

The attraction of cerambycids and other xylophagous beetles, potential vectors of *Bursaphelenchus xylophilus*, to semio-chemicals in Slovenia

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Abstract The attractiveness of different semio-chemicals to potential vectors of the phytoparasitic nematode *Bursaphelenchus xylophilus* was investigated in conifer forests in Slovenia. From 2007 to 2009, the presence of xylophagous beetles in *Pinus nigra*, *P. sylvestris*, *P. halepensis*, *Picea abies* and *Abies alba* stands was assessed at eight locations. Insects were collected at 1-month intervals during the growing season using four cross vane traps per location with a collecting container with propylene glycol and attractants (ethanol+ α -pinene, Pheroprax[®] and Gallowit[®]). The trapped insects represented 24 families of the order Coleoptera, and we identified 94 species. The most numerous group was the weevil subfamily

Scolytinae (76.55% of all insects collected), followed by the family Cerambycidae (8.12%), and the weevil subfamily Curculioninae (1.67%). With regard to species number, the most frequent wood-borers were Cerambycidae (24 taxa), Scolytinae (12 species) and Buprestidae (8 species). The most abundant species was *Spondylis buprestoides*, followed by *Arhopalus rusticus*, *Monochamus galloprovincialis* and *Arhopalus fesus*. At all locations, the largest catch of Cerambycidae occurred in July. The most effective attractant was ethanol+ α -pinene, followed by Gallowit[®]; the least effective attractant was Pheroprax[®]. Among *Monochamus* species, *M. galloprovincialis* represented 17.54%, *M. sutor* 0.09% and *M. sartor* 0.04% of the long-horned beetles collected. *Monochamus* individuals were most numerous in the *P. nigra* stand and were attracted in the greatest numbers by Gallowit[®], followed by ethanol+ α -pinene. The cerambycid catch was highly correlated with the catch of non-target bark beetle predators (Cleridae, Staphylinidae, Histeridae, Trogositidae, Nitidulidae, Rhizophagidae) in the traps.

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Introduction

The most economically important damage caused by wood-boring insects, particularly by long-horned

beetles, is timber degradation due to larvae boring into the sapwood and heartwood. In Europe, timber degradation caused by *Monochamus sutor* (Linnaeus, 1758), *Monochamus sartor* (Fabricius, 1787) and *Tetropium castaneum* (Linnaeus, 1758) affected 120,000 m³ of pine and 225,000 m³ of spruce during the 1990s (Evans *et al.* 2004). However, a few species are capable of attacking and killing living, healthy trees or trees with weakened defences (Hanks 1999; MacLeod *et al.* 2002). Recently, interest in *Monochamus* species has increased because these species are vectors of the pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Nickle, 1970) (Tylenchida: Aphelenchoididae), (Steiner and Buhner 1934), which causes the fatal pine wilt disease of some pine species. The PWN is endemic to North America, and most North American conifers are resistant to it, but numerous conifers from other continents are highly susceptible to this pest (Mamiya 1983; Wingfield *et al.* 1982). Japanese forests suffered extensive damage after the introduction of PWN, which occurred sometime before 1905, when the pine wilt disease was first identified there (Mamiya 2003). Later, extensive damage also occurred in other Asian and European countries, such as Portugal (Sousa *et al.* 2001). Disease expression and tree mortality are associated with the presence of highly susceptible tree species, a suitable vector and appropriate ecological conditions. Studies in regions where pine wilt disease has appeared indicate a close relationship with climatic conditions (Rutherford and Webster 1987). There are no reports of susceptible pines dying of PWN in areas where the mean summer air temperature is less than 20°C, despite the presence of PWN and its vectors (*ibid.*). The greatest damage has been observed in trees stressed by low rainfall and mean air temperatures higher than 25°C for 55 days (Takeshita *et al.* 1975). Pest risk assessments have demonstrated that the nematode can survive in Europe, but extensive damage and tree mortality are likely to be restricted to the warmer southern countries (Evans *et al.* 1996). Non-native (non-European) species of *Monochamus*, which are the vectors of PWN, are therefore listed as I/A1 quarantine organisms for Europe (I/A1 EU list is a list of pests approved by the Council Directive 2000/29/EC whose introduction into, and spread within, all EU member states shall be banned; A1 pests are absent from the EU region) (Smith *et al.* 1992).

With respect to bioclimatic parameters such as temperature, hosts (particularly *Pinus sylvestris* and *P.*

pinaster) and vectors, pine wilt disease has the potential to become a major threat if it is introduced into other European countries. It could become one of the most serious threats to pine forests worldwide in the 21st Century (Økland *et al.* 2010; Tomiczek and Hoyer-Tomiczek 2008).

Many wood-boring insects, such as *Acanthocinus hispanicus* Sama et Schurmann, 1979, *Arhopalus ferus* (Mulsant, 1839), *Rhagium bifasciatum* Fabricius, 1775, *Spondylis buprestoides* (Linnaeus, 1758), *Ips acuminatus* (Gyllenhal, 1827), *I. mannsfeldi* (Wachtl, 1879), *I. sexdentatus* (Börner, 1776), *Orthotomicus erosus* (Wollaston, 1857) and *Pissodes validirostris* (Sahlberg, 1834) are possible vectors for many species of nematodes (PHRAME 2007). Furthermore, some species of the subfamily Scolytinae, such as *Hylurgus ligniperda* (Fabricius, 1792), *Tomicus piniperda* (Linnaeus, 1758) and *O. erosus* have been reported as vectors of some *Bursaphelenchus* species (Skarmoutsos and Skarmoutsos 1999; Sousa *et al.* 2002). Some cerambycid species such as *Arhopalus rusticus* (Linnaeus, 1758) and *Acanthocinus griseus* (Fabricius, 1792), some buprestids (genus *Chrysobotris*) and curculionidae (genera *Pissodes* and *Hylobius*) were found in association with the pine wood nematode (Lint 1988). Any insect that is able to transfer nematodes, particularly members of the genus *Bursaphelenchus*, can be considered a potential vector of the PWN.

