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Metody detekce invazních druhů kůrovců (Curculionidae: Scolytinae)

Disertační práce

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Methods of detection of invasive bark beetles (Curculionidae: Scolytinae)

Doctoral Dissertation

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"Prohlašuji, že jsem disertační práci na téma Metody detekce invazních druhů kůrovců (Curculionidae: Scolytinae) vypracoval samostatně s použitím uvedené literatury a na základě konzultací a doporučení školitele.

Souhlasím se zveřejněním disertační práce dle zákona č. 111/1998 Sb. o vysokých školách v platném znění, a to bez ohledu na výsledek její obhajoby."

V Praze dne

Podpis autora

Poděkování

Touto cestou si dovoluji poděkovat všem spoluautorům odborných článků za jejich podíl na úspěšném přijetí rukopisů a nepřímou spoluúčast na vzniku této disertační práce. Rád bych poděkoval svému školiteli prof. Ing. Bc. Jaroslavovi Holušovi, Ph.D. za odborné vedení disertační práce, věcné konzultace k metodice a pomoc při realizaci analýz. Velké díky patří mé rodině za celkovou podporu během studia.

Abstrakt

Invazní kůrovci představují ohrožení pro lesní ekosystémy, městskou zeleň, biodiverzitu, v jejichž důsledku mohou být velké ekonomické náklady na obranu proti nim.

Disertační práce je rozdělena do čtyř částí. První se věnuje rozšíření stávajících invazních druhů kůrovců v České republice, jmenovitě druhům *Cyclorhipidion bodoanum*, který byl v rámci doktorandského studia zjištěn poprvé v České republice; *Gnathotrichus materiarius*, jehož rozšíření je převážně v Čechách, na Moravě se doposud vyskytuje řídce; *Phloeosinus aubei*, který se ve větší míře více vyskytuje na Moravě než v Čechách, hlavně na jalovcích, ale jako jediný z invazních kůrovců způsobuje větší ekonomické škody v parcích měst na cypřiších a zeravech; *Xyleborinus attenuatus*, který je rozšířen hojně po celé republice; *Xylosandrus germanus*, který je taktéž běžný v celé republice.

Druhá část práce je věnována zjištění vhodných návnad k odchytu invazních kůrovců. Tato část je reprezentována třemi pokusy; (i) použitím průmyslově vyráběných návnad (Trypowit® a Lineatin Kombi®) v dubových porostech, kdy vyšší odchyty měla návnada Lineatin Kombi®, pravděpodobně z důvodu, že obsahuje quaiacol, který zvyšuje afinitu k etanolu; (ii) testováním různých volatilních látek na lákání kůrovce *P. aubei*, kdy bylo zjištěny lepší odchyty na etanol s jalovcovou větévkou než na ostatní druhy návnad; a (iii) použití potencionálního feromonu sulcatol na lákání *G. materiarius*. Sulcatol nebyl potvrzen jako vhodná návnada, mnohem více jedinců se odchytilo na 95% etanol.

Třetí část disertační práce se věnuje studiu, jaké environmentální proměnné ovlivňují výskyt invazních kůrovců. Na základě výsledků lze konstatovat, že vyšší počet ambrosiových kůrovců se vyskytoval v zapojenějších dubových porostech a s větším množstvím odumřelých větví na stromech. Naopak přítomnost nahodilých kůrovcových těžeb a výskyt geograficky nepůvodních jehličnanů nenavyšoval počet invazních kůrovců. Taktéž nebyla zjištěna environmentální proměnná, která by ovlivňovala přítomnost *P. aubei* v jalovcových porostech.

Poslední část disertační práce kompilovala zjištěné výsledky do metodiky monitoringu detekce invazních kůrovců v České republice. Bylo vytipováno 24 lokalit na celém území republiky vhodných k monitoringu invazních kůrovců. Tyto lokality zahrnují mezinárodní letiště, hraniční přechody, botanické zahrady, přirozené vstupní geomorfologické body a velké dřevosklady. Na tyto lokality by se měly umístit tři nárazové lapače ve vzdálenosti 30-50 m od sebe navnaděné etanolem. Vyvěšeny by měly být od poloviny dubna do konce července. Kontrola lapačů bude probíhat každých 14 dní. Celkové počáteční náklady na monitoring včetně nákupu lapačů se pohybují okolo 6 000 €. Další roční náklad je reprezentován pouze nákupem etanolových návnad v hodnotě cca 1 500 €.

Klíčová slova: invazní kůrovci, etanol, monitoring, environmentální proměnné

Abstract

Invasive bark beetles pose a threat to forest ecosystems, urban parks, biodiversity, and as a result represent large economic costs to defend against them.

The PhD thesis is divided into four parts, the first is devoted to the spread of existing invasive species of bark beetles in the Czech Republic, namely the species *Cyclorhipidion bodoanum*, which was discovered for the first time in the Czech Republic as part of the doctoral studies; *Gnathotrichus materiarius*, whose distribution is mainly in Bohemia, is still rarely found in Moravia; *Phloeosinus aubei*, which is more common in Moravia than in Bohemia, mainly on junipers, but as the only invasive bark beetle it causes more economic damage in city parks on cypresses and thujas; *Xyleborinus attenuatus*, which is quite abundant throughout the country; *Xylosandrus germanus*, which is also common throughout the country. The second part of the work is dedicated to finding suitable lures for catching invasive bark beetles. This part was carried out with three trials: using artificially produced lures (Trypowit® and Lineatin Kombi®) in oak trees, where the Lineatin Kombi® bait had higher catches, probably because it contains quaiacol, which increases affinity for ethanol; by testing different volatiles to attract the bark beetle *P. aubei*, better captures were found on juniper twig with ethanol than on other types of volatiles; and the use of the potential pheromone sulcatol to attract *G. materiarius*, sulcatol was not found to be a suitable lure, many more individuals were caught on 95% ethanol. The third part of the dissertation is devoted to finding out what environmental variables influence the occurrence of invasive bark beetles. Based on the results, it can be said that a higher number of ambrosia bark beetles affects a closed canopy and the amount of dead branches on the trees. On the contrary, the presence of random bark beetles cutting, and the occurrence of geographically non-native conifers do not affect the higher abundance of invasive bark beetles. Also, no significant environmental variable influencing the presence of P. *aubei* in juniper stands was found. The last part of the dissertation compiled the obtained results into a methodology for monitoring the detection of invasive bark beetles in the Czech Republic. 24 locations throughout the country suitable for monitoring invasive bark beetles were selected. These locations include international airports, border crossings, botanical gardens, natural entry points and large woodstorages. In these locations, three traps should be placed at a distance of 30-50 m from each other, and ethanol should be used as a lure, as a universal attractant. They should be posted from mid-April to the end of July. Traps should be checked once every 14 days. The total initial costs for monitoring, including the purchase of traps, are around €6,000. Another annual expense is only an item for the purchase of ethanol lures worth approximately €1,500.

Key words: invasive bark beetles, ethanol, monitoring, environmental variables

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1. Úvod

Kůrovci patří taxonomicky do čeledi Curculionidae, podčeleď Scolytinae. V minulosti byli samostatnou čeledí Scolytidae (Latreile 1807), v současné době jsou však na základě larvální morfologie klasifikováni jako podčeleď (Crowson 1967; Lawrence & Newton 1995; Jordal et al. 2014). Celá podčeleď se dělí nadále na triby, z nichž v Evropě se vyskytují: Corthylini, Cryphalini, Crypturgini, Dryocoetini, Ernoporini, Hylastini, Hylesinini, Hylurgini, Hypoborini, Ipini, Phloeosini, Phloeotribini, Polygraphini, Scolytini, Trypophloeini, Xyleborini a Xyloterini (Alonso-Zarazaga et al. 2023). I přes všeobecnou shodu, že kůrovci jsou klasifikováni jako podčeleď, existují význační taxonomové, kteří je nadále považují za čeleď (Bright 2019). Na celém světě se vyskytuje více než 6000 druhů kůrovců, v palearktické oblasti se jich vyskytuje téměř 900 druhů (Knížek & Beaver 2007; Hulcr et al. 2015).

Scolytinae se podle způsobu života dělí na dvě skupiny, na pravé kůrovce a ambrosiové kůrovce. Praví kůrovci se živí floémem nebo méně častěji vytvářejí požerky v běli. Jsou charakterizováni relativně vysokou specificitou hostitele, vzhledem k tomu, že jsou odkázáni na specifické vlastnosti floému, chemické vlastnosti stromu a kvalitu potravy (Gomez et al. 2023). Naproti tomu jednotlivé druhy ambrosiových kůrovců obsazují různé hostitelské dřeviny a živí se symbiotickými houbami, které si vnesou do stěn požerků vytvořených ve dřevě (Hulcr & Skelton 2023). Několik málo známých ambrosiových kůrovců používá metodu „mycocleptism“, to znamená, že nemají mycangium, které se používá k přenosu hub do požerků, a „kradou“ houby jiným ambrosiovým kůrovcům. Vyhloubí matečnou chodbu těsně vedle matečné chodby jiného ambrosiového kůrovce a jeho houby prorostou do matečné chodby mycocleptického kůrovce (Hulcr & Cognato 2010). Ambrosiový kůrovec *Xyleborus ferrugineus* Fabricius, 1801 využívá steroid ergosterol produkovaný houbou *Fusarium solani* (Mart.) Sacc., 1881 ke zvýšení fertility samic a růstu larev (Kok et al. 1970). Některé druhy kůrovců se vyvíjejí v semenech nebo v ovoci, případně v rostlinách (Pfeffer 1955). Tyto různé potravní zvyklosti se odrážejí v diverzitě nepůvodních druhů Scolytinae. Ambrosioví invazní kůrovci vyžadují vhodné klimatické podmínky pro růst svých symbiotických hub a jsou četnější na vlhčích a teplejších lokalitách (Reich et al. 2014; Rassati et al. 2016b; Hulcr & Stelinski 2017), zatímco praví kůrovci jsou méně závislí na podnebí a jsou homogenněji rozšířeni (Marini et al. 2011). Stále však není známo, jak různé potravní preference ovlivňují složení druhové komunity kůrovců. U invazních kůrovců, vzhledem k jejich vysoké hostitelské specificitě, je evidentní, že jejich různé druhové složení závisí na druhové bohatosti dřevin v lesních porostech.

Kůrovci patří mezi nejdůležitější škůdce, kteří ohrožují stabilitu lesních porostů na celém světě. S rostoucím počtem invazních kůrovců je ohrožení lesních porostů ještě vyšší. Invazní kůrovci představují hrozbu nejen pro původní biodiversitu, funkční lesní ekosystémy a ekonomickou produktivitu lesního hospodářství (Brockerhoff et al. 2006a; Aukema et al. 2011; Gohli et al. 2016), ale i pro parky a sady (Francardi et al. 2017; Branco et al. 2019). Invazní kůrovci jsou navíc významnými přenašeči houbových chorob, které způsobují masivní hynutí dřevin. Invazní kůrovec *Pityophthorus juglandis* Blackman, 1928 je přenašečem houby *Geosmithia morbida* M. Kolařík, Freeland, C. Utley & Tisserat, 2010, která způsobuje chorobu Thousand cankers disease na ořešácích (*Juglans* spp.) (Faccoli et al. 2016). Mezi další přenašeče houby *G. morbida* patří v Evropě invazní kůrovci *Xylosandrus crassiusculus* Motschulsky, 1866, *Xylosandrus germanus* Blandford, 1894, *Monarthrum mali* Fitch, 1856 a *Cyclorhipidion bodoanum* Reitter, 1913 (Moore et al. 2019). *C. bodoanum* může být jedním z možných přenašečů závažné choroby dubů Sudden oak death (McPherson et al. 2013). Lýkohub *Dendroctonus valens* LeConte, 1859 je v USA, kde je původní, považován za minoritního škůdce. Naopak v Číně, kde je invazní, způsobuje kalamitní odumírání borovice *Pinus tabuliformis* Carrière, 1867 a je přenašečem několika druhů ophiostomatoidních agresivních hub (Yan et al. 2005; Lu et al. 2009; Min et al. 2009; Marincowitz et al. 2020). Kůrovec *Scolytus multistriatus* Marsham, 1802 je přenašečem hub *Ophiostoma novo-ulmi* Brasier, 1991 a *Ophiostoma ulmi* (Buisman) Nannf., 1934, které způsobují grafiózu jilmů (Basset et al. 1992; Santini & Faccoli 2015). Tento kůrovec je invazní v USA (Chapman 1910) a zároveň byl zpětně reintrodukován do Evropy (Cola 1973; Binazzi et al. 2019). Zpětně reintrodukován z USA, kde byl pravděpodobně již před více než 100 lety zjištěn jako první invazní ambrosiový kůrovec (Rabaglia et al. 2006), do Evropy byl i *Xyleborinus saxesenii* Ratzeburg, 1837, který byl také zpětně importován z Afriky (Cola 1971), kde je taktéž invazní (Wood & Bright 1992). I tyto zpětné reintrodukce reprezentují potencionální nebezpečí, když si kůrovci vytvoří nové symbiózy s lokálními houbami a přenesou je zpět do své původní oblasti (Grubbs et al. 2020). Přenos nepůvodních hub introdukovaných invazními kůrovci na původní kůrovce a jejich další šíření je už zaznamenán (Carrillo et al. 2014). Vlivem změn klimatu se šíří lýkohub *Phloeosinus aubei* Perris, 1855, který je přenašeč houby *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson, 1972 způsobující chorobu „Cypress canker disease“. Ta stojí za masivním odumíráním cypřišů v jižních státech Evropy (Ponchet 1981; Danti & Rocca 2017). Toto houbové onemocnění bylo zjištěno i v České republice (Mertelík & Šindelková 2003).

Invazní kůrovci způsobují po celém světě významné ekonomické škody, které dosahují vysokých finančních hodnot (Dobie 1978; McLean 1985; Wylie et al. 1999; Pimentel et al. 2005; Aukema et al. 2011). Protože i původní kůrovci jsou přenašeči různých druhů hub, např. *Ips acuminatus* Gyllenhal, 1827 je přenašečem více než 50 druhů hub (Davydenko et al. 2017), simultánní efekt vícero invazních druhů, jejich hub a následná interakce se změnou klimatu vytváří stav, u kterého můžeme těžko predikovat vliv na životní prostředí (Lovett et al. 2013).

Kůrovce můžeme najít na všech dřevinách po celém světě. I na jedovatém *Taxus baccata* L., 1753, který byl dlouho považován za dřevinu, která nehostí žádného kůrovce (Pfeffer 1955), byl nakonec zjištěn kůrovec *Hylastes pinicola* Bedel, 1888 (Schedl 1980). Nejčastěji kůrovci provádějí žír pod kůrou a různé druhy napadají všechny části dřeviny, od kořenů a pařezů (rod *Hylastes*), spodní část kmene (c.f. *Dryocoetes autographus* Ratzeburg, 1837, *Hylurgops palliatus* Gyllenhal, 1813, *Dendroctonus micans* Kugelann, 1794), celý kmen (rod *Ips*, *Pityokteines, Scolytus*), vrchol koruny (rod *Orthotomicus*, *Ips*), silnější větve (c.f. *Pityogenes chalcographus* Linnaeus, 1760) až po nejtenčí větévky (rod *Pityogenes*, *Phloeotribus*, *Cryphalus*, *Eidophelus*). V nejslabších větévkách mohou druhy provádět i zralostní žír (*Tomicus minor* Hartig, 1834, *Tomicus piniperda* Linnaeus, 1758, *Phloeosinus thujae* Perris, 1855). I jeden druh kůrovce může napadat různá věková stadia stromů, od sazenic až po dospělé stromy (např. Fiala & Holuša 2021). Jednotlivé druhy kůrovců jsou vázány buď na jehličnany nebo listnáče s jedinou výjimkou. Pouze lýkohub *Polygraphus grandiclava* C.G. Thomson, 1886 se vyvíjí jak na jehličnanech (*Pinus*, *Picea*), tak listnáčích (*Prunus*) (Avtzis et al. 2008). Ambrosioví kůrovci jsou naproti tomu silně polyfágní a řada druhů napadá jak jehličnany, tak i listnáče (Weber & McPherson 1983; Lightle et al. 2007). Preferují však různé dimenze dřevin, od silnějších průměrů kmene, jako např. *Trypodendron lineatum* Olivier, 1800 nebo *Gnathotrichus materiarius* Fitch, 1858, kmeny o průměru okolo 20 cm (*X. germanus, C. bodoanum*) (Brin et al. 2011; Reed et al. 2015; Galko et al. 2019), silnější větve (*X. crassiusculus, Anisandrus dispar* Fabricius, 1792) až po větévky (*Xylosandrus compactus* Eichhoff, 1876, *Xylosandrus morigerus* Blandford, 1894) (Kalshoven 1961; Tanasković et al. 2016; Francardi et al. 2017). Kůrovci napadají nejen dřeviny ale i keře, jako např. nehojný *Phloeotribus rhododactylus* Marsham, 1802, který obsazuje janovec metlatý (*Cytisus scoparius* (L.) Link, 1822) (Fiala & Holuša 2020a), liány, jako např. *Xylocleptes bispinus* Duftschmid, 1825, který provádí žír na plaménku plotním (*Clematis vitalba* L., 1753) (Fiala 2021), na topolech (*Populus* sp.) rostoucí jmelí bílé (*Viscum album* L., 1753) napadá kůrovec *Liparthrum bartschti* Mühl, 1891 (Pfeffer 1941). Z rostlin napadají pryšce (*Euphorbia* sp.) kůrovci *Thamnurgus varipes* Eichhoff, 1878 a *Aphanarthrum alluaudi* Peyerimhoff, 1923 (Schedl 1971; Mandelshtam et al. 2012). Vážným škůdcem kukuřice je kůrovec *Pagiocerus frontalis* Fabricius, 1801 (Okello et al. 1996). Na kaktusech provádí žír kůrovec *Cactopinus hubbardi* Schwarz, 1899 (Bright 1967). Na orchidei *Dendrobium phalaenopsis* Fitzg., 1880 byl v Evropě nalezen invazní kůrovec *X. morigerus* (Reitter 1913). I v semenech se vyvíjejí kůrovci, např. *Hypothenemus hampei* Ferrari, 1867, *Hypothenemus obscurus* Fabricius, 1801 a *X. compactus* se vyvíjejí v zrncích kávy (Greco & Wright 2012, 2015; Vega et al. 2012), kůrovec *Coccotrypes dactyliperda* Fabricius, 1801 je škůdcem datlí (Spennemann 2018). Ve dvouletých šiškách borovice vejmutovky (*Pinus strobus* L., 1753) provádí žír kůrovec *Conophthorus coniperda* Schwarz, 1895 (Hedlin et al. 1980). I neobvyklé materiály mohou sloužit jako potrava pro kůrovce. Z požerků v obalu knihy byl popsán kůrovec *Hypothenemus eruditus* Westwood, 1834, jeden z nejrozšířenějších a nejpočetnějších kůrovců na světě (Kambestad et al. 2017). Tento kůrovec dokáže provádět žír i v uzených rybách nebo slaných broskvích (Madenjian 2020).

Invazní kůrovci se šíří několika způsoby, nejčastěji je to globálním obchodem s dřevěným materiálem (opracované i neopracované dřevo), dřevěnými obaly, plody nebo s živými sazenicemi dřevin (Mathew 1987; Meissner et al. 2008; Pombo et al. 2010; Kangkamanee et al. 2011; Augustin et al. 2012; Brockerhoff & Liebhold 2017; Meurisse et al. 2019). Je potvrzeno i šíření dřevěným materiálem, který byl ošetřen podle mezinárodního standardu ISPM 15 (Haack & Petrice 2009; Haack et al. 2014). V Evropě jsou vstupní branou nejčastěji přístavy na atlantském a středozemním pobřeží (Hagedorn 1910; Hoffmann 1942; Cola 1971, 1973; Faccoli 2008; Faccoli et al. 2009; Moraal 2010; Inghilesi et al. 2013; Rassati et al. 2016a; Binazzi et al. 2019; Branco et al. 2019; Barnouin et al. 2020). V USA v letech 1985-2000 zachytili celkem 6825 exemplářů invazních kůrovců, z toho 1248 kusů bylo zjištěno na letištích, 4973 jedinců v námořních přístavech, 201 kusů na pozemních hranicích, 366 jedinců na speciálních obchodních letištích a 37 kusů bylo zjištěno přímo v zemích, kde vývoz podléhá kontrole předběžného schválení (Haack 2001). Další přesná čísla odchycených kůrovců v amerických přístavech udává Meissner et al. (2008); od 5. července 2006 do 1. ledna 2008 bylo zjištěno 788 kusů invazních kůrovců v 247 případech. Vliv člověka na šíření invazních kůrovců je daleko větší, než vliv klimatu (Gohli et al. 2016; Ward et al. 2019), například zakládání plantáží nepůvodních dřevin zvyšuje riziko zavlečení nepůvodních kůrovců (Lantschner et al. 2017). V ČR se to týká hlavně pěstování borovice černé (*Pinus nigra* Aiton, 1789) a jejích škůdců *Pityogenes bistridentatus* Eichhoff, 1878 a *Orthotomicus robustus* Knotek, 1899, kteří jsou z ČR známi jen z několika lokalit (Pfeffer & Knížek 1996; Urban 2000; Knížek 2006; Knížek & Mertelík 2017; Fiala 2019). Přesto změna klimatu hraje podstatnou roli v rozšíření a přežití populace invazních kůrovců na kontinentech (Rassati et al. 2016b). Změnou klimatu se na sever šíří hostitelské dřeviny, které se původně na daném místě nevyskytovaly (Ge et al. 2017). I kůrovci, kteří se vyskytovali pouze v jižní Evropě se mohou šířit na sever, např. lýkohub *P. aubei*, se šíří ve střední Evropě i do chladnějších lokalit (Fiala & Holuša 2019). Kůrovci se nešíří pouze globálním obchodem, ale i přirozeně, protože tento rod *Phloeosinus* patří mezi dobré letce (Furniss & Furniss 1972). Suchá léta přispívají tomu, že i kůrovci, kteří běžně nevystupují do vysokých nadmořských výšek, se objevují i v alpských polohách (Marini et al. 2012). Naopak i povodně zvyšují možnost napadení stromů kůrovci, protože stromy jsou stresovány nadměrným množstvím stojící vody (Wang et al. 2021). Rychlost šíření se globalizací obchodu významně zrychluje. Vliv na to má i bezcelní kamionová doprava v Evropské unii a pád „železné opony“ (Hulme 2009; Roques et al. 2016). Evropská unie, která stojí za projektem nových typů chráněných územích Natura 2000, přispívá k šíření invazních druhů, protože jsou více přístupná lidem než nedostupné lokality chráněné desítky let (Gallardo et al. 2017). Podstatnou částí boje proti invazním kůrovcům je jejich včasná identifikace, která pomáhá s případnou obranou proti škůdci (Douglas et al. 2009).

V Evropě dochází k častějšímu a častějšímu výskytu nových druhů invazních kůrovců v posledních letech a šíření již zabydlených druhů do nových států (Marchioro et al. 2022; Mas et al. 2023; Sanchez et al. 2023; Saurat et al. 2023). Lze předpokládat, že také v České republice se nové invazní druhy kůrovců budou objevovat častěji. Proto jsme studovali rozšíření etablovaných druhů invazních kůrovců v ČR, při kterém jsme zjistili i nový druh invazního kůrovce pro ČR (Fiala et al. 2021). Stanovení environmentálních faktorů určujících výskyt kůrovců pomůže definovat, na které lokality je nutné se v budoucím monitoringu zaměřit. Srovnáním různých feromonů a volatilních látek je nutné nalézt kombinaci návnad pro celorepublikový monitoring.

Zjištění přítomnosti invazního druhu kůrovce je zásadní podmínkou integrované ochrany lesa. Bez znalostí diverzity kůrovců a jejich potencionálního nebezpečí pro lesní ekosystémy, urbánní zeleň, biodiverzitu a ekonomické škody nemůžeme vytvářet postupy integrované ochrany lesa tak, aby byly účinné, ekonomicky únosné a s nejnižšími vedlejšími efekty.

2. Cíle práce

1. Popis současného rozšíření invazních druhů kůrovců na území České republiky;
2. Nalezení vhodné kombinace volatilních látek nebo feromonů k monitoringu vybraných invazních druhů kůrovců v České republice;
3. Stanovení environmentálních proměnných (zápoj, množství mrtvého dříví, typ lesa, zásoba dřevní hmoty, vzdálenost od zdrojů, geomorfologie terénu, vzdálenost od měst), které ovlivňují abundanci a šíření invazních druhů kůrovců;
4. Definování syntetické metodiky monitoringu invazních druhů kůrovců, které mohou pronikat do České republiky z okolních zemí.

3. Rozbor problematiky

3.1 Semiochemikálie

Semiochemikálie ovlivňující chování hmyzu dělíme na několik druhů: feromony, které zprostředkovávají vnitrodruhovou komunikaci; allelochemikálie, které zprostředkovávají mezidruhovou komunikaci; allomony, které zprostředkovávají mezidruhovou komunikaci, ze které má užitek původce, nikoli příjemce; kairomony (volatilní látky), které zprostředkovávají mezidruhovou komunikaci, ze které má užitek příjemce, nikoli původce; synomony, které zprostředkovávají mezidruhovou komunikaci, ze které mají užitek jak původce tak příjemce (Seybold et al. 2018). Pohlaví, které jako první začíná napadat hostitele vylučuje primární feromony, zatímco druhé pohlaví může uvolňovat dodatečné feromony (Vité & Francke 1976). U polygamních druhů (c.f. *Ips* nebo *Pityokteines*) začínají na hostitelské dřeviny nalétávat samci, u monogamních druhů (c.f. *Dendroctonus*) jsou to naopak samice (Borden 1974). První agregační feromon byl identifikován v roce 1966 z lýkožrouta *Ips paraconfusus* Lanier, 1970 jako směs ipsdienolu, ipsenolu a *cis*-verbenolu, přičemž tyto terpenové alkoholy byly izolovány z požerků (Silverstein et al. 1966). V moderní době se semiochemikálie získávají přímo z dospělců, kteří čerstvě opustili matečné chodby přímo extrakcí nebo záchytem na adsorbenty. Látky jsou analyzovány pomocí plynového chromatografu, a následně se testují na tykadlech kůrovců (González et al. 1994; Kendra et al. 2012; Ranger et al. 2014; Gao et al. 2019). Poté se tyto látky syntetizují a následně testují v polních podmínkách (Pureswaran et al. 2004; Rainho et al. 2021).

