Pterolonche inspersa Staudinger.

INVASIVE SPECIES ATTACKED: Diffuse knapweed (*Centaurea diffusa* Lam.),
Spotted knapweed (*C. biebersteinii* DC.) (potentially) and,
European Spotted knapweed (diploid) (*C. micranthos or C. maculosa ssp. micranthos, a synonym for C. biebersteinii ssp. biebersteinii)*.

TYPE OF AGENT: Root feeding moth

COLLECTABILITY: Passive Distribution

ORIGIN: *Hungary and Austria*

DESCRIPTION AND LIFE CYCLE

Adult:

Pterolonche inspersa are univoltine. Adult moths measure 14-28 mm long, are grey-white coloured and exhibit a silvery sheen on their wings[11,15]. They have narrow wings spanning 1.9-2.5 cm and hold them close to their sides when at rest[7,15]. The mouthparts of the adult moth are characteristically angled downward (S. Cesselli pers. comm. Oct. 2005). Adult moths exit the root through silken chimney tubes, created by the larval stage, from June to early-September while peak emergence occurs in mid-August[6,11]. Mating begins within 24 hours of emergence and 3-9 days later oviposition starts[5,11]. Egg laying occurs mainly from late afternoon through until midnight[8]. An average 142 eggs are laid individually or up to six in a cluster typically on the underside of rosette leaves[7]. Males lived 10-12 days while females lived 15-18 days under laboratory conditions during which the male/female ratio was found to be 1:1.5[6].

Fig. 1. *P. inspersa* adult
(credit: Powell et al. 1994)

Egg:

The black oval eggs are 0.039 x 0.025 mm with a slight depressed centre. They incubate for 12 days at 24.6°C. It is essential the weather remain dry during this time to prevent the egg 'shell' from becoming rubbery which would inhibit the larvae from emerging[7].

Larva:

P. inspersa larvae are pearly-white with inflated segments and small brown head capsules[7,15]. Mature *P. inspersa* larvae found in B.C. are larger and have a very pale blue iridescent sheen compared to the slightly smaller, creamy-white and slightly translucent mature larvae of the other root-feeding moth, *Agapeta zoegana*.

P. inspersa has five larval instars and they feed from September until the following June, about 11 months of the year. New larvae instars mine down the root, feeding mainly on the central portion or, less frequently, under the root epidermis. Their feeding causes galls to form and they leave a silken webbing behind[8,15]. Larvae will undertake cannibalism until a sustainable number remain which limits larval populations within roots. Normally one or two larvae develop on a single root, but as many as four have been observed[15]. However, in B.C. only a single *P. inspersa* larva has been found occupying a diffuse knapweed plant (S. Cesselli pers. comm. Mar 2020). Larvae overwinter as third instars within a silken case they create inside the root. Feeding continues the following spring until pupation. With spring feeding larvae spin a silken tube that lines the feeding area or the gallery their feeding created[6]. The tube, which can measure 2-2.5 mm wide, extends from the galls upward 3-5 cm[6,15]. The tube is often called a "chimney" and provides protection for the larvae and pupae and a means of escape from the woody root by the newly emerging moth[6]. The chimney is tan-coloured, appears grainy and commonly extends beyond the soil surface. It also can be created on the lower stem instead of the root crown (S. Cesselli pers. comm. Oct. 2005). During sunny days the larvae often lie in the tube above the soil surface and move downward when they encounter vibrations, their movement within the tube can cause it to "wave"[7].

Fig. 2. *P. inspersa* larvae in knapweed root

Fig. 3. *A. zoegana* larvae on knapweed root. (credit Powell et al. 1994)

Pupa:

In early to mid-July larvae begin to pupate[4]. Pupation takes about 15 days within the tube[15]. The pupal casing, which is typically discarded inside the root cavern created by the larvae, is transparent and very thin, even papery. The sheen of the moth's silver wings can be seen through the pupal case walls. This is in contrast to *A. zoegana* whose pupal case is opaque and reddish-brown (S. Cesselli pers. comm. Oct. 2005).

Overwintering stage:

The third instar larvae overwinter in a silken case in the root[6].

