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Pheromone-based monitoring of invasive alien insects along the border of Finland and Russia – methods and unintentionally caught species

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Abstract

Global trade provides pathways for the spread of invasive species. To tackle the threat, many countries have designated surveys that are typically conducted at the probable ports of entry. For Finland, the most north-eastern region of the European Union (EU), such site is the border with Russia and the imports of coniferous roundwood and wood chips. In this paper, we describe the monitoring systems based on pheromone-trapping for three EU-wide quarantine pests: *Dendrolimus sibiricus*, *Polygraphus proximus* and *Bursaphelenchus xylophilus*. We also list the non-target species caught in an exploratory survey using pheromone traps. During the three years of survey, no quarantine pests were detected, but 30 other species of insects were caught. Therefore, the monitoring – despite not detecting the target pests – provided information about the abundance of other species. As insect diversity reflects the status of the surrounding environment, the value of such data should be increased via co-operation among research institutes.

Keywords: wood import, invasive alien species, monitoring, pest, forest damage, insect

Introduction

Invasions by non-native species are, to a large extent, results from human activities. While oceans, deserts and mountain ranges can prevent species relocation *per se*, they form no barriers to global trade or human movement. This, in turn, has opened a pathway for species to spread beyond their native ranges (Liebhold et al. 2017). In some cases, they have resulted in mere isolated observations, but in other cases they have devastated entire ecosystems. A recent example is the spread of the pine-wood nematode (*Bursaphelenchus xylophilus* (Steiner et Bührer) Nickle). The species is native to the boreal zones of North America, but is now established in Japan, Portugal and Spain, causing severe damage and mortality to the local *Pinus* species (IPPC 2016).

When the effects of an alien species are severe, they are referred to as invasive alien species (IAS) (EASIN 2021). In general, the chance for an IAS to establish in a new region is low if the climatic or environmental conditions in the new region differ considerably from the origin. Considering this, the northern hemisphere with its vast coniferous forests can be considered particularly vulnerable. For example, the list of IAS that have spread from Asian Russia to European Russia includes 42 species, 23 of which have been categorized as pests (Orlowa-Bienkowskaja 2017). In addition, the potential threat of an IAS should not be overlooked even if the main host species is absent: the four-eyed fir bark beetle *Polygraphus proximus* (Brandford) switched from one *Abies* species to another and into *Pinus* and even *Picea* trees as it reached western Russia (Kerchev 2014). Considering such patterns, Finland has one specific gateway for non-native species to spread: the Trans-Siberian Railway and the imports of roundwood and wood chips it carries. The route has been shown to function not just for transporting the wood, but also the insects within the wood: Siitonen (1990) found 23 bark beetle species from coniferous logs imported from Russia, including potential pests.

IAS monitoring operations are often conducted using pheromone traps (Poland et al. 2018) that not only lure the target species, but numerous other insects as bycatch. Ostrauskas and Ivinskis (2011) caught 32 non-targeted species of moths in their search for the pine-tree lappet *Dendrolimus pini* (Linnaeus) and the Siberian silk moth in Lithuania. Similarly, Jakubikova et al. (2016) found the first ever field-confirmed records of the carnation tortrix (*Cacoecimorpha pronubana* Hübner) from the Czech Republic while conducting pheromone trapping for fruit tortricid moths. Therefore, monitoring of IAS can provide unexpected data on the occurrence and abundance of many other insect species, ones that might not be monitored otherwise.

In this paper, we focus on three EU-wide quarantine pests (EU 2021): the pinewood nematode, the Siberian silk moth *Dendrolimus sibiricus* (Chetverikov), and the four-eyed fir bark beetle. The pinewood nematode (spread to trees via *Monochamus* beetles) causes the pine wilt disease (Futai 2013), the Siberian silk moth larvae feed on needles of a wide variety of *Larix*, *Abies*, *Pinus* and *Picea* trees (Kononov et al. 2016, EFSA 2020), and the four-eyed fir bark beetle is a pest of especially *Abies* species in its native range, but this beetle is also potentially capable of attacking *Pinus*, *Picea*, *Tsuga* and *Larix* species (EFSA 2020a). Here, we describe the trap types and pheromones used in monitoring of these three potential IAS along the Finnish-Russian border. We also list the non-target species that we caught as bycatch during these campaigns. The aim is to: 1) describe the current methodology of how the target IAS are monitored at the Finnish-Russian border, 2) analyse and report the range of non-target species thus far collected in these campaigns as an unintended bycatch, and 3) raise the general awareness of these topical IAS.