Kůrovci používají komplex chemického komunikační systému k vyhledávání nového hostitele, na kterém pak provádějí žír, páření a rozmnožování. Protože potravní zdroj může být nedostupný, dokud hostitel neodumře nebo není jinak oslaben, vyvinul se u kůrovců komunikační systém pomocí feromonů (Tab. A1), které ve výsledku vedou k agregaci populace na novém hostiteli. Tato agregace musí mít dostatečnou intenzitu, aby populace mohla nového hostitele využít (Wood 1982a). Proces kolonizace nového hostitele může být rozdělen do čtyř fází: rozptýlení – začíná opuštěním matečného hostitele a končí reakcí na podněty nového hostitele nebo reakcí na feromony; výběr – začíná odpovědí na podněty hostitele nebo feromonu a končí žírem na hostiteli; koncentrace – začíná reakcí na feromony, které produkují samci nebo samice, kteří provádějí žír na hostiteli a končí v době kdy se již neuvolňují žádné feromony nebo se naopak uvolňují antiatraktanty; udržení – začíná, když je populace dostatečná k zajištění odumření hostitele nebo u druhů, které nezpůsobují odumření hostitele, začíná žírem oplodněných samic. Tato fáze končí ukončením tvorby matečných chodeb a nakladením vajíček (Wood & Bedard 1977; Byers 1989). Někteří autoři tento mechanismus zkracují na tři fáze (definované u kůrovce *Dendroctonus frontalis* Zimmermann, 1868); iniciační napadení, kdy samice při napadení hostitele vylučují frontalin a napadený strom α pinen; masivní napadení, kdy stále samice vylučují frontalin a napadený strom α pinen, ale samci již vylučují verbenon jako repelentní látku; a přesměrování útoku, při němž obě pohlaví už vylučují *endo*-brevicomin, značící, že hostitel je plně obsazen (Vité & Francke 1976).

Mezi nejdůležitější původce kairomonů patří samotné dřeviny. Stresované dřeviny (sucho, mráz, povodně, oheň, poškození způsobené člověkem) intenzivně produkují etanol, který je nejdůležitější volatilní látkou pro ambrosiové kůrovce (Kelsey & Joseph 2003; Ranger et al. 2013, 2018, 2021; Reed et al. 2015; White 2015; Gely et al. 2020). Etanol vylučují stresované dřeviny v kambiu (MacDonald & Kimmerer 1991; Kelsey &

Joseph 2003). Na tyto stresované dřeviny, které vypadají opticky zdravě, nalétávají ambrosioví kůrovci (Kühnholz et al. 2001; Ranger et al. 2015). Mezi další studované vlivy, které podmiňují nálet ambrosiových kůrovců na stromy, je vliv houbových patogenů na strom. Houby můžou ovlivnit atraktivitu hostitele pro kůrovce (Blood et al. 2018; Egonyu & Torto 2018; Fraedrich et al. 2018; Rassati et al. 2020).

Zdravé nestresované stromy etanol nevylučují. Aby se zjistilo, jaká míra etanolu je nutná k úspěšnému náletu ambrosiových kůrovců, používá se metoda injektáže etanolu do stromu. Následně se měří plynovým chromatografem množství vylučovaného etanolu ze stromu. Tato metoda je vhodná k použití stromu, který není v lesním porostu či parku perspektivní, jako stojící lapák. Metoda je účinnější než na strom vyvěsit kapsli s etanolem (Ranger et al. 2010, 2012; Dodds et al. 2016; Klingeman et al. 2017; Reding et al. 2017; Addesso et al. 2019; Rassati et al. 2020).

V poslední době se využívá různé kombinace volatilních látek (Tab. A1), protože se zjistilo, že se tím zvyšuje množství odchytaných kůrovců do lapačů (Schroeder & Lindelöw 1989; Flaherty et al. 2018). Mezi nejvyužívanější kombinace patří etanol + α pinen (Borden et al. 1980; Francardi et al. 2006; Wylie et al. 2008; Miller & Rabaglia 2009; Burbano et al. 2012; Yang et al. 2018; Kamata et al. 2020), etanol + conophthorin (VanDerLaan & Ginzel 2013; Galko et al. 2014; Miller et al. 2015), quercivorol + α copaen (Kendra et al. 2017) nebo etanol + benzaldehyd (Yang et al. 2018). Mnohem méně se jako návnada lapačích používají různé druhy dřevinných olejů, např. copaiba oil, manuka oil, phoebe oil, cubeb oil, ginger oil, cade oil nebo juniper berry oil (Hanula & Sullivan 2008; Hayes et al. 2008; Kendra et al. 2011, 2016; Burbano et al. 2012; Hanula et al. 2013; Johnson et al. 2014), etylen (González & Campos 1996). α pinen lze použít i jako samostatnou složku k odchytu kůrovce *Tomicus destruens* Wollaston, 1865 (Peverieri et al. 2004). Z agregačních feromonů lze k odchytu invazních kůrovců, které se vyskytují v Evropě, použít sulcatol (Flechtmann & Berisford 2003; Miller & Crowe 2020), [S]-*cis*-verbenol + ipsdienol+ ipsenol (EPPO 2014) nebo prenol (Seybold et al. 2015). Částečně lze chytat invazní kůrovce na feromon exo-brevicomin nebo frontalin (Wylie et al. 2008). I někteří ambrosioví kůrovci mají agregační feromony, nejznámější je feromon kůrovců rodu *Trypodendron,* který se jmenuje lineatin (MacConnell et al. 1977; Paiva et al. 1983; Zumr 1983). V případě nouze lze v lapači použít i 40 % vodku jako návnadu pro odchyt kůrovců (Sweeney et al. 2016) (Tab. 1). Experimentálně byly zkoušeny lapače navnaděné etanolem s přidaným LED světlem. Přidané UV světlo (395 nm) nebo zelené světlo (525 nm) zvyšovalo účinnost lapače při odchytu kůrovce *X. crassiusculus* (Gorzlancyk et al. 2013).

Mezi semiochemikálie patří i látky, které naopak mají repelentní účinky na kůrovce. Mezi nejznámější patří verbenon. Působí jako antiagregační feromon na kůrovce rodu *Ips* a *Dendroctonus* a redukuje napadení stromů kůrovci rodu *Xyleborus*, *Xylosandrus* a *Xyleborinus* (Burbano et al. 2012; VanDerLaan & Ginzel 2013; Werle et al. 2019; Rivera et al. 2020; Frühbrodt et al. 2023). Mezi méně používané repelenty patří směs 1-hexanolu a benzylalkoholu a směs metylsalicylátu a salicylaldehydu, které redukují nálet *T. lineatum* o 80 %, respektive o 65 % (Borden et al. 1997, 2001). Pro kůrovce rodu *Tomicus* je repelentní směs alkoholů C_6, C_8 a benzylalkoholu. Tyto alkoholy jsou emitovány listy a kůrou bříz *Betula pendula* Roth, 1788 a *Betula pubescens* Ehrh., 1791, případně osikou *Populus tremula* L., 1753 a bezem černým *Sambucus nigra* L., 1753 (Zhang et al. 1999a; Poland & Haack 2000; Schlyter et al. 2000; Peverieri et al. 2004). Tyto alkoholy dokážou snížit účinnost agregačních feromonů na kůrovce *D. frontalis*, *Ips avulsus* Eichhoff, 1868 a *Ips grandicollis* Eichhoff, 1868 (Dickens et al. 1992). U kůrovce *Ips typographus* Linnaeus, 1758 redukuji nálet až o 70 % (Zhang et al. 1999b). Na samce kůrovce *Pityophthorus ramulorum* Perris, 1856, který přenáší houbu *Fusarium circinatum* Nirenberg & O´Donnell, 1998, která způsobuje chorobu „Pitch canker disease“ na borovicích *Pinus radiata* D.Don, 1836, působí repelentně látka racemic *trans*-conophthorin (López et al. 2013). Někdy látka, která působí jako agregační feromon na jednoho kůrovce, působí repelentně na jiného kůrovce, příkladem může být multistriatin, který je agregační složkou u *S. multistriatus*, ale repelentem pro *Scolytus scolytus* Fabricius, 1775 (Blight et al. 1976) nebo (S)-(-)-ipsenol působící agregačně na kůrovce *Pityokteines curvidens* a repelentně na *I. typographus* (Vité & Baader 1990).

3.2 Typy lapačů a možnosti využití

Pro odchyt kůrovců se používají nejčastěji čtyři typy lapačů. Ve všech případech se jedná o nárazové typy, které jsou pro zvýšení účinku vnazeny feromony nebo kairomony. Jedná se lapače typu Theysohn, Ecotrap, Borregaard (tubusový) a Lindgren. Poslední dva jmenované se mohou použít i pro mokrý typ odchytu. V tomto případě se do odchytové nádoby přidává polypropenglykol, který odchycené brouky konzervuje (Allison & Redak 2017). Bylo zjištěno, že různé druhy kůrovců preferují jiné typy lapačů. *H. eruditus* a *H. obscurus* preferují typ Ecotrap, kůrovec *Cryptocarenus diadematus* Eggers, 1937, *T.*

piniperda, *Hylastes opacus* Erichson, 1836 nebo *Hylurgus ligniperda* Fabricius, 1787 je lépe chytán do typu Lindgren, do typu Theysohn lépe nalétávají kůrovci *Xyleborus affinis* Eichhoff, 1868, *Premnobius cavipennis* Eichhoff, 1878 nebo *Orthotomicus caelatus* Eichhoff, 1868. Typ Borregaard je účinný pro odchyt kůrovce *Hylastes salebrosus* Eichhoff, 1868 a *Hylastes tenuis* Eichhoff, 1868 (Flechtmann et al. 2000; Petrice et al. 2004; Dodds et al. 2010; Hanula et al. 2011; Miller & Crowe 2011; Burbano et al. 2012). Rozdíly lze pravděpodobně vysvětlit funkční plochou každého typu lapače a vizuální atraktivitou každého tvaru lapače pro kůrovce. Vliv má i rychlost letu každého druhu kůrovce, např. lapač typu Theysohn je vhodnější pro pomaleji letící kůrovce (Petrice et al. 2004).

Pro odchyt ambrosiových kůrovců (*A. dispar*, *X. saxesenii*) v ovocných a lískových sadech, případně i v jiných typech porostů, se používají i lepové pasti většinou navnaděné etanolem typu Rebel Rosso a Csalomon Palx (Atkinson et al. 1988; Hayes et al. 2008; Speranza et al. 2009; Saruhan & Akyol 2013; Ak et al. 2014; Salmane et al. 2015; Sarikaya & Saym 2016). Zemní pasti se používají úspěšně na odchyt kůrovců vyvíjejících se v kořenech nebo v pařezech (c.f. *Hylaste*s spp., *D. valens*) (Erbilgin et al. 2001) nebo v pralesích, kde kůrovci létají při zemi (Abreu et al. 2012). Vzhledem k rozšiřující se občanské vědě, lze využít k odchytu kůrovců i jednoduché PET lahve, které si občané mohou snadno vyrobit doma (Steininger et al. 2015; Pajek et al. 2020; Sanguansub et al. 2020; Tarno et al. 2021). Mezi důležité faktory ovlivňující výši a efektivitu odchytů do lapačů patří i výška umístění lapačů nad zemí. Většina kůrovců je chytána do lapačů ve výšce 35-200 cm (Reding et al. 2010; Hanula et al. 2011; Brar et al. 2012; Machado & Costa 2017; Menocal et al. 2018; Ulyshen & Sheehan 2019). Přesto některé druhy lze nachytat i ve výškách nad 10 m (Procházka et al. 2014; Flaherty et al. 2019; Ulyshen & Sheehan 2019).

Vliv na výši odchytu a druhové spektrum má i barva lapače. Tmavé barvy odchytají více kůrovců než světlé. Je to dáno nižší odrazivostí světla od tmavších lapačů (Dubbel et al. 1985; Strom & Goyer 2001; Werle et al. 2014). Umístění lapačů v porostu (v zapojeném lese nebo na hraně lesa) nemá vliv na celkový počet odchycených kůrovců, ale má vliv na počet odchycených druhů kůrovců, který je větší na okraji lesa nebo přilehlé pasece (Dodds 2011). Na odchyt kůrovců do lapačů typu Lindgren může mít vliv i to, zda používáme suchý odchyt nebo mokrý odchyt s nemrznoucí směsí jako náplní. Do lapačů s mokrým odchytem se chytá více druhů, které využívají k náletu na dřeviny etanol, který nemrznoucí směs obsahuje než druhů rodu *Ips*, které využívají jiné agregační feromony (Miller & Duerr 2008).

Umístění návnady v/na lapači má také vliv na počet odchycených jedinců. Návnada umístěná uvnitř lapače zvyšuje počty odchycených kusů kůrovců než návnada umístěná vně (Miller et al. 2013). Experimentálně se používaly pro odchyt kůrovců i lapače s ultrafialovým světlem, které lze spíše použít pro faunistické průzkumy (Frost 1964; Ostmark 1968). Pro faunistické průzkumy se používají i nárazové pasti bez návnad (Faccoli & Rukalski 2004).

Důležité faktory jsou i povětrnostní faktory, jako je teplota, vlhkost, vítr a jeho směr i sluneční radiace, které nakonec určují úspěšnost odchytu kůrovců (Daterman et al. 1965; Beaver & Löyttyniemi 1991; Barclay et al. 1998; Sittichaya et al. 2012; Brockerhoff et al. 2017; Rodríguez et al. 2017; Gugliuzzo et al. 2019; Jones et al. 2019; Monteiro et al. 2019; Chen et al. 2020). Některé druhy kůrovců mají vrchol letu odpoledne, jiné až k večeru (Calnaido 1965; Klimetzek et al. 1989a; Johnson et al. 2016; Menocal et al. 2018).

3.3 Monitoring

Vzhledem k ekonomickým a ekologickým škodám, provádějí některé státy pravidelný monitoring invazních škůdců na svém území. Důvodem je zabránění potencionálnímu nebezpečí, které by mohlo vzniknout. Nejvíc propracovaný systém pravidelného monitoringu mají USA, kde monitoring probíhá už 20 let (Rabaglia et al. 2008). Jejich systém je založen na využití husté síti lapačů typu Lindgren vnazených etanolem, α pinenem+etanolem a ipsdienolem+*cis*-verbenolem+metylbutenolem. Lapače jsou umístěny hlavně u obou pobřeží, ale i ve vnitrozemí USA. Území USA je rozděleno na tři části a každá část je monitorována jednou za tři roky. I připojená zámořská území jako Portoriko nebo Guam se pravidelně zúčastní monitoringu, zde se používají i jiné volatilní látky k odchytu, manuka oil nebo etanol+cubeb oil. Lapače jsou umístěny u mořských přístavů nebo u dřevozpracujícího průmyslu (Rabaglia et al. 2019). Již před započetím tohoto programu docházelo v USA k odchytu invazních kůrovců v přístavech a na letištích (Rabaglia & Cavey 1994; Haack 2001, 2006; Mudge et al. 2001). Data z tohoto monitoringu se využívají pro určování chování invazních kůrovců a modelování jejich šíření v USA (Rassati et al. 2014, 2015, 2016b). V Kanadě proběhly první pokusy detekovat invazní kůrovce na konci 90.let 20. století v okolí Vancouveru. K odchytu byly použity následující látky: etanol, α pinen a atraktanty *I. typographus* (Humble 2001). V Itálii již proběhly pokusy zachytit kůrovce v přístavech v druhé polovině 20. století (Cole 1971, 1973). V současnosti v Itálii monitorují 15 mezinárodních přístavů a jim přilehlých lesních porostů; k odchytu používají lapače typu Lindgren a podobné semiochemikálie jako v USA (etanol, α pinen+etanol a ipsdienol+ipsenol+metylbutenol). Tři lapače umisťují v přístavu a tři lapače do přilehlých lesů. Úspěšnost odchytů byla vyšší v listnatých lesích než v jehličnatých porostech a v přístavech. V přístavech zachytili kůrovce, kteří prozatím nejsou schopni založit stálou populaci v okolních lesích (Rassati et al. 2016a). Snaha o pravidelnější odchyt invazních kůrovců proběhla i na Novém Zélandě. Opět byly použity lapače typu Lindgren s návnadami α pinen+etanol, β pinen+etanol, frontalin+etanol a ipsdienol v přístavech, mezinárodních letištích a v lesích blízko těchto míst. Bylo prokázáno, že tento model monitoringu je úspěšný pro včasnou detekci invazních kůrovců a je zde velká šance následně tyto kůrovce eliminovat z přírody (Brockerhoff et al. 2006b). První pokusy s detekcí invazních druhů na Novém Zélandu proběhly v 80. letech 20. století (Carter 1989). Šířeji zaměřený byl monitoring invazních druhů v Austrálii, kde byly monitorovány i Lepidoptera. Používány byly lepové pasti, lapače typu Lindgren a Ecotrap. Jako návnada byl aplikován etanol, cineol, α pinen, phellandren a směs pinenu, phellandrenu, cineolu, terpenu a cymenu. Lapače byly instalovány u přístavů a letišť a další byly umístěny v zóně do 5 km od těchto přístavů a letišť (Bashford 2012). Ve Francii spustili monitoring invazních tesaříků (Cerambycidae), kde testovali i odchyt na α pinen+etanol do lapačů typu Ecotrap. Lapače umístili do přirozených lesů, a do přístavů, letišť a zahradnictví (Fan et al. 2019). Čína má vytvořený Integrated Pest Management Plan, kde se monitoring provádí na vytýčených plochách různými metodami, od navnaděných lapačů s různými typy semiochemikálií přes světelné pasti až po obyčejnou pochůzku plochou (Anonymus 2009). Zároveň se provádí odchyt kůrovců v přístavech (Lin et al. 2021). Čína má rovněž vytvořenou normu pro kalamitního lýkohuba *P. aubei*, který zde působí vážné škody na cypřiších (Anonymus 2017). Tento lýkohub se vyskytuje jako invazní i v České republice (Fiala & Holuša 2019). V dnešní době, kdy se invazní kůrovci více rozšiřují po světě, objevují se snahy i o zavedení celosvětového monitoringu. Na několika kontinentech proběhly odchyty zároveň ve stejnou dobu za účelem zjištění početnosti kůrovců v dotčených regionech. V pastech byly použity tyto semiochemikálie: α pinen+etanol, α pinen+etanol+ipsdienol+ipsenol+Z-verbenol. Studie je prvním krokem k vývoji mezinárodního monitorovacího protokolu postaveném na odchytu do lapačů navnaděných různými druhy látek (Faccoli et al. 2020). V poslední době je moderní občanská věda, která může přinášet cenné a důležité údaje o výskytu živočichů (Cohn 2008). I při výzkumu invazních kůrovců mohou občané pomoci při monitoringu kůrovců ve svém okolí. Jako lapač lze použít obyčejnou PET lahev (Steininger et al. 2015; Pajek et al. 2020; Sanguansub et al. 2020; Tarno et al. 2021) a jako návnadu 40% vodku (Sweeney et al. 2016). Existují i matematické modely (MaxEnt), které lze použít pro predikci rozšíření invazních kůrovců na základě rozšíření druhu a ekologických podmínek (Örücü et al. 2019; Yu et al. 2019; Sarikaya & Sen 2020) nebo lze použít algoritmy strojového učení (umělé inteligence) k modelování šíření kůrovců (Koreň et al. 2021).

3.4 DNA analýzy a kůrovci

První kůrovci byli popsáni v 10. vydání Systema Naturae v roce 1758: *I. typographus*, *Pityophthorus micrographus* Linnaeus, 1758, *Polygraphus poligraphus* Linnaeus, 1758 a *T. piniperda*. Byli zařazeni do rodu *Dermestes* a čeledi Dermestidae (Linnaeus 1758). Počet popsaných druhů kůrovců se začal pozvolna zvyšovat od poloviny 19. století a svého vrcholu dosáhl v letech 1970-1979 (Hulcr et al. 2015). Tyto druhy byly popisovány na základě morfologie. Tím docházelo k opakovaným popisům stejného druhu, a různá jména musela být následně synonymizována (Hulcr et al. 2015; Jordal & Tischer 2020). Postupně, jak se technika zlepšovala, byli kůrovci popisováni na základě mikroskopické morfologie (Lanier 1970) a na přelomu 20. a 21. století přichází na řadu fylogenetická molekulární analýza (Hulcr et al. 2015).

Na základě studia DNA dochází k zpřesňování jednotlivých tribů a rodů (c.f. Cognato & Sperling 2000; Hulcr et al. 2007; Johnson et al. 2017). Pokud jednotlivé morfologicky popsané druhy kůrovců ukazují 10-12% odlišnost v mitochondriálně enkódovaném cytochromu c oxidase (COI), jsou jedinci považováni a následně popsáni jako nový druh (Cognato et al. 2020). U široce rozšířeného tribu Xyleborini (Cognato et al. 2011) se vyskytují vzhledově podobné druhy a na základě COI jsou popisovány nové druhy, které se pak řadí do skupiny dle základního druhu. Např. od druhu *Xyleborus glabratus* Eichhoff, 1877 byly na základě COI analýzy odděleny *Xyleborus insidiosus* Cognato & Smith, 2019 a *Xyleborus mysticulus* Cognato & Smith, 2019 a jsou řazeny do *X. glabratus* species group (Cognato et al. 2019). Genetické analýzy se používá i při rozlišení příbuzných tribů, tribus Premnobiini byl uznán za samostatný a byl oddělen od tribu

Xyleborini (Cognato 2013). Nebo jsou rody spojovány do jednoho (c.f. Johnson et al. 2020), případně jsou přesouvány do jiných tribů (c.f. Jordal 2021). Genetická analýza se používá i při zjišťování původu druhu vyskytujícího se na určitém území. Bylo zjištěno, že *X. germanus* byl do Evropy zavlečen pouze jedinkrát a evropská populace pochází z jediné lokality v Japonsku, kdežto do USA byl zavlečen ve více vlnách (Dzurenko et al. 2021). To samé bylo zjištěno u druhu *P. juglandis* v Itálii. Jediná introdukce tohoto invazního druhu způsobila rozšíření po celé jižní Itálii (Faccoli et al. 2016). Druh *P. chalcographus* je silně polyfágní, napadá různé jehličnany, i nepůvodní, ale přesto nevykazuje žádné genetické modifikace (Bertheau et al. 2012). Některé druhy kůrovců mají řadu synonym (Alonso-Zarazaga et al. 2023) a genetická analýza může pomoci k tomu, aby byly druhy správně synonymizovány či odhalena kryptická rozmanitost druhu (c.f. Kambestad et al. 2017).

3.5 Invazní kůrovci v České republice

Přestože nemáme mezinárodní mořské přístavy, dovážíme do České republiky v průměru okolo 5 tisíc tun tropického dříví ročně (ČSÚ 2023). I přes toto množství dovezeného dříví nebylo doposud v ČR zjištěno velké množství druhů invazních kůrovců. Zatím má v ČR stálou populaci pouze 6 druhů invazních kůrovců. Další uvedené druhy prozatím nejsou schopny v ČR mít stabilní životaschopnou populaci, především v důsledku klimatu a nedostupností hostitelských dřevin (Hamilton et al. 2019; Spennemann 2019a, 2019b). Pak jsou druhy v ČR, které se vyskytují vzácněji a u kterých faunistickými průzkumy lépe zpřesňujeme jejich oblast výskytu, *Hylastinus fankhauseri* Reitter, 1895, *Pityophthorus micrographus* Linnaeus, 1758, *Pityophthorus morosovi* Spessivtsev, 1926, *Pityophthorus traegardhi* Spessivtsev, 1921, *Pityophthorus balcanicus* Pfeffer, 1940, *Phloeotribus caucasicus* Reitter, 1891, *Trypodendron laeve* Eggers, 1939, *Scolytus koenigi* Schevyrew, 1890 nebo *Xyleborus pfeili* Ratzeburg, 1837 (Pfeffer & Knížek 1989; Knížek 2003, 2006; Müller et al. 2005; Lukášová et al. 2012; Carpaneto et al. 2015; Kašák et al. 2015; Knížek & Liška 2015; Januš 2016; Park et al. 2020; Wermelinger et al. 2020; Týr 2021).

3.5.1 Druhy se stabilními populacemi

Cyclorhipidion bodoanum Reitter, 1913

Ambrosiový kůrovec pocházející z východní Asie byl poprvé Evropě chycen v roce 1960 ve Francii (Schott 2004). V ČR byl poprvé odchycen do lapače s etanolem v roce 2020 v západních Čechách v Přírodní rezervaci Vladař (Fiala et al. 2021). Patří mezi kůrovce, který především preferuje listnaté stromy z čeledi Fagaceae, zvláště duby (McPherson et al. 2013), ale dokáže napadnout i borovice (Lightle et al. 2007). Obsazuje kmínky a větve o tloušťce větší než 10 cm (Brin et al. 2011). Kůrovec je přenašečem houby *Geosmithia morbida*, která způsobuje Thousand cankers disease na ořešácích (Moore et al. 2019) a kolekce hub, které způsobují Sudden oak death (McPherson et al. 2013).

Dryocoetes himalayensis Strohmeyer, 1908

Kůrovec pocházející z Indie byl poprvé zachycen v Evropě roce 1975 ve Francii. V ČR byl poprvé chycen v roce 2009 na jižní Moravě (Knížek 2011) a v Čechách v roce 2018 (Knížek & Kopecký 2021). V Evropě patří mezi škůdce ořešáku královského (*Juglans regia* L., 1753) a ořešáku černého (*Juglans nigra* L., 1753) a je řazen mezi jejich potencionální škůdce (Foit et al. 2017). Kůrovec byl na lokalitách zjištěn pomocí nárazových lapačů (Procházka et al. 2018), údaje o početnostech na lokalitách na Moravě byly zjištěny lapákovou metodou (Kašák et al. 2023). Je vyslovena neověřená hypotéza, že je i škůdcem dubů (Knížek 2011; Procházka et al. 2018). Napadá oslabené ořešáky slabších výčetních tlouštěk v bazální části do výšky 4 m se slabší kůrou (Foit et al. 2017; Kašák et al. 2023).