Many bark and wood-boring insects are known to be attracted by host odors, and commercial baits are based on host monoterpenes (α -pinene) and ethanol (Fan *et al.* 2007; Phillips *et al.* 1988). In North America, Billings and Cameron (1984) and Billings (1985) have demonstrated a kairomonal response of *Monochamus titillator* to blends of bark beetle pheromones acting synergistically with host turpentine. Allison *et al.* (2003) have shown that ipsenol and ipsdienol, aggregation pheromones of *Ips* DeGeer, 1775 species, are highly synergistic with α -pinene and ethanol in attracting *Monochamus clamator* (LeConte, 1852) and *Monochamus scutellatus* (Say, 1824), whereas pheromone compounds from *Dendroctonus* Erichson, 1836 species are not. In Spain, Pajares *et al.* (2004) have studied the effects of bark beetle (*Ips* spp.) pheromone components, released individually (ipsenol) or in blends (ipsenol, ipsdienol, *cis*-verbenol and methyl-butenol), together with host volatiles (turpentine or α -pinene and ethanol) on *Monochamus galloprovincialis* (Olivier,

1795) trap catches. The full blend of the four *Ips* semiochemicals with the host compounds is highly attractive (Pajares *et al.* 2004). The roles of ipsdienol, ipsenol, *cis*-verbenol, methyl-butenol and α -pinene+ethanol as attractants for *M. galloprovincialis* have been field-tested in Spain to obtain an operative kairomonal lure for the management of this species (Ibeas *et al.* 2007). The blend composed of α -pinene, ethanol, ipsenol, ipsdienol and methyl-butenol was very efficient in trapping the cerambycids *M. galloprovincialis*, *Acanthocinus griseus* and *Arhopalus syriacus* (Reitter, 1895) and the scolytids *I. sexdentatus*, *O. erosus* and *H. ligniperda* (Francardi *et al.* 2009). Recently, sex attraction and mating behavior in *M. galloprovincialis* have also been investigated (Ibeas *et al.* 2007, 2009), and considerable progress has been made in the study of *M. galloprovincialis* pheromones. Researchers have found that 2-undecyloxy-1-ethanol is a male-produced aggregation pheromone of the pine sawyer beetle (Pajares *et al.* 2010a). This compound also synergized the attractiveness of the standard kairomonal lure of α -pinene, ipsenol (2-methyl-6-methylene-7-octen-4-ol), and 2-methyl-3-buten-2-ol; in this way it is potentially useful for trap bait and management of *M. galloprovincialis* and pine wilt disease (Ibeas *et al.* 2007; Pajares *et al.* 2004; 2010b).

In this paper, we report the results of field experiments designed to (i) assess the usefulness of lures as tools for evaluating presence and density of xylophagus beetles known as vectors or potential vectors of *Bursaphelenchus* spp. nematodes; (ii) determine the differences in attractiveness of beetles between an ethanol+ α -pinene lure and commercial baits (Pheroprax[®] and Gallowit[®]); and (iii) compare the relative efficiency of different attractants.

Materials and methods

Field tests Sampling was carried out during the growing seasons from June 2007 to November 2009 at eight different sites in western, south-western and central Slovenia. The tested attractants were an ethanol+ α -pinene blend (years 2007–2009) and two commercial baits (years 2008–2009) under different ecological conditions and in different host tree stands. Descriptive details for the sampling locations and sampling periods are summarized in Table 1.

The study area is located in three different ecological regions. In the Submediterranean ecological region, the climate is Submediterranean (average yearly temperature 13.8°C, average growing season temperature 21.6°C, maximum temperature above 27°C, minimum temperature below 16°C, precipitation 1,031 mm). In the Dinaric ecological region, where the influences of Mediterranean and Alpine climates meet, the climate is transitional (average yearly temperature 9.7°C, average growing season temperature 17.4°C, maximum temperature above 23°C, minimum temperature below 12°C, precipitation 1,438 mm). In the Prealpine ecological region, the climate is alpine (average yearly temperature 9.5°C, average growing season temperature 17.8°C, maximum temperature above 24°C, minimum temperature below 12°C, precipitation 1,336 mm) (Statistical Yearbook of the Republic of Slovenia 2000–2010).

We collected entomofauna using black cross vane traps (WitaPrall IntPt–Nassfalle; Witasek Pflanzen-Schutz GmbH, Austria) equipped with collecting container with propylene glycol and attractants. Four traps were placed in each location at a height of about 2 m on self-supporting stages. The distance between the traps was at least 50 m. At each location, one trap contained a blend of ethanol (p.a., Merck) and α -pinene (98%, Sigma-Aldrich), released at about 2 g d⁻¹ at 25–28°C; one contained the pheromone Pheroprax[®] (BASF) (ingredients: 2-methylbut-3-en-2-ol); one contained Gallowit[®] (Witasek PflanzenSchutz GmbH (ingredients: ipsdienol CAS 14434-41-4, ipsenol CAS 60894-96-4, DMWK CAS 115-18-4, *cis*-verbenol CAS 18881-04-4, α -pinene CAS 80-56-8, ethanol CAS 64-17-5); and one, as a control, contained no attractants. Each collecting container was filled with 200 ml of propylene glycol to preserve the collected entomofauna. The samples were collected at 1-month intervals, fixed with 0.1% benzoic acid (p. a., Merck), prepared, identified (determination keys used: Bense 1995; Freude *et al.* 1966; Grüne 1979; Pfeffer 1995; Sama 2002; Löbl and Smetana 2006) and deposited in the entomological collection of BF-Gozd, Ljubljana.

Statistical analysis Variables were $\ln(x+1)$ transformed whenever they were found to be non-normal ($P < 0.05$ in a χ^2 normality test). The data were analyzed using one-, two- and three-way ANOVA. The LSD post-ANOVA test was used to

Table 1 Descriptive details of the sampling locations and sampling periods: location, trap coordinates, dominant tree species, age of dominant tree species (d.t.s.), ecological region, forest community, parent material/soil type, altitude (m a.s.l.), and sampling period (2007, 2008, 2009)