Life Cycle Summary

Activity of interest	Jan-Apr		May		Jun		Jul		Aug		Sep		Oct	
			1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31
Life cycle	overwintering larva		larva / pupa		larva/pupa/adult				adult		overwintering larva			
monitor					larva		larva/pupa		adult/evid		evidence			
collect					larva		larva/pupa		adult					
Notes	*Prefers diffuse knapweed. Adults are difficult to collect. Evidence = chimney formation.*													

EFFECTIVENESS ON HOST PLANT

In Europe, *P. inspersa* is reported to feed on both diffuse and spotted knapweeds but in B.C. it prefers the diploid diffuse knapweed[12] (S. Cesselli pers. comm. Apr. 2016). Larvae feed on roots, interrupting the vascular flow of nutrients to the plants[14]. Larvae that hatch near the center of the plant root mine into the woody core and larvae that hatch near the outer area of the crown feed on the cortex and outer root areas, all of which results in a loss of woody root texture[6]. Roots become spongy and fragile and secondary roots easily break apart[4]. Damaged roots attract other predators which move into the roots and provide secondary attack on the plant[14]. Larval feeding reduces the plants' ability to store nutrients which decreases the plants' height, size, colour and shape of the overall aerial structure and flowering ability[6,15]. In B.C., plants attacked by *P. inspersa* have many withered basal leaves, appear to be shorter than normal and may produce a single bolting stalk or multiple weak bolting stalks, with smaller, or no flowers (S. Cesselli pers. comm. Apr. 2016).

P. inspersa will attack, kill and feed on other larvae (including their own species) they encounter while feeding in the root, particularly the *Sphenoptera jugoslavica* beetle larvae as the location in the root of both agents is most similar. *P. inspersa* can co-exist with *S. jugoslavica* if they feed in different locations in the root: *P. inspersa* feeding in the lower and outer parts of the root will not encounter *S. jugoslavica* feeding in higher portions and the combination of both agent's feeding is thought to be more detrimental on the plant than the beetle alone[6]. Significant mining creates large cavities within the root similar to *S. jugoslavica* and *Cyphocleonus achates* (S. Cesselli pers. comm. Apr. 2016). *P. inspersa*'s carnivorus nature tends to prevent large numbers of larvae from attacking a single plant, however, as diffuse knapweed roots are generally small, the large feeding area of *P. inspersa* causes substantial damage[6]. In a study performed in B.C. by Newman et al. (2011) involving six study sites in the central southern interior, *P. inspersa* was found to co-exist at sites with the seed-feeders *Metzneria paucipunctella*, *Urophora affinis* and *U. quadrifasciata*, and *Larinus minutus* and the root-feeders, *S. jugoslavica*, *C. achates*, and *A. zoegana* although it was absent in the root with the highest count of *S. jugoslavica*. The adult moth's ability to move between plants of low density allows it to exploit sparse diffuse knapweed stands[7].

Fig. 4. Diffuse knapweed with *P. inspersa* larva attack.

The United States Department of Agriculture (USDA) performed the first screening studies of *P. inspersa* moths collected from diffuse knapweed plants in Northern Greece[6]. The screening took place in Rome and lasted from 1979 to 1984. The moth had high attack rates on the European and North American diffuse plants (chromosome complement is diploid with 2n=18)[12, 13]. These moths were imported to the U.S.

However, the moths released into B.C. were studied by Commonwealth Agriculture Bureaux International (CABI) and came from Pills, Hungary with a few from Gant, Austria[2]. *P. inspersa* collected from European *C. maculosa* in Hungary in 1983 were screened for release in Canada. Two strains were therefore suspected, one that prefers diffuse, the other spotted. However, the European spotted knapweed is not genetically the same as the North American spotted knapweed. In surveys, plants collected in the European region that included Hungary, Albania, Bulgaria, Czechoslovakia, Romania and Russian all turned out to be *C. micranthos* or *C. maculosa* ssp. *micranthos*, a synonym for *C. biebersteinii* ssp. *biebersteinii,* and a diploid (2n=18), unlike the spotted knapweed found in North America which is a perennial tetraploid (2n=36) and grows in much drier climates. The North American spotted knapweed is most closely matched to the European *C. biebersteinii* DC which is also a tetraploid. The North American spotted knapweed has also been equated to the European *C. stobe* and *C. maculosa* spp. *rhenana*, which may be a synonym for *C. rhenana Boreau*, but this too is a diploid. In surveys looking for the European tetraploid *C. biebersteinii* DC, there was only one instance it was reported; in a botanical garden in Hungary. It was concluded that studies should proceed with potential agents collected off the European diploid from Hungary as it at least had the closest climate match. The *P. inspersa* collected and studied by CABI was from the diploid European *C. maculosa*[12]. The *P. inspersa* in B.C. prefers diffuse knapweed, also a diploid like its European host plant.

