Distribution and abundance of the alien *Xylosandrus germanus* and other ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) in different forest stands in central Slovenia

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The East Asian ambrosia beetle (*Xylosandrus germanus* – Blandford 1894) is an invasive species that has become successfully established in Europe and North America. In Slovenia, *X. germanus* was first recorded in 2000 in the western part of the country, and since 2008 the species has also been identified in other parts of Slovenia. The first economic damage was recorded in 2016 after a massive attack on recently felled logs of different tree species, spurring research into this non-native invasive species. To examine the distribution and abundance of *X. germanus* compared to other ambrosia beetles and to determine voltinism and the flight period of the species in our climatic conditions, we deployed 19 ethanol-baited traps from March to November 2017 in oak-, beech- and fir-dominated forest stands in central Slovenia. To verify the vertical distribution of *X. germanus*, traps were installed at altitudes ranging from 303 m to 941 m a.s.l. Furthermore, the impact of the ice storm that hit Slovenia in 2014 on the abundance of *X. germanus* was also studied. Non-native *X. germanus* represented 71.8% of the total catch and was significantly more abundant than the other five most common species: *Xyleborinus saxesenii* (20.0%), *Xyleborus monographus* (3.6%), *Anisandrus dispar* (2.5%), *Trypodendron domesticum* (1.2%) and *Trypodendron signatum* (0.6%). *X. germanus* was most abundant in beech-dominated stands, but the differences between forest types were not significant. The species was found along the entire altitudinal gradient. Our results indicate that the swarming of *X. germanus* in lowland forests may already occur by the middle of March. Maximum flying activity was observed in May and early June in forests below 600 m a.s.l. and at the end of May and in June in forests above 700 m a.s.l. Only one generation per year was observed. The ice storm positively affected the abundance of *X. germanus*, especially in areas where sanitary logging was delayed. *Xyleborinus attenuatus* was detected for the first time in Slovenia.

Keywords: *Xylosandrus germanus*, Ambrosia Beetles, Black Timber Bark Beetle, Invasive Species, Habitat Preference, Monitoring, Ethanol-baited Traps

Introduction

Ambrosia beetles are weevils from the Scolytinae and Platypodinae subfamilies (Coleoptera: Curculionidae) that are obligately associated with nutritional fungal symbionts (Hulcr et al. 2015). They usually excavate tunnels in the wood of dead or stressed trees in which they actively cultivate ambrosia fungi. Because of their hidden way of life (most of the time they reside within the wood), they can be easily and imperceptibly transported to new environments (Rassati et al. 2016a). Many species are polyphagous and have haplodiploid reproduction and sibling mating behavior. All these features allow small founder populations to rapidly establish and spread in new environments (Kirkendall 1983). Therefore, ambrosia beetles are one of the most successful groups of invasive species worldwide (Kirkendall & Faccoli 2010).

The black timber bark beetle *Xylosandrus germanus* (Blandford, 1894) is native to East Asia (Kirkendall & Faccoli 2010). In Europe, it was first recorded in 1952 in Germany (Groschke 1953). In the last two decades of the 20th century, the species was also recorded in Austria, Belgium, France, Italy, Poland and Switzerland. After the year 2000 *X. germanus* spread throughout Europe and is now present in Croatia, the Czech Republic, Denmark, Hungary, the Netherlands, Romania, the Russian Federation, Slovakia, Slovenia, Spain, Sweden, Turkey, the United Kingdom and Ukraine (Björklund & Boberg 2017a, EPPO 2018, Galko et al. 2019).

In Slovenia, *X. germanus* was recorded for the first time in 2000. It was found on an infested sweet chestnut tree (*Castanea sativa* Mill.) in the western part of the country (Jurc et al. 2010, Seljak 2013). Since

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2008, the species has also been identified in other parts of Slovenia. It has often been caught in traps intended for monitoring *Monochamus* beetles (Coleoptera: Cerambycidae), and in a few cases, infestations of visually healthy trees have been found (Jurc et al. 2010, 2011, 2012).

The first economic damage was recorded in 2016, when *X. germanus* infested a large amount of timber that was not removed from the forest immediately after logging (Hauptman et al. 2018). In addition to finding *X. germanus* in sweet chestnut trees, we have also found infestations on Norway spruce (*Picea abies* [L.] H. Karst), silver fir (*Abies alba* Mill.), Scots pine (*Pinus sylvestris* L.), common beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* [Mattuschka] Liebl.).

In North America, where the species was introduced in 1932, *X. germanus* is one of the most economically important ambrosia beetle pests in nurseries (Ranger et al. 2016), while in Europe it is considered a secondary pest (Henin & Versteirt 2004, Lakatos & Kajimura 2007). However, in some parts of Europe, *X. germanus* has become one of the most common scolytid species (Grégoire et al. 2001, Henin & Versteirt 2004, Bouget & Noblecourt 2005, Holzinger et al. 2014, Rassati et al. 2016a) and is considered to have the potential to affect native scolytid communities (Henin & Versteirt 2004).

X. germanus is extremely polyphagous (Weber & McPherson 1983). However, in Italy, the forest type was recently found to have a significant effect on the activity-density of the species (Rassati et al. 2016a). The abundance of *X. germanus* in ethanol-baited traps was significantly higher in chestnut-dominated (*C. sativa*) forests than in forests dominated by hop hornbeam (*Ostrya carpinifolia* Scop.) and beech (*F. syl-* vatica*). Furthermore, temperature was also found to have a positive impact on the activity-density of *X. germanus*, meaning that low temperatures limit its spread to high-elevation forests (Rassati et al. 2016a). In Europe an elevation limit of 500 m a.s.l. was proposed by Bruge (1995), but a study from Romania (Olenici et al. 2014) states that *X. germanus* was found at an altitude of 760 to 900 m a.s.l. Similar findings have recently been reported from Slovakia (Galko et al. 2019). The number of generations per year also depends primarily on environmental temperatures. In Central Europe, *X. germanus* is considered to be monovoltine (Bruge 1995, Henin & Versteirt 2004), but in Italy, two generations per year have been observed (Rassati et al. 2016b).

The main aim of this study was to examine the distribution and abundance of *X. germanus* and other ambrosia beetles in different forest stands dominated by different tree species and at different altitudes in the central part of Slovenia. Furthermore, we also wanted to determine voltinism and the flight period of *X. germanus* in our climatic conditions. Forests damaged due to natural disasters are an appropriate habitat for the development of the species (Jurc et al. 2010); therefore, the impact of the 2014 ice storm on the abundance of *X. germanus* was also studied.

Materials and methods

Research area

The study was conducted in 19 different forest stands located in the wider area of the capital city of Ljubljana (Fig. 1). The selected stands are dominated (*i.e.*, more than 50% of trees belong to a given species) by common beech (*F. sylvatica*, 7 stands), sessile oak (*Q. petraea*, 7 stands)

and silver fir (*A. alba*, 5 stands). The stands are located at altitudes ranging from 303 m to 941 m a.s.l. and were therefore divided into three altitudinal groups: (I) lower altitudes, below 450 m a.s.l.; (II) medium altitudes, between 450 and 600 m a.s.l.; (III) higher altitudes, above 700 m a.s.l. (Tab. 1).