Location	Trap coordinate	Dominant tree species ^z	Age of d.t.s. (years)	Ecological region	Forest community ^x	Parent material/soil type ^w	Altitude (m a.s.l.)	Sampling period
1. Dekani	N45° 32' E13° 49'	<i>P. halepensis</i> ^{mon}	70–80	Submed. ^y	<i>Ses. autumn.</i> - <i>Querc. pube.</i>	car. fly./e.b.s.	95 m	03.07–07.11.2007
								20.05–15.10.2008
								22.06–20.10.2009
2. Filipčje brdo	N 45° 43' E 13° 53'	<i>P. nigra</i> ^{mon}	80–90	Submed.	<i>Ses. autumn.</i> - <i>Querc. pube.</i>	lim./e.b.s.	~370 m	03.07–07.11.2007
								20.05–15.10.2008
								22.06–20.10.2009
3. Kozina	N 45° 35' E 13° 55'	<i>P. nigra</i> ^{mon}	60–70	Submed.	<i>Ses. autumn.</i> - <i>Querc. pube.</i>	lim./e.b.s.	~500 m	03.07–07.11.2007
								20.05–15.10.2008
								22.06–20.10.2009
4. Krajna vas	N45° 46' E13° 49'	<i>P. nigra</i> ^{mon}	80–90	Submed.	<i>Ses. autumn.</i> - <i>Querc. pube.</i>	lim./e.b.s.	~269 m	03.07–07.11.2007
								20.05–15.10.2008
								22.06–20.10.2009
5. Kastelec	N45° 34' E13° 52'	<i>P. nigra</i> ^{mon}	50–60	Submed.	<i>Ses. autumn.</i> - <i>Querc. pube.</i>	lim./e.b.s.	308 m	03.07–07.11.2007
								20.05–15.10.2008
								22.06–20.10.2009
6. Podpeč	N45° 58' E14° 26'	<i>Abies alba</i> ^{mix}	60–70	Dinaric	<i>Ab.-Fag.</i>	lim.	341 m	16.06–17.10.2008
								21.05–20.11.2009
								22.05–22.10.2009
7. Idrija	N45° 57' E14° 05'	<i>Picea abies</i> ^{mix}	60–70	Dinaric	<i>Ab.-Fag.</i>	lim.	650 m	16.06–17.10.2008
								21.05–20.11.2009
								22.05–22.10.2009
8. Brdo pri Kranju	N46° 17' E14° 20'	<i>Pinus sylvestris</i> ^{mon}	60–65	Prealpine	<i>Hac.-Fag.</i>	sil.carb.dol. lim./b.s.	338 m	05.07–14.11.2007
								16.07–13.08.2008
								01.07–23.10.2009

^z *P. halepensis*^{mon} - monoculture; *P. nigra*^{mon} - monoculture; *Abies alba*^{mix} - mixed forest with *Fagus sylvatica*; *Picea abies*^{mix} - mixed forest with *Fagus sylvatica*; *P. sylvestris*^{mon} - monoculture)

^y Submed. - Submediterranean

^x *Ses. autumn.-Querc. pube.* = *Seslerio autumnalis-Quercetum pubescentis*; *Ab.-Fag.* = *Abieti-Fagetum*; *Hac.-Fag.* = *Hacquetio - Fagetum*

^w car. fly./e.b.s. = carbonate flysch/eutric brown soil; lim./e.b.s. = limestone/eutric brown soil; lim. = limestone;

sil.carb.dol.lim./b.s. = silicates and carbonate sediment rock, dolomite, limestone/brown soil

detect significant differences between the mean log-transformed values. The variability of the frequency of entomofauna was investigated with respect to

three factors: locality (eight levels: Dekani, Filipčje Brdo, Kozina, Krajna Vas, Kastelec, Podpeč, Idrija and Brdo pri Kranju), attractant (three levels:

ethanol+ α -pinene, Pheroprax® and Gallowit®) and year (three levels: 2007, 2008 and 2009). Chi-square (goodness of fit) test was used to compare differences in capture between traps ($P \leq 0.05$). We also used correspondence analysis (CA) as a descriptive/exploratory technique to analyze the correspondence between the rows (insect species), columns (attractant type) and structure of the frequency data. Analyses were performed using the statistical software Statistica 5 for Windows and Statgraphics Plus (version 5.0; Statistical Graphics Corporation, USA).

Results

Entomofauna collected Twenty-four families of the order Coleoptera were identified: wood-borers (Curculionidae, Cerambycidae and Buprestidae); non-target bark beetle predators (Cleridae, Staphylinidae, Histeridae, Ostomidae, Nitidulidae, Rhizophagidae, Tenebrionidae, Carabidae and Elateridae); and others (Oedemeridae, Dermestidae, Silphidae, Cucujidae, Colydiidae, Melyridae, Scarabaeidae, Catopidae, Erotylidae, Scaphidiidae, Mordellidae and Chrysomelidae). In total, 94 species were identified (Table 2).

The most numerous group was Scolytinae, with 76.55% of the total number of specimens (20,857), followed by Cerambycidae, with 8.12% (2212), Cleridae, with 4.31% (1175), Silphidae, with 3.04% (828), Curculioninae, with 1.67% (455) and Histeridae, with 1.00% (272). The most abundant groups of wood-boring insects were Scolytinae, Cerambycidae, other Curculionidae and Buprestidae. With respect to the number of species identified, the most numerous groups were Cerambycidae (24 taxa), Scolytinae (12 species) and Buprestidae (8 species). In the subfamily Scolytinae, we identified *Hylurgus ligniperda*, *Tomiscus piniperda*, *T. minor*, *Orthotomicus erosus*, *Ips sexdentatus*, *Hylastes attenuatus*, *H. opacus*, *Ips typographus*, *Dryocoetes autographus*, *Xyleborus germanus*, *Gnathotrichus materiarius* and *Taphrorychus villifrons*.

Non-target bark beetle predators represented an important component of the collected material. The most numerous were Cleridae, with 4.31% of the total number of specimens (1175); Histeridae, with 1.00% (272); Elateridae, with 0.66% (179); Tenebrionidae,

with 0.44% (120); Rhizophagidae, with 0.28% (77); Ostomidae, with 0.19% (53); Staphylinidae, with 0.11% (30); and Nitidulidae, with 0.07% (20). Some species belonging to these families play the most significant role in controlling the non-target insect population of bark beetles (Escherich 1923). We collected four species of the family Cleridae (*Thanasimus formicarius*, *T. rufipes*, *T. pectoralis* and *Clerus mutilarius*) and three species of the family Rhizophagidae (one of which was determined as *Rhizophagus depressus*).

We also collected five species of the family Histeridae, six species of the family Elateridae, one species of the family Tenebrionidae (*Hypophloeus linearis*), one species of the family Oedemeridae (*Nacerdes carniolica*), one species of the family Ostomidae (*Nemosoma elongatum*), two unidentified species of the family Staphylinidae, three species of the family Nitidulidae (undetermined) and one species of the family Carabidae (undetermined).