British Columbia History

European surveys for potential biocontrol agents of knapweed began in 1961[12]. Following the establishment of the two seed-feeding *Urophora* flies (*U. affinis* and *U. quadrifasciata*) and the root-feeding beetle *S. jugoslavica*, the root-feeding moth *Pelochrista medullana* failed to establish. It was thought *P. inspersa* would fill this niche[6]. *P. inspersa* collected from European *C. maculosa* in Hungary was screened for release in Canada. Screening studies of *P. inspersa* began in 1983[12]. Shipments coming from Pilis, Hungary with a few from Gant, Austria for release into B.C. took place between 1986 and 1991 and were comprised of combinations of small numbers of adult moths, pupae and several eggs.

Propagation:

The first rearing attempts of *P. inspersa* were made in 1986 onto knapweed species in tented rearing plots at the Kamloops Propagation Facility (KPF). The first tented releases were with 55 + 26 for a total of 81 adult moths into four tents. Nothing was found established the following year and the plots appear to have been

discarded. In 1987, five new tents were seeded with knapweed species and received additional shipped *P. inspersa* (90 pupae, resulting in 28 moths, and 4500 eggs). The eggs were spread into two tents, and the small number of moths that emerged from the pupae went into three additional tents; 14, 7 and 7 moths, respectively. In 1988, the 6 moths that emerged from the five 1987 KPF tents were consolidated into a single tent seeded with spotted knapweed. Additional *P. inspersa* were released at the KPF in subsequent years: 1989 (63 moths), 1990 (25 pupae from which nine moths emerged) and 1991 (52 moths). A few deformities were noted with a small number of moths raised in these tents, for example, some emerged with no wings. After autumn of 1991, there are no further records of *P. inspersa* propagation tents at KPF. There is little detail indicating which knapweed species were used in the tents. It is possible the lack of success of propagation attempts was due to providing *P. inspersa* with spotted instead of diffuse knapweed plants or potentially there were too few moths released into some tents. Whichever may be the cause, there are no records of subsequent collections from the five KPF propagation tents for distribution into the field. Establishment of *P. inspersa* in B.C. was thus reliant on the field releases that took place during the same years of propagation.

As well in 1987, *P. inspersa* was released in the central Kootenays at Selkirk College, Castlegar onto two spotted knapweed sites. Seventy-seven pupae and 2 moths were released into a propagation tent while an additional 5000 eggs and 66 larvae were placed at an open site at the end of the soccer field[2]. The Selkirk sites were monitored frequently between 1988 and 2007. The tent did not remain in place long (dismantled prior to 1992) but in 1988 some root damage and evidence of old presence was found. Records indicate in 2000, chimneys were found at the tent location and at the end of the soccer field on spotted knapweed. These are the only mentions of *P. inspersa* on spotted knapweed in the province.

In 1991, 20 moths were placed onto diffuse knapweed in a release cage at the Agriculture and Agri-Food Canada (AAFC) Research Station in Summerland. There are no subsequent records of this project.

Field sites:

The original field treatment near Osoyoos in 1986 was made onto diffuse knapweed with seven larvae in poor physical condition during a rainy period. It appears to be the only field release made on diffuse knapweed that failed to establish. In 1986, 40 larvae were transferred onto a diffuse knapweed field site in the east Gilpin grasslands in the east Kootenays and a supplemental release of 7000 eggs was added to the same site in 1987. In 1991, two additional releases onto diffuse knapweed were made, one in the Lac du Bois grasslands near Kamloops with 73 adults and 200 eggs and one at the (AAFC) Research Station in Summerland with several hundred eggs placed onto uncaged rosettes surrounding the cages mentioned above. Two additional treatment methods were trialed in 2007 and 2008, both with diffuse knapweed plants: 25 larvae-infested plants collected from Lac du Bois were transplanted into a diffuse knapweed stand east of Kamloops; and six moths emerged from Summerland-collected roots laid in moist soil in wide, flat containers were released north of Kamloops. These latter two sites have also established; however, the moths may already have been present as they could have dispersed from the 1991 Lac du Bois site.