Traps, lures and sampling

In each stand, one black cross-vane panel trap (WitaPrall IntPt - Nassfalle, Witasek PflanzenSchutz GmbH, Germany) was placed in the middle of a plot of at least 2 ha where a particular tree species dominates. However, these plots are a part of a larger forest area where a particular species is the most common tree species. Traps were hung on a tree branch approximately two meters above the ground and at least 50 m from the forest edge. All traps were equipped with a half-liter plastic bottle without a cap, containing 200 ml of 96% ethanol as an attractant. The bottles were attached in the upper half of the traps. Furthermore, wet collecting cups filled with 200 ml of antifreeze (80-98% ethylene glycol – Arteco, Belgium) to preserve the collected entomofauna were positioned at the bottom of the traps. Traps were set in the period between 20 February 2017 and 7 March 2017. On 10 March 2017, traps were visited for the first time, and since then, traps were visited at 14-day intervals until 3 November 2017. Due to the different time settings of the traps, catch data from the period before 10 March 2017 were not included in the analysis.

On each visit, samples were collected from each trap. In total, 17 samples per trap were collected in the entire sampling period. After every sampling, the collecting cups were refilled with fresh ethylene glycol. On each visit, the amount of evaporated ethanol was also measured, and the
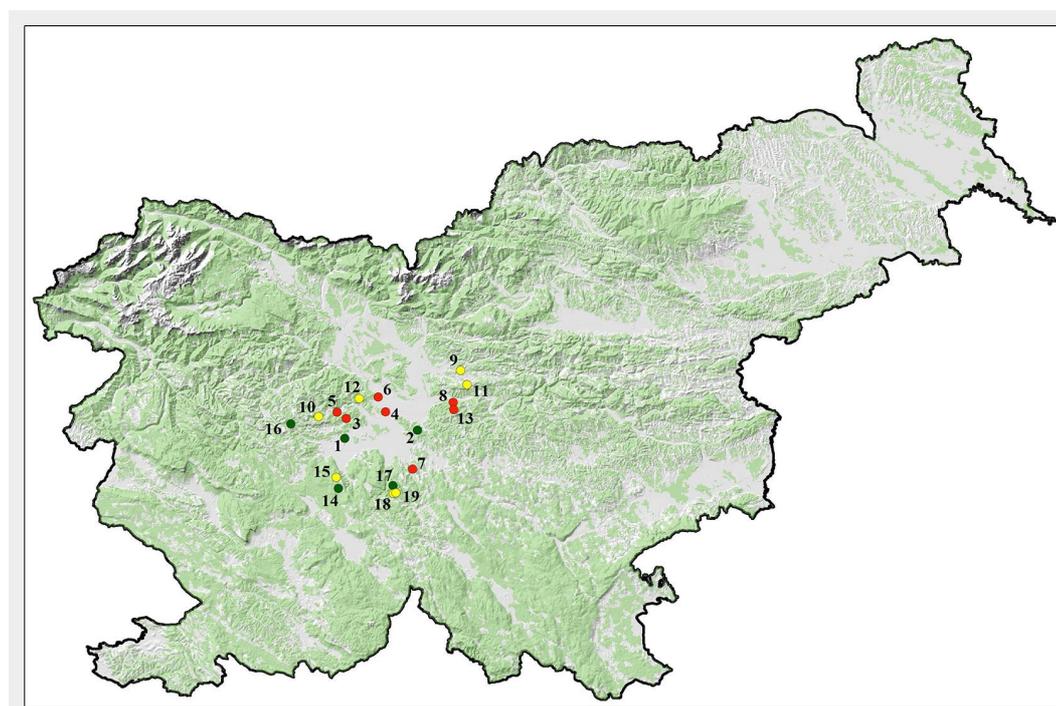

Fig. 1 - Trap locations in stands of common beech (yellow), sessile oak (red) and silver fir (green). The numbers on the map match the stand numbers in Tab. 1.

same amount of fresh pure ethanol was added to the ethanol-containing bottle. The mean release rate of ethanol during the sampling period was 1.65 ml day^{-1}.

Samples were analyzed using an Olympus SZX12® stereo microscope, and ambrosia beetles were identified according to the insect identification keys presented by Grüne (1979), Pfeffer (1995) and Faccoli (2008).

Statistical analyses

For each species, analyses were performed on the total cumulative number of beetles captured during the trapping period per trap. Data were analyzed using the statistical package SPSS® ver. 25 (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was performed to check the normality of the data. For non-normally distributed data, log(x+1) transformations were used to normalize them. A one-way ANOVA was used to test the differences in ambrosia beetle species abundance (only six of the most common species were included in the analysis) and to separately test the differences in the abundance of species between different forest types and between different altitudinal groups. To detect differences in abundance between pairs of samples, Games-Howell *post-hoc* test was used. The correlation between sanitary felling and the abundance of *X. germanus* was tested by the Pearson's correlation coefficient. Data on the quantity of sanitary logging due to the ice storm in 2014 at individual trap locations were obtained from the Slovenia Forest Service database (ZGS 2017).

Results

Distribution and abundance of X. germanus and other ambrosia bark beetles

We trapped eleven species of ambrosia beetles: three non-native and eight native to Europe (Tab. 2). There were significant differences in beetle abundance between the different species (one-way ANOVA: $F_{[5, 108]}$=34.026, p<0.001). Non-native *X. germanus* represented 71.8% of the total catch, and the results of the *post-hoc* test (Tab. 2) show that it was significantly more abundant (p<0.05 – Games-Howell *post-hoc* test) than the other five most common species: *Xyleborinus saxesenii* (Ratzeburg, 1837) at 20.0%, *Xyleborus monographus* (Fabricius, 1792) at 3.6%, *Anisandrus dispar* (Fabricius, 1792) at 2.5%, *Trypodendron domesticum* (Linnaeus, 1758) at 1.2% and *Trypodendron signatum* (Fabricius, 1787) at 0.6%. *X. germanus* was the most abundant species at 18 of the 19 locations (at one location, it was outcompeted by *X. saxesenii*). The other two non-native species were rare. Twelve specimens of *Gnathotrichus materiarius* (Fitch, 1858) and one specimen of *Xyleborinus attenuatus* (Blandford, 1894) were caught. Four species, namely, *X. germanus, X. saxesenii, A. dispar* and *T. signatum*, were caught at all 19 sampled loca-

tions.