Gnathotrichus materiarius Fitch, 1858

Ambrosiový kůrovec pocházející ze Severní Ameriky byl v Evropě zjištěn poprvé v roce 1933 ve Francii (Balachowsky 1949). V ČR byl zjištěn v roce 2005 v západních Čechách (Knížek 2009). Patří mezi polyfágní druhy s vazbou na jehličnany (Kamp 1970). Často bývá chycen v lapačích navnaděných na kůrovce rodu *Ips* (Schneider 1985; Knížek 2009; Mazur et al. 2018). I přes téměř 90leté působení v evropských lesích, nejsou známy rozsáhlejší škody (Mazur et al. 2018).

Phloeosinus aubei Perris, 1855

Lýkohub, který se původně vyskytoval pouze v jižní Evropě, severní Africe, na Blízkém východě a v Číně (Sanyang 1992; Cobos 2011; Beaver et al. 2016). V ČR byl poprvé zjištěn v roce 1947 (Fiala & Holuša 2019). Na původních místech výskytu se jedná po vážného škůdce cypřišů (*Cupressus* spp.) (Cobos 2011; Anonymus 2017). Způsobuje chorobu „Cypress canker disease“ (Ponchet 1981; Danti & Rocca 2017). V ČR napadá hlavně oslabené jalovce obecné (*Juniperus communis* L., 1753). Jeho šíření na sever je pravděpodobně způsobeno změnou klimatu (Bozsik & Szöcs 2017; Fiala & Holuša 2019).

Xyleborinus attenuatus Blandford, 1894

Ambrosiový kůrovec pocházející z Dálného Východu. V Evropě poprvé zjištěn roku 1960 ve Španělsku (Lombardero 1998). V ČR poprvé zjištěn roku 1982 v jižních Čechách (Fiala & Holuša 2023a). Obsazuje hlavně měkké listnáče, ale lze ho chytit i v dubových porostech (Kvamme et al. 2020). Rozšířen je v celé Evropě (Fiala & Holuša 2023a). Tento kůrovec je sekundárním škůdcem, který napadá odumřelé nebo odumírající stromy (Borowski et al. 2012; Skrylnik et al. 2019), je však přenašečem houby *Ambrosiella* sp. (Nakashima et al. 1992).

Xylosandrus germanus Blandford, 1894

Ambrosiový kůrovec pocházející z východní a jihovýchodní Asie (Beaver & Liu 2010) byl v Evropě byl poprvé zjištěn v Německu v roce 1951 (Groschke 1952). V České republice byl zjištěn na hranicích Čech a Moravy v roce 2007 (Knížek 2009). Postupně se rozšířil po celé republice a největší početnosti dosahuje na jižní Moravě (Fiala et al. 2020). I přes výrazně vysoké počty chycených brouků na jižní Moravě nejsou zatím známy ekonomické škody v ČR (Fiala et al. 2020), i když v zahraničí již způsobil technické škody na dříví (Maksymov 1987; Galko et al. 2019). Kůrovec je přenašečem velkého množství hub, které mohou napadat a ohrožovat dřeviny (Kawasaki et al. 2010; Agnello et al. 2017; Ito & Kajimura 2017).

3.5.2 Druhy nevytvářející stabilní populaci

Coccotrypes dactyliperda Fabricius, 1801

První záznam o výskytu tohoto kůrovce v Evropě pochází z přelomu 18. a 19. století z Německa (Fabricius 1801). V ČR byl poprvé zaznamenán v roce 1824 v Praze (Opiz 1824) a v západních Čechách v polovině 19. století (Glückselig & Schoebl 1863). Patří mezi škůdce datlí a arekových ořechů (Spennemann 2019a). Stabilní populace se dokáže v Evropě udržet pouze v jižních státech, kde má dostatek hostitelských dřevin a příznivé teploty pro vývoj. Do ČR je zavlékán jen dovozem datlí a arekových ořechů (Fleischer 1927–1930, Pfeffer 1989). V Národním muzeu v Praze jsou uloženy doklady tohoto kůrovce z lokalit Praha a Brno (Fiala & Holuša observ.).

Hypothenemus areccae Hornung, 1842

Druh pochází z jihovýchodní Asie a patří mezi významné škůdce semen, ořechů, ovoce, čaje a konopí a rozmnožuje se i na sušeném ovoci a chilli papričkách nebo ve větvičkách (Wood 1977; Crosby et al. 1986; Madenjian 2020). Do ČR je zavlékán pouze dovozem kávových bobulí (Fleischer 1927–1930).

Hypothenemus hampei Ferrari, 1867

Tento kůrovec je endemitem Centrální Afriky (Vega et al. 2009, 2012). V současnosti je rozšířen po celém světě, kde se pěstují kávovníky. V těch způsobuje velké ekonomické škody (Vega et al. 2009, 2012), což je jeden z důvodů, proč je káva tak drahá (Hulcr 2019). V Evropě byl poprvé zaznamenán v roce 1867 ve Francii v bobulích kávovníku dovezených z Afriky nebo Saudské Arábie (Waterhouse & Norris 1989). Do ČR je pouze zavlékán dovozem kávových bobulí (Fleischer 1927–1930; Dirlbek & Dirlbek 1956; Fiala & Holuša 2023b).

Hypothenemus setosus Eichhoff, 1868

Kůrovec pochází z Afriky (Hagedorn 1912), kde je škůdcem kávových a kakaových bobů, ovoce, řapíků a větviček cekrópií (Kalshoven 1963; Wood 1982b). Je široce rozšířen v celé tropické oblasti světa (Bright & Torres 2006). Do ČR je pouze zavlékán dovozem kávových bobulí (Fleischer 1927–1930).

Xyleborus affinis Eichhoff, 1868

Ambrosiový kůrovec je původem ze Střední a Jižní Ameriky (Sobel et al. 2018). V Evropě byl poprvé zjištěn v roce 1950 v Belgii v tropickém dřevě dovezeném ze západní Afriky (Schedl 1962). V ČR jeho nález popisují Pfeffer & Knížek (1989) bez udání podrobnějších dat. Většina nálezů z Evropy pochází z importovaného dříví nebo odchytů u přístavů (Cola 1971, 1973; Merkl & Tusnádi 1992; Barnouin et al. 2020). V Rakousku byl odchycen ve volné přírodě v roce 2006 (Holzer 2007). Patří mezi silně polyfágní druhy (Wood & Bright 1992). Je přenašečem houbových chorob „Mango wilt“ a „Laurel wilt“ (Souza et al. 2013; Sobel et al. 2018).

Xyleborus volvulus Fabricius, 1794

Ambrosiový kůrovec pocházející z Jižní Ameriky (Wood 2007). V Evropě zjištěn poprvé v roce 1966 v Itálii (Cola 1971, 1973). V ČR jeho nález popisují Pfeffer & Knížek (1989) bez udání podrobnějších dat. Evropské nálezy jsou z importovaných dřevin z Afriky a Asie zachycených v přístavech (Cola 1971, 1973). Patří mezi polyfágní druhy (Silva et al. 2015). Je přenašečem houbových chorob „Mango wilt“ a „Laurel wilt“ (Menocal et al. 2017; Castrejón-Antonio et al. 2018).

Xylosandrus morigerus Blandford, 1894

Ambrosiový kůrovec pocházející z jihovýchodní Asie (Browne 1961; Hulcr & Cognato 2013). V Evropě byl poprvé zjištěn v roce 1894 v Itálii (Blandford 1894), následně pak roku 1897 ve Francii (Chobaut 1897), poté následovalo Rakousko a ČR (Reitter 1913), Nizozemí, Německo a Velká Británie (Schedl 1962). Dokladový materiál nálezu z Rakouska z Vídně je uložen v Národním muzeu v Praze (Fiala & Holuša observ.). Jedinec nalezený v ČR je nezvěstný. Kůrovec byl v Evropě nalezen ve sklenících pouze na orchideii *Dendrobium phalaenopsis* a po roce 1950s již nebyl v Evropě potvrzen (Schedl 1955). Orchideje jsou také jeho hlavní hostitelskou rostlinou na Nové Guiney (Hulcr & Cognato 2013). recetně se jedná především o škůdce kávovníku, kakaovníku a čajovníku (Browne 1961; Kalshoven 1961; Nakayama & Terra 1986).

4. Metodika

4.1 Stanovení rozšíření invazních druhů kůrovců na území České republiky

Faunistické údaje o invazních kůrovcích v České republice (*Cyclorhipidion bodoanum*, *Gnathotrichus materiarius*, *Phloeosinus aubei*, *Xyleborinus attenuatus* a *Xylosandrus germanus*) byly získány vlastním sběrem, vlastní determinací, excerpcí literatury a ze soukromých a muzejních sbírek. U druhu *P. aubei* bylo navíc využito mapování Natura 2000 pro stanovení vhodných lokalit pro zjišťování výskytu a jednalo se o přírodní biotopy T3.4A (Porosty s význačným výskytem vstavačovitých a s jalovcem obecným), T3.4B (Porosty bez význačného výskytu vstavačovitých a s jalovcem obecným), T8.1A (Porosty s jalovcem obecným suchých vřesovišť nížin a pahorkatin) a T8.2A (Porosty s jalovcem obecným sekundárních podhorských a horských vřesovišť). V roce 2018 bylo navštíveno 27 lokalit, každá lokalita byla kontrolována jednou na výskyt *P. aubei*. U druhů *X. attenuatus* a *G. materiarius* byly zjišťovány navíc detaily odchytu (návnada, datum, typ pasti, dřevina) (příloha 10.1-10.5).

4.2 Nalezení vhodné kombinace volatilních látek nebo feromonů k monitoringu invazních druhů kůrovců v České republice

Odchyt ambrosiových kůrovců pomocí průmyslově vyráběných návnad v dubových porostech (příloha 10.6)

Potencionální využití uměle vyráběných návnad pro odchyt kůrovců rodu *Trypodendron* pro ostatní druhy kůrovců žijících v dubových lesích bylo studováno v roce 2018. V roce 2018 bylo pět párů lapačů typu Theysohn rozmístěno v lesním porostu u obce Peruc (50.3549364N, 13.9686869E; 280-360 m n. m.); v lesích převládaly duby (*Quercus* spp.), ale vyskytoval se v nich také habr (*Carpinus betulus* L., 1753), dřín (*Cornus mas* L., 1753) a lípa (*Tilia cordata* Mill., 1768). Páry lapačů byly od sebe vzdáleny 20 m a jejich vzdálenost činila cca. 200 m od sebe. Pasti byly umístěny na malých mýtinách (<0,5 ha) ve vzdálenosti > 10 m od okraje lesa. Jedna past z každého páru byla navnaděna návnadou Trypowit® (Witasek GmbH, Rakousko) a druhá byla navnaděna návnadou Lineatin Kombi® (Witasek GmbH, Rakousko). Hlavními účinnými látkami v odparníku Trypowit® jsou alfa-pinen a lineatin, v návnadě Lineatin Kombi® to jsou lineatin, quaiacol, nonyl aldehyd, a 3-hydroxy-2-methyl-2-butanon (http://www.witasek.com/). Pasti byly kontrolovány každý týden od 20. března 2018 do 31. července 2018, a návnady byly po 8 týdnech vyměněny. Průměrný počet odchycených kůrovců jednotlivými druhy návnad byl porovnán pomocí Wilcoxonova párového testu v programu Statistica (verze 12.0.).

Úspěšnost různých druhů návnad na Phloeosinus aubei (příloha 10.7)

Experimenty byly provedeny v letech 2020 a 2021 na pěti vybraných lokalitách ve středních a západních Čechách, kde se vyskytovaly hostitelské dřeviny. Přítomnost *P. aubei* byla již dříve potvrzena na všech studovaných lokalitách (Fiala & Holuša 2019; Fiala observ.). Tři z lokalit tvořily otevřené lesy nebo opuštěné pastviny s jalovcem obecným (*Juniperus communis*) : Záborná Lhota (49,7679 N, 14,3120 E, rozloha 1,2 ha, 10 %. zastoupení jalovce, 400 m n. m.); přírodní památka Jalovce na Světovině (49.8774 N, 13.7380 E, 0,9 ha v ploše, 50% zastoupení jalovce, 405 m n. m.); a Přírodní památka Jalovcové stráně nad Vrbičkou (50.1878 N, 13.2772 E, 48 ha plochy, 30% zastoupení jalovce, 520 m n. m.). Zbylé dvě lokality se nacházely v obci Hudlice (Hudlice 1 a Hudlice 2, 49.9624 N, 13.9732 E a 49.9618 N, 13.9616 E, resp. 400 m n. m.), kde došlo k poškození tújí a cypřišů v zahradách obyvatel.

V roce 2020 jsme porovnávali (Experiment 1) atraktivitu a účinnost čtyř druhů návnad pro *P. aubei*. Návnady zahrnovaly α-pinen (jako kontrolu); terpen; směs olejů (cade oil, juniper berry oil a etanol) (vše Fytofarm s.r.o., Slovensko) a jalovcová větev s etanolem. Jako kontrolu jsme použili α-pinen, protože ten je základní sloučeninou vylučovanou jalovci (Foudil-Cherif et al. 2009) a předpokládali jsme, že *P. aubei* bude především přitahován α-pinenem. Návnady byly umístěny do polypropylenových zkumavek (průměr = 23 mm, výška = 49 mm, objem = 12,5 ml, hmotnost bez atraktantu = 3,5 až 4,0 g); každá zkumavka obsahovala 6 ml návnady. Použité větvičky jalovce byly 8-10 cm dlouhé a 3-5 cm široké a byly umístěny zároveň s etanolem UHR (Alpha Scents, Inc., USA). Větve byly odříznuty z jednoho jalovce bezprostředně před pokusem. Všechny čtyři návnady byly umístěny v lapačích na všech pěti lokalitách. Na každé lokalitě jsme umístili 20 černých pastí Ecotrap® se suchým odchytem (Fytofarm spol. s r.o., Slovensko; pět opakování pro každou návnadu); každá past měla záchytnou plochu 5550 cm^2. Pasti byly umístěny ve výšce 130 cm nad zemí pomocí tyčí, podél transektu ve směru východ-západ, s jedním odparníkem nebo větví na past a v 10 m odstupech od sebe.

V roce 2021 jsme provedli následný (Experiment 2) experiment na dvou lokalitách (Hudlice 1 a Jalovce na Světovině). Dvě linie s 10 lapači Ecotrap®. Pasti v rámci jedné linie byly od sebe vzdáleny 10 m a samotné linie byly rovněž vzdáleny 10 m od sebe. Do každé pasti jsme umístili návnadu Hostowit® (Witasek GmbH, Rakousko) a špalíčky z větví jalovce s etanolem. Pasti s návnadami byly rozmístěny koncem dubna a byly v obou pokusech kontrolovány každých 14 dní až do konce června. Nachytaní kůrovci byli zmraženi a následně druhově určeni autorem.

Data jsme analyzovali v programu R 4.2.1 (R Core Team 2022). Abychom zjistili faktory ovlivňující výsledky odchytu, provedli jsme následující kroky: sestavili řadu zobecněných lineárních smíšených modelů (GLMM) pomocí knihovny "lme4" (Bates et al. 2015), Kde lokalita byla zahrnuta jako náhodný efekt, zatímco návnady, datum odběru a jejich interakce byly považovány za fixní efekty. Významnost jednotlivých vysvětlujících proměnných v GLMM byla určena porovnáním modelů s využitím Akaikeho informačního kriterionu (AIC) s odpovídajícími zjednodušenými modely (s vyloučením ohniskové proměnné) nebo nulovým modelem). Problémy s konvergencí algoritmů byly vyřešeny jejich přeškálováním na 100 000 iterací. Tolerance pro určení dosažení konvergence byla v případě penalizovaného iterativně váženého součtu čtverců reziduí nastavena na 1×10^{-3}. K optimalizaci byl použit algoritmus 'nloptwrap' Zpočátku jsme analyzovali rozdíly ve výskytu *P. aubei* mezi čtyřmi typy návnad v experimentu I. V důsledku malého počtu chycených *P. aubei* (zřídka dva exempláře na jedno opakování), jsme se rozhodli zpracovávat údaje jako přítomnost/nepřítomnost, nikoliv jako početnost (podle binomického rozdělení, logistická vazba). Početnost byla považována za váhu, kde váha 1 byla určena pro pasti s 0 nebo 1 odchyceným broukem a váha 2 pro pasti se dvěma brouky. Při sestavování modelu jsme se zaměřili na údaje v prvních třech odběrech vzorků, protože ve čtvrtém termínu nebyly zachyceny žádné exempláře *P. aubei*. Zahrnutí čtvrtého data by zásadně nezměnilo výsledky, ale mohlo by ztížit konvergenci modelového algoritmu. Následně jsme provedli podobnou analýzu, abychom prozkoumali faktory ovlivňující celkovou početnost všech brouků. Pro tuto analýzu jsme využili GLMM s negativním binomickým rozdělením a logaritmickým rozdělením z knihovny "MASS" (Venables & Ripley 2002). Dále jsme použili následující metody, kterými jsme zkoumali druhovou bohatost (GLMM s Poissonovým rozdělením s logaritmickou vazbou). Byly sestaveny odpovídající modely a analyzovány i pro experiment II. Nakonec byla provedena vizualizace dat pomocí knihovny "sciplot" (Morales 2020). Abychom prozkoumali faktory ovlivňující složení společenstva, použili jsme permutační vícerozměrnou analýzu rozptylu s použitím vzdálenosti matic (PERMANOVA) s Bray-Curtisovou distanční maticí. Tato analýza byla provedena pomocí knihovny "vegan" (Oksanen et al. 2022) a použila permutační test s 999 permutacemi. Pro model jsme použili postupný výběr založený na AIC konstrukci modelu. Jako doplněk k PERMANOVA jsme provedli omezené ordinační analýzy: částečnou kanonickou korespondenční analýzu (p-CCA) v případě prvního experimentu a částečnou redundanční analýzu (p-RDA) v případě druhého experimentu. V těchto analýzách se lokalizace a datum odběru vzorků považovaly za kovariáty, přičemž návnada byla považována za vysvětlující proměnnou. Statistická významnost výsledků byla zjišťována pomocí Monte-Carlo permutačního testu s 999 permutacemi. Kromě toho jsme k identifikaci kůrovců, kteří vykazovali významné asociace s konkrétními návnadami.použili víceúrovňovou analýzu vzorů z balíčku "indicspecies" (De Caceres & Legendre 2009).

Využití etanolu a dalších návnad k monitoringu invazních ambrosiových kůrovců v malých populacích (příloha 10.8)

Pokusy byly provedeny na čtyřech lokalitách v západních Čechách v roce 2022. Ve studovaných oblastech byl zjištěn výskyt invazních ambrosiových druhů kůrovců *G. materiarius*, *Xylosandrus germanus*, *Cyclorhipidion bodoanum* a *Xyleborinus attenuatus*. Všechny lokality byly umístěny v jehličnatých lesích, a to Úbočí (50,0259°N, 12,5859°E, 750 m n. m.), Kladská (50,0116°N, 12,6746°E, 865 m n. m.), Žihle (50,0391°N, 13,3520°E, 510 m n. m.) a Kdyně (GPS 49,4024°N, 13,0995°E, 600 m n. m.).

Byla porovnávána atraktivita čtyř návnad k lákání *G. materiarius*: Cembräwit (jako kontrola, Witasek GmbH, Rakousko), α-pinen, etanol UHR (vše Synergy Semiochemicals Corp., USA) a Wood Stainers Lure (směs α-pinenu, etanolu a sulcatolu) (zkráceně WSL) (Alpha Scents, Inc., USA). Na každé lokalitě bylo umístěno 20 pastí Ecotrap® (4 návnady × 5 opakování) (Fytofarm spol. s r.o., Slovensko) rozmístěných v lineárním transektu v desetimetrových intervalech. Tento interval je dostatečný, protože atraktivita lapačů na kůrovce dosahuje jen několik metrů (Duelli et al. 1997). Lapače byly umístěny 130 cm nad zemí pomocí dřevěných kůlů v linii ve směru východ-západ, přičemž v každému lapači byla umístěna jedna návnada. V řadě pastí bylo náhodně rozmístěno pět opakování pro každý typ návnady na každé lokalitě.

Pasti s návnadami byly rozmístěny koncem dubna a kontrolovány každých 14 dní až do poloviny srpna. Nasbíraní brouci byli zmraženi a následně určeni prvním autorem podle Pfeffera (1989).

Data byla zpracována pomocí programu R 4.2.2 (R Core Team 2022). K analýze bohatosti a početnosti kůrovců byly použity negativní binomické smíšené modely s nadbytkem nul z knihovny NBZIMM (Yi 2020). Proměnné zahrnovaly početnost všech kůrovců, druhovou bohatost všech kůrovců, celkovou početnost invazních ambrosiových kůrovců (*C. bodoanum, G. materiarius, X. germanus* a *X. attenuatus*). Jako potenciální vysvětlující proměnné jsme použili typ návnady, den odchytu (dos), kvadratický polynom dos a interakci mezi dos a typem návnady, protože tato analýza se zaměřila na zkoumání vlivu jednotlivých nástrah v závislosti na části odchytu v sezóně. Model zahrnoval jako náhodné členy místo odběru vzorků, jednotlivé plochy vnořené do místa odběru a jednotlivé pasti vnořené do ploch. Tyto komponenty současně definovaly autokorelační strukturu jako "ar1" v modelu.

Pro druhou část analýzy byly použity zobecněné lineární smíšené modely s binomickým rozdělením z knihovny lme4 (Bates et al. 2015). Uvažovanou vysvětlující proměnnou byl typ návnady, zatímco náhodné členy zahrnovaly místo odběru a jednotlivé plochy vnořené do místa odběru. Proměnnými byly podíly jedinců invazních ambrosiových kůrovců ke všem ambrosiovým kůrovcům, kůrovci vázaní na jehličnaté stromy ke všem jedincům. Výsledné modely byly porovnány s příslušnými nulovými modely, aby bylo možné posoudit jejich vhodnost. Pro sledování případné autokorelace byl na reziduích modelu proveden odhad autokovarianční a autokorelační funkce, stejně jako křížové kovariance a korelace. Vzhledem k vzájemné závislosti podílů byly výsledné p-hodnoty upraveny pomocí FDR (False Discovery Rate) korekce. Potenciální problémy s konvergencí algoritmu byly řešeny nastavením maximálního počtu iterací pro model na 10^5, stanovením toleranční úrovně tolPwrss na 10^{-3} pro vyhlášení konvergence v penalizovaném iterativně váženém součtu čtverců reziduí a použitím Gauss-Hermiteova algoritmu. V modelu zkoumajícím poměr jedinců invazních ambrosiových kůrovců ke všem jedincům byla navíc z analýzy vyloučena lokalita odběru Kladská a α-pinen. Toto rozhodnutí bylo učiněno z důvodu absence zaznamenaných invazních ambrosiových kůrovců. V analýzách byla WSL zvolena jako kontrolní skupina vzhledem k nepřítomnosti invazních ambrosiových kůrovců na α-pinenu a jejich omezené početnosti na Cembräwitu.

Ke zjištění, zda má dos a návnada významný vliv na složení společenstev kůrovců, byla použita částečná kanonická korespondenční analýza (p-CCA) z programu Canoco 5.01 (ter Braak & Šmilauer 2012), přičemž jako kovariát byla zahrnuta místa odběru vzorků. Před analýzou byla vzácným druhům snížena váha a výsledky byly vyhodnoceny pomocí Monte-Carlo permutačního testu s 999 permutacemi. K určení indikačních druhů pro každou návnadu byla použita metoda IndVal, která zohledňuje četnost a relativní početnost brouků (Dufrêne & Legendre 1997). Následovala víceúrovňová analýza vzorů pomocí knihoven "indicspecies" (De Cáceres & Legendre 2009) a "labdsv" (Roberts 2019). Většina grafů byla vytvořena pomocí knihovny sciplot (Morales 2020).

4.3 Stanovení environmentálních proměnných, které ovlivňují abundanci a šíření invazních druhů kůrovců

Ambrosioví kůrovci upřednostňují zapojené porosty: Studie v dubových porostech střední Evropy (příloha 10.9)

Studie byla provedena v západních Čechách v nižších nadmořských výškách, kde se nalézají acidofilní dubové porosty. Bylo vybráno deset lokalit tak, aby polovina z nich reprezentovala přestárlé neobhospodařované dubové porosty a polovina mladší hospodářské dubové porosty. Zastoupení dubu na lokalitách bylo větší než 60 %, průměrné srážky se pohybovaly mezi 550-700 mm a průměrná roční teplota je 7,5°C. Na každé lokalitě byl umístěn jeden lapač typu Theysohn navnaděný etanolem. Lapače byly aktivní od začátku dubna do konce srpna roku 2020 a byly vybírány jednou za 14 dní.

Environmentální proměnné byly:

Typ lesa - použili jsme dva typy lesů podle způsobu hospodaření. Dospělé obhospodařované lesy byly dubové porosty s převahou dubu ve stáří 80 až 120 let. Všechny stromy v obhospodařovaném lese byly stejně staré a lesy byly velmi homogenní. Objem mrtvého dříví a odumřelých větví byl malý. Většina nejstarších stromů v neobhospodařovaných lesích byla starší než 120 let a lesy nebyly obhospodařovány posledních 70 let. Lesy v této kategorii byly blízké přirozeným lesům, které zůstaly v západních Čechách. Jediné známky lidských zásahů byla přítomnost několika roztroušených pařezů po předchozích výběrových těžbách. Na rozdíl od lesů hospodářských, neobhospodařované lesy zahrnovaly stromy všech věkových kategorií včetně plošek s mladými stromky. Objem mrtvého dříví a odumřelých větví byl vysoký. Neobhospodařovaný les v Kynžvartu byl upraven na park s travnatými plochami, ale více než 60 % plochy bylo pokryto stromy. Protože struktura tohoto porostu byla podobná starým porostům, zařadili jsme tento porost do neobhospodařovaných lesů. Naproti tomu les v Národní přírodní rezervaci Soos, ačkoli se nachází v chráněném území, byl zařazen do kategorie obhospodařovaných lesů, protože se jednalo o homogenní porost, který byl uměle vysazen.