All field releases have been made on diffuse knapweed save one, near Castlegar. Monitoring records from 2000 have the moth establishing on the spotted knapweed in this area, however, all other field investigations in the province and subsequent monitoring around this site has found *P. inspersa* only to be infesting diffuse knapweed.

Since 1999, dispersal sampling has occurred in the geographic areas of all seven original field releases and found the moth dispersed on diffuse knapweed from the early established sites. In 2000, *P. inspersa* was found at Genelle, 16 km south of the Castlegar site, 13 years after the release. Chimneys found in the Cranbrook District at Pickering Hills in 2001 could have been created by moths migrating from the U.S. as none were released in this geographic area. The potential dispersal distribution of *P. inspersa* in the Kootenays to spring 2003 has been described: in the Arrow-Boundary Forest District from the junction of the Kootenay and Columbia Rivers south to Genelle and from Lone Pine pit west to Gilpin Creek; and in the Rocky Mountain Forest District on the south-facing slopes of Pickering Hills (Val Miller pers. comm. Apr. 2003). Near Kamloops the moth has spread over 20 km in 15 years. Similar monitoring in Summerland has shown dispersal to be less than around Kamloops. Infested with several biological control agent species, diffuse knapweed now occurs in widely spaced patches. In the Okanagan (Summerland area) significant urban development has contributed to the spacing between plant infestations. *P. inspersa* has also dispersed to the east side of Okanagan Lake (S. Cesselli pers. comm. Apr. 2016). In a study performed in B.C. by Newman et al. (2011) of six study sites in the central southern interior, *P. inspersa* was found established at five. The order of abundance of biocontrol agents found over all study sites from lowest to highest counts was: *U. affinis, L. minutus, A. zoegana, C. achates/P. inspersa, S. jugoslavica, U. quadrifasciata, and M. paucipunctella*. *P. inspersa* is generally left to disperse on its own throughout the range of diffuse knapweed in B.C.

HABITAT AND DISTRIBUTION

Native:

P. inspersa's native geographic range is south and southeast Europe. It occurs in Spain, France, southern Russia, Hungary, Turkey, Romania, Bulgaria, Italy and Yugoslavia. Its occurrence ends abruptly in Eastern Europe. It is found in central Hungary, but is scarce in northwest Hungary and Austria. It is notably absent in eastern Romania. *P. inspersa* commonly occurs on diffuse knapweed stands in northern Greece and western Turkey[7].

P. inspersa thrives in hot, dry sites where the plants become drought stressed from June to late-September and have low to moderate plant density[7,14]. Preferred soils are loose compositions of sand or gravel[15].

Fig. 5. *P. inspersa* dispersal site near Grand Forks (Interior Douglas-fir zone)

North America:

It was originally believed the Canadian summers were too moist for significant numbers of eggs to hatch and, therefore, for the moth to thrive[7].

In the U.S.A., *P. inspersa* was first released in 1986 in Idaho, Oreg. and Utah on diffuse knapweed with populations originating from Austria, Hungary and Greece[4,14,16]. In 1988, populations originating from Hungary were released on spotted knapweed in Colo., Mont., and Oreg.[14,16]. In 1990, eggs received from Greece populations were transferred onto squarrose knapweed in Utah, but failed to establish[14,16]. Andreas et al. (2009) reported that the moth has been released in the Pacific Northwest in Oreg., Wash., and Idaho states but it has only survived in Oreg. In Oreg., the moth was found consistently on 20% of the plants at one site, however, *P. inspersa* populations have declined significantly since the *Larinus* species have controlled the diffuse knapweed[15].

Fig. 6. *P. inspersa* release site near Kamloops (Bunchgrass zone)

British Columbia:

Biogeoclimatic Ecosystem Classification Zones

P. inspersa releases have established at five sites on diffuse knapweed in three different biogeoclimatic (BEC) zones in the province (Bunchgrass, Ponderosa pine and Interior Douglas-fir). Subsequent monitoring of these sites has been fairly consistent. Additionally, the natural dispersal of this agent into the different BEC zones has been documented at 58 sites by February 2020, all on diffuse knapweed. The habitat of the dispersal sites may be more indicative of the moth's preferences than the survival, or lack thereof, at release sites. *P. inspersa* has been found dispersed in the Bunchgrass (28 sites); Interior cedar-hemlock (1 site); Interior Douglas-fir (8 sites); and Ponderosa pine (21 sites) biogeoclimatic zones[2,3].