Most of the bark beetle predators belonging to the families Cleridae and Tenebrionidae were attracted by the traps containing Gallowit®.

The most important saproxylic family was Cerambycidae, of which we collected 24 taxa and identified 22 species. The dominant species was *Spondylis buprestoides*, followed by *Arhopalus rusticus*, *M. galloprovincialis*, *Arhopalus ferus*, *Neoclytus acuminatus*, *Rhagium inquisitor* and *Acanthocinus aedilis* (Table 3).

Of the total number of long-horned beetles collected, *M. galloprovincialis* represented 17.54%, *M. sutor* represented 0.09%, and *M. sartor* represented 0.04%.

The month of collection (June, July, August, September and October) was related to the effectiveness of the attractants with respect to the number of Cerambycidae taxa collected ($\chi^2_{12} = 77.48$, $P < 0.05$). The dynamic temporal (monthly and annual) changes in the numbers of Cerambycidae insects (24 taxa) captured by the three attractants are presented in Fig. 1.

In 2007, only ethanol+ α -pinene was used (Gallowit® and Pheroprax® were not used). The line chart shows the direction and intensity of the temporal changes. At all locations, the largest numbers of individuals of Cerambycidae were collected in July. The most effective attractant for Cerambycidae was ethanol+ α -pinene, followed by Gallowit®, and the least effective was Pheroprax® ($P < 0.05$).

Table 2 Collected entomofauna from the order Coleoptera (all periods, all locations, all host trees). Numbers of species and specimens are listed with respect to the attractants used

Taxa (fam., subfam)	Species/ Taxa (No.)	Specimens (No.)						Locations ^y	Host trees ^w
		Ethanol + α -pinene	Gallowit [®]	Pheroprax [®]	Control	Total (No.)	% of total		
Curculionidae	7	98 c ^x	322 d	27 b	8 a	455	1.67	1,3,5,6,7,8	
Scolytinae	12	4,762 b	8,547 d	7,291 c	257 a	20,857	76.55	1,2,3,4,5,6,7,8	1,2,3,4,5
Cerambycidae	24	1,780 d	273 c	97 b	62 a	2,212	8.12	1,2,3,4,5,6,7,8	1,2,3,4,5
Cleridae ^z	4	209 c	754 d	190 b	22 a	1,175	4.31	1,2,3,4,5,6,7,8	1,2,3,4,5
Silphidae	3	38 a	414 d	242 c	134 b	828	3.04	1,2,3,4,5,6,7,8	1,2,3,4,5
Histeridae ^z	5	178 b	87 a	2	5	272	1.00	1,2,3,4,5,6,7,8	1,2,3,4,5
Elateridae ^z	6	78 c	50 b	26 a	25 a	179	0.66	1,2,3,4,5,6,7,8	1,2,3,4,5
Tenebrionidae ^z	1	44 a	76 b	0	0	120	0.44	2,3	2,3
Oedemeridae	1	81 a	0	0	0	81	0.30	1,2,5,6	1,2,3
Rhizophagidae ^z	3	64 b	8 a	1	4	77	0.28	1,2,3,4,5,6	1,2,3
Ostomidae ^z	1	40 b	6 a	1	5	53	0.19	2,3	2,3
Buprestidae	8	9 a	12 a	13 a	2 a	36	0.13	1,2,5,6	1,2,3
Staphylinidae ^z	2	17 a	11 a	2	0	30	0.11	1,3,4,5,6,7,8	1,3,4,5
Nitidulidae ^z	3	15 a	3	2	0	20	0.07	3,4,5,7	1,3,4
Cucujidae	1	8	0	0	0	8	0.03	3,6	1,3
Dermestidae	1	6	2	0	0	8	0.03	1,2,3,4,5,8	1,2,3,5
Melyridae	2	1	8	0	0	9	0.03	3	3
Catopidae	2	0	8 a	2	0	10	0.04	3	3
Scaphidiidae	1	0	0	2	0	2	0.01	3	3
Mordellidae	1	1	1	1	0	3	0.01	3	3
Scarabaeidae	2	0	2	0	2	4	0.01	3	3
Chrysomelidae	1	0	2	0	0	2	0.01	3	3
Colydiidae	1	0	1	0	0	1	0.00	3	3
Erotylidae	1	0	0	1	0	1	0.00	3	3
Carabidae ^z	1	1	0	0	0	1	0.00	2	2
Coleoptera (undetermined- destroyed in traps)	/	440 d	169 c	75 a	119 b	803	2.95		
Total	94	7,870 b	10,756 d	7,976 c	645 a	27,247	100		

^zBark beetle predators (Escherich 1923)^yLocations: 1- Kastelec, 2- Dekani, 3- Brdo pri Kranju, 4- Filipčje brdo, 5- Kozina, 6- Krajna vas, 7- Podpeč, 8- Idrija^xNumber of individuals captured; a,b,c,d = observed frequencies for considered taxa marked with different letters are significantly different compared to each other ($P \leq 0.05$; Chi-square, goodness of fit test); observed frequencies that are not marked are not eligible for the test (expected frequencies calculated by test were smaller than 5.0)^wHost trees: 1- *Pinus nigra*, 2- *Pinus halepensis*, 3- *Pinus sylvestris*, 4- *Abies alba*, 5- *Picea abies*

The three-factor ANOVA considering locations, attractants and years provides insight into the entomofauna collected in this study. There were significant differences between localities ($F=5.55$, $df=7$, $P<0.05$) and attractants ($F=14.27$, $df=2$, $P<0.05$).

The variability of the entomofauna among three selected localities (5-Kastelec, 1-Dekani and 8-Brdo pri Kranju) and 2 years (2008 and 2009) was also examined by ANOVA. The attractants differed significantly ($F=16.68$, $df=2$, $P<0.05$). The least effective attractant was Pheroprax[®].

