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A monitoring network for the detection of invasive ambrosia and bark beetles in the Czech Republic: principles and proposed design

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Invasive bark beetles pose a threat to native biodiversity and to functional ecosystems and the economic productivity of forests, parks, and orchards. In the Czech Republic, there are six species of invasive ambrosia and bark beetles with a stable natural population, and it can be assumed that other invasive species that will be found. In the Czech Republic, there are no guidelines or methods for the early detection of invasive ambrosia and bark beetles. We propose monitoring at a total of 24 locations considering the following: (i) monitoring approaches used in other countries; (ii) identified entrance gates of invasive ambrosia and bark beetles found in the Czech Republic; (iii) presumed invasive species that occur in surrounding countries and are expanding their range; (iv) substances attractive to all the above mentioned species; (v) commonly available traps; and (vi) minimization of operating costs. Most of the chosen locations are located on the state borders and in river valleys, which are probably the entrance gates to the Czech Republic for invasive ambrosia and bark beetles. In addition, two large timber warehouses where international trade takes place, all international airports and three botanical gardens with tropical greenhouses were selected. Three Theysohn or Ecotrap impact traps should be installed every year at all locations. Traps should be baited with ethanol and exposed from mid-April to the end of July and should be checked every 2 weeks.

KEYWORDS
Cyclorhipidion bodoanum*, *Dryocoetes himalayensis*, *Gnathotrichus materiarius*, *Phloeosinus aubei*, *Xyleborinus attenuatus*, *Xylosandrus germanus

1. Introduction

Invasive ambrosia and bark beetles (further BB) represent a threat to biodiversity, functional ecosystems, and the economic productivity of forestry (Brockerhoff et al., 2006; Aukema et al., 2011; Gohli et al., 2016), as well as to parks and orchards (Francardi et al., 2017; Branco et al., 2019; Fiala et al., 2022). BB are important vectors of fungal diseases that cause massive tree death. The simultaneous effect of several invasive species, their symbiotic fungi, and the subsequent interaction with climate change creates a situation in which it is difficult to predict the future impact of ambrosia and bark beetles on the environment (Lovett et al., 2013). Early detection is key to controlling BB because only then can a real integrated pest management (IPM) strategy be developed (Brockerhoff et al., 2006, 2010; Douglas et al., 2009; Samons, 2022).

Bark beetles spread in several ways, the most common being the global trade in wood material (treated and untreated wood), wooden packaging, and fruits or live seedlings of various non-native trees (Mathew, 1987; Meissner et al., 2008; Pombo et al., 2010; Augustin et al., 2012; Brockerhoff and Liebhold, 2017; Meurisse et al., 2019). It has also been confirmed that they can be introduced with wooden material that has been treated according to the international standard ISPM 15 (Haack and Petrice, 2009; Haack et al., 2014). In Europe, ports on the Atlantic and Mediterranean coasts are most often the gateway (Hagedorn, 1910; Hoffmann, 1942; Schedl, 1962; Cola, 1971, 1973; Faccoli, 2008; Moraal, 2010; Inghilesi et al., 2013; Rassati et al., 2015; Binazzi et al., 2019; Branco et al., 2019; Barnouin et al., 2020). Another entry point is botanical gardens, where non-native ambrosia and bark beetles may be introduced when expanding collections of exotic trees (Chobaut, 1897; Merkl and Tusnádi, 1992; Schuler et al., 2023).

Due to climate change, the host tree species are spreading northwards into areas where they did not originally occur (Ge et al., 2017). Even ambrosia and bark beetles, which are only found in southern Europe, may spread north; e.g., the bark beetle *Phloeosinus aubei* Perris, 1855 has spread to colder areas in Central Europe (Fiala and Holuša, 2019). Ambrosia and bark beetles not only spread through global trade but also naturally, as some are good flyers (Nilssen, 1984; Jones et al., 2019). Dry summers contribute to the appearance of ambrosia and bark beetles in alpine locations, even though they do not normally ascend to high altitudes, also (Marini et al., 2012).

However, the influence of humans on the spread of BB is far greater than the influence of climate (Gohli et al., 2016; Ward et al., 2019). Establishing plantations of non-native trees increases the risk of introducing non-native ambrosia and bark beetles (Lantschner et al., 2017). In Central Europe, this mainly concerns the cultivation of black pine (*Pinus nigra*) and bark beetles, which feed on it; *Pityogenes bistridentatus* Eichhoff, 1878 and *Orthotomicus robustus* Knotek, 1899 are found in several areas in the Czech Republic (Pfeffer and Knížek, 1996; Urban, 2000; Knížek, 2006; Knížek and Mertelík, 2017; Fiala et al., 2022). Climate change may help the maintenance of populations of BB on continents (Rassati et al., 2016a).

Most ambrosia and bark beetles are native to temperate and subtropical forests, so they represent the greatest danger for southern Europe due to a similar climate; hence, damage is most concentrated here (Pennacchio et al., 2004, 2012; Alfaro et al., 2007; Francardi et al., 2017; Leza et al., 2020). In the more northern countries of Europe, only damage by the ambrosia beetle *Xylosandrus germanus* Blandford, 1894 has been recorded (Maksymov, 1987; Graf and Manser, 2000; Galko et al., 2019).

Due to the economic and ecological damage caused by ambrosia and bark beetles, some governments perform regular monitoring of BB in their territory. This is helpful for identifying risk in a timely manner. There have been several monitoring attempts, of which baited traps are the most effective and least expensive method (Poland and Rassati, 2019).

