistle species (Table 1). The summarized sites do not depict all ranges of these criteria the agent may currently or eventually occupy.

Table 1. Monitoring results of *Rhinocyllus conicus* as of March 2015 (BCMFLNRO 2015)

Monitoring Results	Bull	Canada	Marsh	Nodding	Plumeless	Scotch
# Releases	7	25	27	33	3	2
# Monitored Positive		2	20	4		
# Releases Monitored Negative		1	1	8		2
# Releases Not Monitored	7	22	6	21	3	
# Dispersal Sites	17	30	1	19	9	

Growing Season:

The length of daylight affects the potential for a second generation within a year. In Canada, when F1 generation adults had emerged by mid-June when the day length was more than 16 hours, the weevils were observed to be mating (Gassmann and Louda 2001). As noted above, successful establishment of *R. conicus* at a site and on thistle species is significantly influenced by the availability of flower buds when the weevil is ready to oviposit. This availability can be influenced by the growth habit of the plant, by the elevation or latitude it grows at and climatic variations. See also the Life Cycle Summary section above.

Site Size:

R. conicus have a reputation for seeking out isolated plants (Phillipps 1982). In B.C., the range of site sizes of releases is 0.0009 to 120.6 ha while the weevil dispersed on its own onto sites in the range of 0.0001 to 20 ha, of which 97% were under 4.5 ha, which is likely a reflection of the either the plant growth habit or sampling bias.

Plant Density:

R. conicus favours dense stands over widely spaced or scattered patches (Harris 2007). In B.C., *R. conicus* has established on sites with a plant density range of 1 plant/m^2 to greater than 10 plants/m^2. Establishment has been variable at release sites within this range: of the sites monitored, 0.8% of releases at sites with 2-5 plants/m^2 established; while 30.6 and 16.7% of releases established at sites with each of 6-10 and greater than 10 plants/m^2, respectively. Additionally, 35% of the locations *R. conicus* dispersed to in this range were recorded on sites with 1 plant/m^2 while 30.1% were recorded on 2-5 plants/m^2, 25% were recorded on 6-10 plants/m^2 and 0.9% were recorded on sites with greater than 10 plants/m^2. Density can also be described by Distribution Codes which combine density and cover and have a range of codes from 1 to 9 where 1 is a single occurrence of a plant and 9 is a continuous dense occurrence of a species (B.C. Ministries of Environment and Forests 1990). The weevil has established at sites with a distribution range of 1 to 9. While the release treatments have been placed at sites with a distribution code of 4 or higher, the weevil's self-dispersal has occurred relatively consistently across all sites from 1 to 8 with the highest percent (20.6%) occurring with a distribution code of 2. *R. conicus* does not appear to be influenced by plant density. Note that this summary includes all thistle species, some of which typically grow in denser patches than others (e.g. Canada thistle).

Ground Cover:

In B.C., *R. conicus* exists at sites with ground cover ranging from bare soils, to grazed forage and to significant ground cover with debris.

Competing Vegetation

Although *R. conicus* has not been witnessed in B.C. to be influenced by competition, a decline in nodding thistle was slower at sites in Virginia U.S.A with little competing vegetation. In particular competition by grass was found important to restrict thistle growth and infestations (Gassmann and Kok 2002).

Slope:

In B.C., the range of slopes of both established release sites and subsequent sites the weevil has dispersed to on its own is 0 to 45% slope.

Aspect:

In B.C., the aspect range of both currently established release sites and subsequent sites the weevil has dispersed to on its own is 0 to 338^o.

Elevation:

Host thistles preferentially grow at elevations below 600 m in Europe (Gassmann and Louda 2001). Plants growing at high elevations can have late flowering periods that may not be synchronized with the weevil's requirement of flower buds during its oviposition period. In B.C., the elevation range of both established release sites and subsequent sites the weevil has dispersed to on its own is 1 to 1575 m. In the U.S.A., weevils were found at 1829 m elevation over the Bozeman Pass in the Bridger Range in Montana and flying weevils were found at higher elevations in several canyons (Rees 1977).

Temperature:

According to a phenological model that describes the relationship between *R. conicus* and nodding thistle in Virginia, temperature may have a significant effect on the synchronization and, therefore, the level of attack of *R. conicus* on its host plant (Gassman and Louda 2001). The maximum rate for egg development was found to be above 31^{o}C but 36^{o}C was too high. The minimum temperature for egg hatching was found to be 11^{o}C. The temperature of 15^{o}C was determined to be below the threshold for the larval and pupal stages whereas 16-20^{o}C was found to be optimum. However, sustained high temperatures >24 ^{o}C can cause the roots to rot and plants to become chlorotic before flowering is complete, thus negatively affecting larval development. Larvae die in aborted and desiccated heads when temperatures reach 31^{o}C. The speed the plant matures and the rate of growth of the weevil in its various stages must be synchronized. Temperature affects development time and survival through to adulthood. If plants advance too quickly, they will not supply the larvae with food reserves at critical stages in development such as arriving inside the receptacle to begin feeding or preparing to pupate. When the temperatures are favourable more reproductive success is achieved and more weevils are available to attack the host (Smith and Kok 1985). Dry, hot sites with rapid temperature increases in early summer prevent optimal larval development and seed destruction and therefore, locations with moderate temperatures are more effective (Coombs et al. 1996). Weevils require a diapause period which is best supported by lower temperatures and shorter photophases (even no light). Where temperatures do not decrease or resume (26-32^{o}C), weevils will not enter diapause or will break it and begin feeding, copulating and ovipositing. Most weevils in a laboratory situation died after breaking diapause and feeding (Kok 1979). Sites near Edmonton, Alberta (measured between 1987 and 2006) that have been sources of B.C. weevils, and a subsequent site in the mid-east portion of B.C. (measured 1993 to 2006) have somewhat more extreme habitat conditions than the southern portion of B.C. Temperatures in these areas have been: mean temperature of 3.5 ^{o}C near Edmonton and 5 ^{o}C in mid-east B.C.; mean maximum 9.67 and 10.65 ^{o}C; mean minimum -2.7 and -0.75 ^{o}C; extreme maximum 32.2 and 32 ^{o}C; and extreme minimum -35.3 and -32.2 ^{o}C, respectively (Government of Canada 2015).

Moisture Regime:

Some moisture is required in diapause. In a laboratory situation where weevils were stored with senescent heads that provided no moisture, the weevils experienced high mortality by December and none survived through to April. Field conditions typically provide the slight moisture required (Kok 1979). Sites near Edmonton, Alberta (measured between 1987 and 2006) that have been sources of B.C. weevils, and a subsequent site in the mid-east portion of B.C. (measured 1993 to 2006) have somewhat more extreme habitat conditions than the southern portion of B.C. Total annual precipitation in these areas have been 441.9 mm near Edmonton and 697 mm in mid-east B.C. and total rainfall has been 355.8 mm and 533.8 mm, respectively (Gov. of Canada 2015).

Soil Moisture:

Laboratory studies on soil moisture and the addition of fertilizer revealed that *R. conicus* survival and weight was higher in conditions that were optimal for the plant. Moisture was proposed to be more important than fertilizer as it allows the plants to take up the nutrients that the weevils feed upon and conversely, dry conditions could potentially cause mortality (Dowd and Kok 1983). Gassmann and Louda (2001) note spring soil moisture effects the degree of larval aggregation in the heads; with higher spring soil moisture, there would be less larval aggregation and vice versa.

Soil Texture and Compactness:

No data on soil texture and compactness has been collected, however, as for all agents that overwinter in the soil or litter, it is critical that litter be present and if not, the soil is not so compact that the weevils cannot burrow into it.

Snow Cover:

Sites near Edmonton, Alberta that have been sources of B.C. weevils, and a subsequent site in the mid-east portion of B.C. have somewhat more extreme habitat conditions than the southern portion of B.C. Total snowfall near Edmonton (measured between 1987 and 2006) has been 86.8 cm and in mid-east B.C. (measured 1993 to 2006) has been 163.2 cm (Gov. of Canada 2015).

Disturbance:

Wind and rain were found to be the primary cause of egg and larvae mortality early in their development in Virginia (58.3%), while parasitism, larval crowding and other unknown factors were responsible for late larval and pupal mortality (9.7%) (Gassmann and Louda 2001). Eight species of parasites were found with *R. conicus* in a study area in Wyoming. All were considered general feeders and attack other insects as well. The range of parasitism by any of these species in Wyoming was low, from 0.00% to 1.33% while other U.S.A. sites investigated ranged from 0.00% to 7.03%. Generally, the higher numbers of parasites found on the weevils are first within the peduncles (up to 18.92% in California), then in the flowers (0.56% in Wyoming) where the terminal peduncle or flower had higher rates of attack than in the secondary and tertiary peduncle or flower. The parasites in North America feed on a wide range of hosts so their numbers do not build on *R. conicus* (Littlefield 1991). In Virginia, native parasites kill 3% of larvae in nodding heads, 16% in plumeless heads, and 19% in nodding stems (Harris 2005).

Feeding by *T. horridus* can delay flowering by approximately a week, therefore, having a negative effect on *R. conicus*' oviposition window. As well, *T. horridus* can create shorter plants and prevent flowering, both results having a negative effect on *R. conicus* (J. Nehols personal communication 2010). *T. horridus* can also cause the thistle to branch and create multiple, shorter stems, however, the resulting flowers are weaker (Coombs et al. 1996). It was shown that high populations of *T. horridus* can decrease the effect of *R. conicus*, however, as *T. horridus* contributes to the control of nodding thistle, the combined effort of both agents is higher than the individual agents acting alone (J. Nehols personal communication 2010).