X. germanus was most abundant in beech-dominated stands (Fig. 2) and at medium altitudes (Fig. 3). Although the mean catch in beech stands was almost three times larger than that in oak stands and more than seven times larger than that in fir stands, the differences were not significant (one-way ANOVA: $F_{[2, 16]}$=3.346, p=0.061). Similarly, the mean catch at medium altitudes was more than three times larger than that at lower altitudes and more than four times larger than that at higher altitudes, but again, the differences were not significant (one-way ANOVA: $F_{[2, 16]}$=1.829, p=0.193). However, it should be pointed out that 47.3% of *X. germanus* specimens were caught at one beech stand

at a medium altitude. If we eliminate this location from the analysis, the differences between altitudes were even more non-significant (p=0.506 – Games-Howell *post-hoc* test), while the differences between different forest types remained at a similar level (p=0.063). Although the differences were not significant (oak-fir: p=0.122; beech-fir: p=0.063 – Games-Howell *post-hoc* test), fir stands proved to be the least suitable for *X. germanus*. The abundance of the species in oak and beech stands was quite similar (p=0.568 – Games-Howell *post-hoc* test), especially if we removed the extreme beech location from the data (p=0.909 – Games-Howell *post-hoc* test).

Significant differences in the abundance of *X. saxesenii* between different forest

Tab. 1 - Data on trap locations.

Stand no.	Local unit	Latitude	Longitude	Dominant tree species	Altitude (m a.s.l.)	Altitude group
1	Vrhnika	45°59′52″	14°21′42″	*Abies alba*	303	I
2	Domžale	46°01′03″	14°35′24″	*Abies alba*	328	I
3	Vrhnika	46°00′20″	14°20′11″	*Quercus petraea*	329	I
4	Ljubljana	46°03′27″	14°29′20″	*Quercus petraea*	335	I
5	Ljubljana	46°02′32″	14°21′58″	*Quercus petraea*	344	I
6	Ljubljana	46°05′26″	14°27′57″	*Quercus petraea*	357	I
7	Škofljica	45°55′53″	14°34′32″	*Quercus petraea*	413	I
8	Domžale	46°04′45″	14°42′07″	*Quercus petraea*	452	II
9	Domžale	46°08′59″	14°43′31″	*Fagus sylvatica*	499	II
10	Ljubljana	46°02′45″	14°16′42″	*Fagus sylvatica*	502	II
11	Domžale	46°07′07″	14°44′41″	*Fagus sylvatica*	529	II
12	Ljubljana	46°05′12″	14°24′20″	*Fagus sylvatica*	574	II
13	Domžale	46°03′48″	14°42′14″	*Quercus petraea*	589	II
14	Vrhnika	45°53′14″	14°20′35″	*Abies alba*	713	III
15	Vrhnika	45°54′42″	14°20′06″	*Fagus sylvatica*	749	III
16	Ljubljana	46°01′45″	14°11′29″	*Abies alba*	801	III
17	Škofljica	45°53′41″	14°30′49″	*Abies alba*	860	III
18	Škofljica	45°52′39″	14°30′53″	*Fagus sylvatica*	900	III
19	Škofljica	45°52′43″	14°31′24″	*Fagus sylvatica*	941	III

Tab. 2 - Species of ambrosia beetles caught in ethanol-baited traps. The total and mean number ± standard error (SE) of individuals and number of sites (out of 19) where each species was trapped are shown. Means followed by the same letter were not significantly different (p>0.05) after Games-Howell *post-hoc* test. (*): Non-native species.

| Species	No. of sites	Total no. of individuals	Mean ± SE
Xylosandrus germanus *	19	67592	3557.5 ± 1611.5 [a]
Xyleborinus saxesenii	19	18825	990.8 ± 220.7 [b]
Xyleborus monographus	14	3385	178.2 ± 138.1 [c]
Anisandrus dispar	19	2336	122.9 ± 20.5 [d]
Trypodendron domesticum	17	1127	59.3 ± 15.9 [c,d]
Trypodendron signatum	19	558	29.4 ± 5.3 [d]
Xyleborus dryographus	11	208	10.9 ± 4.5
Trypodendron lineatum	6	59	3.1 ± 1.5
Gnathotrichus materiarius *	4	12	0.6 ± 0.4
Xyleborus cryptographus	1	1	0.1 ± 0.1
Xyleborinus attenuatus *	1	1	0.1 ± 0.1
Total	19	94104	4952.8 ± 1802.9

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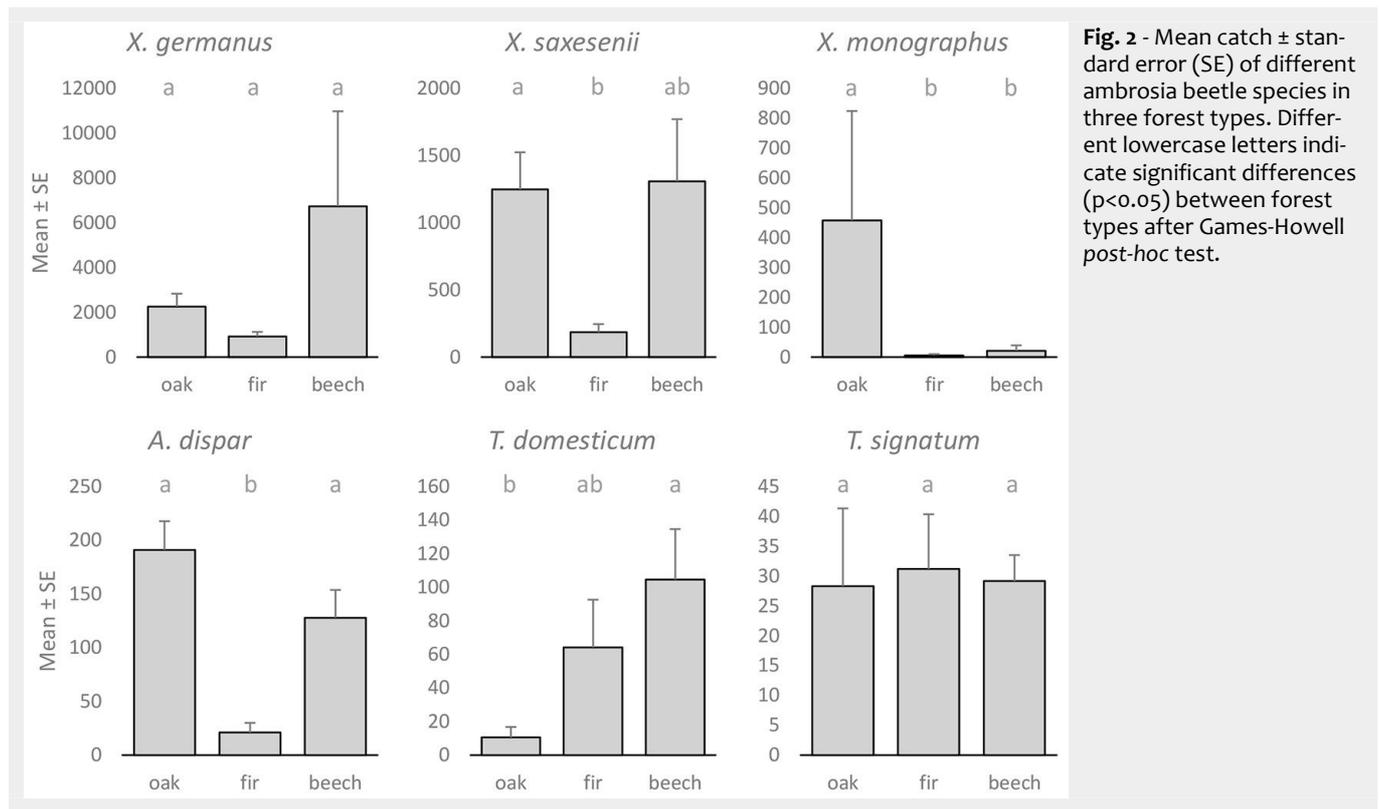
Fig. 2 - Mean catch ± standard error (SE) of different ambrosia beetle species in three forest types. Different lowercase letters indicate significant differences (p<0.05) between forest types after Games-Howell *post-hoc* test.