Table 3 Taxa of the family Cerambycidae from all locations according to the attractants used (ethanol+ α -pinene, Gallowit[®] or Pheroprax[®]) and on all host trees

Taxa	Ethanol + α -pinene	Gallowit [®]	Pheroprax [®]	Control	Total (No.)	Locations ^z	Host trees ^y
<i>Spondylis buprestoides</i> (Linnaeus, 1758)	1,043 d*	80 c	32 b	14 a	1,043 d	1,2,3,4,5,6	1,2,3
<i>Arhopalus rusticus</i> (Linnaeus, 1758)	360 b	7 a	8 a	13 a	360 b	1,2,3,4,5,6,7,8	1,2,3,4,5
<i>Monochamus galloprovincialis</i> (Olivier, 1795)	44 a	56 a	5	1	44 a	1,2,3,4,5,6	1,2,3
<i>Arhopalus ferus</i> (Mulsant, 1839)	71 b	2 a	0	0	71 b	1,2,3,4,5,6	1,2,3
<i>Neoclytus acuminatus</i> (Fabricius, 1775)	42 b	11 a	4 a	10 a	42 b	1,2,3,4,5,6	1,2,3
<i>Rhagium inquisitor</i> (Linnaeus, 1758)	24 a	42 b	0	0	24 a	1,2,3,4,5,6	1,2,3
<i>Acanthocinus aedilis</i> (Linnaeus, 1758)	3	30 a	20 a	0	3	1,2,3,4,5,6	1,2,3
<i>Leiopus nebulosus</i> (Linnaeus, 1758)	2	19 a	14 a	0	2	1,2	1,2
<i>Stictoleptura rubra</i> (Linnaeus, 1758)	2	3	1	3	2	1,2,3	1,2,3
<i>Acanthocinus griseus</i> (Fabricius, 1792)	1	3	1	0	1	2,3	2,3
<i>Arhopalus</i> sp.	1	1	0	2	1	2	2
<i>Prionus coriarius</i> (Linnaeus, 1758)	3	1	0	0	3	6	1
<i>Tetropium castaneum</i> (Linnaeus, 1758)	3	0	0	0	3	8	5
<i>Leiopus</i> sp.	0	0	2	0	0	1	1
<i>Tetropium fuscum</i> (Fabricius, 1787)	1	1	0	0	1	3	3
<i>Xylotrechus arvicola</i> (Olivier, 1795)	2	0	0	0	2	6	1
<i>Monochamus sutor</i> (Linnaeus, 1758)	0	2	0	0	0	2	2
<i>Lepturalia nigripes</i> (De Geer, 1775)	0	0	0	1	0	4	1
<i>Clytus arietis</i> (Linnaeus, 1758)	0	0	0	1	0	4	1
<i>Exocentrus adpersus</i> Mulsant, 1846	0	0	0	1	0	4	1
<i>Monochamus sartor</i> (Fabricius, 1787)	0	1	0	0	0	1	1
<i>Purpuricenus kaehleri</i> (Linnaeus, 1758)	1	0	0	0	1	6	1
<i>Rutpela maculata</i> (Poda, 1761)	1	0	0	0	1	1	1
<i>Strangalia melanura</i> (Linnaeus, 1758)	0	1	0	0	0	3	3
SUM	1,604 d	260 c	87 b	46 a	1,604 d		
Cerambycidae (undetermined or destroyed in traps)	176 b	13 a	10 a	16 a	176 b		
Total (no.)	1,780 d	273 c	97 b	62 a	1,780 d		

*Number of individuals captured; a,b,c,d = observed frequencies for considered taxa marked with different letters are significantly different compared to each other ($P \leq 0.05$; Chi-square, goodness of fit test); observed frequencies that are not marked are not eligible for the test (expected frequencies calculated by test were smaller than 5.0)

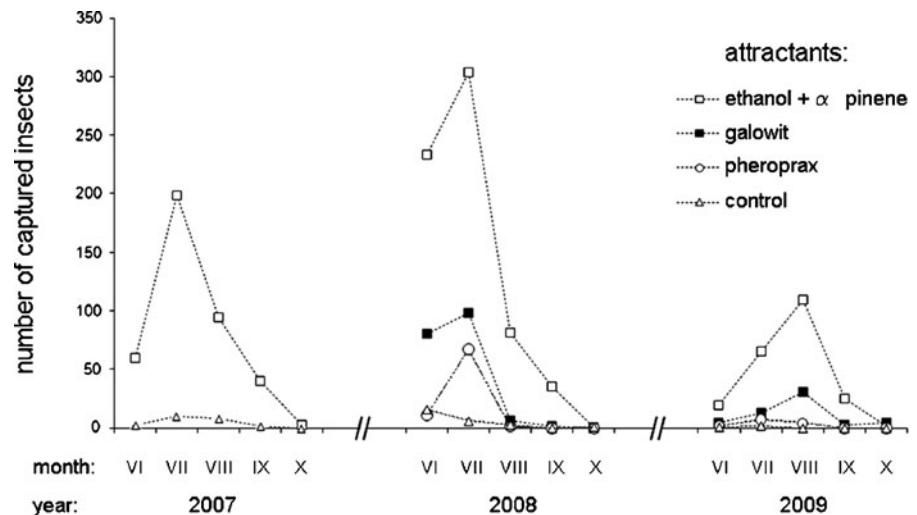
^zLocations: 1- Kastelec, 2- Dekani, 3- Brdo pri Kranju, 4- Filipčje brdo, 5- Kozina, 6- Krajna vas, 7- Podpeč, 8- Idrija

^yHost trees: 1- *Pinus nigra*, 2- *Pinus halepensis*, 3- *Pinus sylvestris*, 4- *Abies alba*, 5- *Picea abies*

Interactions between localities and attractants ($F=3.40$, $df=4$) and between localities and years ($F=6.56$, $df=2$) were statistically significant ($P < 0.05$). The variability in the entomofauna among four selected localities (2-Filipčje Brdo, 3-Kozina, 4-Krajna vas and 6-Podpeč) over 1 year (2008) showed no significant differences between localities or attractants and no significant interaction effects ($P > 0.05$). The variability of the

entomofauna at site 7 (Podpeč) over 2 years (2008 and 2009) also did not differ significantly with respect to attractants, years or their interaction ($P > 0.05$). The variability of the entomofauna at site 8 (Idrija) over 1 year (2009) also did not differ significantly between attractants ($P > 0.05$). The variability of the entomofauna among six selected localities (5-Kastelec, 1-Dekani, 8-Brdo pri Kranju, 2-Filipčje Brdo, 3-Kozina and 4-Krajna vas) over 1 year

Fig. 1 Dynamic temporal changes in Cerambycidae captured by different attractants



(2007) showed a statistically significant difference among sites. Two localities where the dominant tree species were *P. sylvestris* (8-Brdo pri Kranju) and *P. halepensis* (1-Dekani) were characterized by their depauperate entomofauna. Conversely, the localities dominated by *P. nigra* (particularly 2-Filipčje Brdo and 4-Krajna vas) were rich in entomofauna.