Nodding thistle plants regrow if they are damaged or insufficiently treated with herbicide or mechanical treatment. Their response is to grow more stems and more flowers. *R. conicus* do not illicit this reaction from the plants (Phillipps 1982). However, the weevil is negatively affected by these activities.

Harris (2005) reports that the early bloom stage may be treated with 2,4-D to decrease seed production without adversely affecting the weevil. Yet, Rees (1977) reports that larvae are susceptible to herbicide treatment, particularly when the number per seed head increases. Higher mortality was found in seed heads with higher numbers of larvae when herbicide was applied.

R. conicus is susceptible to mowing. Generally all stages of the larvae die from mowing but many of the pupae and adults may survive. Grazing and trampling cause low weevil numbers as shoot development is retarded and fewer oviposition locations are available.

Agent Handling

Collecting:

Overwintered adults can be collected in the spring or newly emerged adults can be collected in August. Spring collections are recommended for most agents to allow for acclimation to the site and synchronization with the host plant in more favourable conditions. August collections may include weevils that still exist on the site from the previous year but will soon perish. The adults can be aspirated or swept off plants during bright sunny and warm days. However, thistle plants do not fare well from sweeping and create significant debris in the sweep nets. Adults can also be extracted from seed heads in August or allowed to exit from collected heads on their own within field cages.

If adults are kept in artificial conditions through their diapause period, they will require slight moisture. In a laboratory situation, 40-48% survival was achieved with weevils kept in 0-10 hours of photoperiod and provided slight moisture with an artificial diet which is comparable to field cage survival (Kok 1979). Field cages may be easier to manage when handling agents for collections held throughout diapause.

Containers carrying weevils following collection should be kept in cool locations and, when transported, within coolers also housing ice packs wrapped in paper towel to absorb unwanted moisture. Weevils should be released as soon as possible following collections; 1-2 days is recommended, particularly in the spring when the agents will be/are ovipositing. However, at this time, little redistribution of *R. conicus* continues to occur for most invasive thistles established within B.C.

For more detailed information on collecting, shipping and releasing methods and equipment, please refer to the document Biocontrol Agent Handling Techniques, for the collecting, shipping and releasing in B.C. which is located at www.for.gov.bc.ca/hra/Plants/publications.htm#operationalFieldGuides.

Releasing:

Establishment of weevil populations has been proven more efficient with spring releases (Laing and Heels 1978). The timing is very important for releasing *R. conicus* as the weevils' life cycle stage must be synchronized with the plants at the new site. If the weevils oviposit onto buds that are too young, the buds tend to become necrotic and, thereafter, die before the larvae can complete full development. If the weevils oviposit onto buds that are too advanced, the buds tend to bloom and desiccate before the larvae can complete full development (Smith and Kok 1985).

Monitoring:

In New Zealand weevils were found in a 50 km radius only six years after release (Phillipps 1982). In Montana, the weevils were found to have dispersed over a 1280 km^2 area in five years from 1969 to 1974 but in Virginia they were found dispersed 1.6 km after three years and 32 km after six years (Rees 1977; Gassmann and Kok 2002).

Eggs may be observed on flower bracts in mid-June. Larvae, pupae and new adults can be observed by opening seed heads: in the latter half of June to mid-July; in the latter half of July; and in August, respectively. Seed heads may also be collected and the adults allowed to exit on their own within a container if unclear on larvae identification. Care is needed in order not to spread resulting seed. Adults can be observed or swept off plants during bright sunny and warm days in August, although thistle plants do not fare well from sweeping and leave significant debris in the nets.

NOTES

- New Zealand found that *R. conicus* negatively affects the first of two generations of the fly *Urophora solstitialis* (attacks both nodding and plumeless thistles) occurring in that country when the two species overlap (Groenteman et al. 2011). However, in Ontario, *U. solstitialis* has generally displaced *R. conicus* from plumeless thistle heads (Harris 2005). There has been a single release of this fly in B.C. onto plumeless thistle, yet, it has never been recovered. At the time of the *U. solsitialis* release, records showed there was an abundance of frass and *R. conicus* weevils present on seed heads at the site (B.C. Ministry of Forests, Lands and Natural Resource Operations 2015*).*

- *R. conicus* and *T. horridus* have been observed to co-exist on nodding and plumeless thistles. In B.C., *R. conicus* also shares thistle sites with the adventive foliar feeding leaf beetle *Cassida rubiginosa*, the native foliar feeder moth, *Vanessa cardui* (painted lady) and the native bud feeding moth *Platyptila spp.* (artichoke plume moth).

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