Fig. 7. *P. inspersa* dispersal site, 440 m from release site at Summerland (Ponderosa pine zone)

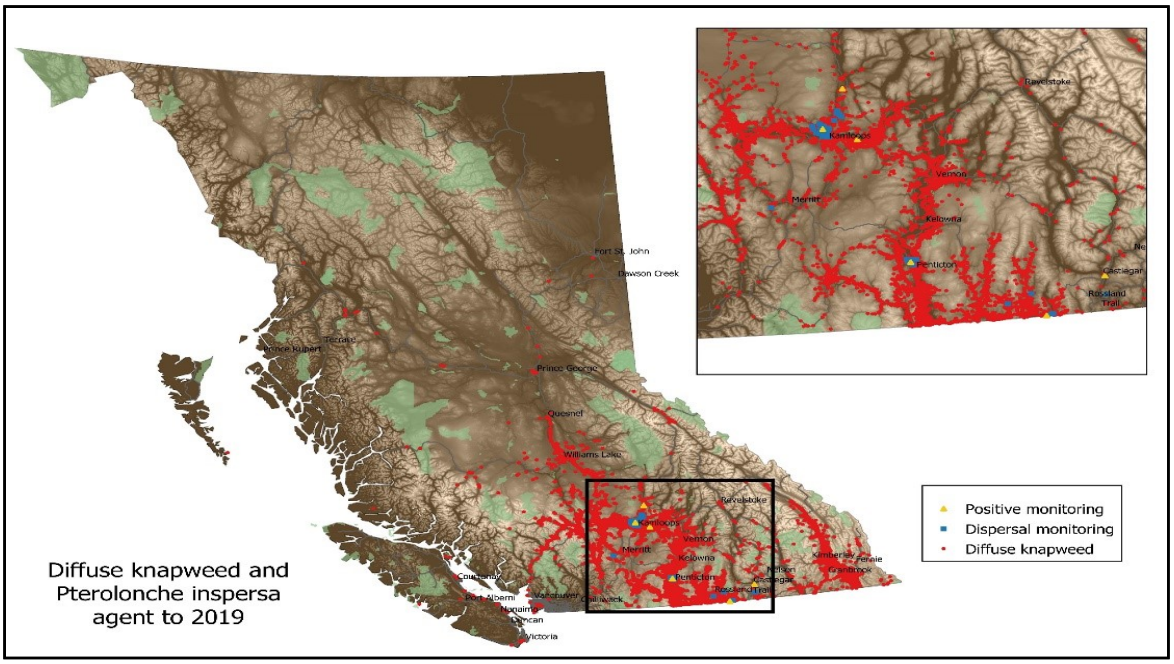

Fig. 8. *P. inspersa* establishment in relation to diffuse knapweed locations in B.C. to 2019[2].

Site Criteria

Site criteria have been summarized from the literature and for existing B.C. field sites. In B.C., *P. inspersa* has been field released onto 7 sites: 6 and 1 of diffuse and spotted knapweed sites, respectively, of which 5 and 0 have been recorded as established. Additionally, the moth has self-dispersed to 58 sites, all of diffuse knapweed[2]. The summarized sites do not depict all ranges of these criteria the agent may currently or eventually occupy.

Growing Season

Females require rosette or basal leaves to lay their eggs upon[7]. Through trial and error, it was found that plants with chimneys present typically had at least a few green or dried basal leaves present. Plants with very few withered, dried, or absent basal leaves were less likely to be infested with *P. inspersa* (S. Cesselli pers. comm. Oct. 2005).

Site Size

The range of site sizes of the five established releases is 0.0625 to 12.5270 ha. *P. inspersa* dispersed on its own onto sites in the range of 0.0001 to 2 ha with 96.5% of the sites comprised of areas of 0.5 ha and smaller[7].

Plant Density

In Northern Greece, the rate of *P. inspersa* attack appeared correlated to knapweed density. High plant density infestations had low attack rates (10%) while the average was 20 to 30% attack and the highest rate of attack found (75%) was in infestations with the lowest plant density, less than 1 plant/m^2[2,6,7].