Monochamus spp. To analyze the presence of *Monochamus* spp., we used the localities where insects were collected over 3 years (5-Kastelec, 1-Dekani and 8-Brdo pri Kranju, 2007–2009). The affinity between the attractants and insects was evaluated through a CA (Fig. 2). Correspondence analysis is a statistical visualization method for picturing the associations between the levels of a two-way contingency table (Michaud *et al.* 2010; Ribeiro-Troian *et al.* 2009). The goal is to have a global view of the data that is useful for interpretation. Often, no more than two dimensions are needed to display most of the variability in the table. CA visualized the association between the categories of the row (insect species) and/or column (attractant type) variables summarized by the cell frequencies (number of captured insects). The distance between any two categories is a measure of their similarity (mutual affinities). The proximity between a point and a triangle on the figure is interpreted as the affinity of the insect for a given attractant. For example, the points labeled 59, 130, 134 and α -pine-09 (*Monochamus* sp., *Monochamus* sp., *M. galloprovincialis* and ethanol+ α -

pinene 2009, respectively) are all very close together on the picture showing affinity of these insects for a ethanol+ α -pinene in 2009 (Fig. 2). The most numerous were *Monochamus* individuals in the stand where *P. nigra* was the dominant tree species (*i.e.*, 5-Kastelec) ($\chi^2_2 = 15.9$, $P < 0.05$). Observations at six localities (5-Kastelec, 1-Dekani, 8-Brdo pri Kranju, 2-Filipčje Brdo, 3-Kozina and 4-Krajna vas) over 2 years revealed that the largest numbers of *Monochamus* individuals were again found in stands where *P. nigra* was the dominant tree species ($\chi^2_6 = 64.51$, $P < 0.05$).

Affinity between the attractant and insects was summarized through the CA into two dimensions (two axes) which explained 58% of the total variation (Fig. 2). The closeness between the points and a triangle on the figure is interpreted as the affinity of the insect to a given attractant. Regarding attractants, the greatest number of Cerambycidae specimens were attracted by the traps with ethanol+ α -pinene. The largest numbers of *Monochamus* specimens were attracted by the traps containing Gallowit® (galo-08, galo-09) followed by ethanol+ α -pinene (a-pine-09). Excluding the control traps, the fewest *Monochamus* individuals were attracted by the traps containing Pheroprax® ($\chi^2_2 = 17.72$, $P < 0.05$). There was a strong correlation between the numbers of *Monochamus* individuals and the numbers of non-target bark beetle predators (Cleridae, Staphylinidae, Histeridae, Trogositiidae, Nitidulidae and Rhizophagidae) in the traps ($r = 0.89$, $P < 0.05$). Most of the bark beetle