Since BB are spreading increasingly around the world, there have also been efforts to introduce global monitoring. Observations were made on several continents at the same time to determine the abundance of ambrosia and bark beetles in the affected regions. The following semiochemicals were used in the traps: α-pinene + ethanol and α-pinene + ethanol + ipsdienol + ipsenol + Z-verbenol. The study is the first step toward the development of an international monitoring protocol based on trapping in traps baited with different types of substances (Faccoli et al., 2020).

There are six species of BB in the Czech Republic with a stable population in the wild (Knížek, 1988; Procházka et al., 2018; Fiala and Holuša, 2019; Fiala et al., 2020, 2021), and other species can be expected to occur in this territory (Gebhardt, 2014; Gebhardt and Doerfler, 2018). In the Czech Republic, there are no guidelines or methods for the early detection of BB. In addition, approximately half of the records of new species of ambrosia and bark beetles for the Czech Republic were accidental; the species were caught by amateur entomologists, and there was a delay of approximately 1–3 years between detection and publication (cf. Knížek, 2009a,b, 2011; Knížek and Kopecký, 2021). An extreme example is a report published 18 years after the species *Pityophthorus balcanicus* Pfeffer, 1940 was captured (Knížek and Liška, 2015). Therefore, it is necessary to create a stable network of traps for monitoring invasive species of ambrosia and bark beetles. To determine the methodology, several experiments were carried out in the Czech Republic, providing basic knowledge about the spread of BB and their bionomics in the Czech Republic (Fiala and Holuša, 2019, 2020; Fiala et al., 2020; Holuša et al., 2021; Fiala et al., 2023).

The aim of this work is to propose a methodology for monitoring BB based on the following:

(i) monitoring approaches in other countries;
(ii) the entrance gates of the existing species of BB found in the Czech Republic;
(iii) presumed species that occur in surrounding countries and are expanding their range;
(iv) substances attractive to all of the above;
(v) commonly available traps;
(vi) minimization of operating costs.

1.1. Monitoring methods in North America

In Canada, the first attempts to detect BB were made at the end of the 1990s in the vicinity of Vancouver. The following substances were used for trapping: ethanol, α pinene, and attractants (*cis*-verbenol, ipsdienol, and methylbutenol) for *Ips typographus* Linnaeus, 1758 (Humble, 2001). Ethanol and α-pinene are kairomons for many ambrosia and bark beetles (Schroeder and Lindelöw, 1989). After that, long-term monitoring began, and was carried out in the period from 2000 to 2021. Each year between 2000 and 2011, six Lindgren funnel traps were installed at each of 63–80 locations (ports, industrial zones, and wood processing industries). Traps at each location included three baited with ethanol + α-pinene and *cis*-verbenol + ipsdienol + methylbutenol and three baited with ethanol alone. Since 2012, another trap baited with ethanol + C6-ketol + C8-ketol as aggregation pheromones have been added to longhorned beetles (see Hanks et al., 2019). Since 2015, traps for longhorned beetles have been baited with the combination of racemic (E,Z)-fuscumol + racemic

(E,Z)-fuscumol acetate + ethanol and the combination of ipsenol + monochamol + α-pinene + ethanol. During the experiment, seven species of BB were captured, of which three species were new to Canada (Thurston et al., 2022).

The most sophisticated system of regular monitoring is carried out in the US, where monitoring has been ongoing for 20 years (Rabaglia et al., 2008). Even before the start of this program, BB were caught in ports and airports in the US (Rabaglia and Cavey, 1994; Haack, 2001, 2006; Mudge et al., 2001). The American system is based on a dense network of Lindgren funnel traps lured with ethanol, α-pinene + ethanol, and ipsdienol + cis-verbenol + methylbutenol, each separately. Traps are located mainly along both ocean coasts but also in the interior of the US. The US territory is divided into three parts, and each part is monitored once every 3 years. Even connected overseas territories such as Puerto Rico or Guam regularly participate in monitoring, where other volatile substances are also used for captures, such as manuka oil or ethanol + cubeb oil. Traps are located at seaports or at companies in the wood processing industry (Rabaglia et al., 2019). Data from this monitoring are used to determine the behavior of BB and to model their spread in the US (Rassati et al., 2016a). During the evaluation of this program (Rabaglia et al., 2019), ethanol was found to be the most suitable for trapping BB, while trapping with *Ips* lures was not effective for BB. Specific substances can be used to target selected BB (Hartshom et al., 2021).

1.2. Monitoring methods in Australia and New Zealand

Efforts to detect BB has also taken place in New Zealand. The first attempts to develop invasive species monitoring were in the 1980s (Hosking and Gadgil, 1987; Carter, 1989). Lindgren funnel traps with baits of α-pinene + ethanol, β-pinene + ethanol, frontalin + ethanol, and ipsdienol were also used in ports, international airports, and forests near these locations. This monitoring model has been proven to be successful in the early detection of BB, and it has, therefore, a good chance of eliminating these ambrosia and bark beetles (Brockerhoff et al., 2006). There was also an experimental trial to detect damage by invasive pests using field observations (car and walking) in New Zealand. Virtually no difference in results was found between these two methods (Bulman et al., 1999).