Materials and methods

Study sites

The monitoring was conducted during the summers of 2019–2021 at six sites in the district of North Karelia, Eastern Finland (Figure 1).

The trapping sites (Table 1) were selected based on: 1) vicinity to a site where roundwood from Russia is imported or stored, and 2) their forest structure (matching the known host-tree requirements of the target species).

Trapping methods

Insect trapping was conducted using pheromone-baited traps. To collect pine-wood nematode data, its vector species, the *Monochamus* beetles, were sampled and nematodes were extracted from them in the laboratory (see details further below). *Monochamus* sampling was done with the Galloprotect Pack pheromone by SEDQ, which includes both pheromones and kairomones. The Siberian silk moth was lured with the ISCALureIT630 pheromone by ISCA Technologies. The four-eyed fir bark beetle was lured by a combination of two pheromones placed in the same trap: *Ips sexdentatus* (Börner) combo pheromone by Synergy Semiochemicals Corp. and the P573-Lure by Chemtica (originally meant for *Polygraphus poligraphus* L.). Figure 2 illustrates the traps as they were set in the field.

The *Monochamus* (for pine wood nematode data) and *D. sibiricus* traps were set to hang in three trees at each site at ca. 7–10 m height. This is not optimal for catching *Monochamus* species (Foit et al. 2019), but we could not reach higher due to the structure of the surrounding trees. The funnels in *Monochamus* traps were lubricated with Synergy Semiochemical's EZ Fluon to prevent the beetles from escaping (Alvarez et al. 2014). Additionally, the white containers at the bottom of the *Monochamus* traps (Figure 2b) were equipped with fresh pine twigs to provide food for the trapped insects; the detection of the pine wood nematode (see below) requires the *Monochamus* vectors to be alive. Altogether, eight *Monochamus* traps were placed at sites 1 (3 traps), 2 (3 traps) and 4 (2 traps), and fourteen *D. sibiricus* traps were placed at all sites (three on sites 1 and 2, two on the others). For *P. proximus*, twelve traps were set in trapping sites 1–4 in a shape of a triangle, ca. 5–10 meters apart (one set of three traps per trapping site). In each trapping year, traps for *Monochamus* species and *D. sibiricus* were set in the last week of June, and *P. proximus*