In B.C., *P. inspersa* appears to reflect the findings in Greece and have the ability to establish and disperse on a limited supply of preferred target plants in desired habitats (S. Cesselli pers. comm. Apr. 2016). The range of site plant density of the five established releases is less than or equal to (<=) 1 plant/m^2 to 6-10 plants/m^2. *P. inspersa* has dispersed onto sites with a plant density range of <= plant/m^2 to greater than (>) 10 plants/m^2. Ten percent of the locations *P. inspersa* dispersed to in this range were recorded on sites with <= 1 plants/m^2 while 81% were recorded on 2-5 plants/m^2, 2% were recorded on 6-10 plants/m^2 and 7% of sites had a density of greater than 10 plants/m^2 [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 (MOE/MOF 1990). The moth has established at release and dispersal sites with a distribution range of 2 to 8 and 2 to 9, respectively. Establishment at various distribution codes of releases is: 60% at distribution code 2 and 40% at 7. Establishment at various distribution codes of dispersal sites is: 12% at distribution code 2; 19% at 3; 52% at 4; 3% at 5; 2% at 6; 5% at 7; 5% at 8; and 2% at 9[2].

Ground Cover

In B.C., higher numbers of *P. inspersa* moths have been found in infestations that lack ground litter (S. Cesselli pers. comm. Oct. 2005).

Fig. 9. Ground cover at established *P. inspersa* release site near Kamloops.

Competing Vegetation

In B.C., few to no moths have been found on diffuse plants among healthy stands of grass (S. Cesselli pers. comm. Oct. 2005). However, the effectiveness of biocontrol agents in general is enhanced with the pressures of competing vegetation.

Slope

To date, the range of recorded slopes of sites the moth has dispersed to on its own is 0 to 40% slope while the range of established release sites is 0 to 30% slope. The site with 40% slope has an aspect of 268° and the site with 30% slope has an aspect of 180°. The majority (96.5%) of sites have a slope of 20% or less while only 2 sites (3.5%) have slopes of 30 and 40%[2].

Aspect

The aspect range of both currently recorded established release sites and subsequent sites the moth has dispersed to on its own is 0 to 332°, essentially it establishes in all directions[2].

Elevation

In B.C., the elevation range of both recorded established release sites and subsequent sites *P. inspersa* has dispersed to is 346 to 905 m[2].

Temperature

In B.C., *P. inspersa* has been observed to thrive in hot, dry sites such as around Summerland, Naramata and Lac Du Bois/Kamloops but not as well in cooler sites such as higher elevations above Kamloops grasslands (S. Cesselli pers. comm. Oct. 2005). It prefers a climate similar to the Mediterranean and may not do well in harsh winters[4].

Fig. 10. *P. inspersa* exit hole in top of chimney.

Moisture Regime

As mentioned previously, it is important for summer conditions to be dry during egg incubation and hatching stages to ensure the egg does not become leathery and prevent the larvae from emerging[7]. Sites with aspen, Douglas-fir or lodgepole pine growing in preferred BEC zones containing subzones with relatively higher moisture are potentially less suitable to the target plant as well as the moth.

Soil Texture and Compactness

In Greece, *P. inspersa* is found on diffuse knapweed growing commonly in sandy or gravelly/cobbled soils[6]. European preferred soils are loose compositions of sand or gravel[15]. In the B.C. Gilpin grassland, *P. inspersa* has been found in cobbled soil (V. Miller pers. comm. Apr. 14, 2003). It has often been found on well-drained soils that have been composed of rock, gravel or silt and even compacted. Plants growing in clay-loam soils have also been found infested (S. Cesselli pers. comm. Oct. 2005).

Disturbance

In a study performed in B.C. by Newman et al. (2011), of the five out of six study sites where *P. inspersa* was found in the central southern interior, *P. inspersa* counts were highest at the non-grazed site.

Agent Handling

Collecting

Fig. 11. *P. inspersa* adult resting on chimney

Due to its unremarkable colouring, *P. inspersa* resembles many other moth species. It is difficult to identify, observe and track, therefore, in B.C. *P. inspersa* is generally left to self-disperse throughout the diffuse knapweed range. If collections are required, they are instead done with transplanted infested plants. Transplanting is labour-intensive, particularly given the small number of *P. inspersa* larvae infesting each root and may also result in the transfer of other agents. This activity should not be performed past the end of June as adult moths exit the plants in July, leaving behind an empty root and chimney. Prior to collection, it should be determined that enough plants with chimneys are present to warrant collection and exit holes are not already visible at the top of the chimneys, or if the chimney is too small to see this clearly, a couple of roots should be cut open to verify the biocontrol agent is still present. The plants are dug up and placed in temporary pots with a small amount of water. The plants can also have the soil removed and moist paper towel wrapped around the roots. The top two-thirds of the plants are cut off to decrease transplant stress. The pots can either be placed in a cage or large container with good air flow but with mesh openings to keep the moths confined, or dug directly into field sites. If kept in a cage, the emerging moths are thereafter collected and placed in containers kept in cool locations and when transported, within coolers housing ice packs wrapped in paper towel to absorb unwanted moisture. The moths need to be released as soon as possible due to their short life span during which they mate and oviposit eggs at the new site.