The monitoring of invasive species in Australia was broader; Lepidoptera was also caught. In sticky traps, Lindgren and Ecotrap. Ethanol, cineole, α-pinene, phellandrene, and a mixture of pinene, phellandrene, cineole, terpene, and cymene were used as bait. Traps were placed near ports and airports, and others were placed in a zone within 5 km of ports and airports (Bashford, 2012). The following baits were also tested in Brisbane harbor from 2006 to 2007: ipsenol, ipsdienol, frontalin, exo-brevicomin, and a combination of ethanol and α-pinene; a total of 29 species of ambrosia and bark beetles were caught (Wylie et al., 2008). In Tasmania, a method of static traps baited with a combination of α-pinene and ethanol was developed to monitor BB in *Pinus radiata* plantations (Bashford, 2008). These attempts subsequently developed into massive permanent monitoring throughout Australia (Carnegie et al., 2018, 2022; Carnegie and Nahrung, 2019).

1.3. Monitoring methods in Asia

In China, an IPM plan has been created and monitoring is carried out in designated areas using various methods, from baited traps with different types of semiochemicals to light traps to simply patrolling the area (Anonymus, 2009). At the same time, ambrosia and bark beetles are caught in ports (Lin et al., 2021). China also has an IPM standard for *P. aubei*, which causes serious damage to cypress trees there (Anonymus, 2017).

Other maritime countries also monitor BB in ports. In Japan, BB have been monitored in ports since the 1950s (Murayama, 1957; Schedl, 1966, 1969, 1970; Browne, 1980a,b; Ohno, 1989). In South Korea, BB were also monitored in harbors as early as the late 1970s (Choo et al., 1981; Choo and Woo, 1983; Choi et al., 2003).

1.4. Monitoring methods in Europe

In Italy, BB have long been monitored in ports (Cola, 1971, 1973). In total, 15 international ports and their adjacent forest stands are monitored; for trapping, Lindgren funnel traps and semiochemicals similar to those in the USA, ethanol, α pinene + ethanol, and ipsdienol + ipsenol + methylbutenol, are applied. Three traps were placed in the harbor, and three traps were placed in the adjacent forests. More species were found in deciduous forests than in coniferous stands. Invasive species richness was higher in forests than in harbors. The ambrosia and bark beetles were caught in the harbors, and were not yet able to establish a permanent population in the surrounding forests (Rassati et al., 2015). At Malpensa International Airport, the capture of invasive beetles in PET bottles was successfully tested using the following baits: apple cider vinegar, red wine, and 80% ethanol (Ruzzier et al., 2021).

Monitoring of invasive longhorned beetles (Cerambycidae) was launched in France, where they also tested trapping with α pinene + ethanol in Ecotrap traps. The traps were placed in natural forests and then in ports, airports, and orchards (Fan et al., 2019).

In Lithuania, as part of prevention, the bark beetle *Dendroctonus rufipennis* Kirby, 1837 was monitored in 2000 in the port of Klaipeda, near the Vaidotai railway station and along forest roads. *D. rufipennis* was not detected (Ostrauskas and Ferenca, 2010). In the period from 2002 to 2005, further monitoring was carried out at the borders, again in the port of Klaipeda, and at temporary wood warehouses, but no BB were caught. Lures α-pinene, myrcene, and *cis*-verbenol were used in Lindgren funnel traps (Ostrauskas and Tamutis, 2012).

Extensive monitoring of invasive species took place in Great Britain between 2013 and 2017. Lindgren funnel traps and cross-vane panel traps were placed in different types of forests near the ports. Ethanol and ethanol + α-pinene were used as bait. A total of three species of BB, *Cyclorhipidion bodoanum*, *Gnathotrichus materiarius*, and *X. germanus*, were captured (Inward, 2020).

2. Invasive species of ambrosia and bark beetles in the Czech Republic and expected invasive species

In the Czech Republic, there are six species of BB with a stable natural population: *C. bodoanum* Reitter, 1913, *Dryocoetes himalayensis* Strohmeyer, 1908, *G. materiarius* Fitch, 1858, *P. aubei*, *Xyleborinus attenuatus* Blandford, 1894, and *X. germanus* (Knížek, 1988; Procházka et al., 2018; Fiala and Holuša, 2019; Fiala et al., 2020, 2021, 2023). Furthermore, several introduced species that could not form a stable population due to an unfavorable climate or absence of host plants were found in the territory of the Czech Republic: *Coccotrypes dactyliperda* Fabricius, 1801, *Hypothenemus areccae* Hornung, 1842, *Hypothenemus hampei* Ferrari, 1867, *Hypothenemus setosus* Eichhoff, 1868, *Xyleborus affinis* Eichhoff, 1868, *Xyleborus volvulus* Fabricius, 1794, and *Xylosandrus morigerus* Blandford, 1894 (Reitter, 1913; Fleischer, 1927–1930; Pfeffer and Knížek, 1989).

New invasive species of ambrosia and bark beetles which are already present in Germany may be expected to invade the Czech Republic. These include, *Xyloterinus politus* Say, 1826, which was detected in Bavaria in 2014 (Gebhardt and Doerfler, 2018), and *Cyclorhipidion pelliculosum* Eichhoff, 1878, which was found in Baden-Württemberg in 2013 (Gebhardt, 2014). The greatest economic danger to tree species in the Czech Republic is the bark beetle *Pityophthorus juglandis* Blackman, 1928, which has been spreading in Italy since 2013 and is a carrier of the serious fungal disease, thousand cankers disease (Montecchio and Faccoli, 2014). From the east, we can expect an invasion of the bark beetle *Polygraphus proximus* Blandford, 1894, which spreads from Siberia toward the west, and its harmfulness is comparable to that of *I. typographus* (Peña et al., 2020). Therefore, a pest risk analysis was developed for both species (EPPO, 2014, 2015).