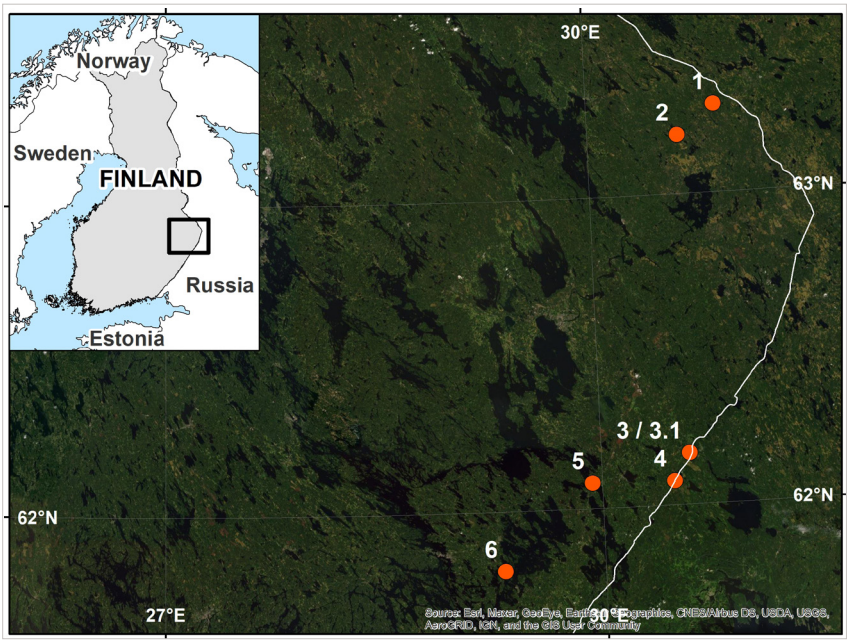

Figure 1. Locations of the six trapping sites overlaid on a SPOT satellite image (natural colour)

Table 1. Information on the trapping sites and forest characteristics at the site

Site	Trapping periods	Characteristics	Dominant tree species
1	Summers 2019–2021	Forest. Traps located next to a storage site for Russian roundwood	Scots pine, average diameters 15–20 cm
2	Summers 2020–2021	Fresh clear-cut. Located next to a road used for transporting Russian roundwood. The same clear-cut was not used twice; in 2021 the traps were moved to a clear-cut in the same area (about 1000 m away), surrounded by the same forests	Scots pine. The clear-cut, and the surrounding mature forests (average diameters 25–30 cm)
3	Summer 2019	Forest. Traps located near a train terminal used to store Russian roundwood	Scots pine, average diameters 20–25 cm, understorey of Norway spruce
3.1.	Summer 2021	The train terminal near site 3. Traps located 20 m away from the tracks via which roundwood is transported	No forest in the near vicinity
4	Summer 2020	Fresh clear-cut. Located near the Russian border, 50 m away from it	Scots pine. The clear-cut, and the surrounding mature forests (average diameters 20–25 cm)
5	Summer 2019	Forest stand	Siberian larch (*Larix sibirica*), average diameters > 40 cm
6	Summer 2019	Arboretum	Siberian larch (*Larix sibirica*), Siberian fir (*Abies sibirica*), average diameters > 40 cm