Redukovaná plocha – Redukovaná plocha byla vypočtena jako celková plocha porostu vynásobená zakmeněním a procentuálním zastoupením dubů. Údaje byly získány z lesních hospodářských plánů.

Objem dubového dříví - Údaje o objemu dubového dříví (m^3/ha) byly získány z lesních hospodářských plánů.

Objem mrtvého dříví - Objem mrtvého dříví byl kvantifikován na každé lokalitě na pěti plochách o10 m^2. Byly změřeny průměry a délky odumřelých stromů a odumřelých větví.

Množství odumřelých dubových větví - Byly stanoveny počty velkých odumřelých větví na 10 dubech umístěných v linii procházející každou lokalitou; hodnoty byly následně vyjádřeny jako průměrný počet odumřelých větví na strom. Transekty byly umístěny v centrální části každé lokality a byly přibližně 50 m dlouhé. Odumřelé větve dubů zahrnovaly veškeré stojící a ležící odumřelé dřevo o průměru větší než 7 cm a s tvrdou konzistencí.

Zápoj - Zápoj na každé studijní ploše byl zjištěn fotografováním oblohy ze země přímo vzhůru. Obloha byla vyfotografována na deseti místech ve vzdálenostech mezi sebou 20 m. Fotografovaná plocha činila přibližně 200 m^2. Fotografie byly analyzovány na procentuální zastoupení bílé (obloha) a černé (koruny stromů) pomocí softwaru ImageJ (v.1.47). Procento plochy oblohy, které bylo na fotografiích černé, bylo považována za ekvivalentní procentuálnímu zapojení koruny.

Vzdálenost od hranice porostu - Vzdálenost každého lapače od nejbližší hranice porostu (nikoliv okraje lesa, porostem se rozumí homogenní lesní porost).

Vliv environmentálních proměnných na početnost ambrosiových kůrovců byl hodnocen pomocí algoritmu random forest s použitím podmíněných hodnot inferenčních stromů jako základních proměnných, které jsou k dispozici v balíčku „party“ (funkce cforest, 10 000 vygenerovaných stromů) v softwaru R 4.0.2 (The R Foundation, Vídeň, Rakousko). Tato metoda byla použita proto, že je vysoce efektivní pro vyhodnocování významu vysvětlujících faktorů, dokáže pracovat s různými typy proměnných a je robustní s ohledem na významnost vysvětlujících proměnných. Protože některé testované vysvětlující proměnné vykazovaly v našem souboru dat multikolinearitu, byl proveden podmíněný výpočet důležitosti (možnost conditional = TRUE). Kromě toho byl použit nestranný model random forest (možnost control = cforest_unbiased), protože testované prediktory byly zároveň kvantitativní i kategoriální proměnné. Závislá proměnná byla v modelech reprezentována počtem jedinců a druhů ambrosiových kůrovců v konkrétních vzorcích (vzorek = kůrovci chycení v jedné pasti během dvoutýdenního období). Statistická významnost ($\alpha = 0,05$) každé vysvětlující proměnné byla vyhodnocena pomocí algoritmu výběru atributů založeného na permutaci, který je uveden v balíčku Boruta. Nakonec byl zjištěn mezní vliv vybraných významných proměnných na počet odchycených jedinců a druhů ambrosiových kůrovců, byl zviditelněn pomocí částečné závislosti pomocí balíčku pdp (funkce partial následovaná plotPartial).

Ordinační analýzy vztahu mezi početností ambrosiových kůrovců (tj, druhovým složením jejich společenstev) a charakteristikami lesních porostů byly provedeny v programu Canoco 5 (Wageningen University & Research, Wageningen, Nizozemsko). Na základě předběžné analýzy dat byla použita analýza redundance (RDA). Údaje o početnosti druhů byly logaritmicky transformovány a centrovány podle druhů. Po globálním Monte-Carlo permutačním testu (10 000 permutací) (zahrnujícího všechny dostupné vysvětlující proměnné) se potvrdilo, že byla potvrzena celková významnost vztahu mezi reakčními a vysvětlujícími proměnnými (pseudo-F = 4,2, p = 0,001), byl proveden další výběr vysvětlujících proměnných k identifikaci environmentálních charakteristik lesních porostů, které jsou nejtěsněji spojeny s výskytem ambrosiových kůrovců a druhovou bohatostí.

Původní a invazní kůrovci ohrožují exotické jehličnany v lázeňských městech (příloha 10.10)

Experiment byl proveden v západních Čechách v lázeňském trojúhelníku Františkovy Lázně, Mariánské Lázně a Karlovy Vary, který je zařazen do UNESCO. Na 12 lokalitách byly prováděn sběr kůrovců v požercích na geograficky nepůvodních jehličnanech, lokality byly jak lesní, tak parkové.

Ordinační analýza pro vztah mezi relativní početností kůrovcem napadených stromů a proměnnými prostředí (popsané níže) byla provedena v programu Canoco 5. Závislá proměnná byla relativní četnost stromů napadených kůrovcem. Stromy napadené *Phloesinus* spp., které napadají pouze jalovce (*Juniperus*) a *Thuja* spp., nebyly do analýzy zahrnuty. Použili jsme semikvantitativní stupnici pro hodnocení počtu kůrovcem napadených nepůvodních jehličnanů v dané lokalitě. Stupnice byla založena na našem pozorování, že pravděpodobnost napadení stromu kůrovcem je vyšší, pokud je strom na lokalitě, kde je mnoho potenciálních hostitelských stromů, než když je na lokalitě pouze jeden strom. Pro daný druh kůrovce je stupnice od 1 do 3, přičemž 1 znamená, že byl napaden jeden strom, 2 znamená, že bylo napadeno 2-5 stromů a 3 znamená, že bylo napadeno > 6 stromů na dané lokalitě. Bylo použito následujících devět proměnných prostředí hodnoceny: typ lokality (lázeňský park nebo lázeňský les); rozloha lokality; vzdálenost od nejbližšího hospodářského lesa napadeného kůrovcem; procento potenciálních hostitelských stromů, které jsou nepůvodními jehličnany (*Abies*, *Picea*, *Pinus*, *Pseudotsuga* a *Tsuga*) na lokalitě; a procento potenciálních hostitelských stromů, které jsou původními jehličnany. Příspěvek prediktorů byl hodnocen pomocí kanonické korespondenční analýzy (CCA) s dopředným výběrem vysvětlujících proměnných.

Výskyt invazního kůrovce Phloeosinus aubei v jalovcových porostech v České republice (příloha 10.3)

Vztah mezi přítomností/nepřítomností *P. aubei* a zeměpisnou délkou byl analyzován pomocí logistického modelu (Quassiho-Newtonova metoda odhadu). K porovnání byly použity Mannovy-Whitneyho U-testy následujících proměnných mezi lokalitami s výskytem a bez výskytu *P. aubei*: nadmořská výška, rozloha lokality, vzdálenost od místa výskytu *P. aubei* od další nejbližší lokality *J. communis* a zastoupením jalovců. GLM analýzy (Poissonovo rozdělení, LN spojovací funkce) byly použity k určení vztahů mezi "dobou nálezu" a charakteristikami lokality uvedenými výše a managementem lokality (obhospodařovaná vs. neobhospodařovaná). Jak již bylo uvedeno dříve, doba nálezu sloužila jako náhradní měřítko *P. aubei* populační hustoty. Všechny testy byly provedeny pomocí softwaru Statistica 12.0 (StatSoft ČR, s.r.o.; Praha, Česká republika).

4.4 Metodika monitoringu invazních kůrovců

Návrh monitoringu invazních kůrovců v České republice je sofistikovaný kompilát výsledků předchozích studií (viz přílohy) a analýzy prováděných monitoringů na celém světě (Brockerhoff et al. 2006b, Bashford 2012, Rassati et al. 2016a, Rabaglia et al. 2019, Inward 2020, Lin et al. 2021, Thurston et al. 2022). Na základě těchto zjištěných údajů (jako jsou hraniční přechody, přirozené migrační trasy, mezinárodní letiště, botanické zahrady a dřevosklady) byly vybrány lokality a typ návnady pro monitoring v České republice (příloha 10.11).

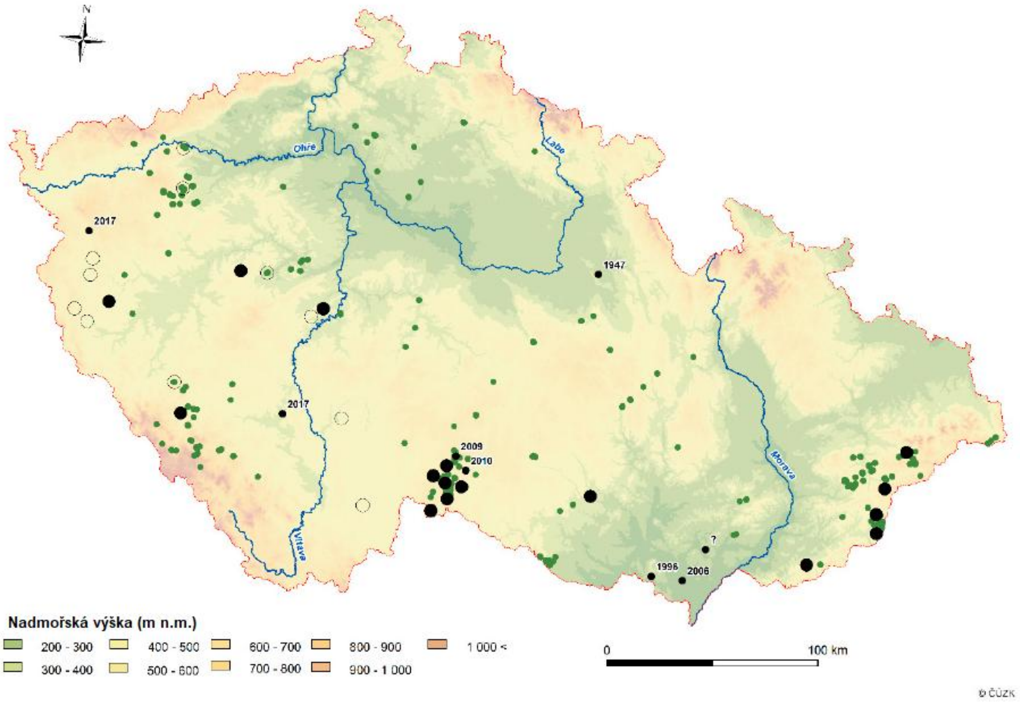
5. Výsledky

Výsledky byly publikovány v 11 vědeckých publikacích. Pro přehlednost jsou tyto výsledky sdruženy podle jednotlivých cílů a tyto cíle jsou i souhrnně diskutovány. Články jsou zahrnuty do příloh této disertační práce.

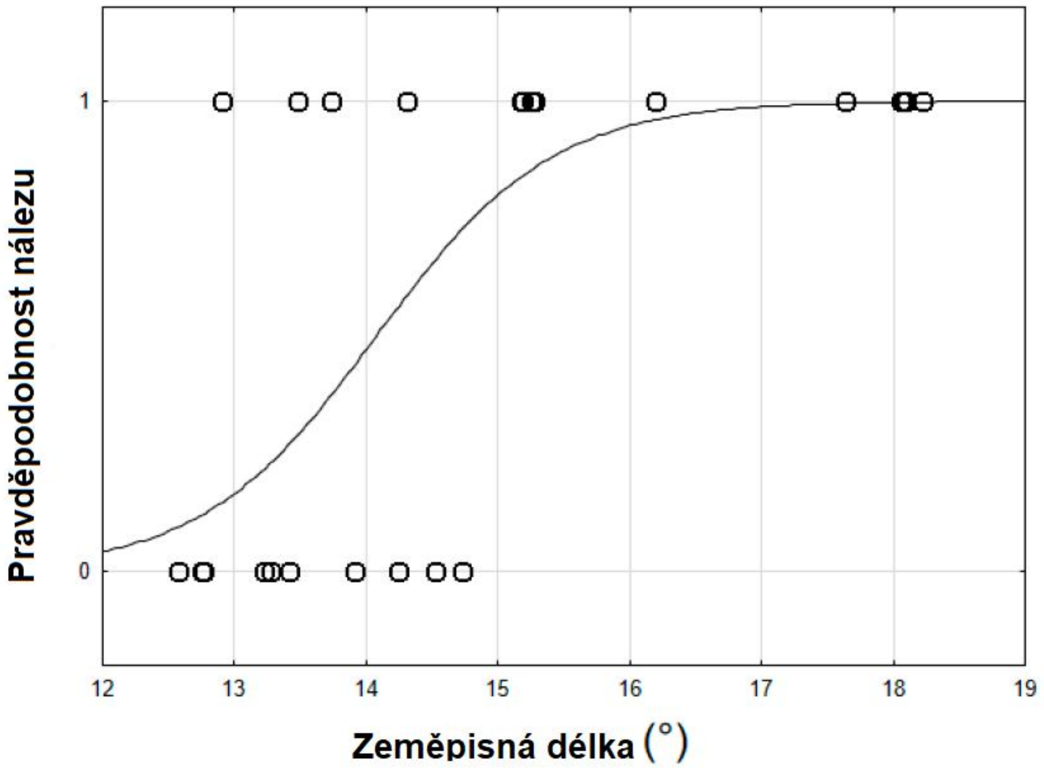
5.1 Stanovení rozšíření invazních druhů kůrovců na území České republiky

Ze 6 druhů invazních kůrovců na území České republiky bylo rozšíření v ČR stanoveno u 5 druhů. U druhu *Dryocoetes himalayensis* bylo rozšíření zpracováno jinými autory specializujícími se na tento druh (Procházka et al. 2018; Kašák et al. 2023). Pro účely disertační práce bylo tedy zpracováno rozšíření u *Phloeosinus aubei* (Fiala & Holuša 2019; příloha 10.3), *Xylosandrus germanus* (Fiala et al. 2020; příloha 10.5), *Gnathotrichus materiarius* (Fiala et al. in prep.; příloha 10.2) a *Xyleborinus attenuatus* (Fiala & Holuša 2023a; příloha10.4). Během výzkumu byl zjištěn i nový druh invazního kůrovce pro ČR, *Cyclorhipidion bodoanum* (Fiala et al. 2021; příloha 10.1).

P. aubei byl poprvé zjištěn v ČR v roce 1947 u Pardubic. Jeho výskyt je početnější ve směru na východě a klesá směrem na západu (Obr. 1, 2) (Fiala & Holuša 2019).

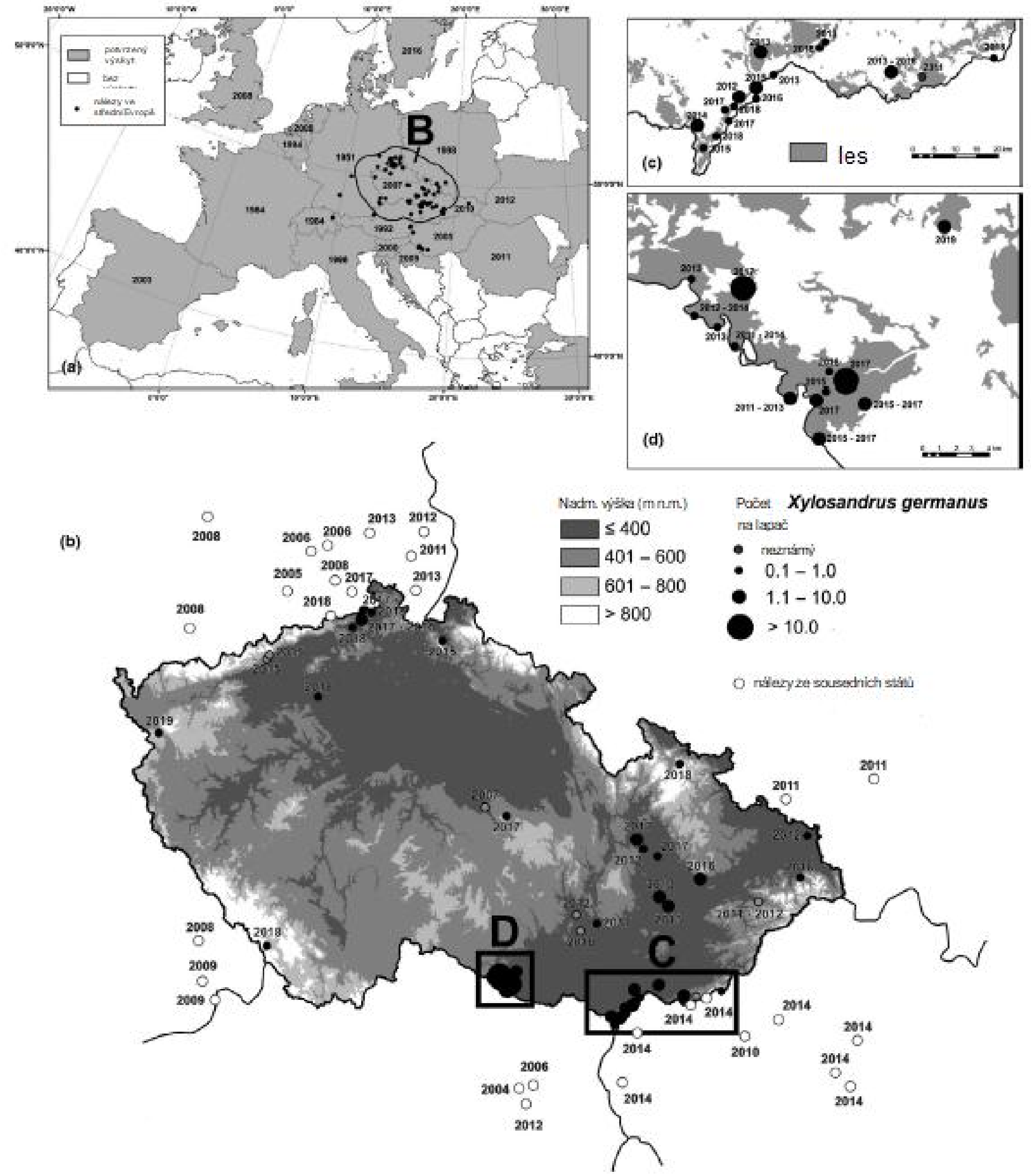


Obr. 1 Výskyt *Phloeosinus aubei* v České republice. Nálezy do roku 2017 jsou zobrazeny malými černými kroužky s rokem nálezu, nálezy v roce 2018 jsou zobrazeny velkými černými kroužky. Lokality bez potvrzení *P. aubei* v roce 2018 jsou zobrazeny prázdnými kroužky. Malé zelené kroužky jsou biotopy s jalovcem na základě mapování Natura 2000.

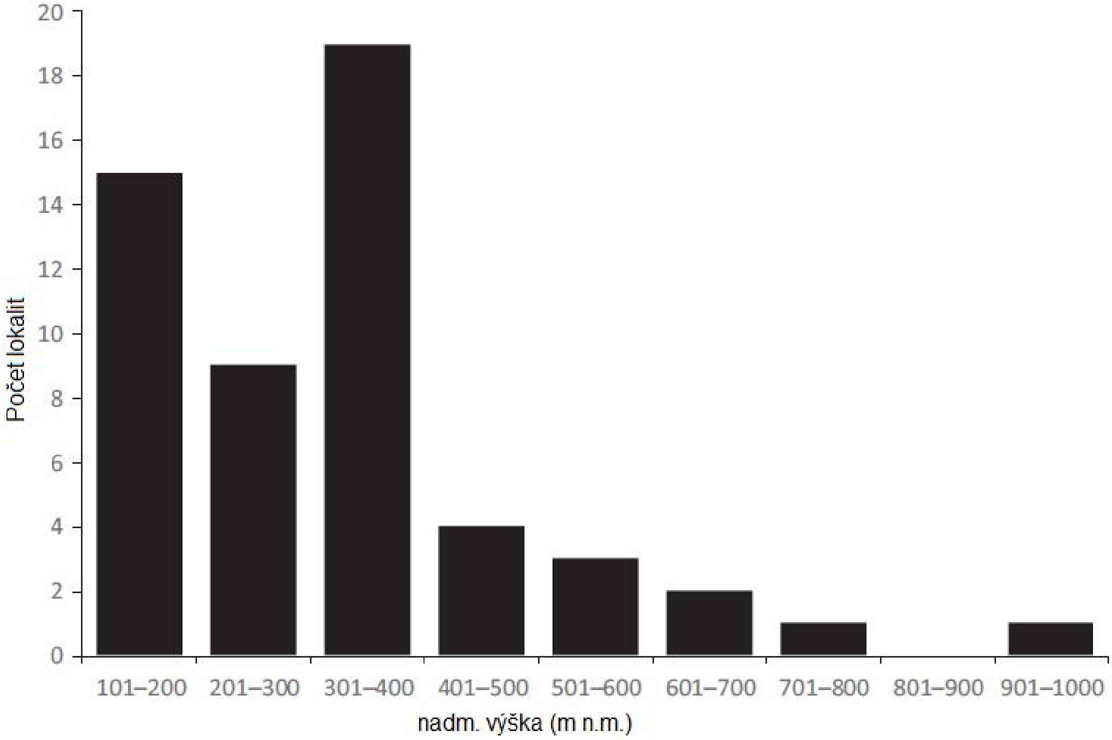


Obr. 2 Závislost výskytu *Phloeosinus aubei* v České republice na zeměpisné délce. Na ose *y* je 0 nepřítomnost a 1 přítomnost *P. aubei* na lokalitě.

Kůrovec *X. germanus* je rozšířen po celé republice, nejvíce na jižní Moravě. Na základě nálezů ze sousedních států je zřejmé, že se tento druh se do České republiky šíří ze severozápadního Německa, ze severního Rakouska a ze Slovenska (Obr. 3). Většina nálezů z ČR pochází z nadmořských výšek nižších než 400 m (Obr. 4) (Fiala et al. 2020).

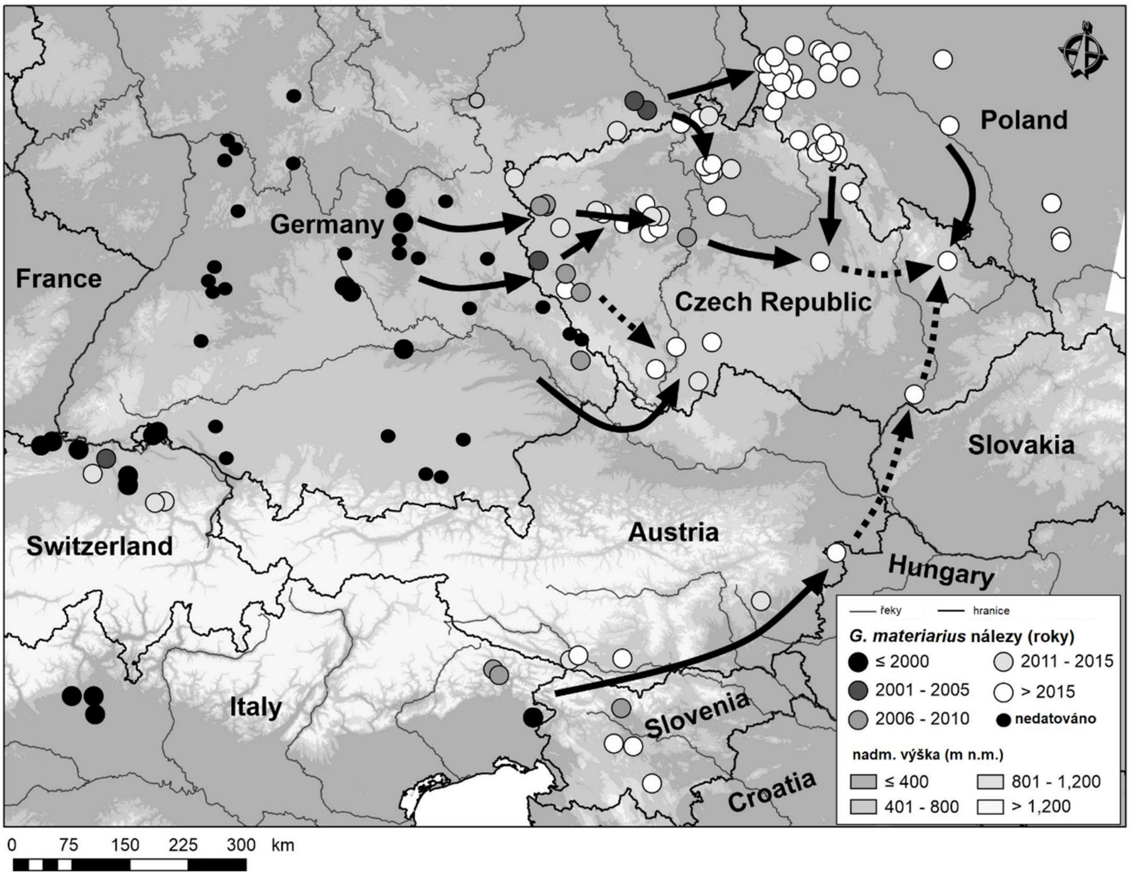


Obr. 3 Rozšíření *X. germanus* v ČR včetně 100 km bufferu okolo hranic ČR (a), nálezy s uvedením roku v ČR a v okolních státech (b), detail s nálezy podél hranic se Slovenskem (c), detail s nálezy v Národním parku Podyjí podél hranice s Rakouskem (d).