Releasing

Infested plants have been loosely transplanted, sometimes in small groups (e.g. five plants), into shovel-sized holes and backfilled. The soil does not get packed down and the chimneys are kept above the soil line for adults to emerge in a few weeks. If moths are reared from cages, the speed and timing of their release is critical. A minimum of 75 plants with strong evidence of *P. inspersa* larvae or pupae presence or 75-100 moths should be released at new sites.

Monitoring

Due to the indiscriminate colouring of *P. inspersa*, the moths are camouflaged well amongst dry plant stalks and litter and difficult to distinguish from other brown or grey-coloured moths. It requires practice to identify this moth which is present when the plants have begun to dry slightly into hues of gray. Visual searches, particularly among rosettes and plants still bearing basal leaves during the time period for oviposition can often yield no sign of the moths. When they are located on plants, they are commonly observed facing downward. Grasshoppers and leafhoppers are plentiful during this time period and can interfere with searching for *P. inspersa* when plants are accidentally bumped or purposefully stirred in attempts to cause the moths to fly. When *P. inspersa* does fly, it takes short, erratic low flights close to the vegetation which makes them difficult to track and pinpoint their whereabouts (S. Cesselli pers. comm. Oct. 2005). Additionally, the moths may be difficult to see as they lay their eggs from late afternoon until midnight[8].

Attacked plants appear weakened, stunted, less branched and have fewer flowers than healthy diffuse knapweed. Evidence of *P. inspersa* infestation has been found on plants with significant basal leaves, however, not on plants with bushy basal leaves; this may be a result of unfavourable site conditions. Roots may be excavated for larvae presence or feeding evidence. Roots infested by *P. inspersa* will often look swollen or 'bulbous'. Frass left behind by larvae feeding is light brown, dry and abundant, often loosely packed into the feeding cavity. The transparent pupal case of *P. inspersa* is typically found inside the chimney or within the cavity created by larvae feeding or even on the soil surface if it clings to the moth as it crawls from the chimney. Monitoring for chimney evidence can be performed from July to October, but it is best done before the rainy season as mud can cover the basal leaves and make the chimneys difficult to find. To determine if diffuse knapweed roots are still infested, examine the end of the chimney for the presence, or lack thereof, of characteristic exit holes (S. Cesselli pers. comm. Oct. 2005).

When examining root crowns for *P. inspersa's* chimneys, it is important to distinguish these from *A. zoegana*'s silk feeding tubes. *A. zoegana* spin their silk underground on the outer portion of the root where they feed or within the root, dependent on the availability of root tissue for the larva to consume. The whole or a portion of the tube is attached to the root as is *P. inspersa*'s erect chimney but a times *A. zoegana*'s tube may be found

lying horizontal on the ground as it lacks the firm structure of the *P. inspersa* chimneys. For the most part, the current season's *A. zoegana* feeding tubes remain highly flexible and can easily bend without breaking or tearing open and feel rubbery, like latex, while the *P. inspersa* chimneys feel rough as though covered in sand. Additionally, *P. inspersa*'s chimney is usually pale, unlike when *A. zoegana*'s feeding tube extends above the soil it darkens over time to a rich dark-brown colour, somewhat shiny, and dries, becoming rather brittle by autumn. Furthermore, when *A. zoegana* exit their feeding tube, they will often still have their reddish-brown pupal case stuck to them which they shed on the basal leaves, the soil, or at the emergence point from their silken feeding tube (S. Cesselli pers. comm. Oct. 2005).

Fig. 12. *A. zoegana* tube detached from host knapweed root.

Fig. 14. Discarded pupal casing of *A. zoegana* at the crown of a diffuse knapweed plant.

Fig. 13. *P. inspersa* chimney attached to host knapweed plant.