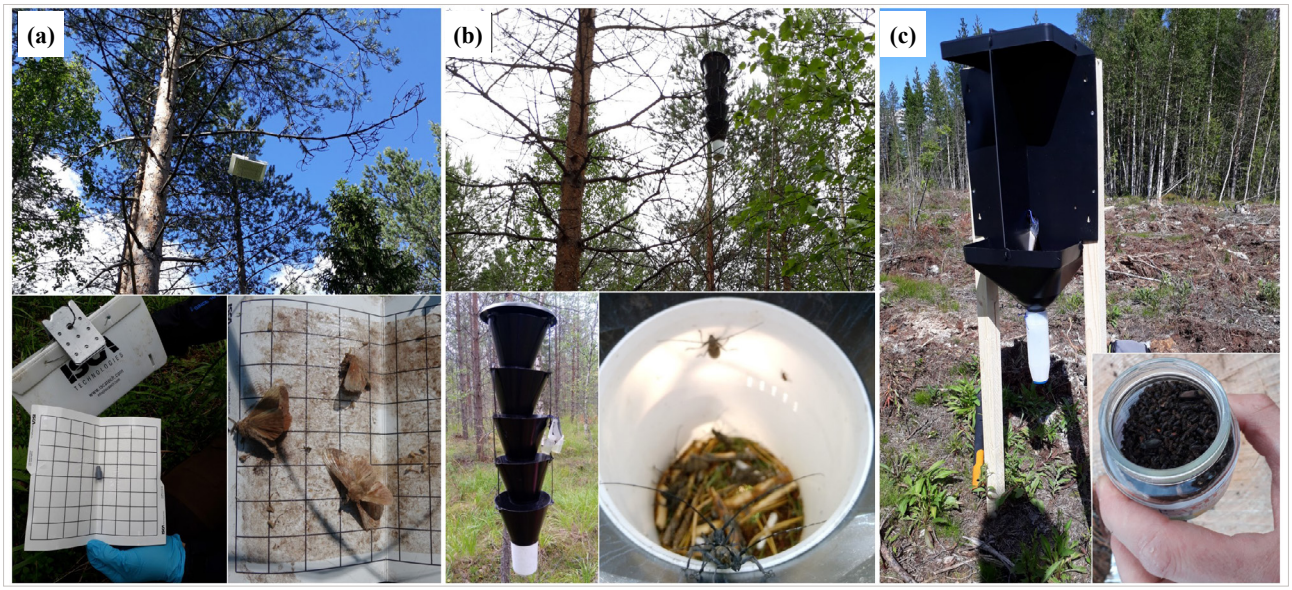

Figure 2. Types of traps used in this survey. The Delta (ISCA Technologies) was used for *D. sibiricus* (a), the Multitrap 5-unit Funnel Trap (Synergy Semiochemicals) for *Monochamus* sp. (b) and the WitaPrall Ecco Dry Trap (Witasek PflanzenSchutz GmbH) for *P. proximus* (c)

traps were set in mid-May. All traps were kept in place until the end of August during all trapping years. Traps to collect *Monochamus* species and *D. sibiricus* were emptied at ca. one-week intervals and the *P. proximus* traps at ca. four-week intervals. The collected insects were stored in dry, closed containers and sent within 24 h from each emptying occasion to the laboratories of the Finnish Food Authority, where they were identified at the species or genus level by entomologists based on their morphological features.

The *Monochamus* species are known to be able to carry various *Bursaphelenchus* nematodes of which the *B. mucronatus* Mamya et Enda is native and common in Finland. The *B. mucronatus* was thus serving as a control for the ability of the protocol to detect the nematodes carried by *Monochamus* beetles, and thus also the potential IAS, *B. xylophilus*. The nematodes were extracted from crushed *Monochamus* beetles using the Baermann funnel method (Kusumoto et al. 2014) and the two *Mucronatus* species were then distinguished from one another with microscopes based on their morphological features (Braasch 2004). The data collected from the trapping campaigns were processed with the R software environment (R Core Team 2022).

Results

Caught species per trap/pheromone type

No target IAS were found during the surveys. Altogether, 31 species of insects were recorded from the traps and identified at the species level (Table 2).

Table 2. List of caught species

Pheromone (target species)	Order: Family	Species (catch) *	Catch site						
			1	2	3	3.1	4	5	6
ISCALure IT630 (*D. sibiricus*)	Lepidoptera: Lasiocampidae	*Dendrolimus pini* (87)	X	.	X	.	X	X	X
	Lepidoptera: Lymantriinae	*Lymantria monacha* (50–70)	.	.	.	.	.	X	X
SynergyCombo (*I. sexdentatus*)	Blattodea: Ectobiidae	*Ectobius sylvestris*	X	.	.	.	.	.	.
	Coleoptera: Cerambycidae	*Rhagium inquisitor*	X	.	.	.	.	.	.
+	Coleoptera: Cleridae	*Thanasimus formicarius*	X	X	.	.	X	.	.
Chemtica P573 (*P. poligraphus*)	Coleoptera: Elateridae	*Ampedus balteatus*	X	X	.	.	X	.	.
	Coleoptera: Elateridae	*A. nigrinus*	X	X	.	.	X	.	.
...	Coleoptera: Elateridae	*A. pomorum*	X	.	.	.	.	.	.
...	Coleoptera: Elateridae	*Dalopius marginatus*	X	.	.	.	.	.	.
...	Coleoptera: Elateridae	*Selatosomus melancholicus*	X	.	.	.	.	.	.
...	Coleoptera: Elateridae	*Serica brunnea*	X	.	.	.	.	.	.
...	Coleoptera: Pissodini	*Pissodes pini*	.	.	.	.	X	.	.
...	Coleoptera: Scolytinae	*Crypturgus hispidulus*	X	.	.	.	X	.	.
...	Coleoptera: Scolytinae	*C. pusillus*	X						
...	Coleoptera: Scolytinae	*Dryocoetes autographus*	.	X	.	.	.	.	.
...	Coleoptera: Scolytinae	*Hylastes brunneus*	X	X	.	.	X	.	.
...	Coleoptera: Scolytinae	*H. cunicularius*	X	.	.	.	.	.	.
...	Coleoptera: Scolytinae	*Ips duplicatus*	X	X	.	.	X	.	.
...	Coleoptera: Scolytinae	*I. typographus*	X	X	.	.	X	.	.
...	Coleoptera: Scolytinae	*Orthotomicus laricis*	X	.	.	.	.	.	.
...	Coleoptera: Scolytinae	*O. proximus*	.	.	.	.	X	.	.
...	Coleoptera: Scolytinae	*O. suturalis*	X	.	.	.	.	.	.
...	Coleoptera: Scolytinae	*Pityogenes chalcographus*	X	.	.	.	X	.	.
...	Coleoptera: Scolytinae	*P. quadridens*	.	X	.	.	.	.	.
...	Coleoptera: Scolytinae	*Polygraphus poligraphus*	X	.	.	.	.	.	.
...	Coleoptera: Scolytinae	*P. subopacus*	X	.	.	.	.	.	.
...	Coleoptera: Scolytinae	*Tomicus minor*	X	.	.	.	.	.	.
...	Coleoptera: Scolytinae	*Trypodendron lineatum*	.	X	.	.	X	.	.
Galloprotect pack (*Monochamus* sp.)	Coleoptera: Cerambycidae	*M. galloprovincialis* (127)	X	X	.	.	.	.	.
	Coleoptera: Cerambycidae	*M. sutor* (35)	X	X	.	X	X	.	.
...	Coleoptera: Cerambycidae	*M. sartor urussovii* (16)	X	X	.	X	X	.	.