types (one-way ANOVA: $F_{[2, 16]}=4.235$, p=0.033) and different altitudes (one-way ANOVA: $F_{[2, 16]}=7.839$, p=0.004) were found. The species was least common in fir stands (Fig. 2) and at higher altitudes (Fig. 3). Significant differences in abundance between fir and oak stands (p=0.009 – Games-Howell *post-hoc* test) and lower and higher altitudes (p=0.019 – Games-Howell *post-hoc*

test) were also observed.

X. monographus was almost exclusively trapped in oak-dominated stands (Fig. 2) and at lower altitudes (Fig. 3). Nearly 95% of all *X. monographus* specimens were trapped in oak-dominated stands; however, 78.0% were trapped at only one location. Differences in abundance between forest types were significant (one-way

ANOVA: $F_{[2, 16]}=5.911$, p=0.012). Furthermore, significant differences between different altitudes (one-way ANOVA: $F_{[2, 16]}=11.581$, p=0.001) were also observed.

The abundance of *A. dispar* in fir-dominated stands was significantly lower than that in oak- (p=0.009 – Games-Howell *post-hoc* test) and beech-dominated stands (p=0.019 – Games-Howell *post-hoc* test)
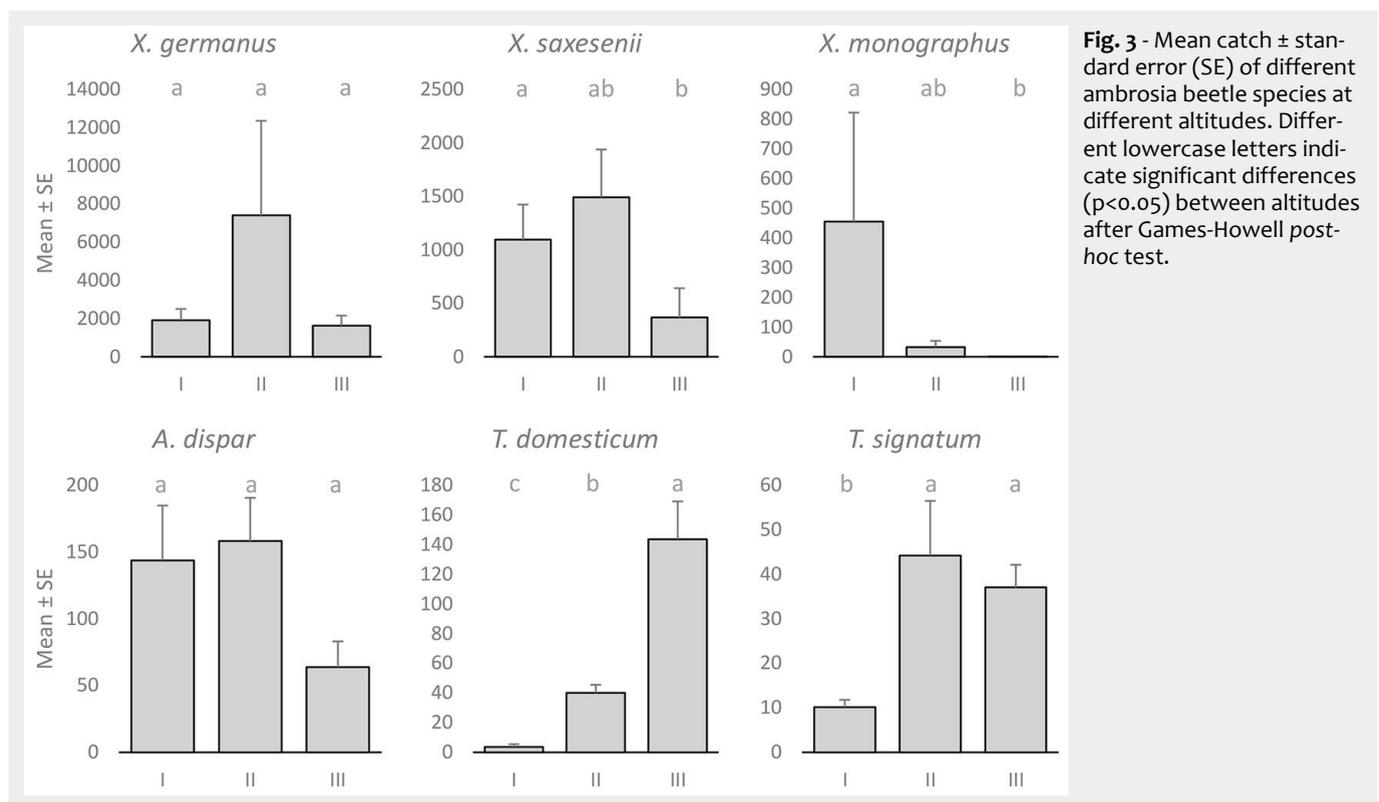

Fig. 3 - Mean catch ± standard error (SE) of different ambrosia beetle species at different altitudes. Different lowercase letters indicate significant differences (p<0.05) between altitudes after Games-Howell *post-hoc* test.