The MaxEnt algorithm can be used to model the spread of invasive species around the world. For the invasive ambrosia beetle *Xylosandrus compactus* Eichhoff, 1876, which occurs in southern Europe (Pennacchio et al., 2012; Barnouin et al., 2020; Leza et al., 2020; Riba-Flinch et al., 2021), with the continuation of average climatic values from 1970 to 2000, *X. compactus* is predicted to find suitable ecological conditions for development in southern Moravia (which is the warmest region of the Czech Republic) by 2050 (Urvois et al., 2021).

2.1. Basic points for determining the monitoring methodology of invasive ambrosia and bark beetles in the Czech Republic

Since 2020, efforts have been underway to determine the possible entry gates and directions of expansions of BB in the Czech Republic (**Figure 1**; Fiala and Holuša, 2019; Fiala et al., 2020, 2021, 2022, 2023). Potential types of volatile substances that could be used for monitoring were compared to find the simplest monitoring method (Fiala and Holuša, 2020; Fiala et al., 2023).

The Czech Republic has no seaports, but has five international airports (Prague, Brno, Ostrava, Pardubice, and Karlovy Vary; **Table 1**) and many road and rail border crossings with foreign countries. Therefore, global trade is a possible reason for the flight activity of individual invasive species when entering the Czech Republic. In 2022, 302,640 tons of wood materials with a size larger than 6 mm were imported from all over the world into the Czech Republic, of which 4,993 tons were tropical wood of all kinds (ČSÚ, 2023).

The invasive ambrosia beetle *X. germanus* in the middle of the Czech Republic in 2007 (Knížek, 2009a) was first found near the largest wood warehouse of Stora Enso in Ždírec nad Doubravou, similar to the invasive sawfly *Urocerus albicornis* Fabricius, 1781, was found on the grounds of the Kronospan wood processing plant in Jihlava (Háva and Holuša, 2019). The occurrence in botanical gardens through the importation of live exotic plants has only been demonstrated once in the Czech Republic, in the case of *X. morigerus* (Reitter, 1913); however, this does not mean that other introductions have not occurred and escaped notice. The ambrosia beetle *G. materiarius* was first found through flight monitoring near the border with Bavaria in western Bohemia (Knížek, 2009a). Likewise, the spreading of *X. germanus* in northern Bohemia and southern Moravia (Fiala et al., 2020) or *D. himalayensis* in southern Moravia (Procházka et al., 2018) is a result flight of beetles.

Most of the BB were found near the borders with Germany and Austria (cf. Fiala et al., 2021; **Figure 1**). This is logical because most of the BB in Europe have been detected near seaports in western and southern Europe. The main entry points were clearly identified as river valleys and border crossings (Fiala et al., 2020, 2023).

2.2. Results of case studies in the Czech Republic

In 2021, two experiments were conducted to detect BB: (i) the capture of ambrosia and bark beetles at a warehouse of tropical wood imported from Central Africa in Pilsen – Doubravka town[1] and (ii) the capture of ambrosia and bark beetles in the Botanical Garden in Prague – Troja with a tropical greenhouse, where tropical trees are brought in every year. This botanical garden is the largest in the Czech Republic, and its tropical greenhouse offers vegetation of dry tropics and subtropics, lowland rainforest, and tropical forests of high mountains.[2]

No invasive bark beetle was caught near Pilsen (**Appendix Table 1**); only the bark beetle *Lymantor coryli* Perris, 1855, which is rarely found throughout Europe, was detected (Fiala, 2021). No bark beetles were caught in the tropical greenhouse, but the two BB, *X. germanus* and *D. himalayensis*, were caught at the edge of oak forests (**Appendix Table 2**).

At the same time, at the end of 2021, 13 companies involved in the coffee trade in the Czech Republic were asked to cooperate to detect the occurrence of introduced species of ambrosia and bark beetles damaging coffee beans. Several samples of damaged beans were obtained, and the bark beetle *H. hampei* (**Figure 2**) from Brazil, Colombia, and India (**Appendix Table 3**) was detected