Note: * Only available for Monochamus and Dendrolimus samples. *L. monacha* were recorded for a designated survey conducted by author MM at the same time. For different bark beetle species, the catches varied between "one and dozens", but no accurate counting was done (*see* Discussion).

All of the caught *Monochamus* specimens were tested negative for carrying the quarantine species *B. xylophilus*, but individuals from each *Monochamus* species were tested positive for *B. mucronatus,* further proving that they can work as vectors for *Bursaphelenchus* nematodes. In addition to a total of 31 identified insect species, representatives of the families Curculionidae, Dermestidae, Dytiscidae, Formicidae, Hydrophilidae, Leiodidae, Noctuidae, Staphylinidae, Tabanidae and the arthropod group Collembola were captured but not identified at the species level. Of the counted *Monochamus* beetles, 57 were captured in the year 2019 (45 *M. galloprovincialis* (Oliver), 8 *M. sutor* (Linnaeus) and 4 *M. urussovii* (Fischer)), 41 in 2020 (22 *M. galloprovincialis*, 15 *M. sutor* and 4 *M. urussovii*) and 80 in 2021 (60 *M. galloprovincialis*, 12 *M. sutor* and 8 *M. urussovii*).

On the other hand, *Dendrolimus* catches were zero in 2021, two were caught in 2020 and 85 in 2019. This pattern is likely explained by an unusually cold weather during the flight period of *D. pini* in 2020, whereas for the other insects (bark beetles, *Monochamus* species) the two years did not differ notably in relation to catch size. Then the year 2020 could have affected the local breeding success, resulting in zero catches during 2021 also. The exact number of caught *Lymantria monacha* Linnaeus moths in the *D. sibiricus* traps was not attainable due to shattered insects, resulting in a coarse estimate of 50–70 individuals.

Discussion and conclusions

No quarantine species were caught in the traps, but altogether over 30 other insect species were caught. The shortcoming of the study was that, with a few exceptions, the numbers of caught individuals had not been recorded at the species level. The only insects whose numbers were recorded were the original targets of the IAS monitoring: *Dendrolimus* moths (which were identified as *D. sibiricus* or *D. pini*) and *Monochamus* beetles (tested for whether they carried *B. xylophilus* or *B. mucronatus*). For the rest,

only occurrences had been recorded because the Finnish Food Authority focuses on IAS only. In the future, the counting of non-target species should be set as an additional research goal due to the diversity of the bycatch.