Fig. 4 - Relative share of the catch (number of caught specimens in particular period and particular altitude group / number of caught specimens in entire monitoring period at particular altitude group) of *X. germanus* in different periods and at different altitudes.
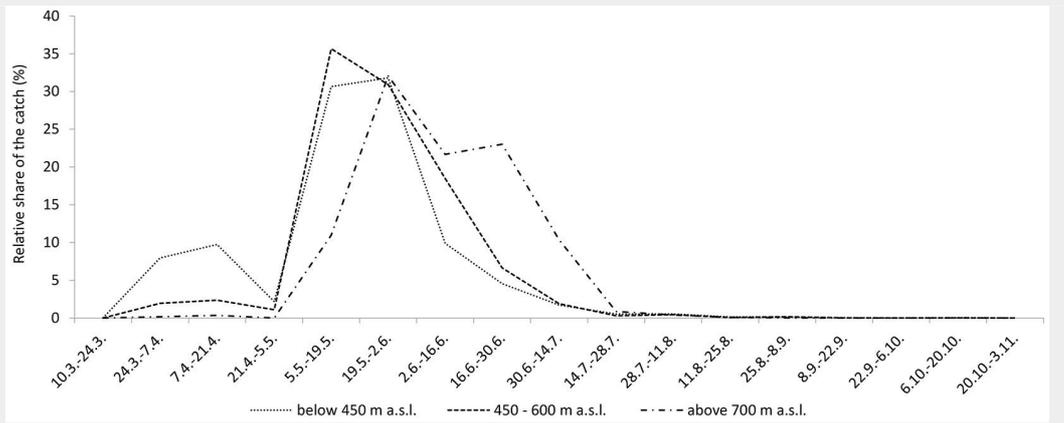
(Fig. 2). The species was less abundant at higher altitudes (Fig. 3), but the differences between different altitudes were not significant (one-way ANOVA: $F_{[2, 16]}=1.421$, p=0.270).

Both *Trypodendron* species, *T. domesticum* and *T. signatum*, were more abundant at higher altitudes (Fig. 3). The catch of *T. domesticum* at higher altitudes was significantly larger than that at medium (p=0.002 – Games-Howell *post-hoc* test) and lower altitudes (p<0.001 – Games-Howell *post-hoc* test), and the difference was also significant between medium and lower altitudes (p=0.001 – Games-Howell *post-hoc* test). The abundance of *T. signatum* at lower altitudes was significantly lower than that at medium (p=0.007 – Games-Howell *post-hoc* test) and higher altitudes (p<0.001 – Games-Howell *post-hoc* test), but there was no significant difference (p=0.989 – Games-Howell *post-hoc* test) between medium and higher altitudes. The catch of *T. signatum* was evenly distributed between different forest types (Fig. 2), while a significant difference (one-way ANOVA: $F_{[2, 16]}=6.794$, p=0.007) in the abundance of *T. domesticum* between different forest types was found. There was a significant difference between beech- and oak-dominated stands (p=0.002 – Games-Howell *post-hoc* test).

Flight period of X. germanus

The first *X. germanus* specimens were trapped in the first monitoring period (Fig. 4), between 10 and 24 March, but only in three stands at lower altitudes. In the second monitoring period, the species was trapped at 12 additional locations. At the locations above 800 m a.s.l., *X. germanus* appeared in the third monitoring period (2 locations) or, even later, in the fifth monitoring period (2 locations).

The flight period at the end of April was interrupted due to cold and wet weather (Fig. 4). At lower and medium altitudes, peak flight activity appeared between 5 May and 2 June. At higher altitudes (above 700 m a.s.l.), peak flight activity appeared between 19 May and 2 June, but the catch was relatively large until the end of June. By the end of July, more than 99.0% of all

X. germanus specimens had been trapped. A second peak in flight activity, which would indicate the development of the second generation, was not observed. However, *X. germanus* specimens were still sporadically caught in September and October.

Ice storm impact on the abundance of X. germanus

A catastrophic ice storm damaged Slovenian forests in February 2014. The positive correlation between the amount of sanitary felling in the period from 2014 to 2017 and the abundance of *Xylosandrus germanus* was not significant (one-tailed Pearson's correlation coefficient: r=0.308, p=0.10). However, if we excluded the year 2014 and considered only the period from 2015 to 2017, the positive correlation was higher and significant (one-tailed Pearson's correlation coefficient: r=0.41, p=0.04). Furthermore, the catch of *X. germanus* at one beech stand was extremely large. If we remove this data, the positive correlation was even higher and more significant (one-tailed Pearson's coefficient: r=0.481, p=0.022).

Discussion

Less than 10 years after its first discovery, *X. germanus* is already the most abundant ambrosia beetle species in the research area. The species was trapped at all 19 sampled locations. It was the most common species at all locations except one and represented 71.8% of all ambrosia beetles caught in this study. Similar dominance has also been reported for other parts of Europe. In Belgium, *X. germanus* constituted 79.6% and 84.0% of all trapped scolytids in two monitoring studies in beech forest stands near Brussels (Grégoire et al. 2001). In a large monitoring project of saproxylic beetles near Vienna in Austria, *X. germanus* represented 70% of all identified individuals (Holzinger et al. 2014). Most recently, in a study located in northeastern Italy, *X. germanus* was the most commonly trapped ambrosia bark beetle (58.7% – Rassati et al. 2016a). Our results refer only to ambrosia bark beetles (Tab. 2); however, if we include the data on other beetles, individuals

of *X. germanus* represented 71.6% of all bark beetles and 65.4% of all beetles. In addition to Curculionidae, Salpingidae was the most commonly trapped beetle family in our study.

Klimetzek et al. (1986) tested different ethanol release rates and found a positive correlation between the ambrosia beetle catch and ethanol release rates. However, some authors have reported that high ethanol release rates may have an inhibitory effect and reduce the catch of ambrosia beetles (Salom & McLean 1990, Ranger et al. 2011, Galko et al. 2014). The release rate in our study was relatively high and could have had a negative impact on some species. However, based on unpublished data from experiments in which we used non-baited traps, we believe that a lower ethanol release rate would not have affected the proportion of *X. germanus* in the catches.

As in some other studies in Europe (Markalas & Kalapanida 1997, Galko et al. 2014, Rassati et al. 2016a), *X. saxesenii* was the most common native ambrosia beetle. It was especially abundant at lower and medium altitudes, but the proportion of this species in the catch was significantly reduced at higher altitudes (Fig. 3). In contrast, the catch of *T. domesticum* at higher altitudes was significantly larger than that at medium and lower altitudes, and therefore, at the three higher locations, *T. domesticum* was the most common native species. However, when interpreting the results for *T. domesticum*, it is necessary to stress that we missed the beginning and the peak of flight activity at lower and medium altitudes. In the local literature (Titovšek 1988, Jurc 2005), there is information that the species begins to fly in the second half of March, when ambient temperatures reach 13-14 °C, but our study (especially trap catches before 10 March 2017 – data not included in analysis) showed that in the lowlands, *T. domesticum* already had a peak occurrence before 10 March. According to temperature data from nearby meteorological stations (ARSO 2018), it is assumed that the temperature swarming threshold for *T. domesticum* is lower than that mentioned before, and it could be sim-

ilar to that in Croatia, where it was found that flight activity starts when daily temperatures rise above 9 °C (Franjević 2013). However, the abundance of *T. domesticum* and, to a lesser extent, *T. signatum* in our lowland forests is certainly greater than our results showed.