1 www.exoticke-drevo.com

2 https://www.botanicka.cz/en

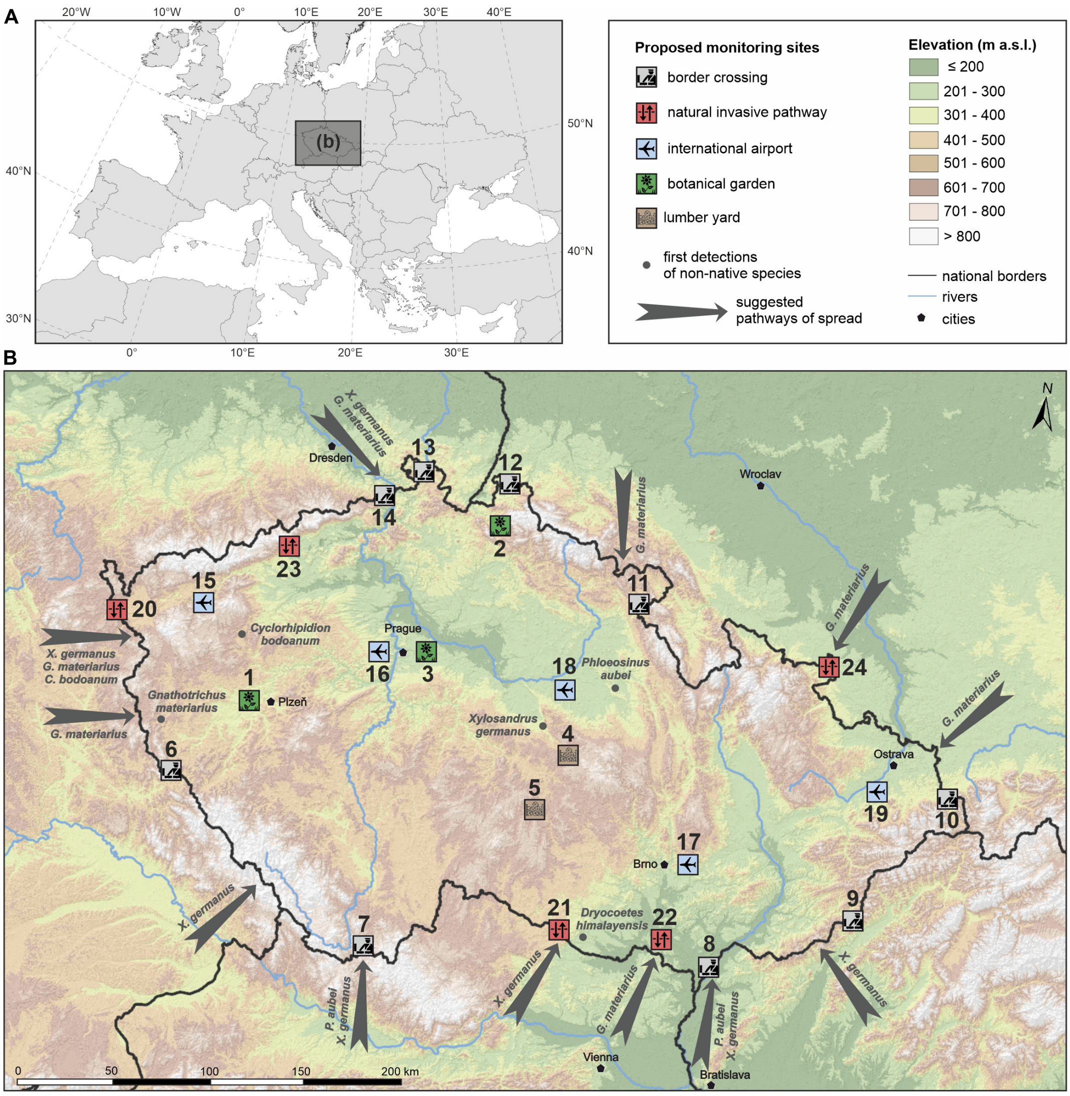

FIGURE 1
The position of the Czech Republic in Europe **(A)** and the possible entry gates, places of first detections, and a proposal for monitoring locations for invasive ambrosia and bark beetles in the Czech Republic **(B)**.

by the occurrence several dead individuals in the Czech Republic. However, *H. hampei* does not pose a danger, even to undamaged coffee stocks, as its stages do not survive the Central European climate (Jaramillo et al., 2009). It can be speculated that beetles may, however, introduce various fungal and bacterial infections into uninfected beans (Damon, 2000; Jaramillo et al., 2006).

3. Proposal of a methodology for the detection of invasive species of ambrosia and bark beetles in the Czech Republic

The selection of locations is based on possible entry points such as border crossings, border river valleys, international airports, large timber warehouses, and botanical gardens; at the same time, these points will be used to monitor already established species whose abundance is still very low (Procházka et al., 2018; Fiala and Holuša, 2019, 2020; Fiala et al., 2020, 2021, 2022; Holuša et al., 2021). For the purposes of regular and permanent monitoring of BB, we therefore propose the following locations (**Table 1** and **Figure 1**). A quarter of the locations are in protected areas; there is sufficient dead wood, and there are overgrown stands that provide a suitable environment for the development of ambrosia and bark beetles (Lee et al., 2019; Fiala et al., 2021).

Some invasive bark beetles are polyphagous, such as *X. germanus* (Weber and McPherson, 1983) and *X. politus* (MacLean and Giese, 1967), and can attack both coniferous and deciduous trees; some attack only deciduous trees, such as *X. attenuatus* (Kvamme et al., 2020), or only conifers, such as *G. materiarius* (Kamp, 1970). The representation of tree species is not significant for ambrosia and bark beetle monitoring because the type of forest has no effect on the abundance of beetles (Bouget et al., 2008). Therefore, the type of forest in which the trap is placed is not important, although a mixed forest with different tree

FIGURE 2
Dead individual of bark beetle *H. hampei* found in damaged coffee bean introduced to the Czech Republic.

species is preferable. We prefer oak forests, in the vicinity of which there are also conifers. In the Czech Republic, almost all forests are cultural, and conifers grow even at low altitudes. Therefore, choosing a combination of forests at the different locations was straightforward (**Table 1**).

Most BB in Europe are ambrosia species (Alonso-Zarazaga et al., 2023), and in our study in oak forests in western Bohemia, we found that ambrosia beetles had a higher abundance with a greater canopy cover, due to the wetter microclimate and greater amount of dead wood (Holuša et al., 2021). The influence of the close canopy on the abundance of ambrosia and bark beetles was also confirmed by Menocal et al. (2022). Therefore, forests with close canopy is generally preferred, although we are aware that *C. bodoanum* seems to prefer open forests (Fiala et al., 2021).