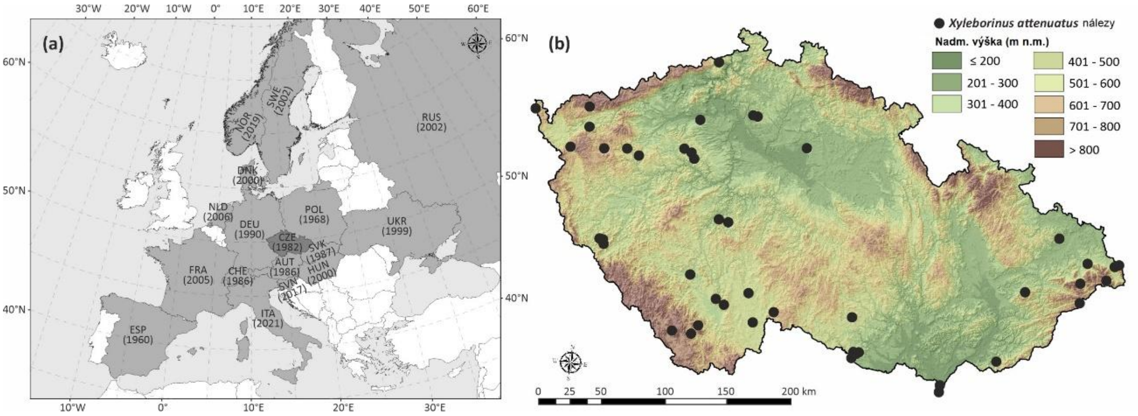
Obr. 4 Výšková distribuce *Xylosandrus germanus* v ČR v letech 2007 až 2019.

Kůrovec *G. materiarius* je rozšířen především na území Čech, na Moravě se vyskytuje sporadicky (Obr.5). Do Čech se rozšířil z Německa, na Moravu pravděpodobně pronikl přes Rakousko a Maďarsko z jihu a ze severu z Polska (Obr.5) (Fiala et al. in prep.)



Obr. 5 Rozšíření druhu *Gnathotrichus materiarius* v ČR, Rakousku, Německu, Maďarsku, severní Itálii, Polsku, Slovinsku a Švýcarsku se směry předpokládaného šíření (plné šipky jsou směry šíření, a čárkované šipky jsou předpokládané směry).

Kůrovec *X. attenuatus* byl poprvé zjištěn v ČR v roce 1982 v jižních Čechách. Ze získaných dat je vidět, že vyskytuje hlavně v Čechách. Je zřejmé, že území Moravy je obsazeno v menší míře (Obr. 6). Vzhledem k tomu, že determinace druhu je obtížná, protože tento druh je zaměnitelný s druhem *Xyleborinus saxesenii*, je pravděpodobnější, že se vyskytuje na Moravě ve stejné míře, jen druh není správně determinován (Fiala & Holuša 2023a).

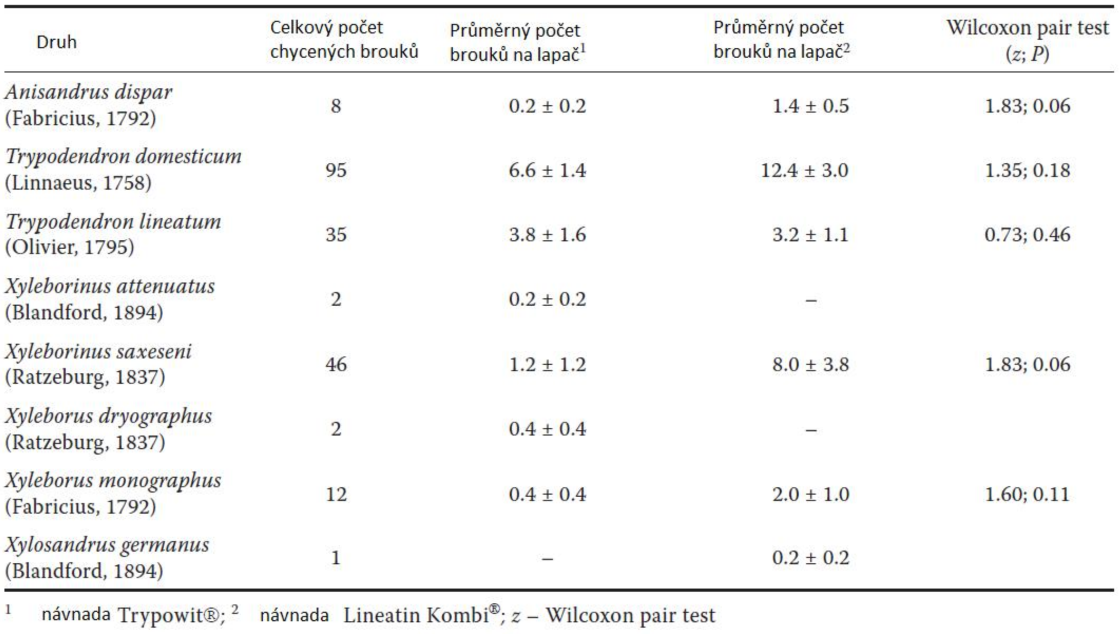


Obr. 6 Známé rozšíření *Xyleborinus attenuatus* v Evropě s rokem prvonálezu a lokality v ČR.

Při výzkumu byl v roce 2020 zjištěn nový druh kůrovce pro ČR, *Cyclorhipidion bodoanum* v PR Vladař v západních Čechách (Fiala et al. 2021). Je sekundárním škůdcem vázaný hlavně na duby (*Quercus* spp.). Během dalšího výzkumu bylo zjištěno jeho rozšíření na širším území západních Čech (Žihle, Kdyně a Zbiroh) (Fiala et al. 2023a, 2023b).

5.2 Nalezení vhodné kombinace volatilních látek nebo feromonů k monitoringu invazních druhů kůrovců v České republice

Odchyt ambrosiových kůrovců na uměle vyrobené návnady, Trypowit® a Lineatin Kombi®, byl testován v dubových porostech. Celkově se nachytalo nejvíce jedinců kůrovce *Trypodendron domesticum*, ale početnost všech chycených ambrosiových kůrovců byla vyšší v lapačích návnaděných Lineatin Kombi® (Tab. 1) (Fiala & Holuša 2020b; příloha 10.6).

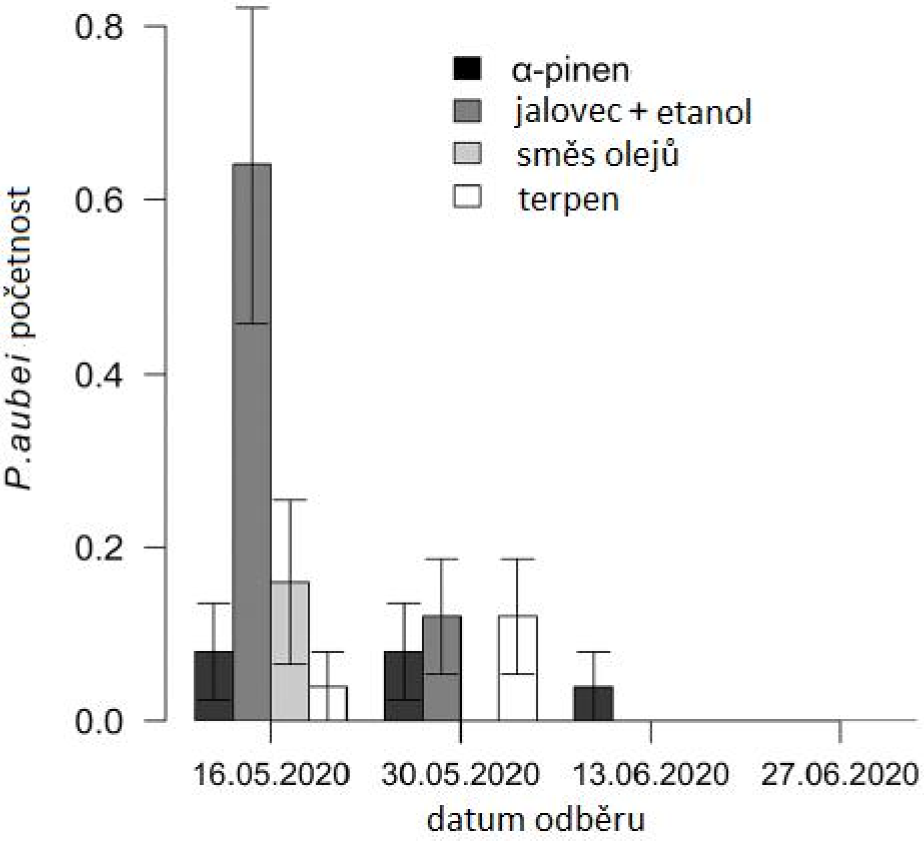


Druh	Celkový počet chycených brouků	Průměrný počet brouků na lapač[1]	Průměrný počet brouků na lapač[2]	Wilcoxon pair test (*z*; *P*)
Anisandrus dispar (Fabricius, 1792)	8	0.2 ± 0.2	1.4 ± 0.5	1.83; 0.06
Trypodendron domesticum (Linnaeus, 1758)	95	6.6 ± 1.4	12.4 ± 3.0	1.35; 0.18
Trypodendron lineatum (Olivier, 1795)	35	3.8 ± 1.6	3.2 ± 1.1	0.73; 0.46
Xyleborinus attenuatus (Blandford, 1894)	2	0.2 ± 0.2	–	
Xyleborinus saxeseni (Ratzeburg, 1837)	46	1.2 ± 1.2	8.0 ± 3.8	1.83; 0.06
Xyleborus dryographus (Ratzeburg, 1837)	2	0.4 ± 0.4	–	
Xyleborus monographus (Fabricius, 1792)	12	0.4 ± 0.4	2.0 ± 1.0	1.60; 0.11
Xylosandrus germanus (Blandford, 1894)	1	–	0.2 ± 0.2	

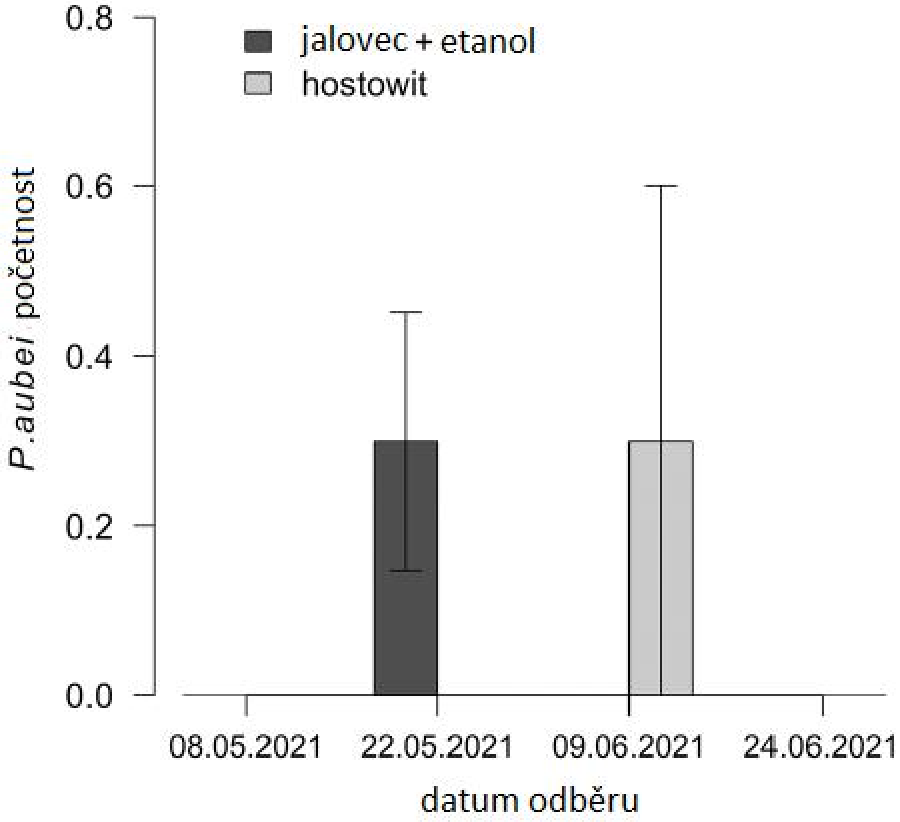
[1] návnada Trypowit®; [2] návnada Lineatin Kombi®; *z* – Wilcoxon pair test

Tab. 1 Celkový počet ambrosiových kůrovců chycených do lapačů Theysohn, průměrný počet chycených do lapače s návnadou Trypowit® nebo Lineatin Kombi® a statistické porovnání obou návnad.

Při dalším pokusu jsme testovali několik volatilních látek na odchyt kůrovce *P. aubei*. Bylo použito pět návnad: α pinen, terpen, směs cade oil, juniper berry oil a etanolu v poměru 1:1:1 a etanol s jalovcovým špalíčkem. V prvním experimentu byla afinita studována na pěti lokalitách. Na každé lokalitě bylo umístěno 20 lapačů typu Ecotrap, každá návnada měla pět opakování. Druhý experiment byl uskutečněn na dvou lokalitách. Na každé loaklitě bylo umístěno 10 lapačů typu Ecotrap a jako návnada byl použit Hostowit® a špalíček z větve jalovce s etanolem. Každá návnada měla pět opakování. I přes nižší počty chycených cílových jedinců nejúčinnější byla návnada etanol s jalovcovou větvičkou (Obr. 7). Atraktivita této návnady byla vyšší ve srovnání s odchyty na α pinen, ale výsledky byly srovnatelnější s odchyty na terpeny, Hostowit® a směsí olejů (Obr. 8) (Fiala et al. 2023a; příloha 10.7).

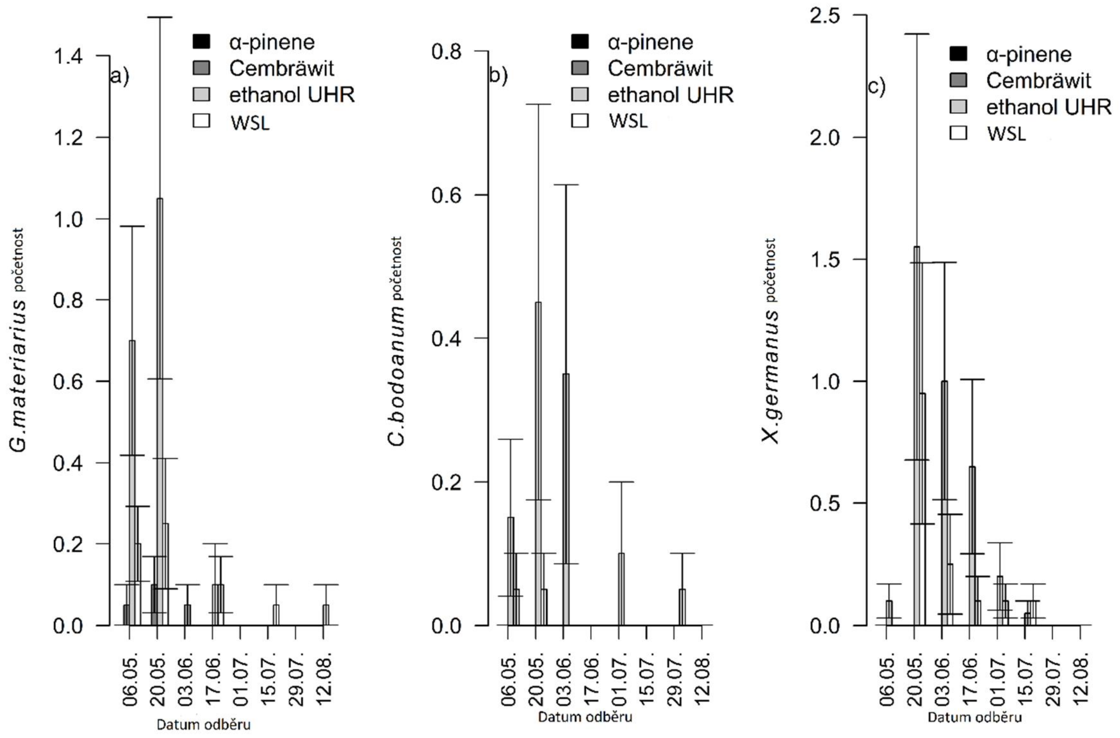


Obr. 7 Početnost *Phloeosinus aubei* podle data a návnad v Experimentu I



Obr. 8 Početnost *Phloeosinus aubei* podle data a návnad v Experimentu II

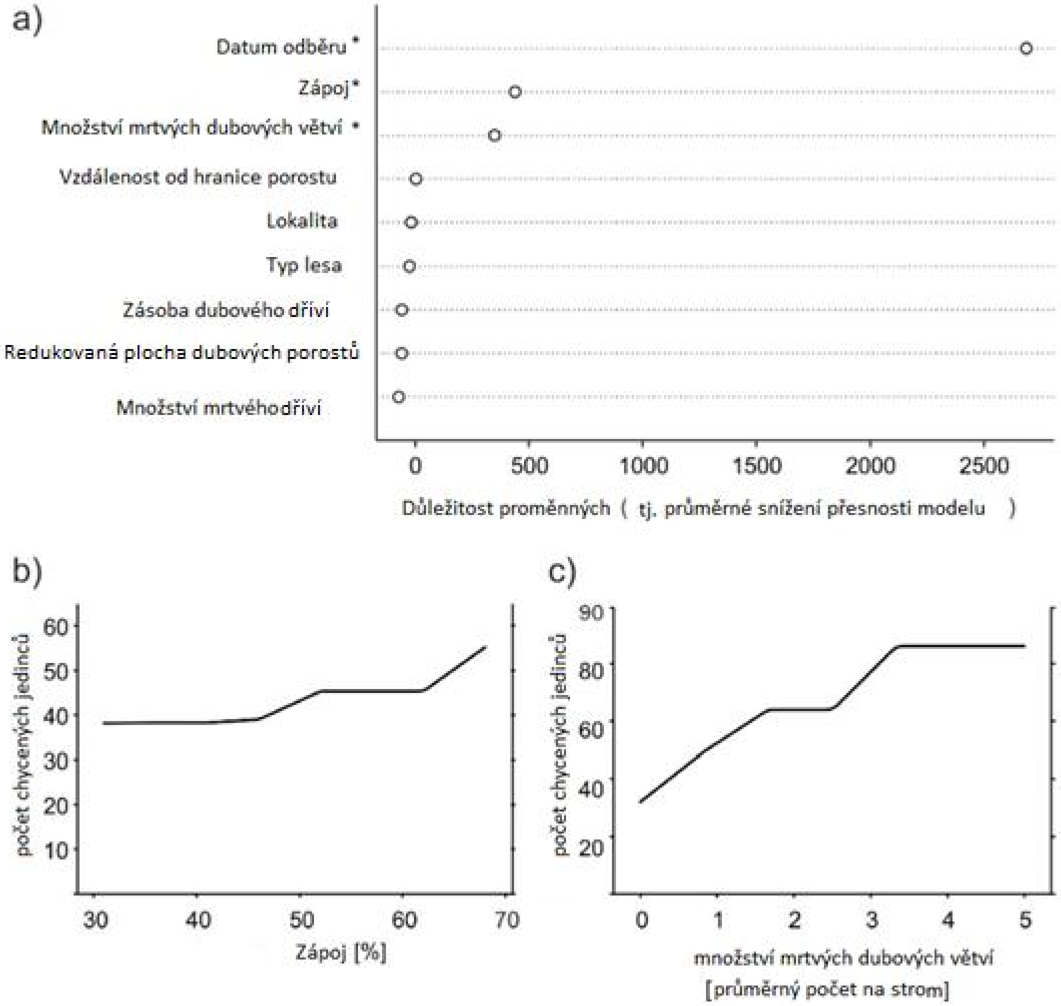
Cílem dalšího pokusu bylo zjistit, zda lze použít feromon sulcatol k monitoringu ambrosiového kůrovce *Gnathotrichus materiarius*. Na čtyřech lokalitách bylo použito 20 lapačů typu Ecotrap$^{®}$ a čtyři druhy návnad: Wood Stainers Lure (směs sulcatolu, α pinenu a etanolu v poměru 1:1:1; zkratka „WSL“), α pinen, etanol UHR a Cembräwit. Každá návnada měla na lokalitě pět opakování. Většina jedinců *G. materiarius* byla chycena na samotný etanol než na WSL, několik jich bylo chyceno na Cembräwit a žádný na α pinen. Při pokusu byly odchyceny další dva druhy invazních kůrovců (*C. bodoanum, X. germanus*), které signifikantně reagovaly na etanol (Obr. 9) (Fiala et al. 2023b; příloha 10.8).



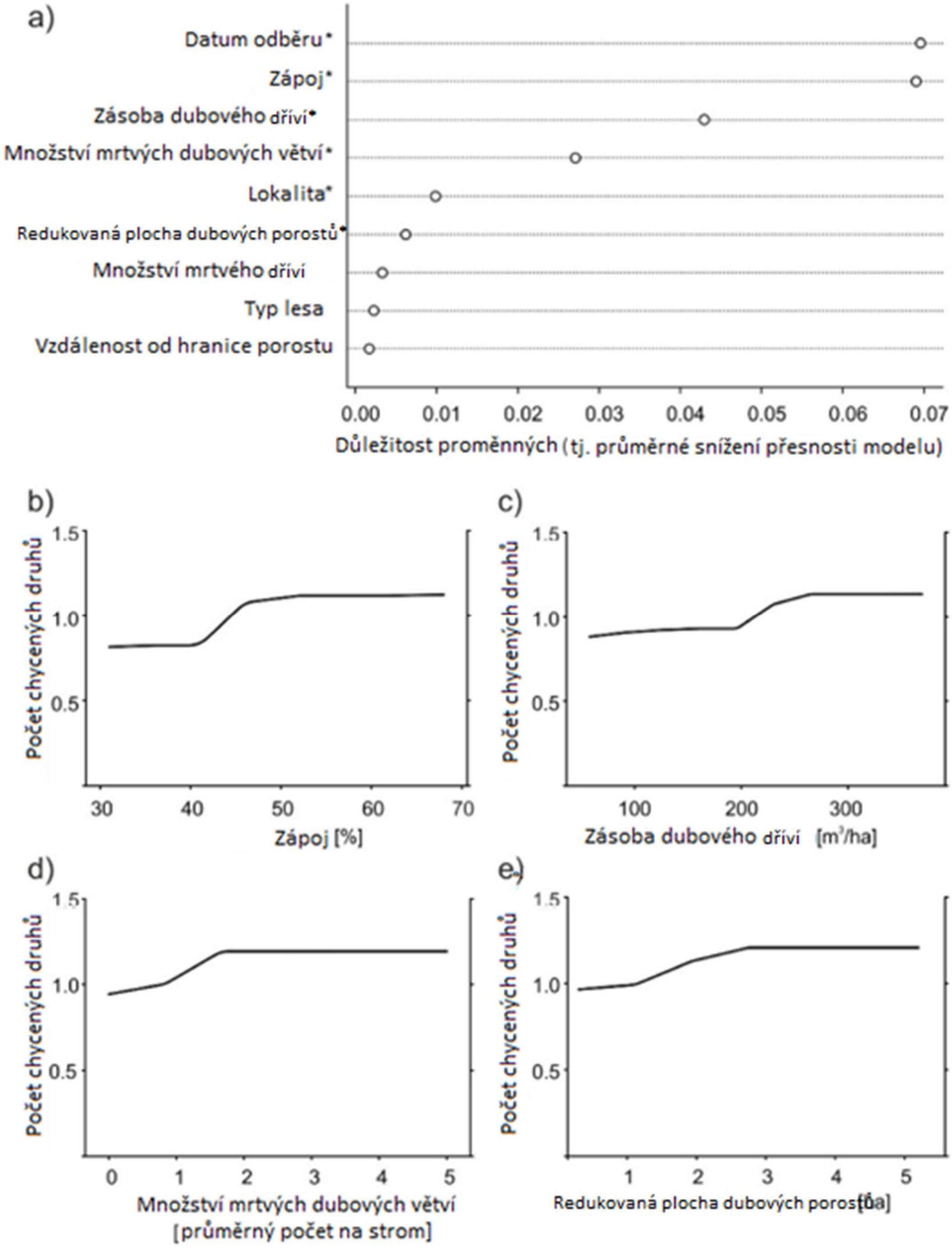
Obr. 9 Počet ambrosiových invazních kůrovců chycených do lapačů na různé druhy návnad, *Gnathotrichus materiarius* (a), *Cyclorhipidion bodoanum* (b) a *Xylosandrus germanus* (c).

5.3 Stanovení environmentálních proměnných, které ovlivňují abundanci a šíření invazních druhů kůrovců

Při prvním studiu byl na 10 lokalitách v dubových porostech použit lapač Theysohn navnazený etanolem ke zjištění ekologických proměnných vhodných pro šíření invazních kůrovců. Na každé lokalitě byly sledovány tyto proměnné: datum odběru, zápoj, množství mrtvých dubových větvích na stromech, vzdálenost od hranice porostu, lokalita, typ lesa (hospodářský a bezzásahový), zásoba dubového dříví, redukovaná plocha a množství mrtvého dříví. Jako signifikantní proměnné pro celkovou abundanci kůrovců byly zjištěny zápoj a množství mrtvých dubových větví na stromech (Obr. 10). Jako signifikantní proměnné pro druhovou početnost byly potvrzeny zápoj, zásoba dubového dříví na hektar, množství mrtvých dubových větví na strom a redukovaná plocha dubového porostu (Obr. 11) (Holuša et al. 2021; příloha 10.9).

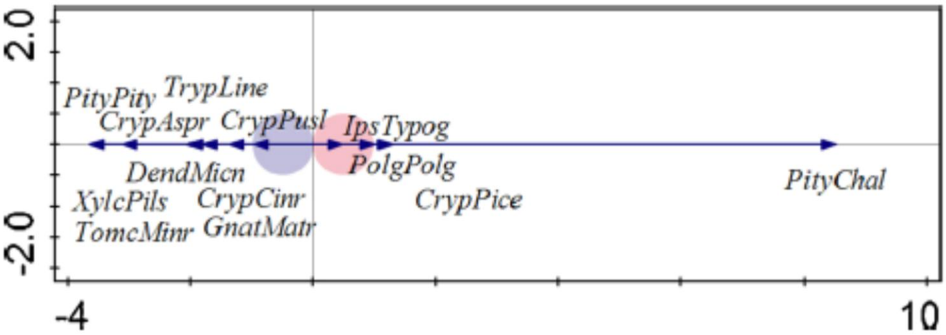


Obr. 10 Výsledky random forest regression závislosti počtu jedinců ambrosiových kůrovců na studovaných faktorech: (a) významnost proměnných. Proměnné s významným vlivem ($p < 0,05$) jsou označeny hvězdičkou (*); (b,c) závislosti počtu jedinců ambrosiových kůrovců chycených do pastí během roku na zápoji (b) a množství mrtvých dubových větvích (c).