In North America *X. germanus* did not exhibit flight activity until one or two days with a maximum daily temperature of at least 20 °C (Reding et al. 2013). According to data from the official meteorological station in Ljubljana (ARSO 2018), similar maximum daily temperatures were also measured in the second half of the first monitoring period (between 10 and 24 March). This turned out to be sufficient to induce flight activity in the first *X. germanus* specimens in nearby oak stands with a southern exposition. In the middle of the second monitoring period (between 24 March and 6 April), maximum daily temperatures also exceeded 20 °C at higher altitudes, and the first *X. germanus* specimens were also trapped at all other traps located below 800 m a.s.l. On this basis, we assume that the temperature threshold of 20 °C is also valid in our climatic conditions.

The flight activity of beetles varies from season to season due to different weather conditions in every year. However, Graf & Manser (2000) reported that the flight activity of *X. germanus* in Switzerland started at the beginning of May, with a maximum in June and July. Similar observations were also made in Romania (Olenici et al. 2014). Earlier flight activity of the species was observed in Croatia (Franjević et al. 2016), where swarming began in the beginning of April, with peak flight activity already occurring at the end of April. On the basis of our results, it could be argued that the swarming of the species in lowland forests may take place by the middle or end of March. Maximum flight activity in our study was observed in May and early June in forests below 600 m a.s.l. and at the end of May and in June in forests above 700 m a.s.l. However, we assume that the cold and wet weather at the end of April prevented an even earlier occurrence of peak flight activity and that the peak occurrence of *X. germanus* could occur as soon as the end of April, as formerly recorded in Croatia.

In Central Europe *X. germanus* has one generation per year (Bruge 1995, Henin & Versteirt 2004), while in Italy two generations per year have been observed (Rassati et al. 2016b). The first peak of occurrence in Italy is in the middle of May and the second at end of July or at the beginning of August (M. Faccoli, personal communication). Because we observed the first peak in May, we also expected the occurrence of a second peak in July or August, but it obviously did not appear (Fig. 4). Therefore, we assume that only one generation per year develops in central Slovenia.

Minimum winter temperatures are pre-

sumably the key limiting factor for the survival of *X. germanus* (Bruge 1995, Henin & Versteirt 2004, Galko et al. 2019). According to Slovenian Environment Agency data (Cegnar 2017), January 2017 was the coldest January in the last three decades. The average monthly temperature in Ljubljana was -3.1 °C, which is 3.5 °C lower than the average of the period 1981-2010. The abundance of *X. germanus* in our traps after such exceptional conditions indicates that winter temperatures in the lowlands of Slovenia are most likely not a real limiting factor for the species. However, at higher altitudes, conditions are more extreme, and the vertical spread of the species is certainly limited. In our research, *X. germanus* was found along the entire altitudinal gradient between 303 and 941 m. Our results thus confirm the findings from Slovakia (Galko et al. 2019) and Romania (Olenici et al. 2014), where the species appeared at altitudes of approximately 900 m. However, the catch of *X. germanus* in the trap at the highest altitude (941 m a.s.l.) was relatively large (2060 specimens). We therefore assume that we did not reach the upper boundary of the appearance of the species in Slovenia. In Slovakia, *X. germanus* was found to attack spruce logs at an altitude of 1020 m, but it has not yet been found in forests above 1100 m a.s.l. (Galko et al. 2019).

In Slovakia, altitude had a significant effect and forest type (oak, beech, spruce) had a nonsignificant effect on the abundance of *X. germanus* (Galko et al. 2019). However, the results from Slovakia suggest that beech forests between approximately 500 and 700 m a.s.l. are optimal for the species. Warmer and dryer oak forests at lower altitudes, and cool submontane mountain forests are presumably less suitable for the beetle. Our findings are to some extent also in accordance with the suggested optimal site conditions. The abundance of *X. germanus* in our study was also greatest at medium altitudes (450-600 m a.s.l.) and in beech stands (Fig. 2, Fig. 3). However, the differences between altitude groups and forest types in our study were not significant. Our results actually show that lower elevation oak forests are equally suitable for *X. germanus* compared to higher elevation beech forests, while fir stands proved to be the least suitable for *X. germanus*. The abundance of *X. germanus* in Italy (Rassati et al. 2016a) was significantly higher in sweet chestnut-dominated forests than in hop-hornbeam- and beech-dominated forests. Therefore, the relatively large admixture of sweet chestnut in the oak stands in our study could have contributed to the large abundance of the species in oak stands. Indeed, ongoing research work in Slovenia also shows the massive presence of *X. germanus* in other forest types, demonstrating that the species is a habitat generalist.

In most cases, *X. germanus* attacks hosts that are stressed, dying or recently dead

(Ranger et al. 2016). Forests damaged by natural disasters are therefore a suitable environment for the development of the species (Jurc et al. 2010). In February 2014, Slovenia was hit by an ice storm that damaged approximately 9.3 million m³ of trees over an area of more than 600,000 ha (ZGS 2014). The forests in our research area were also damaged, but to varying degrees. The measure of damage is the amount of sanitary logging carried out in the period after the ice storm. If we take into account the entire period (2014-2017), the positive correlation between the amount of sanitary felling and the abundance of *X. germanus* is weak to moderate and not significant. However, if we exclude 2014, the positive correlation is higher and significant. Thus, we can conclude that the ice storm had a positive effect on the abundance of the species, especially in areas where sanitary logging was delayed.

In addition to *X. germanus*, two other non-native ambrosia beetle species were trapped in our study. *G. materiarius* is native to North America and was introduced to Europe in the 1930s (Balachowsky 1949). In Slovenia, it was first found in 2003 during an inventory of fauna at Brdo pri Kranju (Geister 2004). Since then, it has often been found in traps intended for monitoring of *Monochamus* beetles in pine stands around Slovenia (Jurc et al. 2012). Damage caused by this species has not yet been recorded. To our knowledge, the specimen of *X. attenuatus* caught in our study is the first finding of this species in Slovenia. It was trapped in the period between 14 and 28 July in an oak-dominated forest in the outskirts of Ljubljana. In another monitoring program that took place in 2018, another specimen of *X. attenuatus* was caught in an ethanol-baited trap located near Tolmin in the western part of Slovenia. However, this species was first found in Europe in 1987 (Knížek 1988). Its exact distribution is not clear because of confusion with the closely related *X. saxesenii*; nevertheless, it is estimated to be widely distributed within the EU (Björklund & Boberg 2017b).

Conclusions

The spread of *X. germanus* in Europe has accelerated since 2000 (Galko et al. 2019). Based on the results of this study and observations in other parts of Slovenia, we can conclude that the species has become established in Slovenia. Moreover, as in some other countries in Europe (Grégoire et al. 2001, Holzinger et al. 2014, Rassati et al. 2016a, Galko et al. 2019), monitoring programs using ethanol-baited traps show that *X. germanus* has become a dominant ambrosia beetle species regardless of forest type. Findings of the species at higher altitudes (Olenici et al. 2014, Galko et al. 2019, and this study) indicate the vertical spread of the species in Europe, which is most likely the result of climate change.