We also tested substances suitable for trapping BB. Factory-produced pheromones were suitable for trapping ambrosia and bark beetles of the genus *Trypodendron*; we found one specimen of *X. germanus* (Fiala and Holuša, 2020). Among volatile substances, we found the best combination of ethanol and juniper twigs suitable for trapping bark beetles *P. aubei* (Fiala et al., 2023). We found ethanol to be the most suitable for *G. materiarius* (Fiala et al., 2023). Likewise, *C. bodoanum* was captured in ethanol (Fiala et al., 2021), and although *D. himalayensis* and *X. germanus* were captured in impact traps as such, they were also captured in ethanol (Procházka et al., 2018; Hauptman et al., 2019a; Fiala et al., 2020; **Appendix Table 2**). *X. attenuatus*, like the ambrosia bark beetle, was attracted to ethanol (Galko et al., 2014).

Although sulcatol, which is considered a potential aggregation pheromone of *G. materiarius*, was expected to be successful (Flechtmann and Berisford, 2003), it was not the best lure tested in Central European conditions. The combination of sulcatol and ethanol resulted in the capture of a significantly greater number of beetles of *Gnathotrichus* sp. (McLean and Borden, 1977). However, in our case, ethanol alone captured more beetles than the combination of baits. Ethanol also significantly attracted other invasive ambrosia beetles, *C. bodoanum*, *X. germanus*,

TABLE 1 Proposed localities for permanent monitoring of invasive ambrosia and bark beetles (types of protected areas of the Czech Republic: NP, National Park; NPR, National Nature Reserve; PP, Nature Monument; PR, Nature Reserve).

No.	Monitoring locations	GPS	Reason for location selection and inclusion
1	Zoologická a botanická zahrada Plzeň	49.7595N, 13.3598E	Botanic garden
2	Botanická zahrada Liberec	50.7768N, 15.0768E	Botanic garden
3	Pražská botanická zahrada	50.1224N, 14.4138E	Botanic garden
4	Ždírec	49.7022N, 15.8088E	Wood storage
5	Jihlava	49.4219N, 15.6050E	Wood storage
6	Česká Kubice	49.3643N, 12.8522E	Border crossing
7	PP Horní Malše	48.6553N, 14.4575E	Border crossing
8	Tvrdonice	48.7504N, 17.0210E	Border crossing
9	PP Okrouhlá	49.0466N, 18.0576E	Border crossing
10	Třinec	49.6795N, 18.6930E	Border crossing
11	Hronov	50.4776N, 16.2129E	Border crossing
12	PR Meandry Smědé	50.9808N, 15.0345E	Border crossing
13	Velký Šenov	50.9960N, 14.4053E	Border crossing
14	Hřensko	50.8730N, 14.2392E	Border crossing
15	Karlovy Vary	50.1998N, 12.9028E	International airport
16	Praha Ruzyně	50.1244N, 14.3054E	International airport
17	Brno	49.1606N, 16.6602E	International airport
18	Pardubice	50.0203N, 15.7153E	International airport
19	Ostrava	49.6981N, 18.1397E	International airport
20	PR Rathsam	50.1013N, 12.2485E	Assumed migration path
21	NP Podyjí	48.8495N, 15.8835E	Assumed migration path
22	NPR Děvín	48.8587N, 16.6511E	Assumed migration path
23	NPR Jezerka	50.5433N, 13.4844E	Assumed migration path
24	PP Osoblažský výběžek	50.3032N, 17.7005E	Assumed migration path

X. attenuatus, and other species of native ambrosia and bark beetles. Ethanol attracts both ambrosia and bark beetles *X. politus* and *C. pelliculosum*, which are already present in Germany (Ranger et al., 2011, 2014). Ethanol generally has a better capture ratio of invasive ambrosia beetles than the other substances (Fiala et al., 2023). Ethanol has long been known to be the main volatile substance on ambrosia and bark beetles (Kelsey and Joseph, 2003; Ranger et al., 2013, 2019).

For capturing and monitoring the dangerous invasive species *P. juglandis*, ethanol is also a suitable substance (Roling and Kearby, 1975). However, in acute situations, the monitoring network can be extended by adding a trap with the aggregation pheromone prenol, which was detected in this bark beetle (Seybold et al., 2015). Ethanol can also be used to detect *P. proximus*, although the beetles will most likely be caught in small quantities, as it reacts mainly to *cis*-verbenol, ipsdienol, and ipsenol (EPPO, 2014), like *I. typographus* (Schlyter et al., 1987). If the occurrence of *P. proximus* in the vicinity of the Czech Republic has already been predicted, the monitoring network can be expanded by adding another trap to the monitoring location with one of the industrial attractants containing *cis*-verbenol.

We propose total of 24 monitoring locations. Most of them are located at the border crossings of the Czech Republic and in river valleys, which are probably the entrance gates to the Czech Republic of BB (**Figure 1**). In addition, two large timber warehouses in which international trade takes place were selected (Žemlička, 2012), along with all international airports and three botanical gardens with tropical greenhouses. The latter locations cover a variety of modes of invasion by ambrosia and bark beetles: natural dispersal by the flight abilities of ambrosia and bark beetles and spread by global trade (**Table 1**).

We designed specific locations so that they were easily accessible in forests and were warmer locations of southern exposures. We selected overgrown forests near state borders or places that represent a "steppingstone," as in the case of point 22, NPR Děvín (a woven area in an agricultural landscape), and point 23, NPR Jezerka (located on the migration route along the Ohøe River valley). From airports and large timber warehouses, we assume that bark beetles will fly to the nearest forest stands. Botanical gardens have the character of open forests and are mostly surrounded by forests, so localities in the territory of the garden have been suggested.