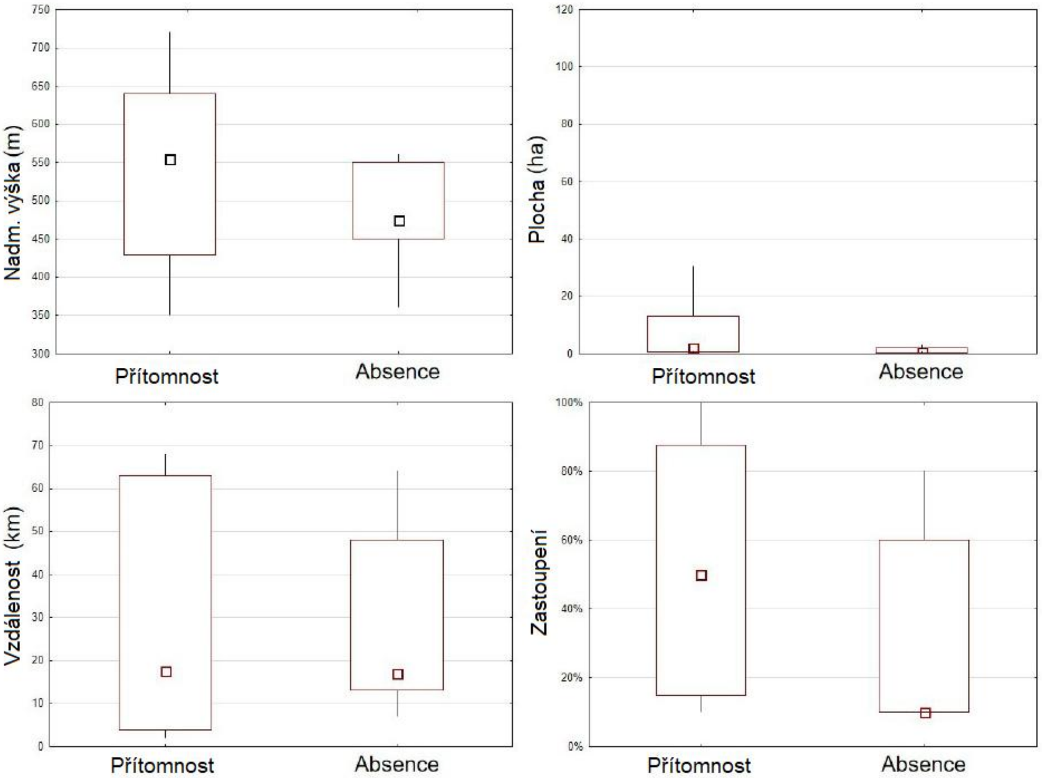
Obr. 11 Výsledky random forest regression průměrného počtu druhů ambrosiových kůrovců zachycených v jedné pasti na studovaných faktorech: a) významnosti proměnných. Proměnné s významným vlivem ($p < 0,05$) jsou označeny hvězdičkou (*); (b-e) závislost počtu ambrosiových kůrovců odchycených během osmi dvoutýdenních období od dubna do srpna na zápoji (b), zásobě dubového dříví (c), množství mrtvých dubových větví (d) a redukované ploše dubových porostů (e).

V dalším experimentu byl studován vliv geograficky nepůvodních dřevin v parcích a lesích v okolí lázeňského trojúhelníku (Františkovy Lázně, Mariánské Lázně a Karlovy Vary) na výskyt invazních kůrovců. Žádný vliv nebyl zjištěn (Obr. 12) (Fiala et al. 2022; příloha 10.10)



Obr. 12 Biplot T-hodnoty kanonické korespondenční analýzy s výsledky regresí početnosti kůrovců v parku na území lázeňského města (červený kruh) a v lese přiléhající k lázeňskému městu (modrý kruh). (*Cryphalus asperatus –CrypAspr; Cryphalus piceae –CrypPice; Crypturgus cinereus –CrypCinr; Crypturgus pusillus –CrypPusl; Dendroctonus micans –DendMicn; Gnathotrichus materiarius –GnatMatr; Ips typographus –IpsTypog; Pityogenes bistridentatus –PityBist; Pityogenes chalcographus –PityChal; Pityophthorus pityographus –PityPity; Polygraphus poligraphus –PolgPolg; Tomicus minor –TomcMinr; Trypodendron lineatum –TrypLine; Xylechinus pilosus –XylcPils*).

V posledním studiu bylo zjišťováno, jaké faktory ovlivňují výskyt a možnost detekce kůrovce *P. aubei* na lokalitách s výskytem jalovce obecného. Žádná použitá proměnná (nadmořská výška, plocha, vzdálenost lokality od další lokality s výskytem jalovce a zastoupení jalovce na ploše) nebyla signifikantní k detekci *P. aubei* na lokalitách (Obr. 13) (Fiala & Holuša 2019; příloha 10.3).



Obr. 13 Faktory lokalit s přítomností a absencí *Phloeosinus aubei*: nadmořská výška, rozloha, vzdáleností od nejbližšího území s jalovcem a zastoupení jalovce na ploše. Malé čtverce označují mediány, krabicové grafy označují 25 % a 75 % kvartily a čáry označují minimální a maximální hodnoty.

5.4 Metodika monitoringu invazních kůrovců

Na základě výše uvedených výsledků a tří dalších menších experimentů byl sestaven návrh metodiky monitoringu invazních kůrovců v ČR. Bylo vytipováno 24 lokalit po celé ČR, které zastupují hlavní zjištěné vstupní body invazních kůrovců, a to hraniční přechody, přírodní vstupní body (hlavně údolí řek), mezinárodní letiště, botanické zahrady a velké dřevosklady (Obr. 14, Tab. 2). Do každého z těchto bodů jsou navrženy tři lapače s návnadou etanol, která je univerzální pro odchyt invazních kůrovců. Lapače by měly být od sebe vzdáleny 30 až 50 m a kontrolován jednou za 14 dní od poloviny dubna do konce července. Etanol by měl být vyměněn na začátku června. Odebrané vzorky by měly být zmraženy a následně odeslány k determinaci odborníkům. Celkem by mělo být postaveno 74 lapačů a použito 144 etanolových návnad. Odhadované náklady na první rok i s nákupem lapačů se pohybují mezi 3200 až 6000 € dle typu lapače a na další roky to jsou pouze prakticky náklady na etanolové návnady ve výši cca 1500 €.

Takto koncipovanou monitorovací síť lze jednoduše zařadit do dlouhodobé monitorovací sítě na *Ips duplicatus* C.R. Sahlberg, 1836 (Fiala & Holuša 2023b; příloha 10.11).

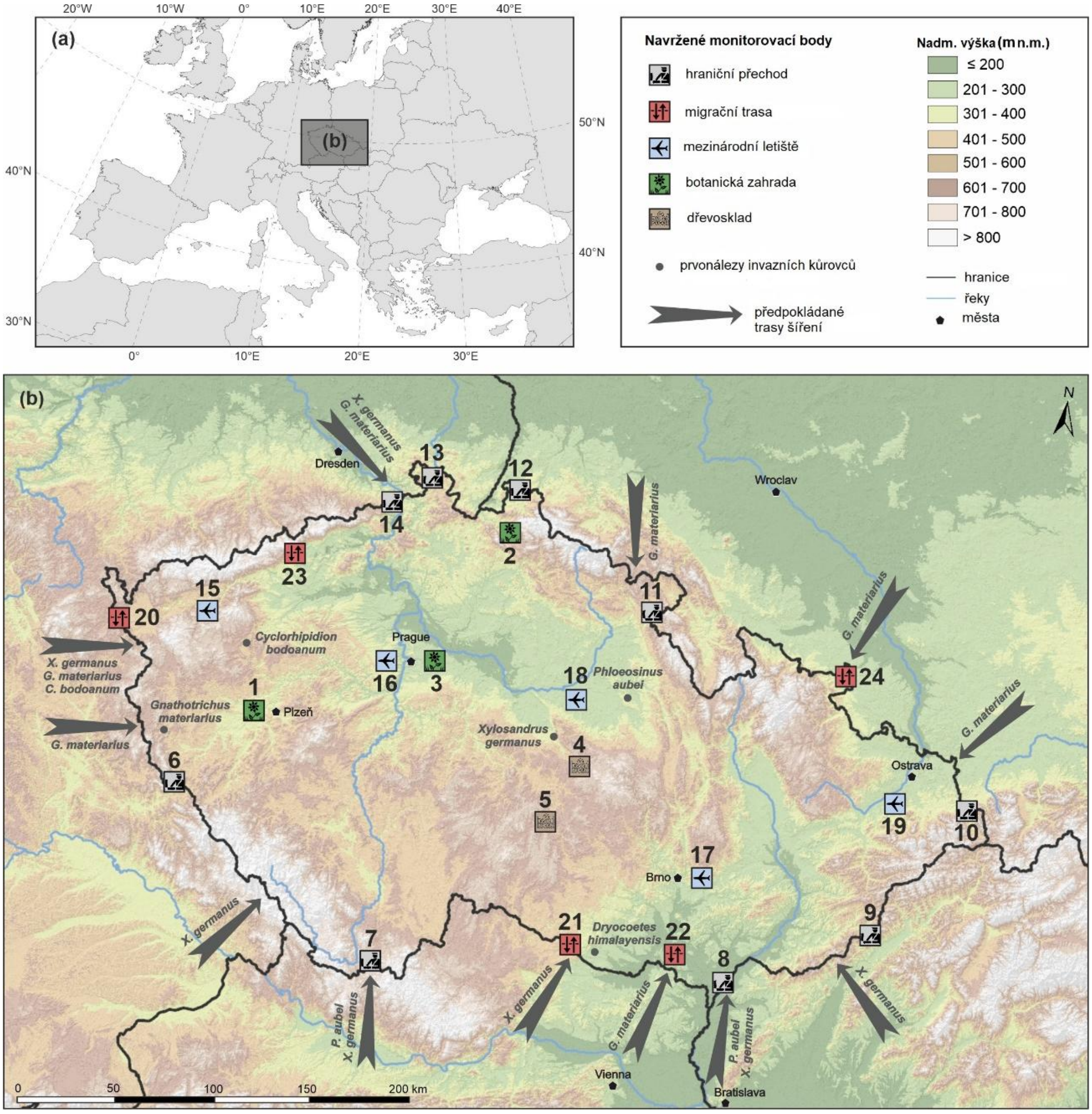


Fig. 14 Pozice České republiky v Evropě (a), možné vstupní brány, lokality prvonálezů a návrh monitorovacích bodů na invazní kůrovce v ČR (b).

Tab. 2 Návrh monitorovacích bodů invazních kůrovců

Č.	Monitorovací bod	GPS	Důvod výběru lokality
1	Zoologická a botanická zahrada Plzeň	49.7595 N, 13.3598E	Botanická zahrada
2	Botanická zahrada Liberec	50.7768 N, 15.0768E	Botanická zahrada
3	Pražská botanická zahrada	50.1224 N, 14.4138E	Botanická zahrada
4	Ždírec	49.7022 N, 15.8088E	Dřevosklad
5	Jihlava	49.4219 N, 15.6050E	Dřevosklad

Č.	Monitorovací bod	GPS	Důvod výběru lokality
6	Česká Kubice	49.3643 N, 12.8522E	Hraniční přechod
7	PP Horní Malše	48.6553 N, 14.4575E	Hraniční přechod
8	Tvrdonice	48.7504 N, 17.0210E	Hraniční přechod
9	PP Okrouhlá	49.0466 N, 18.0576E	Hraniční přechod
10	Třinec	49.6795 N, 18.6930E	Hraniční přechod
11	Hronov	50.4776 N, 16.2129E	Hraniční přechod
12	PR Meandry Smědé	50.9808 N, 15.0345E	Hraniční přechod
13	Velký Šenov	50.9960 N, 14.4053E	Hraniční přechod
14	Hřensko	50.8730 N, 14.2392E	Hraniční přechod
15	Karlovy Vary	50.1998 N, 12.9028E	Mezinárodní letiště
16	Praha Ruzyně	50.1244 N, 14.3054E	Mezinárodní letiště
17	Brno	49.1606 N, 16.6602E	Mezinárodní letiště
18	Pardubice	50.0203 N, 15.7153E	Mezinárodní letiště
19	Ostrava	49.6981 N, 18.1397E	Mezinárodní letiště
20	PR Rathsam	50.1013 N, 12.2485E	Předpokládaná migrační trasa
21	NP Podyjí	48.8495 N, 15.8835E	Předpokládaná migrační trasa
22	NPR Děvín	48.8587 N, 16.6511E	Předpokládaná migrační trasa
23	NPR Jezerka	50.5433 N, 13.4844E	Předpokládaná migrační trasa
24	PP Osoblažský výběžek	50.3032 N, 17.7005E	Předpokládaná migrační trasa

6. Diskuse

6.1 Stanovení rozšíření invazních druhů kůrovců na území České republiky

Kůrovec *P. aubei* je původem z jižní Evropy, severní Afriky, Blízkého Východu a Číny (Balachowsky 1949; Beaver et al. 2016). Jeho šíření směrem na sever je způsobeno změnou klimatu (Bozsik & Szöcs 2017) a globálním obchodem (Meurisse et al. 2019). Rozšíření *P. aubei* mimo změny klimatu a globálního obchodu napomáhá i poškozování hostitelských dřevin spárkatou zvěří, které stromy oslabuje a *P. aubei* tím získává lepší podmínky k osídlení nového území (Fiala & Holuša 2019).

Obecně hlavním důvodem pro šíření ambrosiových invazních kůrovců je globální obchod, protože ambrosioví kůrovci nejsou zdatní letci. Jejich dolet se pohybuje mezi 0,5 až 2 km za rok (Salom & McLean 1989; Grégoire et al. 2001). Proto trvalo několik desítek let, než invazní druhy kůrovců pronikly do ČR, *G. materiarius* přes 70 let, *C. bodoanum* přes 60 let a *X. germanus* přes 50 let (Knížek 2009; Fiala et al. 2021). *C. bodoanum* má prozatím jen omezené rozšíření v západních Čechách, protože pronikl do ČR teprve recentně (Fiala et al. 2021). Ostatní druhy ambrosiových kůrovců jsou rozšířeny po celé ČR (Fiala et al. 2020, in prep.; Fiala & Holuša 2023a). V plošném rozšíření není zřejmý vzorec, výsledky pravděpodobně svědčí jen o větší či menší míře prozkoumanosti jednotlivých oblastí a publikování těchto výsledků (Fiala & Holuša 2022). V případě invazního druhu *X. attenuatus* se na znalostech o rozšíření projevuje i chybná determinace a záměna s podobným druhem *Xyleborinus saxesenii* (Fiala & Holuša 2023a).

Invazní druhy do Čech pronikají hlavně z Německa a Rakouska, na Moravu z Čech, Rakouska, Polska a v případě *X. germanus* i ze Slovenska (Fiala & Holuša 2023b). Tyto různé směry jsou způsobeny centrální polohou ČR v Evropě, na rozdíl od západní Evropy, kde vstupní brány představují především námořní přístavy (Rassati et al. 2016a; Binazzi et al. 2019; Branco et al. 2019; Barnouin et al. 2020).

6.2 Nalezení vhodné kombinace volatilních látek nebo feromonů k monitoringu invazních druhů kůrovců v České republice

Atraktivita etanolu pro lákání ambrosiových kůrovců je dlouho známa (Kühnholz et al. 2001; Kelsey & Joseph 2003). Lineatin Kombi® obsahuje quaiacol, který zvyšuje afinitu k etanolu (Karlsson & Friedman 2017) a nejspíše proto bylo na tuto návnadu chyceno více ambrosiových kůrovců než na Trypowit®. I pro ostatní kůrovce, jako je třeba *Pityophthorus juglandis* nebo *Polygraphus proximus*, lze použít etanol jako návnadu, i když jejich odchyty jsou málo početné (Roling & Kearby 1975; EPPO 2014). Etanol může sloužit jako minoritní složka k feromonům, které jsou hlavním prostředkem k jejich odchytu. Role minoritních volatilních složek není pořádně prostudována (Kalinová et al. 2014). Přidání etanolu ke špalíčku z větve jalovce obecného pro *P. aubei* může být právě proto vhodnější než použití samotného etanolu nebo samotné větvičky. Špalíček však sesychá a emituje tak menší množství monoterpenů a i výše odchytu kůrovců se tím snižuje (Långström & Hellqvist 1983). Avšak přidání špalíčku k etanolu eliminuje větší spotřebu průmyslově vyráběných návnad jako je třeba Hostowit®, který nalákal stejné množství *P. aubei*.

Sulcatol je považován za možný feromon pro *G. materiarius* (Flechtmann & Berisford 2003). Ale odchyty byly vyšší na etanol než na sulcatol, který byl složkou návnady WSL. Tento výsledek může být vysvětlen jiným poměrem (S)-(+)- a (R)-(-)-sulcatolu v návnadě WSL než použili při pokusu Flechtmann & Berisford (2003). V USA taktéž na sulcatol nebyl chycen žádný *G. materiarius* (Miller & Crowe 2020). Etanol je celkově atraktivnější pro invazní ambrosiové kůrovce, kteří se v ČR vyskytují a zároveň i pro invazní kůrovce, kteří byli zatím zjištěny v Německu (*Xyloterinus politus* a *Cyclorhipidion pelliculosum* (Ranger et al. 2011, 2014).

6.3 Stanovení environmentálních proměnných, které ovlivňují abundanci a šíření invazních druhů kůrovců

V rámci řešení dizertační práce bylo zjištěno, že početnost ambrosiových kůrovců byla významně vyšší v lesích s větším zápojem korun a množstvím odumřelých dubových větví. Zvýšení těchto dvou proměnných také zvýšilo druhovou diverzitu ambrosiových kůrovců, což naznačuje, že procento a množství odumřelých dubových větví může ovlivňovat nejen druhy, ale i jejich početnost. To se týká početnosti *T. domesticum*, která je korelována s množstvím odumřelých dubových větví. Vysoké procento zápoje je známkou stabilního a vlhkého prostředí, které je vhodné pro růst ambrosiových hub (Renaud et al. 2011; Flerchinger et al. 2015). Protože ambrosioví kůrovci potřebují tyto houby jako zdroj potravy pro svůj vývoj, vyskytují se jak houby, tak brouci častěji ve vlhčích a teplejších lokalitách než na sušších a chladnějších lokalitách (Reich et al. 2014; Rassati et al. 2016c; Hulcr & Stelinski 2017). Kromě toho ambrosioví kůrovci obecně dávají přednost osídlování nižších částí korun stromů, takže většina kůrovců se chytá do pastí ve výšce 35-200 cm (Reding et al. 2010; Hanula et al. 2011; Brar et al. 2012; Menocal et al. 2018). Objem dubového dříví a redukovaná plocha, kterou duby zabírají, představují množství a rozmístění hostitelů ambrosiových kůrovců. Ambrosioví a další kůrovci jsou závislí na efemérních a zpravidla rozptýlených substrátech pro rozmnožování (Eidmann 1985; Forsse & Solbreck 1985), a proto je zřejmé, že se početnost jedinců a druhů ambrosiových kůrovců na lokalitě bude zvyšovat s tím, jak bude koncentrace hostitelských dřevin narůstat. Početnost kůrovců s dostupností zdrojů narůstá (Bussler et al. 2011; Morin et al. 2015). Jak bylo dříve zjištěno, početnost invazních ambrosiových brouků *X. attenuatus* a *C. bodoanum* pozitivně korelovala s plochou obsazenou duby, protože oba tyto druhy žijí na dubech a dalších listnatých stromech (Lassauce et al. 2012; Fiala & Holuša 2020b). Počet odchycených druhů ambrosiových brouků byl významně ovlivněn také velikostí plochy, což stále souvisí s dostupností zdrojů. Na druhou stranu vzdálenost od hranice porostu neměla žádný vliv ani na druhové spektrum ani na počet odchycených kůrovců, což svědčí o tom, že pro sledování početnosti druhů ambrosiových brouků postačí jedna past na studijní ploše. To je vysvětlitelné tím, že kůrovci byli lákáni do pastí návnadou, kterou byl v této studii etanol. Většina jedinců, kteří se vylíhli na daném místě, budou mít pravděpodobně tendenci vyvíjet se na stejném místě, ale někteří jedinci se rozptýlí a budou hledat nová místa s vhodnými zdroji (Forsse & Solbreck 1985; Byers 1999; Jones et al. 2019).

Naše hypotéza, že větší podíl původních jehličnanů v parcích lázeňských měst by podporoval větší počet napadených stromů, se nepotvrdila. Důvodem je to, že kůrovci nerozlišují mezi původními a nepůvodními druhy dřevin. Taktéž nebyl prokázán vliv geograficky nepůvodních dřevin na výskyt invazních druhů kůrovců, protože i výskyt *P. aubei* může být vázán na zastoupení jalovce obecného v zahradách obyvatelstva (Fiala & Holuša 2019). Nicméně vysoký podíl listnatých stromů, jako je bříza, topol, nebo jasan, vylučují těkavé látky, které mnoho kůrovců odpuzují (Byers et al. 1998; Zhang et al. 1999a). Protože je podíl listnatých stromů v parcích a městských lesů v lázeňských městech vysoký a početnost kůrovců byla nízká, listnaté stromy v těchto lokalitách mohly potenciálně chránit jehličnany.

V České republice se *P. aubei* pravděpodobně šíří směrem na západ, protože jeho početnost klesala od východu na západ. Všechny studované proměnné (nadmořská výška, rozloha lokality, vzdálenost od další lokality s nejbližším výskytem jalovce a zastoupení jalovce) měly velké rozmezí hodnot v lokalitách s výskytem *P. aubei* a bez něj. V souladu s tím nelze tyto proměnné použít k prognóze dalšího šíření a zdá se, že pouze přítomnost hostitelských stromů je pro ně důležitá. Silný vliv hostitele je pro kůrovce typický (Bussler et al. 2011). To, že *P. aubei* je schopen detekovat své hostitele, potvrzuje skutečnost, že "doba nalezení" nesouvisela s proměnnými hostitele (rozloha, vzdálenost od nejbližšího jalovce a hustota). Kůrovci rodu *Phloeosinus* mají dobré disperzní schopnosti a mohou se uletět více než 24 km (Furniss & Furniss 1972). Schopnost disperze a detekce hostitelů podporuje šíření invazních druhů v krajinné matrici (Chase et al. 2017). Vzhledem k roztříštěnosti a malým populacím jalovce je tok genů pravděpodobně omezený, což může mít vliv na vývoj populací jalovce obecného i tam, kde dochází k produkci životaschopných semen (Van der Merwe et al. 2000; Provan et al. 2008; Vanden-Broeck et al. 2011). Starší porosty *J. communis* v České republice jsou ve špatném stavu a na mnoha místech jsou zarostlé. Faktory, které oslabují stromy, zvyšují šíření *P. aubei* a *P. thujae*, ale přesné kvantifikace obou druhů je obtížná, protože jejich požerky jsou si velmi podobné. Klima a počasí mohou mít na stromy přímý vliv, protože sucho může stromy oslabit a předurčují je k napadení kůrovcem a patogeny. Klima může mít také přímý vliv na hmyz, protože se jedná o malé poikilotermy s omezenou schopností termoregulace (Huey et al. 1999). *P. aubei* je typickým případem tohoto modelu a mohou jej následovat i další druhy ze středomořských zemí. Změna klimatu může mít za následek i šíření hmyzích druhů z opačného směru. *Ips duplicatus*, původní kůrovec na smrku ztepilém ve Skandinávii, se v současné době šíří do střední, východní a jižní Evropy. Výskyt je podporován výsadbou smrku ztepilého na nevhodných lokalitách, která se projevuje zejména v důsledku fyziologickým oslabením stromů a následným výskytem houbových patogenů na hostitelských stromech (Holuša et al. 2010).

6.4 Metodika monitoringu invazních kůrovců

Někteří invazní kůrovci jsou polyfágní, např. *X. germanus* (Weber a McPherson 1983) a *X. politus* (MacLean a Giese 1967), a mohou napadat jak jehličnany, tak i listnaté stromy. Někteří napadají pouze listnaté stromy, např. *X. attenuatus* (Kvamme et al. 2020) nebo pouze jehličnany, např. *G. materiarius* (Kamp 1970). Zastoupení dřevin je pro monitoring invazních kůrovců nevýznamné, protože druh lesa nemá na početnost brouků žádný vliv (Bouget et al. 2008). Většina invazních kůrovců v Evropě jsou ambrosiové druhy (Alonso-Zarazaga et al. 2023) a v naší studii v dubových lesích v západních Čechách, jsme zjistili, že ambrosioví kůrovci měli vyšší početnost v lesích s větším zápojem korun, a následně vlhčím mikroklimatem a větším množstvím mrtvého dříví (Holuša et al. 2021). Vliv většího zápoje na početnost ambrosiových kůrovců potvrdili také Menocal et al. (2022). Zjistili jsme, že pro odchyt kůrovců *P. aubei*, je nejlepší kombinace etanolu a jalovcových větviček vhodnou (Fiala et al. 2023a). Etanol jsme shledali také jako nejvhodnější pro odchyt *G. materiarius* (Fiala et al. 2023b). Stejně tak *C. bodoanum* byl odchycen na etanol (Fiala et al. 2021) a přestože *D. himalayensis* a *X. germanus* byly odchyceny do nárazových pastí jako takových, byly chyceny také na etanol (Procházka et al. 2018; Hauptman et al. 2019; Fiala et al. 2020). *X. attenuatus*, jako další ambrosiový kůrovec je přitahován etanolem (Galko et al. 2014). Etanol přitahuje i invazní kůrovce *X. politus* a *C. pelliculosum*, kteří jsou již etablovány v Německu (Ranger et al. 2011, 2014).

Etanol má obecně lepší poměr záchytu invazních kůrovců než ostatní látky (Fiala et al. 2023b).