Despite the widespread and massive

presence of the species in our forests, damage to standing trees, with rare exceptions for individual trees, has not yet been recorded. In fact, it is very difficult to find signs of the presence of the species in the forest, although we know that the *X. germanus* population is large. Therefore, despite the damage recorded in 2016 when *X. germanus* infested a larger amount of timber that was not removed from the forest immediately after logging (Hauptman et al. 2018), we agree with Lakatos & Kajimura (2007) who stated that *X. germanus* in Europe is a secondary pest that does not cause remarkable damage. However, there are some examples of beetle-fungus symbioses that have shifted from being a secondary pest on a wide range of hosts to becoming a primary pest on a narrow range of hosts in their introduced ranges (Hulcr & Dunn 2011). In some regions of North America, a similar shift may have already occurred for *X. germanus* (Björklund & Boberg 2017a); therefore, there is a risk that the species will become an important pest in European orchards, plantations, nurseries and vineyards, as is currently the case in North America (Galko et al. 2019). Furthermore, with climate change, we can expect more stressful events and large-scale disruptions in forests and, consequently, more attacks of *X. germanus* on weakened or fallen trees.

Maintaining tree health is essential to prevent *X. germanus* attacks (Ranger et al. 2016). This is, for example, easier to achieve in forest nurseries than in forest stands. The most important measure to prevent attacks and economic damage in forests is to transport felled logs from the forests immediately after harvesting and to store the wood in places that allow for proper drying (Graf & Manser 2000). Another option is to carry out logging, transport and storage of lumber outside of the main flight period of the species (Franjević et al. 2016).

Acknowledgements

References

ARSO (2018). Meteorological data of the Environmental Agency of the Republic of Slovenia. Lubiana, Slovenia, web site. [online] URL: https://meteo.arso.gov.si

Balachowsky A (1949). Coléopteres Scolytides [Bark beetles]. Faune de France 50: 1-320. [in French]

Björklund N, Boberg J (2017a). Rapid pest risk analysis *Xylosandrus germanus*. Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 22.

Björklund N, Boberg J (2017b). Rapid pest risk analysis *Xyleborinus attenuatus*. Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 17.

Bouget C, Noblecourt T (2005). Short-term development of ambrosia and bark beetle assemblages following a windstorm in French broad-leaved temperate forests. Journal of Applied Entomology 129 (6): 300-310. - doi: 10.1111/j.1439-0418.2005.00970.x

Bruge H (1995). *Xylosandrus germanus* (Blandford, 1894) (Belg. sp. nov.) (Coleoptera Scolytidae). Bulletin et Annales de la Société Royale Belge d'Entomologie 131: 249-264. [online] URL: http://www.cabdirect.org/cabdirect/abstract/19961103560

Cegnar T (2017). Podnebne razmere v januarju 2017 [Climate in January 2017]. Naše okolje. Mesečni bilten Agencije RS za okolje 24 (1): 3-24. [in Slovenian]

EPPO (2018). *Xylosandrus germanus*. EPPO Global Database, European and Mediterranean Plant Protection Organization, Paris, France, web site. [online] URL: http://gd.eppo.int/taxon/XYLBGE/distribution

Faccoli M (2008). First record of *Xyleborus atratus* Eichhoff from Europe, with an illustrated key to the European Xyleborini (Coleoptera: Curculionidae: Scolytinae). Zootaxa 1772: 55-62. - doi: 10.11646/zootaxa.1772.1.2

Franjević M (2013). Bivoltinism of European hardwood ambrosia beetle *Trypodendron domesticum* in Croatian lowland oak stands of Jastrebarski Lugovi. Šumarski list 137 (9-10): 495-498.

Franjević M, Poršinsky T, Duka A (2016). Integrated oak timber protection from ambrosia bark beetles: economic and ecological importance in harvesting operations. Croatian Journal of Forest Engineering 2016 (37): 353-364. [online] URL: http://hrcak.srce.hr/173835

Galko J, Nikolov C, Kimoto T, Kunca A, Gubka A, Vakula J, Zúbrik M, Ostrihon M (2014). Attraction of ambrosia beetles to ethanol baited traps in a Slovakian oak forest. Biologia 69: 1376-1383. - doi: 10.2478/s11756-014-0443-z

Galko J, Dzurenko M, Ranger CM, Kulfan J, Kula E, Nikolov C, Zubrik M, Zach P (2019). Distribution, habitat preference, and management of the invasive ambrosia beetle *Xylosandrus germanus* (Coleoptera: Curculionidae, Scolytinae) in European forests with an emphasis on the West Carpathians. Forests 10 (1): 10. - doi: 10.3390/f10010010

Geister I (2004). Popis rastlin in živali na Brdu pri Kranju [An inventory of plants and animals of Brdo near Kranj]. Kronika (Ljubljana) 52 (2): 273-284. [in Slovenian]

Graf E, Manser P (2000). The imported Japanese scolytid beetle *Xylosandrus germanus*: biology and damage potential for stored round timber compared to *Xyloterus lineatus* and *Hylecoetus dermestoides*. Schweizerische Zeitschrift fur Forstwesen 151 (8): 271-281. - doi: 10.3188/szf.2000.0271

Groschke F (1953). Der "schwarze Nutzholz-borkenkäfer", *Xylosandrus germanus* Blandf., ein neuer Schädling in Deutschland [The "black ambrosia" *Xylosandrus germanus* Blandf., a new pest in Germany]. Zeitschrift für Angewandte Entomologie 34: 297-302. [in German] - doi: 10.1007/BF02007076

Grégoire JC, Piel F, De Proft M, Gilbert M (2001). Spatial distribution of ambrosia-beetle catches: a possibly useful knowledge to improve mass-trapping. Integrated Pest Management Reviews 6 (3-4): 237-242. - doi: 10.1023/A:1025723402355

Grüne S (1979). Handbuch zur Bestimmung der Europäischen Borkenkäfer [Brief illustrated key to european bark beetles]. Verlag M & H Schaper, Hannover, Germany, pp. 184. [in German]

Hauptman T, Pavlin R, Jurc M (2018). Ambrozijski podlubnik (*Xylosandrus germanus*) [Ambrosia bark beetle (*Xylosandrus germanus*)]. Gozdarski vestnik 5/6 (76): 3-4. [in Slovenian]

Henin JM, Versteirt V (2004). Abundance and distribution of *Xylosandrus germanus* (Blandford 1894) (Coleoptera, Scolytidae) in Belgium: new observations and an attempt to outline its range. Journal of Pest Science 77: 57-63. - doi: 10.1007/s10340-003-0030-5

Holzinger WE, Frie T, Holzer E, Mehlmauer P (2014). Xylobionte Käfer (Insecta: Coleoptera part.) in Wäldern des Biosphärenparks Wienerwald (Österreich: Niederösterreich, Wien) [Saproxylic Beetles (Insecta: Coleoptera part.) in forests of the Biosphere Reserve Wienerwald (Austria: Lower Austria, Vienna)]. Wissenschaftliche Mitteilungen des Niederösterreichischen Landesmuseums, St. Pölten 25: 331-362. [in German]