Three traps at each location is sufficient (Rassati et al., 2015; Thurston et al., 2022). In the Czech Republic, two types of impact traps are used; both are inexpensive and commonly available. They are easy to install and do not catch large numbers of non-target insects (Lubojacký and Holuša, 2014; Galko et al., 2016). The traps can be a Theysohn slot type, which is the most widely used in forestry in the Czech Republic (Zahradník and Zahradníková, 2016), or impact type Ecotrap, from which it is easier to extract the caught beetles. They can be disassembled after each season and stored in a much smaller space than the Theysohn traps.

These types of traps are primarily intended for catching economically important bark beetles that are attracted by specific pheromones (Flechtmann et al., 2000; Šramel et al., 2021); however, they can also be used to capture invasive species without any

TABLE 2 Basic costs of operating the proposed monitoring network of invasive species of ambrosia and bark beetles in the Czech Republic (prices for the year 2023 in €) [energy costs (freezer), human fieldwork and labor costs, and determination costs are not included].

Numbers of traps	Cost per trap	At total for all traps	Number of ethanol lures	Cost per lure	At total for all lures	The total postage for all locations	At total
72	60[1]/22[2]	4,320[1]/1,584[2]	144	10.20	1,469	150	5,939[1]/3,203[2]

Additional years can be calculated without the cost of traps.
[1]Theysohn trap.
[2]Ecotrap.

problems (Holuša et al., 2021; Fiala et al., 2023). Different species of ambrosia and bark beetles are found to prefer different types of traps. *Dryoxylon onoharaense* Murayama, 1934, an invasive species also found in Europe (Marchioro et al., 2022), or *G. materiarius* prefer the Ecotrap type. In contrast, bark beetles *X. affinis* and *Premnobius cavipennis* Eichhoff, 1878 prefer the Theysohn type (Flechtmann et al., 2000; Dodds et al., 2010; Miller and Crowe, 2011).

Each trap is baited with ethanol, which is universal for catching ambrosia and bark beetles (Rassati et al., 2016b; Chen et al., 2021). Traps should be placed between 30 and 50 m apart (Niemeyer, 1997; Rassati et al., 2014). Ethanol is also partly attractive to common species of ambrosia and bark beetles that live on conifers (Fiala et al., 2023). Traps should be operated from mid-April to the end of July, as the flight activity of ambrosia and bark beetles decreases in August (Fiala et al., 2023). Traps are checked once every 14 days, and the collected samples are then stored in the freezer for later determination. Ethanol should be changed in early June since the evaporators are active for approximately 60 days.[3]

In total, there are only 72 traps (e.g., three traps at 24 locations), which represent 144 ethanol lures per year (**Appendix 4**). Given that the Czech Republic is a small country, the number of locations is small, and monitoring should be carried out annually. Since most of the locations are forested, we suggest, if agreeable, partnering with the local forest administration of Forest of the Czech Republic (LČR, s.p., in Czech), a company that manages more than 50% of the Czech Republic's forest stands and has cooperation with the Forest Advisory Service (Lesní ochranná služba in Czech) of Forestry and Game Management Research Institute (FGMRI, VÚLHM in Czech) Jíloviště at Prague, capital of the Czech Republic. In total, the LČR manages thousands of trappers throughout the country every year. The traps that we suggest, slightly more than 70 traps, are not difficult to manage because foresters move around the forests every day. Similarly, workers at the botanical gardens and timber warehouses move around daily and can send samples for determination. The average catch per trap in the world varies between 200 and 500 specimens, similarly in the Czech Republic it is between 50 and 500 specimens (**Appendix Table 5**).

The entire organization of monitoring corresponds to the activity and assignment of the Forest Advisory Service. The Forest Advisory Service deals with research, expert, and monitoring activities in forest protection against biotic pests. It monitors the occurrence of the bark beetle *Ips duplicatus* Sahlberg, 1836, every year. This monitoring has been ongoing for a total of 25 years, and during this period, a total of approximately 400 traps baited with *I. duplicatus* were placed around the country (Holuša et al., 2010; Knížek and Liška, 2022). The traps were checked by foresters, and beetles were collected and sent to FGMRI for determination. In Central Europe, other forest research institutes have also been involved in monitoring BB, e.g., in Slovenia (see Hauptman et al., 2019a), Slovakia (see Galko et al., 2014), and Latvia (see Ostrauskas and Tamutis, 2012); however, these were one-time events.

Our proposed monitoring of BB can be easily merged with the existing monitoring of *I. duplicatus*. It involves incorporating only 72 traps. The Forest Advisory Service would purchase ethanol vaporizers for cooperating entities and provide basic operator training; however, it is also possible to use a recorded instructional video. The total volume of all samples from the three traps does not exceed 1 dm^3, so workers can place it in closed cans in any freezer where the insects will be frozen. It is necessary to determine the entire material of beetles into species by a specialist because data will be obtained on several species of ambrosia and bark beetles, especially rare ones (Fiala, 2021; Holuša et al., 2021; Fiala and Nakládal, 2022; Fiala et al., 2023).