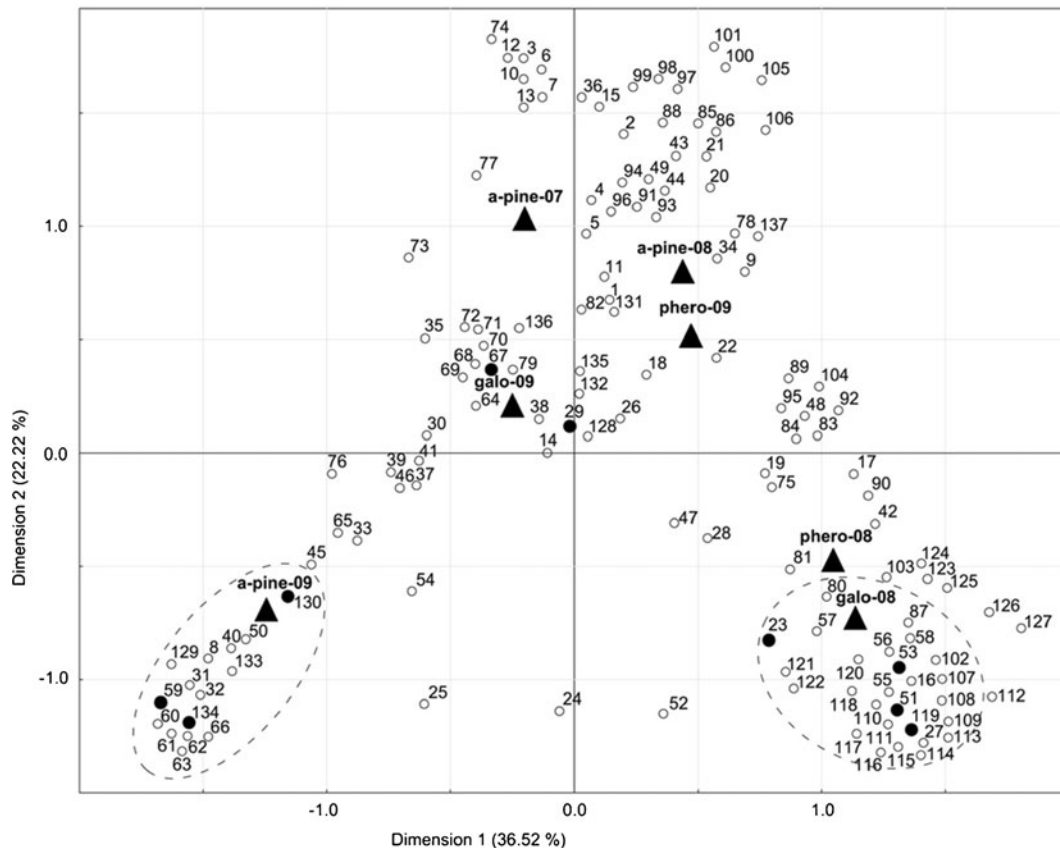


Fig. 2 Two-dimensional plot of the correspondence analysis (CA): entomofaunal samples attracted by ethanol+ α -pinene, Gallowit® and Pheroprax®. Legend: **Triangles represent** the attractants (a-pine-07, ethanol+ α -pinene in 2007; a-pine-08, ethanol+ α -pinene in 2008; a-pine-09, ethanol+ α -pinene in 2009; galo-08, Gallowit® in 2008; galo-09, Gallowit® in 2009; phero-08, Pheroprax® in 2008; phero-09, Pheroprax® in 2009); **Black points represent** *Monochamus*. Beetle taxa: 1. *Spondylis buprestoides*, 2. *Arhopalus rusticus*, 3. *Arhopalus ferus*, 4. *Stictoleptura rubra*, 5. *Dryocoetes autographus*, 6. *Nacerdes (Xanthochroa) carniolica*, 7. *Spondylis buprestoides*, 8. *Arhopalus rusticus*, 9. *Neoclytus acuminatus*, 10. *Nemosoma elongatum*, 11. Histeridae, 12. *Hypophloeus linearis*, 13. Catopidae, 14. *Lepturalia nigripes*, 15. *Hypophloeus linearis*, 16. Staphylinidae, 17. *Rhagium inquisitor*, 18. *Thanasimus formicarius*, 19. Elateridae, 20. Rhizophagidae, 21. Cucujidae, 22. Silphidae, 23. *Monochamus galloprovincialis*, 24. *Stictoleptura rubra*, 25. Curculionidae, 26. Buprestidae, 27. *Monochamus sartor*, 28. *Acanthocinus aedilis*, 29. *Monochamus sp.*, 30. *Leiopus nebulosus*, 31. *Prionus coriarius*, 32. *Rutpela maculata*, 33. Cleridae, 34. *Leiopus sp.*, 35. *Arhopalus rusticus*, 36. *Acanthocinus griseus*, 37. *Ips typographus*, 38. *Clytus arietis*, 39. Histeridae, 40. *Nemosoma elongatum*, 41. *Hypophloeus linearis*, 42. Elateridae, 43. *Rhagium sp.*, 44. *Tomicus piniperda*, 45. *Hylurgus ligniperda*, 46. *Clerus mutillarius*, 47. *Thanasimus formicarius*, 48. Silphidae, 49. *Tomicus sp.*, 50. *Xyleborus germanus*, 51. *Monochamus sutor*, 52. *Neoclytus acuminatus*, 53. *Monochamus sp.*, 54. *Rhizophagus depressus*, 55. *Stictoleptura rubra*, 56. Buprestidae, 57. Cleridae, 58. Scarabaeidae, 59. *Monochamus sp.*, 60. *Ips sexdentatus*, 61. *Orthotomicus erosus*, 62. *Hylastes attenuatus*, 63. *Tomicus minor*,

64. *Leiopus nebulosus*, 65. Cucujidae, 66. Carabidae, 67. *Monochamus galloprovincialis*, 68. *Acanthocinus aedilis*, 69. Dermestidae, 70. *Acanthocinus griseus*, 71. *Thanasimus sp.*, 72. Staphylinidae, 73. *Arhopalus rusticus*, 74. *Corymbia rubra*, 75. *Hylastes opacus*, 76. *Xylotrechus arvicola*, 77. *Exocentrus adspersus*, 78. Histeridae, 79. Staphylinidae, 80. *Thanasimus formicarius*, 81. *Thanasimus rufipes*, 82. Silphidae, 83. *Tetropium fuscum*, 84. *Acanthocinus aedilis*, 85. *Criocephalus rusticus*, 86. *Paromalus parallelepipedus*, 87. *Dasytes niger*, 88. *Dalopius marginatus*, 89. *Melanotus rufipes*, 90. *Ampedus balteatus*, 91. *Rhizophagus depressus*, 92. *Rhizophagus ferrugineus*, 93. *Rhizophagus perforatus*, 94. *Silvanus bidentatus*, 95. *Tomoxia biguttata*, 96. *Phyllobius oblongus*, 97. *Dryophthorus corticalis*, 98. *Hylobius abietis*, 99. *Pissodes piniphilus*, 100. *Gnathotrichus materiarius*, 101. *Taphrorychus villifrons*, 102. *Necrophorus vespilloides*, 103. *Oecepoma thoracica*, 104. *Pityophagus ferrugineus*, 105. *Pissodes piceae*, 106. *Pissodes pini*, 107. *Strangalia melanura*, 108. *Paromalus parallelepipedus*, 109. *Cylister angustatum*, 110. *Necrophorus vespillo*, 111. *Necrophorus humator*, 112. *Sciodrepoides watsoni*, 113. *Amphicyllis globus*, 114. *Epuraea pygmaea*, 115. *Cerylon sp.*, 116. *Tribolium castaneum*, 117. *Geotrupes niger*, 118. *Rhyncholus sculpturatus*, 119. *Monochamus sutor*, 120. *Acanthocinus griseus*, 121. *Paromalus flavicornis*, 122. *Dryophthorus corticalis*, 123. *Catops sp.*, 124. *Scaphisoma agaricinum*, 125. *Diplocoelus fagi*, 126. *Prionychus ater*, 127. *Acanthocinus griseus*, 128. *Thanasimus pectoralis*, 129. *Purpuricenus kaehleri*, 130. *Monochamus sp.*, 131. Cleridae, 132. Elateridae, 133. *Stictoleptura rubra*, 134. *Monochamus galloprovincialis*, 135. Curculionidae, 136. *Rhagium inquisitor*, 137. *Epuraea sp.*

predators were also attracted by the traps containing Gallowit® (Fig. 2).

Discussion

Considering all of the collected entomofauna, we found statistically significant differences between localities and attractants but no differences between years, indicating that a 3-year monitoring period is sufficiently long to gain insight into the prevailing species composition (complexity) in the field (Table 2).

There were statistically significant differences between localities. Two localities where the dominant tree species were *P. sylvestris* (8-Brdo pri Kranju) and *P. halepensis* (1-Dekani) were characterized by their depauperate entomofauna. Conversely, the localities with *P. nigra* (particularly 2-Filipčje Brdo and 4-Krajna vas) were rich in entomofauna. These differences are not dependant solely on the dominant tree species, but also on other characteristics of the sites, such as soil conditions and management of the stand. Interactions between localities and attractants, and between localities and years were statistically significant ($P < 0.05$).