Hulcr J, Dunn RR (2011). The sudden emergence of pathogenicity in insect-fungus symbioses threatens naive forest ecosystems. Proceedings of the Royal Society of London B: Biological Sciences 278 (1720): 2866-2873. - doi: 10.1098/rspb.2011.1130

Hulcr J, Atkinson T, Cognato A, Jordal B, McKenna D (2015). Morphology, taxonomy and phylogenetics of bark beetles. In: "Bark Beetles: Biology and Ecology of Native and Invasive Species" (Vega FE, Hofstetter RW eds). Academic Press, San Diego, CA, USA, pp. 41-84. - doi: 10.1016/B978-0-12-417156-5.00002-2

Jurc M (2005). Gozdna zoologija (univerzitetni učbenik) [Forest Zoology (University textbook)]. Bitehniška fakulteta, Oddelek za Gozdarstvo in obnovljive gozdne vire, Ljubljana, Slovenia, pp. 348. [in Slovenian]

Jurc M, Zavrtanik Z, Reščič M (2010). Tujerodni podlubnik *Xylosandrus germanus* se širi v gozdovih Slovenije [The non-native bark beetle *Xylosandrus germanus* is spreading in the forests of Slovenia. Novice iz varstva gozdov 3: 10-13. [in Slovenian] [online] URL: http://www.zdravgozd.si/nvg/prispevek.aspx?idzapis=3-8

Jurc M, Borkovič D, Pavlin R, Meterc G (2011). Xylosandrus germanus (Blandfort, 1894) (Curculionidae: Scolytinae) in Slovenia. In: Proceeding of the "SIEEC 22, Symposium Internationale Entomofaunisticum Europae Centralis". Varaždin (Croatia), 26 June - 03 July 2011. Gradski muzej, Varaždin, Croatia, pp. 33-34.

Jurc M, Bojovic S, Fernaández MF, Jurc D (2012). The attraction of cerambycids and other xylophagous beetles, potential vectors of Bursaphelenchus xylophilus, to semiochemicals in Slovenia. Phytoparasitica 40: 337-349. - doi: 10.1007/s12600-012-0234-4

Kirkendall LR, Faccoli M (2010). Bark beetles and pinhole borers (Curculionidae, Scolytinae, Platypodinae) alien to Europe. ZooKeys 56: 227-251. - doi: 10.3897/zookeys.56.529

Kirkendall LR (1983). The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). Zoological Journal of the Linnean Society 77: 293-352. - doi: 10.1111/j.1096-3642.1983.tb00858.x

Klimetzek D, Köhler J, Vité JP, Kohnle U (1986). Dosage response to ethanol mediates host selection by "secondary" bark beetles. Naturwissenschaften 73 (5): 270-271. - doi: 10.1007/BF00367783

Knízek M (1988). *Xyleborus alni* Niijima, 1909. Acta Entomologica Bohemoslovaca 85: 396.

Lakatos F, Kajimura H (2007). Occurrence of the introduced *Xylosandrus germanus* (Blandford, 1894) in Hungary - A genetic evidence (Coleoptera: Scolytidae). Folia Entomologica Hungarica 68: 97-104. [online] URL: http://www.cabdirect.org/cabdirect/abstract/20083055832

Markalas S, Kalapanida M (1997). Flight pattern of some Scolytidae attracted to flight barrier traps baited with ethanol in an oak forest in Greece. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz 70 (3): 55-57. - doi: 10.1007/BF01996922

Olenici N, Kníek M, Olenici V, Duduman ML, Biris IA (2014). First report of three scolytid species (Coleoptera: Curculionidae, Scolytinae) in Romania. Annals of Forest Research 57: 89-97. - doi: 10.15287/afr.2014.196

Pfeffer A (1995). Zentral- und westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae) [Central and western Palearctic bark and ambrosia beetles (Coleoptera: Scolytidae, Platypodidae)]. Pro Entomologia, Naturhistorisches Museum, Basel, Switzerland, pp. 310. [in German]

Ranger C, Reding M, Gandhi K, Oliver J, Schultz P, Cañas L, Herms D (2011). Species dependent influence of (-)-α-pinene on attraction of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) to ethanol-baited traps in nursery agroecosystems. Journal of Economic Entomology 104 (2): 574-579. - doi: 10.1603/EC10243

Ranger CM, Reding ME, Schultz PB, Oliver JB, Frank SD, Addesso KM, Chong JH, Sampson B, Werle C, Gill S, Krause C (2016). Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. Journal of Integrated Pest Management 7 (1): 25. - doi: 10.1093/jipm/pmw005

Rassati D, Faccoli M, Battisti A, Marini L (2016a). Habitat and climatic preferences drive invasions of non-native ambrosia beetles in deciduous temperate forests. Biological Invasions 18 (10): 2809-2821. - doi: 10.1007/s10530-016-1172-8

Rassati D, Lieutier F, Faccoli M (2016b). Alien wood-boring beetles in Mediterranean regions. In: "Insects and Diseases of Mediterranean Forest Systems" (Payne TD, Lieutier F eds). Springer International Publishing, Cham, Switzerland, pp. 293-327. - doi: 10.1007/978-3-319-24744-1_11

Reding ME, Ranger CM, Oliver JB, Schultz PB (2013). Monitoring attack and flight activity of *Xylosandrus* spp. (Coleoptera: Curculionidae: Scolytinae): the influence of temperature on activity. Journal of Economic Entomology 106 (4): 1780-1787. - doi: 10.1603/EC13134

Salom SM, McLean JA (1990). Flight and landing behavior of *Trypodendron lineatum* (Coleoptera: Scolytidae) in response to different semiochemicals. Journal of Chemical Ecology 16 (8): 2589-2604. - doi: 10.1007/BF01017481

Seljak G (2013). Dinamika vnosa tujerodnih fitofagnih žuželk in pršic v Slovenijo [The dynamics of introduction of alien phytophagous insects and mites in Slovenia]. Acta entomologica Slovenica 21 (2): 85-122. [in Slovenian]

Titovšek J (1988). Podlubniki (Scolytidae) Slovenije: obvladovanje podlubnikov [Bark beetles (Scolytidae) of Slovenia: control of bark beetles]. Zveza društev inženirjev in tehnikov gozdarstva in lesarstva Slovenije, Gozdarska založba, Ljubljana, Slovenia, pp. 128. [in Slovenian]

Weber BC, McPherson JE (1983). World list of host plants of *Xylosandrus germanus* (Blandford) (Coleoptera: Scolytidae). The Coleopterists' Bulletin 37: 114-134. [online] URL: http://www.jstor.org/stable/4008002

ZGS (2014). The operational plan for the sanitation of the damaged forest after the sleet event in February 2014. Slovenian Forest Service, Ljubljana, Slovenia, pp. 66.

ZGS (2017). Timber: database of tree felling. Slovenian Forest Service, Ljubljana, Slovenia. [CD-ROM]