The *Monochamus* pheromone was luring three *Monochamus* species. *Monochamus galloprovincialis* was the most frequent catch, but *M. sutor* and *M. urussovii* were also collected regularly (Table 2). As individuals from all three species were tested positive for *B. mucronatus,* they seem to be suitable vectors for *B. xylophilus* as well (Akbulut and Stamps 2012). More importantly, the employed method evidently works for trapping of living *Bursaphelenchus* nematodes via *Monochamus* vectors. Of the sites where *Monochamus* were trapped, fresh clear-cuts (sites 2 and 4 in Figure 1) were as good as a timber storage site (site 1 in Figure 1), where the inflow of fresh roundwood (both Scots pine and Norway spruce) is regular throughout the whole snow-free season. For site 1, the stored material (roundwood logs with intact bark) is known to be used for breeding by *M. galloprovincialis* whereas the scent of fresh resin from the clear-cuts at sites 2 and 4 works as a kairomone (Tomminen 1993), which is likely to explain the patterns.

Fresh clear-cuts (cut in the preceding winter) and the timber-storage site 1 proved to be good for catching bark beetles as well and overall, the majority of our non-target species were caught from the bark beetle traps with a combination of *I. sexdentatus* and *P. poligraphus* pheromones. For future surveys, the development of a designated pheromone for *P. proximus* is something to look forward. As the number of the non-target species was relatively high especially in the clear-cut sites, a question rises whether they were lured in by the pheromones or caught by accident. In general, fresh clear-cuts are ideal for catching bark beetles as the smell of fresh resin attracts them and the fresh clear-cuts typically have logging residues (treetops and branches) where many of the caught species (*Pityogenes* sp., *Poligraphus* sp. for instance) breed in. Therefore, many of the bark beetle species may have flown into the traps by mere accident as they were present in the clear-cuts in the first place, instead of having been lured into the traps by the sexual pheromones of another species. Yet, the luring power of the pheromone for the target families was also evident by catches from two *Ips* and *Polygraphus* species. The traps also caught six species of Elateridae click beetles. The family hosts omnivorous species that may prey on bark beetle larvae and Elaterid adults are also commonly found in pheromone traps designed to lure *Ips typographus* (Linnaeus) (Valkama et al. 1997).

The glue-based traps for *D. sibiricus* were luring predominantly *D. pini*. The efficacy of the pheromone for *D. pini* was evident, as in most cases the first *D. pini* individuals appeared on site only seconds after the envelope containing the pheromone was opened (M. Melin, pers. obs.). Although the pheromone was working well, the used trap type proved to be suboptimal for collecting large numbers of moths as birds can (and were seen to) pick the moths from the glue plate (M. Melin, pers. obs.). The *L. monacha* catches in the *D. pini* traps came from sites 5 and 6 (Figure 1). The *L. monacha* catch of ca. 50–70 individuals suggests that they were lured in by the pheromone. For future surveys, testing of different types of funnel traps for catching *Dendrolimus* moths would seem ideal as catches exceeding 900 *L. monacha* individuals per funnel in one season have been reported in Finland (Melin et al. 2020). Therefore, a well-functioning funnel-type trap could result in higher catches of *Dendrolimus* individuals as well.

In conclusion, based on past studies (Ostrauskas and Ivinskis 2011, Jakubikova et al. 2016) as well as the present results, the usefulness of IAS monitoring could be increased by accurate documentation of the unintentionally caught species, since these were frequent especially when trapping was conducted in fresh clear-cuts. Furthermore, insects from ten other families that were not identified at the species level occurred in the present data. A more accurate documentation would call for more intensive co-operation between research institutions and plant health authorities. The latter is mostly interested in IAS, but the former is interested in patterns and trends of any caught species and the diversity of the catches. As the monitoring sites are not static in time, but are rather guided by the ports of entries, monitoring data can bring updates on the range of various insect species. The importance of this should not be undermined as insects are commonly used indicators of the status of their environments and changes therein (Schowalter 2019).

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