Navrhujeme celkem 24 monitorovacích míst. Většina z nich se nachází na hraničních přechodech a v údolích řek, které jsou pravděpodobně vstupními branami do České republiky (Obr. 14). Kromě toho jsou to dva velké dřevosklady, ve kterých probíhá mezinárodní obchod (Žemlička 2012), spolu se všemi mezinárodními letišti a tři botanické zahrady s tropickými skleníky. Jmenované lokality reprezentují různé způsoby šíření invazních kůrovců, tedy přirozené šíření letem a šíření prostřednictvím globálního obchodu (Tab. 2). Na každé lokalitě stačí nainstalovat tři lapače (Rassati et al. 2016a; Thurston et al. 2022). V České republice se používají dva typy lapačů; oba jsou levné a běžně dostupné. Snadno se instalují a nezachytí velké množství necílových hmyzu (Lubojacký & Holuša 2014; Galko et al. 2016). Lapače mohou být štěrbinové typu Theysohn, které jsou nejčastěji používané v lesnictví v České republice (Zahradník a Zahradníková 2016) nebo nárazové pasti typu Ecotrap, ze kterých je snazší extrahovat odchycené brouky. Po každé sezóně je lze rozebrat a uskladnit v mnohem menším prostoru než pasti typu Theysohn. Lapače by měly být umístěny v rozmezí 30 až 50 m (Niemeyer 1997; Rassati et al. 2014). Lapače by měly být aktivní od poloviny dubna do konce července, protože letová aktivita kůrovců klesá v srpnu (Fiala et al. 2023b). Pasti se kontrolují jednou za 14 dní a odebrané vzorky se poté uloží do mrazáku pro následnou determinaci. Etanol by měl být vyměněn na začátku června, protože odparníky jsou aktivní přibližně 60 dní.

7 Závěr a doporučení pro praxi

V České republice je šest druhů invazních kůrovců již trvale usídleno, z nich pouze *P. aubei* působí škody v okrasné městské zeleni; ostatní nemají prozatím větší hospodářský význam nebo způsobují minoritní technické škody na dříví. Čtyři druhy však patří mezi ambrosiové kůrovce, kteří přenášejí různé druhy hub. Tyto houby mohou způsobovat větší problémy v lesním hospodářství.

Proto je včasné zjištění invazního druhu kůrovce zásadní podmínkou integrované ochrany lesa. Bez znalostí potencionálního nebezpečí nově objevených kůrovců pro lesní ekosystémy, urbánní zeleň, biodiverzitu a ekonomické škody nemůžeme vytvářet postupy integrované ochrany lesa tak, aby byly účinné, ekonomicky únosné a s nejnižšími vedlejšími efekty.

V proběhlých terénních experimentech bylo zjištěno, že na možný výskyt invazních kůrovců má významný vliv vyšší zapoj korun a množství mrtvého dříví v korunách stromů. Z toho vyplývá, že invazní kůrovce bychom měli monitorovat v zapojených listnatých nebo smíšených porostech. Nejvhodnější jsou tedy maloplošně zvláště chráněná území, kde je větší pravděpodobnost dostatku mrtvé dřevní hmoty a větší druhová diverzita dřevin.

Při dalších experimentech bylo zjištěno, že nejdůležitější volatilní látkou schopnou zachytit více druhů a vyšší počet jedinců invazních kůrovců je etanol. Odchyty byly větší jak při experimentu u *P. aubei*, tak u *G. materiarius*. Na posledně jmenovaný druh neúčinkuje potencionální agregační feromon sulcatol. I odchyty na průmyslově vyráběné odparníky byly vyšší u odparníku Lineatin Kombi®, který obsahuje quaiacol. Etanol proto můžeme klasifikovat jako univerzální atraktant pro monitoring invazních druhů kůrovců v ČR.

Námi navrhovaná metodika monitoringu invazních druhů kůrovců je robustní metoda, která přináší jednoduchost, efektivitu a nízkonákladovost do iniciačních stádií integrované ochrany lesa. Metoda se dá zakomponovat do sítě lapačů na *I. duplicatus*, který provozuje Výzkumný ústav lesního hospodářství a myslivosti, případně se dají aplikovat do sítě lapačů, které instalují Lesy České republiky, s.p. Metodika monitoringu se dá v případě možnosti rozšířit o další lapače, pokud by byla nutnost monitorovat i jiné druhy kůrovců, pro které není úplně vhodný etanol, např. *P. proximus* nebo *P. juglandis*. Metodika není náročná na čas při kontrolách, ale nějaký čas by trvala determinace. Odhadem by bylo na determinaci odesláno 500 sběrů. Tato doba by se snížila, kdyby se zvětšil počet determinátorů mezi které by se odběry rozdělili. V ČR zatím není dostatek zkušených entomologů, kteří by se věnovali Scolytinae. Přitom podčeleď Scolytinae je nejvýznamnější hmyzí skupina, která způsobuje největší škody v lesním hospodářství.

8 Appendix

Tab. A1 Přehled známých semiochemikálií evropských druhů kůrovců. * - Invazní druh v Evropě. Tučně jsou vyznačeny agregační feromony.

Druh	**Semiochemikálie**	**Zdroj**
*Ambrosiophilus atratus**	etanol	Oliver & Mannion 2001
Anisandrus dispar	etanol	Montgomery & Wargo 1983
	α pinen	Schroeder & Lindelöw 1989
*Anisandrus maiche**	3-hydroxyhexan-2-one+etanol	Sweeney et al. 2016
Cryphalus piceae	**E-conophthorin**	Kohnle et al. 1992
Crypturgus cinereus	ipsenol	Terren & De Simon 1983
	ipsdienol	
	S-fuscumol	Sweeney et al. 2016
	1S-α pinen	
	1R-α pinen	
	1S-β pinen	
	S-3-caren	
	R-limonen	
	α terpinolen	
	etanol	
Crypturgus hispidulus	etanol	Sweeney et al. 2016
	(3R)-3-hydroxyoctan-2-one	
	(3S)-3-hydroxyoctan-2-one	
Crypturgus subcribrosus	S-fuscumol	Sweeney et al. 2016
	1S-α pinen	
	1R-α pinen	
	1S-β pinen	
	S-3-caren	
	R-limonen	
	α terpinolen	
	etanol	
*Cyclorhipidion bodoanum**	etanol	Oliver & Mannion 2001
	α copaen	Kendra et al. 2011
	(3R)-3-hydroxyoctan-2-one	Sweeney et al. 2016
	(3S)-3-hydroxyoctan-2-one	
*Cyclorhipidion pelliculosum**	etanol	Oliver & Mannion 2001
	etanol+conophthorin	Miller et al. 2015
Dendroctonus micans	5S7S-conophthorin	Zhang et al. 2002
	E-conophthorin	
	ipsdienol	Andersson 2012
	exo-brevicomin	
Dryocoetes autographus	α pinen	Rudinsky 1966
	β pinen	

Druh	**Semiochemikálie**	**Zdroj**
	camphen	
	***exo*-brevicomin**	Kohnle 1985
	***endo*-brevicomin**	
	etanol	Lindelöw et al. 1993
	turpentin	
Dryocoetes hectographus	(3R)-3-hydroxyoctan-2-one	Sweeney et al. 2016
	(3S)-3-hydroxyoctan-2-one	
	etanol	
Dryocoetes villosus	etanol	Markalas & Kalapanida 1997
*Gnathotrichus materiarius**	ipsdienol	Valkama et al. 1997
	metylbutenol	
	cis-verbenol	
	α pinen	Erbilgin et al. 2003
	lanieron	
	sulcatol	Flechtmann & Berisford 2003
	etanol+conophthorin	Miller et al. 2015
Hylastes attenuatus	ipsenol	Terren & De Simon 1983
	ipsdienol	
	etanol+α pinen	Francardi et al. 2006
Hylastes brunneus	α pinen	Schroeder & Lindelöw 1989
	etanol	
	turpentin	Lindelöw et al. 1993
	S-fuscumol	Sweeney et al. 2016
	1S-β pinen	
	S-3-caren	
	R-limonen	
	α terpinolen	
Hylastes cunicularius	α pinen	Schroeder & Lindelöw 1989
	etanol	
	turpentin	Lindelöw et al. 1993
Hylastes opacus	etanol	Schroeder & Lindelöw 1989
	turpentin	Lindelöw et al. 1993
	nonanal	De Groot & Poland 2003
	α pinen	Petrice et al. 2004
	β pinen	
Hylastes pinicola	α pinen	Brockerhoff et al. 2006b
	etanol	
Hylastinus obscurus	metyl salicylat	Kamm & Buttery 1984

Druh	**Semiochemikálie**	**Zdroj**
	metyl benzoat	Tapia et al. 2007
	(E)-2-hexenal	
Hylesinus crenatus	E-conophthorin	Zhang et al. 2002
Hylesinus varius	nonan-2-one	Francke et al. 1979
	E-conophthorin	
	exo-brevicomin	
	etanol	Kohnle 1985
Hylurgops palliatus	β pinen	Volz 1988
	α terpinolen	
	myrtenol	
	α pinen	Schroeder & Lindelöw 1989
	etanol	
	S-3-caren	Byers 1992
	(3R)-3-hydroxyoctan-2-one	Sweeney et al. 2016
	(3S)-3-hydroxyoctan-2-one	
Hylurgus ligniperda	metylbutenol	Serez 1987
	ipsdienol	
	cis-verbenol	
	etanol	Markalas & Kalapanida 1997
	α pinen	Petrice et al. 2004
	β pinen	
	α pinen+etanol+ipsenol+ipsdienol	Francardi et al. 2006
Hylurgus micklitzi	metylbutenol	Serez 1987
	ipsdienol	
	cis-verbenol	
Hypothenemus eruditus	etanol	Reding et al. 2011
*Hypothenemus hampei**	etanol	Silva et al. 2006
	metanol	
	metylcyclohexan	Mendesil et al. 2009
	etylbenzen	
	3-etyl-4-metylpentan-1-ol	
	nonane	
	5S7S-conophthorin	Jaramillo et al. 2013
	2S5S-chalcogran	
	1,6-dioxaspirodecan	
	metyl 3-etyl-4-metylpentanoat	
Ips acuminatus	**ipsdienol**	Vité et al. 1972
	ipsenol	
	***cis*-verbenol**	Bakke 1978
	metylbutenol	Gavyalis et al. 1981

Druh	**Semiochemikálie**	**Zdroj**
Ips amitinus	**R-ipsdienol**	Francke et al. 1980
	ipsenol	
	isomyrcenol	
	cis-verbenol	Symonds & Elgar 2004
	trans-verbenol	
	verbenon	
	myrtenol	
	amitinol	
	metylbutenol	
Ips cembrae	**ipsenol**	Stoakley et al. 1978
	ipsdienol	
	3-metyl-3-buten-1-ol	
	2-fenyletanol	Zhang et al. 2000
	geraniol	
	ipsenon	
	ipsdienon	
	myrtenol	
	verbenon	
Ips duplicatus	**ipsdienol**	Bakke 1975
	metylbutenol	Gavyalis et al. 1981
	cis-verbenol	
	E-myrcenol	Byers et al. 1990
	5S7S-conophthorin	Zhang et al. 2002
	E-conophthorin	
	2-fenyletanol	Symonds & Elgar 2004
	isomyrcenol	Zhang et al. 2007a
Ips sexdentatus	**ipsdienol**	Vité et al. 1972
	ipsenol	
	metylbutenol	Vité et al. 1974
	cis-verbenol	
	S-myrtenol	Francke et al. 1995
	2-m-tolylpropan-2-ol	
	***trans*-myrtanol**	
	2-fenyletanol	
	3-caren-10-ol	
	ipsdienon	
	myrtenal	
	S-m-mentha-4,6-dien-8-ol	
	α pinen+etanol+ipsenol+ipsdienol	Francardi et al. 2006
*Ips subelongatus**	5S7S-conophthorin	Zhang et al. 2002
	E-conophthorin	

Druh	**Semiochemikálie**	**Zdroj**
	ipsenol	Zhang et al. 2007b
	R-ipsdienol	Chen et al. 2016
Ips typographus	**α pinen**	Rudinsky et al. 1971
	β pinen	
	limonen	
	ipsdienol	Vité et al. 1972
	ipsenol	
	***cis*-verbenol**	
	***trans*-verbenol**	
	metylbutenol	Krawielitzki et al. 1977
	myrtenol	Schlyter et al. 1987
	***trans*-myrtenol**	
	2-fenyletalon	
	camphen	Francke et al. 1995
	verbenen	
	S-3-caren	
	myrcen	
	α terpinen	
	β phellandren	
	γ terpinen	
	p cymen	
	α terpinolen	
	3-metyl-7-metylen--1,3E8-nonatrien	
	trans-sabinen hydrat	
	trans-pinocamphon	
	isopinocamphon	
	longifolen	
	bornyl acetat	
	trans-pinocarveol	
	estragol	
	α caryophyllen	
	terpinyl acetat	
	5S7S-conophthorin	Zhang et al. 2002
	E-conophthorin	
	3-methyl-3-buten-2-ol	Symonds & Elgar 2004
	monochamol+ipsenol+α pinen+etanol+metylbutenol	Flaherty et al. 2019
*Monarthrum mali**	etanol	Oliver & Mannion 2001
	α copaen	Kendra et al. 2011

Druh	**Semiochemikálie**	**Zdroj**
	sulcatol	Miller & Crowe 2020
Orthotomicus erosus	**ipsdienol**	Giesen et al. 1984
	metylbutenol	
	cis-verbenol	Mendel 1988
	5S7S-conophthorin	Zhang et al. 2002
	E-conophthorin	
Orthotomicus mannsfeldi	**R-ipsdienol**	Kohnle et al. 1993
	S-ipsenol	
	amitinol	Symonds & Elgar 2004
Orthotomicus suturalis	ipsdienol	Valkama et al. 1997
	metylbutenol	
	cis-verbenol	
Phloeosinus armatus	terpinen-4-ol	Chararas et al. 1980
	α terpineol	
Phloeosinus aubei	α-thujon+β-thujon	Bozsik et al. 2016
	(-)-terpen-4-ol	
	(+)-camphor	
	(-)-*cis*-4-thujanol	
	juniper oil	
	korean thuja oil	
*Phloeotribus liminaris**	etanol	Reding et al. 2011
Phloeotribus rhododactylus	**E-conophthorin**	Zhang et al. 2002
Phloeotribus scarabaeoides	etylen	González & Campos 1996
	α pinen	Szauman-Szumski et al. 1998
	β pinen	
	decanal	
	undecanal	
	decan-2-one	
	nonan-2-one	
	undecan-2-one	
Phloeotribus spinulosus	metylbutenol	Valkama et al. 1997
	cis-verbenol	
	ipsdienol	
	etanol	Sweeney et al. 2016
Pityogenes bidentatus	2-fenyletanol	Francke et al. 1995
	etyl dodecanoat	
	grandisol	
	myrtenol	
	isogeraniol	

Druh	**Semiochemikálie**	**Zdroj**
	verbenon	
	borneol	
	cis-verbenol	
	trans-verbenol	
	trans-pinocarveol	
	myrtenal	
	grandisal	Byers et al. 2013
	citronellol	
	geraniol	
	hexan-1-ol	
Pityogenes calcaratus	*cis*-verbenol	Mendel 1988
	metylbutenol	
	2-fenyletanol	Francke et al. 1995
	etyl dodecanoat	
	grandisol	
	citronellol	
	isogeraniol	
	verbenon	
	borneol	
	trans-verbenol	
	trans-pinocarveol	
Pityogenes chalcographus	**chalcogran**	Francke et al. 1977
	hexan-1-ol	
	metylbutenol	Gavyalis et al. 1981
	cis-verbenol	
	ipsdienol	
	chalcogran+terpen+verbenol+metylbutenol	Kohnle 1985
	chalcogran+terpen+verbenol+metylbutenol+*endo*-brevicomin	
	1S-α pinen	Byers et al. 1988
	1S-β pinen	
	metyl(E,Z)-2,4-decadienoat	
Pityogenes quadridens	ipsdienol	Terren & De Simon 1983
	ipsenol	
	chalcogran	Francke et al. 1995
	acetoin	Byers et al. 2013
	hexan-1-ol	
	cis-verbenol	
	trans-verbenol	
	grandlure	
	citronellol	

Druh	**Semiochemikálie**	**Zdroj**
	isogeraniol	
	geraniol	
	(E)-2-(3,3-dimetylcyclohexyliden)-etanol	
	etyl dodecanoat	
	2-fenyletanol	
	myrtenol	
	dodecyl acetat	
Pityokteines curvidens	**ipsenol**	Harring 1978
Pityokteines spinidens	**ipsenol**	Harring 1978
	ipsdienol	
Pityokteines vorontzovi	**ipsenol**	Harring 1978
	ipsdienol	
*Pityophthorus juglandis**	etanol	Roling & Kearby 1975
	prenol	Seybold et al. 2015
	α pinen	Blood et al. 2018
	β pinen	
Pityophthorus pityographus	chalcogran	Heuer & Vité 1984
	pityol	Francke et al. 1987
	grandisol	
Pityophthorus ramulorum	**E-pityol**	López et al. 2011
	rac-*trans*-pityol	López et al. 2013
Polygraphus poligraphus	frontalin	Heuer & Vité 1984
	R-terpen-4-ol	Schurig et al. 1985
	1R2S-grandisol	Rahmani et al. 2019
*Polygraphus proximus**	[S]-*cis*-verbenol+ipsdienol+ipsenol	EPPO 2014
	(3R)-3-hydroxyoctan-2-one	Sweeney et al. 2016
	(3S)-3-hydroxyoctan-2-one	
Polygraphus punctifrons	**R-terpen-4-ol**	Rahmani et al. 2019
	1R2S-grandisol	
Pteleobius vittatus	metylbutenol+*cis*-pityol+*cis*-vittatol	Klimetzek et al. 1989b
Scolytus amygdali	**(3S,4S)-4-metylheptan-3-ol**	Zada et al. 2004
	(3S,4S)-4-metylhexan-3-ol	
Scolytus intricatus	(E)-2-hexenal	Vrkočová et al. 2000
	hexenal	
	(Z)-3-hexenyl acetat	
	E,E-α farnesen	
	anisol	
	trans-β ocimen	
	α copaen	
	β caryophyllen	

Druh	**Semiochemikálie**	**Zdroj**
	5S7S-conophthorin	Zhang et al. 2002
Scolytus laevis	(3R,4S)-4-metylheptan-3-ol	Anderbrant et al. 2010
Scolytus mali	5S7S-conophthorin	Zhang et al. 2002
Scolytus multistriatus	vanillin	Meyer & Norris 1967
	syringaldehyd	
	p-hydroxyacetofenon	Baker et al. 1968
	4-hydroxybenzaldehyd	
	o-hydroxybenzalkohol	
	4-metyl-3-heptanol	Pearce et al. 1975
	multistriatin	
	cubeb oil	
	limonen	Blight et al. 1980
	hexanal	Dickens et al. 1990
	hexan-1-ol	
Scolytus pygmaeus	**multistriatin**	Deventer & Minks 1977
	4-metyl-3-heptanol	
Scolytus ratzeburgi	5S7S-conophthorin	Zhang et al. 2002
Scolytus rugulosus	5S7S-conophthorin	Zhang et al. 2002
Scolytus scolytus	**4-Methyl-3-heptanol+cubeb oil**	Blight et al. 1978
	5S7S-conophthorin	Zhang et al. 2002
Scolytus triarmatus	(3R,4S)-4-metylheptan-3-ol	Anderbrant et al. 2010
Taphrorychus bicolor	**acetofenon**	Kohnle et al. 1987
	2-etyl-1,5-dimetyl-6,8-dioxabicyclo(3.2.1)-octan	Francke et al. 1995
	bicolorin	Astashko et al. 2013
Tomicus minor	α terpineol	Kangas et al. 1970a
	cis-carveol	
	trans-carveol	
	***trans*-verbenol**	Lanne et al. 1987
Tomicus piniperda	benzoic acid	Yasunaga 1962
	α terpineol	Kangas et al. 1970a
	cis-carveol	
	trans-carveol	
	verbenon	Kangas et al. 1970b
	***cis*-verbenol**	
	***trans*-verbenol**	
	3-caren	Byers et al. 1985
	1R-α pinen	
	1S-α pinen	
	α terpinolen	
	β pinen	Volz 1988
	myrtenol	

Druh	**Semiochemikálie**	**Zdroj**
	hexan-1-ol	Poland & Haack 2000
	(E)-2-hexen-1-ol	
	(Z)-2-hexen-1-ol	
	(Z)-3-hexen-1-ol	
	nonanal	Song et al. 2005
Trypodendron domesticum	etanol	Kerck 1972
	3-hydroxy-3-metylbutan-2-one	Francke & Heeman 1974
	metyl tetradecanoat	Francke & Heyns 1974
	etyl palmitat	
	lineatin	Martikainen 2000
Trypodendron laeve	etanol	Sweeney et al. 2016
	(3R)-3-hydroxyoctan-2-one	
	(3S)-3-hydroxyoctan-2-one	
Trypodendron lineatum	α pinen	Rudinsky 1966
	β pinen	
	limonen	
	camphen	
	myrcen	
	etanol	Moeck 1970
	3-hydroxy-3-metylbutan-2-one	Francke & Heeman 1974
	1,3-dimetyl-2,9-dioxabicyclo[3.3.1]nonane	Heeman & Francke 1976
	lineatin	MacConnel et al. 1977
	metylbutenol	Gavyalis et al. 1981
	cis-verbenol	
	ipsdienol	
	exo-brevicomin+etanol+terpen	Kohnle 1985
	frontalin	Setter & Borden 1992
	fenyletanol	Andersson 2012
Trypodendron signatum	salicylic acid	Choo et al. 1988
	turpentin	
	propan-2-ol	
	eugenol	
	lineatin	Martikainen 2000
	etanol	Galko et al. 2014
*Xyleborinus attenuatus**	etanol	Flaherty et al. 2019
Xyleborinus saxesenii	etanol	Montgomery & Wargo 1983
	etanol+α pinen	Petrice et al. 2004
	etanol+β pinen	

Druh	**Semiochemikálie**	**Zdroj**
	etanol+conophthorin	Miller et al. 2015
	etanol+benzaldehyd	Yang et al. 2018
*Xyleborus affinis**	etanol	Oliver & Mannion 2001
	α copaen	Kendra et al. 2011
*Xyleborus bispinatus**	α copaen	Kendra et al. 2017
Xyleborus dryographus	etanol	Galko et al. 2014
*Xyleborus ferrugineus**	etanol	Oliver & Mannion 2001
	α copaen	Kendra et al. 2011
Xyleborus monographus	etanol	Galko et al. 2014
	3-hydroxyhexan-2-one+3-hydroxyoctan-2-one+2,3-hexanediol+etanol	Flaherty et al. 2019
Xyleborus pfeilii	etanol	Galko et al. 2014
*Xyleborus volvulus**	α copaen	Kendra et al. 2011
	etanol	Abreu et al. 2012
Xylechinus pilosus	(3R)-3-hydroxyoctan-2-one	Sweeney et al. 2016
	(3S)-3-hydroxyoctan-2-one	
	1S-α pinen	
	1R-α pinen	
	1S-β pinen	
	S-3-caren	
	R-limonen	
	α terpinolen	
Xylocleptes bispinus	**ipsenol**	Klimetzek et al. 1989a
	ipsenon	
*Xylosandrus compactus**	cuelure	Uchida et al. 2003
	etanol+α pinen	Miller & Rabaglia 2009
	etanol+ginger oil	Burbano et al. 2012
	etanol+manuka oil	
*Xylosandrus crassiusculus**	etanol	Reding et al. 2011
	etanol+ginger oil	Burbano et al. 2012
	etanol+manuka oil	
	etanol+α pinen	
	etanol+conophthorin	VanDerLaan & Ginzel 2013
	quercivorol+α copaen	Kendra et al. 2017
*Xylosandrus germanus**	etanol	Reding et al. 2011
	etanol+conophthorin	VanDerLaan & Ginzel 2013

Druh	Semiochemikálie	Zdroj
	3-hydroxyhexan-2-one+3-hydroxyoctan-2-one+2,3-hexanediol+fuscumol+fuscumol acetát+etanol	Flaherty et al. 2019
	monochamol+ipsenol+fuscumol+fuscumol acetát+α pinen+etanol	
*Xylosandrus morigerus**	eugenol	Nakayama & Terra 1986
*Xyloterinus politus**	etanol	Oliver & Mannion 2001

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10 Seznam příloh

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10.8 Using ethanol and other lures to monitor invasive ambrosia beetles in endemic populations: case study from the Czech Republic.

10.1 Continued eastward spread of the invasive ambrosia beetle *Cyclorhipidion bodoanum* (Reitter, 1913) in Europe and its distribution in the world.

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Rapid Communication

Continued eastward spread of the invasive ambrosia beetle *Cyclorhipidion bodoanum* (Reitter, 1913) in Europe and its distribution in the world

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Abstract

Ambrosia beetles, including *Cyclorhipidion bodoanum*, are frequently introduced into new areas through the international trade of wood and wood products. *Cyclorhipidion bodoanum* is native to eastern Siberia, the Korean Peninsula, Northeast China, Southeast Asia, and Japan but has been introduced into North America, and Europe. In Europe, it was first discovered in 1960 in Alsace, France, from where it has slowly spread to the north, southeast, and east. In 2020, *C. bodoanum* was captured in an ethanol-baited insect trap in the Bohemian Massif in the western Czech Republic. The locality is covered by a forest of well-spaced oak trees of various ages, a typical habitat for this beetle. The capture of *C. bodoanum* in the Bohemian Massif, which is geographically isolated from the rest of Central Europe, confirms that the species is spreading east. The species probably spread naturally from Germany, but the period of establishment is difficult to estimate. Although the spread seems to be slow i.e. the beetle required about 60 years to spread from the borders of France and Switzerland to Bohemia, *C. bodoanum* may have spread more quickly but remained undetected in the newly invaded areas.

Key words: biological invasions, oak forest, pest, Scolytinae

Introduction

Cyclorhipidion bodoanum (Reitter, 1913) belongs to the "ambrosia" group of bark beetles. Members of this group have developed symbiotic relationships with fungi and are sometimes referred to as "fungus-farmers". The term "ambrosia beetle" is an ecological classification describing the larval and adult habit of feeding on mutualistic fungi in woody host tissue. Ambrosia beetles includes species in both the Scolytinae and in the distantly related Platypodidae. The ambrosia fungi are transported by the adult beetles in specialised structures (mycangia) or on the body surface; as the beetle tunnels into the host, they deposit the fungus on the gallery walls (Kirkendall et al. 2015).