Analysis of the variability of the entomofauna among three localities (5-Kastelec, 1-Dekani and 8-Brdo pri Kranju) over 2 years (2008 and 2009) showed statistical differences among the attractants. Considering all of the collected entomofauna, the least effective was Pheroprax®, and there was no difference between ethanol+ α -pinene and Gallowit®. The collected species of Cerambycidae represent more than 10% of all known species of this family in Slovenia, for which 213 species of Cerambycidae are listed (Breljih *et al.* 2006). This could be considered high, since no specific pheromones for this group are known and only kairomonal attraction is applicable. Several species of bark beetles were attracted *e.g.* *Hylurgus ligniperda*, *Tomicus piniperda*, as well as wood-borer beetles (*e.g.* *Spondylis buprestoides*, *Arhopalus ferus*, *Acanthocinus aedilis*, *Rhagium inquisitor*, *Hylobius* spp., *Pissodes* spp.) which are proven vectors of *Bursaphelenchus* spp. nematodes, and are thus also probable potential vectors for *B. xylophilus* (Table 3) (Lint 1988; Petrice *et al.*

2004; Sweeney *et al.* 2007). *Arhopalus ferus*, which is widespread in the Iberian Peninsula and in Slovenia, has been reported to carry *B. xylophilus* in Japan. *Spondylis buprestoides* is another common species found on recently felled *Pinus* and *Abies* trees (Vives 2000) and has been reported to carry *B. xylophilus* in Japan (Kobayashi *et al.* 1984). Species of the subfamily Scolytinae, such as *Hylurgus ligniperda*, *Tomicus piniperda* and *Orthotomicus erosus*, which occur in Slovenia, have also been reported as vectors of some *Bursaphelenchus* species (Skarmoutsos and Skarmoutsos 1999; Sousa *et al.* 2002). It is urgent to test other potential vectors for the presence of *B. xylophilus* in infected areas. If other vectors are proved to exist in Europe, the suppression or mitigation of pine wilt disease could be designed, which would have greater probability of success than has occurred with suppression of *Monochamus* spp. vectors only.

The largest quantity of *Monochamus* specimens was attracted by the traps containing Gallowit®, followed by ethanol+ α -pinene, and the smallest amount was collected in the traps containing Pheroprax®. Studies of the attractiveness of pine volatiles (monoterpene components of resin and ethanol) to various xylophagous species have shown that some of them are attractive to *Monochamus* spp. and other cerambycids, aiding them in locating suitable host material; further studies also revealed the synergistic effects of some of these volatile elements, *e.g.* ethanol+ α -pinene or bark beetles pheromones, in attractive blends (Allison *et al.* 2003; Fan *et al.* 2007; Pajares *et al.* 2004). Our results confirm these findings, because Gallowit®, containing mostly bark beetles pheromones and volatile components of conifers (ipsdienol, ipsenol, cis-verbenaol, α -pinene and ethanol), attracted most *Monochamus* species and most bark beetles in traps.

Data from the collection of the Natural History Museum (NHM) of Slovenia indicate that *M. galloprovincialis* feeds on *Pinus* (*P. sylvestris* and *P. nigra*) but seldom on *Picea*; *M. sartor* feeds on *Picea* but very rarely on *Abies* and *Pinus*; *M. sutor* feeds on *Picea* and occasionally on *Abies*; and *M. saltuarius* feeds on *Picea* and sometimes on *Pinus*. In Slovenia, *M. sartor* is frequent, and its population is stable; *M. galloprovincialis* is rare but increasing. *M. sutor* is frequent, and its population

has been increasing since 1980; *M. saltuarius* is rare but has increased recently (Breljih *et al.* 2006). According to our results, *M. galloprovincialis* is more often found in monocultures of *P. nigra*, followed by monocultures of *P. sylvestris* and *P. halepensis*, where it feeds on mentioned hosts. This is in contrast to indications that the reproductive potential of *M. galloprovincialis* under laboratory conditions has a significantly greater positive effect if bred in *P. sylvestris* than in *P. nigra* (Akbulut 2009). Our result could be the effect of influences other than that of the prevailing tree species and could depend on the climate influences and management of the stand and the consequent amount of suitable breeding material for *Monochamus* beetles.

In *P. sylvestris*, *M. galloprovincialis* has been experimentally bred in France, and French populations of *M. galloprovincialis* have higher fecundity and longevity compared with Portuguese populations (Koutroumpa *et al.* 2008). Palatability tests have been conducted by Austrian researchers, who found that *M. galloprovincialis* could be successfully fed on *P. sylvestris* and that *M. sutor* and *M. sartor* could be successfully fed on *Picea abies* (Hoyer-Tomiczek and Tomiczek 2005). In Slovenia, *M. sartor* was found in stands of *P. sylvestris* and *P. nigra*, and *M. sutor* was found in a stand of *P. nigra*. *P. sylvestris* and *P. nigra* are extremely sensitive hosts to the PWN (PHRAME 2007) and the presence of three possible vector species in stands increases the probability for successful and rapid PWN spread, if it is introduced in Slovenia.

The population density of *Monochamus* beetles in the Iberian Peninsula appears to be high in comparison with Slovenia. However, this difference may be due to the trapping method used. Our traps were installed approximately 2 m above the ground. In Portugal, 2.7% of the specimens were captured at the base of the trunk, 20.9% were captured on the trunk, 41.8% were captured in the lower canopy and 34.5% were captured in the canopy (PHRAME 2007). This difference may also be due to the intensive forest protection measures adopted in most conifer stands in Slovenia, which include the removal of injured and dead trees in due time. *M. sutor* and *M. sartor* are more frequent in the colder and more northern parts of Slovenia. High population densities of

M. galloprovincialis in Austria have been observed only in forests that are in poor condition due to environmental or biotic conditions (PHRAME 2007).

Gallowit[®] was better at attracting bark beetles, bark beetle predators and curculionids in comparison with attracting cerambycids. The correlation with non-target bark beetle predators (Cleridae, Staphylinidae, Histeridae, Trogositidae, Nitidulidae and Rhizophagidae) in the traps was high. Most bark beetle predators were attracted by the traps containing Gallowit[®], rendering this attractant unsuitable for practical use due to its negative impact on bark beetle predators. As expected, Pheroprax[®] was not successful in attracting *Monochamus*, because it is designed for the attraction of bark beetles.

Finally, we conclude that the bioclimatic and other conditions found in Slovenia, such as the temperatures, the hosts and the vector populations, are suitable for the invasion and propagation of the nematode (Evans *et al.* 1996). Consequently, clarifying the attraction of cerambycids and other xylophagous beetles, and the potential vectors of *B. xylophilus* to semiochemicals, is essential for the evaluation of the probable routes of introduction, the possibilities and the speed of spread of PWN. This information will be useful for designing survey and control programs if the PWN is introduced into Slovenia or other nearby countries.

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