Due to the importance of early detection of invasive species of ambrosia and bark beetles, the economic costs are minimal (**Table 2**) compared to the damage that can occur. In the US, the annual loss associated with all invasive species is estimated at $120 billion (Pimentel et al., 2005). In Europe, the loss caused by all invasive species is estimated to be hundreds of millions of € per year (Vilà et al., 2010); e.g., for invasive longhorned beetles of the genus *Anoplophora*, the cost of eliminating one infested hectare of vegetation is $25,000 (Anonymus, 2014). Estimated economic loss to landowners exceeded hundreds of dollars per hectare for invasive pests in *Pinus taeda* Linnaeus, 1753 stands in the southern US when no monitoring was performed (Susaeta et al., 2016). When carrying out integrated protection, the cost is less than the loss of value of the wood (Franjević et al., 2016). At the same time, lures require smaller financial expenditure than the human labor associated with the control of traps (Šramel et al., 2021).

4. Conclusion

The proposed monitoring method based on commonly used traps in selected locations (entrance gates at borders, wood warehouses, tropical greenhouses, and airports) is necessary because we BB have already been detected in the Czech Republic. Therefore, it is necessary to monitor these species and be able to detect new ones. Ethanol is effective for capturing the species that have already been detected, and the method is inexpensive. The method can be implemented by the research institute for monitoring pests. The monitoring results can inform the professional actions of the Central Institute for Supervising and

3 www.e-econex.net

Testing in Agriculture and for the targeted eradication of invasive species, as required by EU regulations.

Author contributions

TF and JH contributed to the conception and design of the study and wrote the first draft of the manuscript. Both authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix

APPENDIX TABLE 1 Detection of ambrosia and bark beetles according to the type of bait at a tropical wood warehouse in Pilsen.

Species	Ethanol	Ethanol + α-pinen	Ethanol + E-conophthorin
Anisandrus dispar Fabricius, 1792	1		
Hylastes attenuatus Erichson, 1836	1	1	
Hylesinus varius Fabricius, 1775	1		
Lymantor coryli Perris, 1853	1		
Scolytus rugulosus P.W.J. Müller, 1818	1		
Tomicus piniperda Linnaeus, 1758		3	
Xyleborinus saxesenii Ratzeburg, 1837	2		1

In Plzeò – Doubravka (GPS 49.7622N, 13.4095E), three Lindgren funnel traps with wet capture and ethanol, ethanol + α-pinene and ethanol + E-conophthorin were used as bait. Trapping took place from mid-April to mid-July, and beetles were collected once a month (det. T. Fiala, M. Knížek).

APPENDIX TABLE 2 Detected species of ambrosia and bark beetles in the Prague-Troja Botanical Garden (GPS 50.1224N, 14.4139E).

Species	Number of specimens
Anisandrus dispar Fabricius, 1792	599
Dryocoetes himalayensis Strohmeyer, 1908	**1**
Dryocoetes villosus Fabricius, 1792	12
Ernoporus tiliae Panzer, 1793	1
Pityogenes chalcographus Linnaeus, 1761	1
Polygraphus grandiclava C.G. Thomson, 1886	4
Scolytus rugulosus P.W.J. Müller, 1818	5
Xyleborinus saxesenii Ratzeburg, 1837	367
Xyleborus dryographus Ratzeburg, 1837	70
Xyleborus monographus Fabricius, 1792	44
Xylocleptes bispinus Duftschmid, 1825	1
Xylosandrus germanus Blandford, 1894	**1**

Theysohn traps baited with ethanol were used at the Troy Botanical Garden. Ten traps were placed in nature near the tropical greenhouse, and two traps were placed inside the tropical greenhouse. Trapping was performed from mid-April to mid-August, and beetles were collected at 2-week intervals (det. T. Fiala, M. Knížek). Invasive species are in bold.

APPENDIX TABLE 3 The presence of feeding and the detected numbers of *Hypothenemus hampei* Ferrari, 1867 in samples of ten coffee beans imported to the Czech Republic from seven countries in 2021–2022 (det. T. Fiala).

Country of origin	Brazil	Brazil, region São Paulo	Colombia	Ethiopia, region Yirgacheffe	Ethiopia, region Guji	India, region Tamil Nadu	Salvador
Presence of feeding	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Numbers of beetles	1	0	2	0	0	1	0

Appendix 4 | Basic monitoring design.

- Twenty-four localities
- Three traps per locality, 30–50 m each other
- Each trap baited with ethanol
- Traps checked once every 14 days

APPENDIX TABLE 5 Overview of the number of scolytines caught by trap in the Czech Republic and in the world.

Country	Year	Traps/Sites	Lures	Total *Scolytinae*	Numbers of invasive species/Specimens	References
United States	2001–2005	1,240/310	Variable	250,000+	24/?	Rabaglia et al., 2008
	1985–2000	?/97	Variable	6,825	67/2,737	Haack, 2001
	2007–2016	4,320/1,440	Variable	840,000+	28/456,000+	Rabaglia et al., 2019
Italy	2009–2011	72/4	Variable	1,043	4/30	Rassati et al., 2014
	2012	90/15	Variable	40,473	11/406	Rassati et al., 2015
Czech Republic	2020	10/10	Ethanol	4,179	3/24	Holuša et al., 2021
	2022	20/4	Ethanol	1,176	4/186	Fiala et al., 2020
	2018	1/1	Ethanol	124	0/0	Fiala, 2019
Slovenia	2017	19/19	Ethanol	94,104	3/67,605	Hauptman et al., 2019b
Slovakia	2010–2012	53/1	Ethanol mixture	24,705	2/561	Galko et al., 2014