REFERENCES

1. Andreas, J. E.M. Coombs, G.L. Piper, M. Schwãrzlander, J. Milan. 2009. Biological control. 2010 PNW Weed Management Handbook http://www.google.ca/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&ved=0ahUKEwj9-4yzv6DLAhWFXBQKHTXKAxIQFggxMAM&url=http%3A%2F%2Fwww.eduinca.net%2Felibrary%2Fru%2Fbo

ok%2Fdownload%2Fid%2F8242&usg=AFQjCNECRl9XxhUM9azTaywml8XU9CGIeQ (Accessed: February 26, 2016).
2. [BCMFLNRO] BC Ministry of Natural Resource Operations. 2015. Invasive alien plant program application (IAPP) – a database for invasive plant data in B.C. B.C. Min. of Nat. Resour. Operations. www.for.gov.bc.ca/hra/plants/application.htm . (Accessed: 2015 and February 2020).
3. British Columbia Ministries of Environment and Forests. 1990. Procedures for environmental monitoring in range and wildlife habitat management. Habitat Monitoring Committee, Victoria B.C.
4. Campobasso, G., E. Coombs, R. Lang, G. Piper, C. Quimby, N. Rees, R. Sobhian, and J. Story. 1996. *Pterolonche inspersa*. Sect. II, The knapweeds. In: Biological control of weeds in the west. N.E. Rees, P.C. Quimbly Jr., G.L. Piper, E.M. Coombs, C.E. Turner, N.R. Spencer, and L.V. Knutson, (editors). Western Soc. Weed Sci.
5. Campobasso, G., R. Sobhian, L. Knutson, A.C. Pastorino and P.H. Dunn. 1994. Biology of *Pterolonche inspersa* (Lep.: Pterolonchidae), a biological control agent for *Centaurea diffusa* and *C. maculosa* in the United States. Entomophaga, Sept. 1994 Vol. 39 Issue 3: 377-384.
6. Dunn, P.H., S.S. Rosenthal, G. Campobasso and S.M. Tait. n.d. A petition for the release of *Pterolonche inspersa* STGR. (Lepidoptera: Pterolonchidae) in the United States as a biological control agent for *Centaurea diffusa* Lam. U.S. Dept. Agric.
7. Harris, P. 2005. Classical biological control of weeds established biocontrol agent *Rhinocyllus conicus* Froel. Seed-head weevil. Gov. of Can., Agriculture and Agri-Food Canada. Updated August 3, 2005. http://res2.agri.ca/lethbridge/weedbio/agents/arhicon_e.htm (Accessed: February 15, 2007).
8. Heppner, J.B. 1997. Immature stages of the Mediterranean knapweed borer, *Pterolonche inspersa* (Lepidoptera:Pterolonchidae). Holarctic Lepidoptera, 4(2): 63-66.
9. Lang, R.F. n.d. *Pterolonche inspersa* Lepidoptera: Pterolonchidae. Biological Control A guide to natural enemies in North America Cornell Univ. http://biocontro.entomology.cornell.edu/weedfeed/pterolonche.php (Accessed February 26, 2016).
10. Newman, R.F., S. Turner, B.M. Wallace and S. Cesselli. 2011. The decline of diffuse knapweed in British Columbia. B.C. Min. of Nat. Resour. Operations Tech. Rep. 065.
11. Powell, G.W., A. Sturko, B. Wikeem and P. Harris. 1994. Field guide to the biological control of weeds in British Columbia. B.C. Min. For. Res. Prog.
12. Schroeder, D. unpubl. data, 1983. Proposal to screen additional insects in Europe for the biological control of spotted knapweed in North America. CIBC European Station, Delemont Switzerland.
13. Watson, A.K. and A.J. Renney. 1974. The biology of Canadian weeds. *Centaurea diffusa* and *C. maculosa*. Can. J. Plant Sci. 54:687-701.
14. Winston, R., C. Bell Randall, R. De Clerck-FLoate, A. McClay, J. Andreas and M. Schwartzlander. 2016. Field guide for the biological control of weeds in the northwest. For. Health. Tech. Enterprise Team.
15. Winston, R., M. Schwa̋rzlander, C.B. Randall, R Reardon. 2012. Biology and biological control of knapweeds. For. Health Tech. Enterprise Team. 70-71; 80-81.
16. Winston, R., M. Schwartzlander, H.L. Hinz, M.D. Day, M.J.W. Cock and M.H. Julien. 2014. Biological control of weeds a world catalogue of agents and their target weeds, 5th edition. For. Health Tech. Team.