Ambrosia beetles are frequently introduced into new areas through the international trade in wood and wood products (Brockerhoff and Liebhold 2017). When novel species of host trees are encountered by the beetles, the

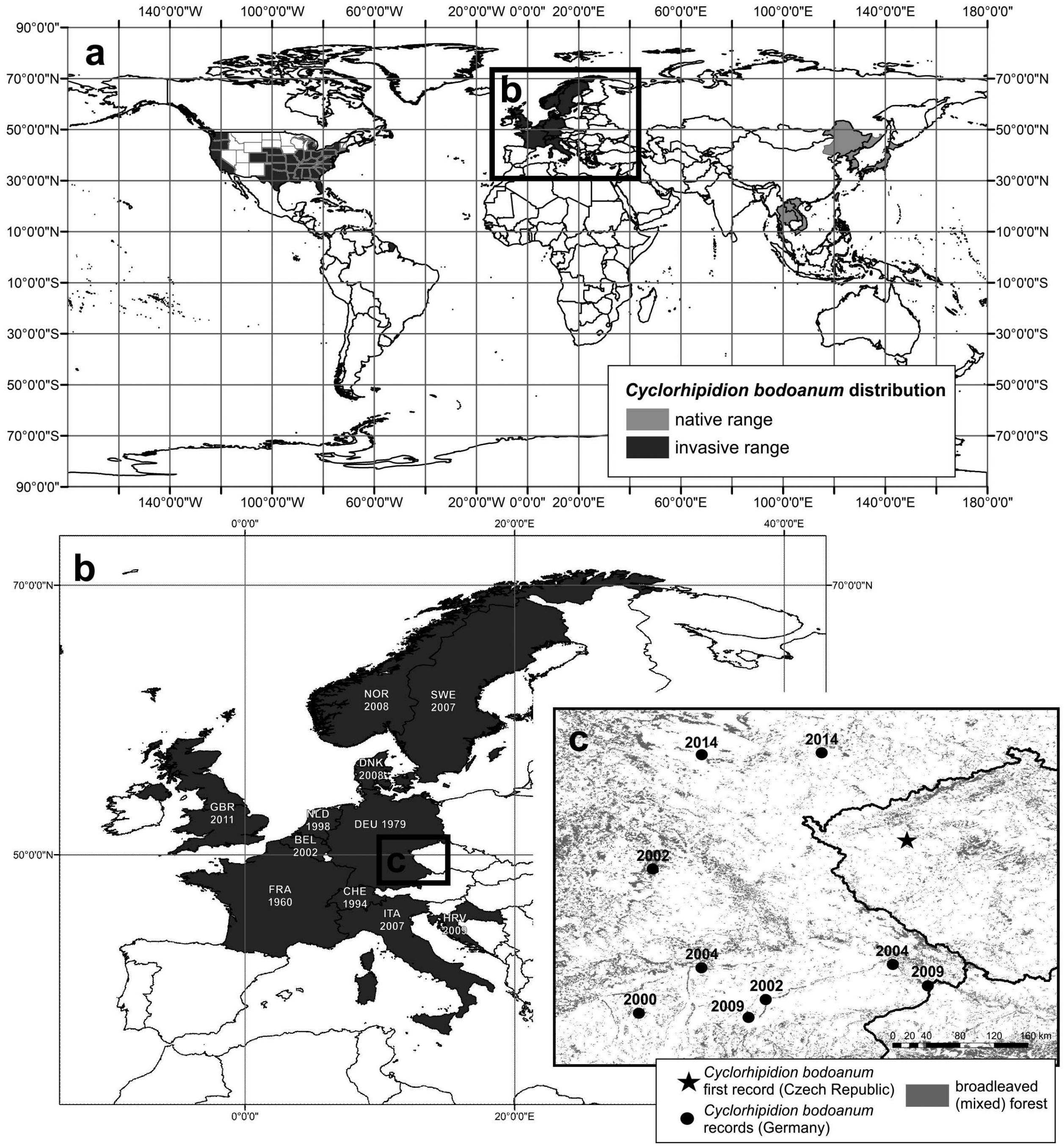

Figure 1. Distribution of *Cyclorhipidion bodoanum* throughout the world (a) with the years of its discovery in European countries (b) and in German localities near the Czech locality (indicated by a star) of the current study (c).

trees can be "naïve" and susceptible to attack, enabling previously harmless scolytine species to become damaging, or the ambrosia fungi to become pathogenic (Kirkendall and Faccoli 2010; Mayers et al. 2015). Although 30 non-native bark beetles are registered in Europe (Alonso-Zarazaga et al. 2017; Dodelin and Saurat 2017; Dodelin 2018), we know relatively little about their spread and recent distribution.

Cyclorhipidion bodoanum is native to eastern Siberia (Figure 1), where it was first described as *Xyleborus bodoanus* (Reitter, 1913) and then as *X. punctulatus* (Kurentsov, 1948). It is also widespread in the Korean Peninsula

Figure 2. The protected area of Vladař where a Theysohn® trap was suspended. Photographs of the forest interior in spring (top) and in autumn (bottom). Photographs by Přemysl Tájek.

and Northeast China (Reitter 1913; Park et al. 2020), in Southeast Asia and Taiwan (Beaver et al. 2014; Smith et al. 2020) and in Japan, where it was reported as *Xyleborus misatoensis* (Nobuchi, 1981) (Smith et al. 2020).

The beetle was also introduced into North America (Figure 1), where it was described as *Xyleborus californicus* (Wood, 1975), before being synonymised and moved into the genus *Cyclorhipidion* by Knížek (2011). The male of *C. bodoanum* was misidentified and described as *Xyleborus peregrinus* Eggers, 1944 (Bense and Schott 1998).

Cyclorhipidion bodoanum has been introduced into Europe (Wood 1975; Vandenberg et al. 2000; Kirkendall and Faccoli 2010; Gomez et al. 2018; Sanchez et al. 2020) and is now established and widespread in Western Europe (Figure 1; Kirkendall and Faccoli 2010; Alonso-Zarazaga et al. 2017).

The aim of the work was (i) to report the spread of C. *bodoanum* into a geographically isolated part of Central Europe and (ii) to review its recent worldwide distribution.

Materials and methods

In 2020, an ethanol-baited Theysohn® insect trap was deployed in the Vladař Nature Reserve (Figure 2) near the town of Žlutice (50.0755; 13.2094) in the Bohemian Massif (Czech Republic). The highest peak in the locality is 693 m a.s.l., and the trap was placed at 605 m a.s.l.

The locality lies in a rain shadow and has a relatively warm and very dry climate (Tolasz 2007). The southern slopes of Vladař support deciduous trees, which grow in Hercynian oak groves, dry acidophilous oak groves, and gravel forests of the *Tilio-Acerion* association (Figure 2).

The trap was suspended from a rope tied between two trees, with the trap base approximately 150 cm above the ground. The 96% ethanol in the trap was contained in a 50-ml polypropylene screw-top container that had four 3-mm-diameter holes in its lid; the ethanol was released from the trap at a rate of approximately 200 mg/day at 15 °C.

The ethanol bait was placed in the trap at the beginning of April. Beetles were collected from the trap every 2 weeks, and the lure and preservative fluid was changed. The collected insects were transferred to 96% ethanol, and two specimens were identified as *C. bodoanum* by the second author.

Results and discussion

In Europe, *C. bodoanum* was first discovered in 1960 in Alsace, France (Schott 2004). It was subsequently discovered in Germany in 1979 (Schott 2004), Switzerland in 1994 (Sanchez et al. 2020), the Netherlands in 1998 (Vorst et al. 2008), Belgium in 2002 (Henin and Nageleisen 2005), Italy in 2007 (Audisio et al. 2008), Sweden in 2007 (Lindelöw 2012), Denmark in 2008 (Pedersen et al. 2010), Norway in 2008 (Kvamme and Lindelöw 2014), Croatia in 2009 (Donji Lozac, 31 July 2009, Åke Lindelöw lgt., coll., Miloš Knížek det.) (Knížek 2011), and Britain in 2011 (Telfer 2019) (Figure 1). As indicated in the previous sentence, the beetle has been spreading to the east and north, and findings in other countries can therefore be expected. It does not appear to have been introduced to Spain or Portugal and is still advancing only from the west to the east and the north.

The species was also mentioned from Austria (Alonso-Zarazaga et al. 2017), but there are no official records in that country (Kirkendall and Faccoli 2010; Holzschuh *pers. comm.*; Zábranský *pers. comm.*). Its occurrence in Austria must be confirmed, although its spread to that country is very likely.

In the current study, two specimens of *C. bodoanum* (Figure 3) were collected from the Vladař Nature Reserve in the Bohemian Massif (Figure 2) on 15 April 2020. The morphological characteristics of these specimens correspond with those of the species. The collected specimens are about 2 mm long (the body length of the species ranges from 1.93 to 2.30 mm (Hoebeke et al. 2018; Knížek *pers. obs.*) and are pale yellow-brown in colour (Figure 3). From a dorsal view, the heads of the specimens are concealed under the pronotum. The basal half of the pronotum is semi-dull, shagreened, and very finely punctated (Figure 3). The frontal part is declivous, tuberculate, with a finely granulate frontal margin. The punctation of elytra is dense, without distinct striae; the postero-lateral margins of the elytral declivity,

Figure 3. Dorsal and lateral photographs of a *Cyclorhipidion bodoanum* female collected in the Vladař locality (the Czech Republic). Photographs by Zbyněk Kejval.

to the elytral apex, are rounded; the whole elytra is covered with dense pubescence, which becomes longer posteriorly; the elytral declivity is dull, flattened, and not impressed between interstriae 1 and 3; the declivital strial punctations are large, shallow, and distinct, with reticulate interior surfaces, and are separated by less than their diameter; the denticles on declivital interstriae 1 and 3 are small and more or less uniform in size.

Cyclorhipidion bodoanum is a polyphagous species that is typically associated with Fagaceae and especially *Quercus* (Wood 1982; McPherson et al. 2008; Blaschke and Bussler 2012), but has also been reported from walnut trees (*Juglans* sp.) (Seybold et al. 2016), chestnut trees (*Castanea*) (Bussler and Immler 2007), and *Pinus* (Pinaceae) and *Populus* trees (Salicaceae) (Lightle et al. 2007). It is usually found on weakened or stressed hosts and attacks trunks > 10 cm in diameter (Brin et al. 2011). *C. bodoanum* prefers forests with well-spaced trees (Bouget et al. 2013) or parks dominated by oak (Lee et al. 2019). The latter characteristics are descriptive of the current study's locality, which is an oak forest with well-spaced trees of various ages (Figure 2).

Cyclorhipidion bodoanum is a secondary pest (Lassauce et al. 2012) but increasing levels of damage from this and other ambrosia beetles can be expected because climate change will likely result in more frequent drought stress and "windblows" and increases in the number of beetle generations per year (Wainhouse and Inward 2016). A warmer, wetter climate is a good predictor of the establishment of exotic ambrosia beetles, probably because such conditions favour the growth of their symbiotic fungi (Marini et al.

2011). *Cyclorhipidion bodoanum* is a carrier of the fungus *Geosmithia morbida* M. Kolařík, Freeland, C. Utley & Tisserat, 2010, which causes a canker disease on walnut (*Juglans* sp.) (Moore et al. 2019), and also of the fungi *Mucor racemosus* f. *racemosus* Fresen., 1850, *Hypocrea lixii* Pat., 1891, *Trichoderma viride* Pers., 1794, *Pleosporaceae* sp. (McPherson et al. 2013), and *Sporothrix stenoceras* (Robak) Z.W. de Beer, T.A. Duong & M.J. Wingf., 2016 (Gebhardt et al. 2005).

McPherson et al. (2008) note that *C. bodoanum* attacks *Quercus* trees previously attacked by pathogenic fungi, resulting in the spread of decay fungi and increased tree mortality. Because *C. bodoanum* attacks relatively thick wood, economic damage may occur (Grégoire et al. 2001). On the other hand, it prefers open, non-commercial oak forests, and extensive damage to commercial forests therefore seems unlikely.

Cyclorhipidion bodoanum was probably introduced into Continental Europe and Britain with timber products or perhaps sapling trees (Schott 2004; Lee et al. 2019). The beetle has spread from its places of introduction (Bouget and Noblecourt 2005), and the finding in the Bohemian Massif, which is geographically isolated by border mountains (Ore Mts., Giant Mts. and Bohemian Forest) from the rest of Central Europe (Chytrý 2012), confirms its continued spread to the east. The species probably spread naturally to the Czech Republic from Germany, but the unintentional introduction by humans could not be also excluded (Figure 1; Table S2). Year of establishment in the Bohemian Massif is difficult to estimate. The beetle seems to spread only slowly, e.g., it required about 60 years to spread from the borders of France and Switzerland to Bohemia. However, the spread may have been faster if the beetle was simply overlooked for long periods.

It is possible that *C. bodoanum* appeared in Bohemia only recently. Although we do not have a single monitoring program for the spread of invasive species, there are number of approaches for monitoring saproxylic species. We have recently summarized the detection of *Xylosandrus germanus* (Blandford, 1894) in dozens of field searches that used hundreds of barrier traps in the Czech Republic (Fiala et al. 2020); all of the scientists involved in the experiments were experts in bark beetles and failed to detect *C. bodoanum*. We therefore speculate that *C. bodoanum* appeared in the Czech Republic only recently.

As noted earlier, invasive bark beetles are being spread by global trade, with huge movements of wood products, live plants, and unprocessed wood occurring between continents (Brockerhoff and Liebhold 2017). Individuals on new continents are first introduced in ports and airports (Haack 2001; Loreto 2015) and from there they can spread across the continents. In the USA, the spread of *C. bodoanum* began on the west coast of California and then stopped at the edge of the Rocky Mountains as a geographical barrier (Liebhold and Tobin 2008). Another wave of spread in the USA began in the ports of Texas and continued toward the east coast

(Figure 1; Table S1). In Europe, proliferation was initially limited to western countries, but after the creation of the European Union (which accelerated trade and abolished tariffs on all products including wooden products) and the fall of the “Iron Curtain”, the spread of bark beetles gained momentum (Roques et al. 2016).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Years when *Cyclorhipidion bodoanum* was discovered in the USA.

Table S2. Years when *Cyclorhipidion bodoanum* was discovered in the Germany, near of the border with the Czech Republic.

Appendix 1. References to Table S1.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Fiala_etal_SupplementaryMaterial.xlsx

10.2 The invasive ambrosia beetle *Gnathotrichus materiarius* (Coleoptera: Curculionidae) in Central Europe.

The invasive ambrosia beetle *Gnathotrichus materiarius* (Coleoptera: Curculionidae) in Central Europe

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Abstract

Gnathotrichus materiarius Fitch, 1858 is an invasive bark beetle that colonizes conifers and has spread widely in Europe. The beetle was introduced from North America to Western Europe, where it was first detected in France in 1933. In countries of Western and Northern Europe, the first detections were found at ports or airports, which probably served as sources of further spread. *Gnathotrichus materiarius* spread eastward to the Czech Republic and other countries in Central Europe from Germany and Poland and spread northward from Italy to Slovenia. The presence of wilting spruces, outbreaks dominated by *Ips typographus* Linnaeus, 1758, and the subsequent transport of has probably accelerated the spread of *G. materiarius*. It is possible that *G. materiarius* was imported with timber to Austria, the Czech Republic, and Poland.

Although *G. materiarius* has been present in Europe for almost 100 years, and even though its host trees include *Picea* and *Pinus* spp., which are abundant in Central European forests, no significant damage caused by this beetle has been detected or reported. *Gnathotrichus materiarius* is a typical secondary pest in that it multiplies on decaying trees or trees infested and killed by other bark beetle species. The best method for monitoring and detecting the presence of *G. materiarius* is the use of ethanol-baited traps. Cutting beetles out of stumps and logs is time-consuming and technically demanding because the beetles need to be cut out of the wood or preserved in emergent traps.

Key words: damage; ethanol; lure; spreading; traps

Biological invasions are a major threat to biodiversity, agriculture, and human health. Invasive populations can be the source of additional new introductions, leading to a self-accelerating process whereby invasion begets invasion. There are several possible reasons why some invasive species benefit. Some species escape from native predators or parasites, others are aided by human-caused disturbances that disrupt native communities. In agriculture, the main pests of temperate crops are non-native species, and the combined costs of pest control and crop losses represent constraints on food, fiber, and feed production. Invasion prevention is much less expensive than post-entry control (Mack et al. 2000).

Bark beetles are among the most abundant and ecologically most important forest arthropods (Martikainen et al. 1999). Invasive bark beetles are of considerable importance in forests (Kirkendall and Faccolli 2010). Invasive bark beetles can pose a threat not only to native biodiversity, ecosystem functions, and the economic productivity of forests (Brockerhoff et al. 2006, Aukema et al. 2011) but can also threaten parks and orchards (Francardi et al. 2017, Fiala et al. 2022). Some invasive bark beetles can be important vectors of fungal diseases that cause massive tree death (Montecchio and Faccoli 2014).

There are 29 known invasive bark beetle species in Europe (Alonso-Zarazaga et al. 2022), and many have been in Europe for decades without causing significant damage. One of the invasive bark beetles, *Gnathotrichus materiarius* Fitch, 1858, is currently spreading from Western Europe to the East. Many authors have assumed that *G. materiarius* would cause economic damage (Table 1) because its host trees include *Picea* and *Pinus*, which represent high percentages of trees in Central European forests (Table 2). Although, *G. materiarius* has had relatively little economic importance in America and Europe, we can't be sure that its status will not change. Some species of marginal economic importance, after being introduced, began to play a significant role in the process of dying trees and forest stands (Kirkendall and Faccoli 2010, Ploetz et al. 2017, Valenta et al. 2017).

The data from this species are scattered in several papers and, with few exceptions, there are no studies focused on this species (e.g., Witkowski et al. 2022). In this article, we summarize the knowledge on the bionomics, distribution, and abundance of this species in Central Europe, incorporating observations and learnings of entomologists. The pest status, routes of spread, potential risk, and monitoring possibilities are discussed.

Life History

Gnathotrichus materiarius (Fig. 1) is one of the fungus-farming weevils. This group of commonly called ambrosia beetles evolved a nutritional mutualism with fungi (Hulcr et al. 2007). All ambrosia beetles are dependent on the presence of woody plants, which are typically unhealthy or recently dead and in which the beetles form their tunnel systems ("galleries"). In those galleries, ambrosia beetles actively farm one or several fungal mutualists, which serve as their essential food source (Hulcr et al. 2007). *Gnathotrichus materiarius* belongs to bark beetles of the tribe Corthylini, whose members may be paraphyletic or polyphyletic and appear to have evolved different symbioses with fungi (Hulcr and Stelinski 2017). *Gnathotrichus materiarius* introduces the fungus *Ambrosiozyma monospora* (Saito) Van der Walt, 1972 into the wood, which is then fed upon by the beetle larvae (Batra 1963). The lack of host specificity in terms of tree species contributes to the invasiveness of ambrosia beetles in many forest ecosystems (Kirkendall et al. 2008).

The species was discovered under the bark of *Pinus sylvestris* and was described as *Xyleborus duprezi* Hoffmann, 1936. This taxonomic designation was subsequently revised to *Paraxyleborus duprezi* Hoffmann, 1942; then to *Gnathotrichus duprezi* Hoffmann, 1947; and finally, to the synonymization of *Gnathotrichus materiarius* (Balachowsky 1948).

Gnathotrichus materiarius is polyphagous on conifers. Its main host trees include conifers of the genera *Abies*, *Larix*, *Picea*, *Pinus*, *Tsuga*, and *Pseudotsuga* (Blackman 1931, Hirschheydt 1992, Mazur et al. 2018). The beetle seems to prefer pine over spruce and pine with thin rather than rough bark (A. Mazur, personal observation). The beetle attacks freshly felled or dead wood (Fig. 2a) and stumps (Fig. 2b-c). On the stumps, they dig not only through the bark but also through the cut (Fig. 2d). Standing trees are inhabited mainly in the lower part, up to about 2 m high, which is covered with thick bark (Ryan et al. 2012). The lying wood is inhabited along its entire length, regardless of the thickness of the bark (A. Mazur, R. Witkowski. personal observation). The wood moisture content at the time of settlement and its maintenance throughout the period of beetle development is a key determining feature on the reproductive success of ambrosia beetles (Capecki 1967), including *G. materialius*. Our observations support the fact that the beetles prefer to occupy wood felled in autumn, like ambrosia beetles of the genus *Trypodendron* (Borden 1988).

Beetles of *G. materiarius* occur the whole year but in Europe the flight of adults starts in early May (Witkowski 2020). *Gnathotrichus materiarius* is a diploidal species. The sex ratio in the population is almost equal and males initiate attacks on host trees. Unmated males release the potential aggregation pheromone, sulcatol, for a minimum of 12 days. Males stop producing sulcatol 24 hours after mating with females. Independent females are unable to initiate galleries and no sulcatol was detected in their head space and hindgut (Flechtmann and Berisford 2003). It is a monogamous species, with no sibling mating nor asexual reproduction, which distinguishes *G. materiarius* from the Xyleborini tribe species (Mazur et al. 2018; Kirkendall 1983; Flechtmann and Berisford 2003, Jordal and Cognato 2012).

The first instar larvae appear in the second half of May (Fig. 3a). Larval development takes 4-6 weeks. A pupal stage lasts 10 days (Fig. 3b). The offspring beetles emerge in mid-July. The beetles of F1 generation swarm in late July and early August, and larval development of their offspring lasts until the beginning of September. Adults of F2 generation overwinter in wood (Witkowski 2020). The sex ratio is 1:1 (Doom 1967), and the system of galleries is like those of *Trypodendron lineatum* Olivier, 1800. The female first creates the radial entrance gallery, which reaches a depth of 0.5–3.0 cm, and then creates 1–6 mother galleries; about one-third of those galleries follow the course of annual rings, and the others intersect annual rings in various ways (Doom 1967, Kamp 1979, Mazur et al. 2018).

Global Distribution

Gnathotrichus materiarius originated in North America (Blackman 1931); occurrence in the Antilles has not been confirmed because the original records are based on erroneous determinations (Bright 2019). In Europe, *G. materiarius* was first detected along the English Channel in France in 1933 (Balachowsky 1949). In other countries of Western and Northern Europe, the first detections were at ports (Belgium, Finland, the Netherlands, Spain, Sweden, and the United Kingdom) or airports (Germany, Italy, and Switzerland) (Fig. 4, Table 3), which were probably sources of dissemination in those same countries. Currently, the eastern border of its spread runs through Finland, Poland, the Czech Republic, Hungary, Slovenia, and Italy (Fig. 4).

Spread in Central Europe

In Germany, *G. materiarius* has been recently recorded throughout the country except in Brandenburg. The beetle has been detected in many locations in the Middle Rhine Valley (Baden-Württemberg) and in the north of the country (Mecklenburg) (Bleich et al. 2021). From Germany, *G. materiarius* spread east to the Czech Republic, where it was first detected in 2005 in the western part of Bohemia, near the border with Bavaria (Knížek 2009) (Fig. 4). It is likely that *G. materiarius* spread naturally into the Czech Republic from other directions, i.e., into southern Bohemia from northern Austria or Bavaria and into northern Bohemia from Saxony (Fig. 5). The beetle spread from Saxony to southern Poland where it was first reported from Poland (Witkowski et al. 2016). However, it is possible that it spread to Poland from northern Germany, where *G. materiarius* was detected as early as the 1990s (Esser and Schneider 2002).

Once introduced into Poland, *G. materiarius* spread widely in that country from 2015 to 2019; at present, the 37 known localities in Poland (Supp. S1) are mainly located in the southwestern part of the country (Fig. 5). The beetle is now spreading eastwards (Mazur et al. 2018), and it is possible that it spread from southern Poland to the eastern Czech Republic (Fig. 4). However, it could also have spread into the eastern Czech Republic from Bohemia in the western Czech Republic (Fig. 4). Spread from southern Moravia is unlikely because, despite a number of entomological surveys, *G. materiarius* has not yet been found in central Moravia and further spread through southern Moravia was not confirmed despite several surveys using mainly flight interception traps or hand collecting (Unar et al. 2021, unpublished survey by Jiří Procházka). In 16 years, the beetle spread throughout the Czech Republic, and a total of 35 localities have been found (up to November 2021) (Supp. S1, Fig. 5). Only two localities are known in Moravia, of which the oldest record is from 2015 in South Moravia. Before 2015, the species probably did not occur there, because it was not detected in South Moravian floodplain forests before 2015 despite extensive passive capture of beetles (including bark beetles) in flight interception traps (Schlaghamerský 2000, Procházka et al. 2018). *Gnathotrichus materiarius* was also not detected in the Bílé Karpaty Mts. (eastern Moravia) in 2015 despite substantial research (Račanský 2019). Given the year of its detection, *G. materiarius* probably spread to south Moravia from the south of Austria (see below).

Gnathotrichus materiarius probably spread from northern Italy into Slovenia, and into Carinthia, where it was first reported beyond the Alps in 2012 (Aurenhammer et al. 2015). Because the spread of invasive insects is often blocked by geographical barriers (Liebhold and Tobin 2008, Wan and Yang 2016), *G. materiarius* could have been artificially introduced to the Alps. Across Austria, only four localities are known (Supp. S1, Fig. 5), which are distributed along the foothills of the Alps towards the Pannonia, where it penetrated western Hungary. The beetle has been known around Sopron since 2016 (Lakatos 2019) (Fig. 5). *Gnathotrichus materiarius* does not seem to be spreading further into Hungary because it has not been detected in central Hungary (F. Lakatos, observ.). It is possible that it spread to South Moravia from western Hungary, where it was found in 2015. The species probably already occurs in western Slovakia, where it could spread to South Moravia (Fig. 5). Molecular analysis may be needed to gain further insight into the colonization history of *G. materiarius.*

Factors favoring the spread

In several places the abundance of *G. materiarius* has been sharply increased recently. In 2017, numerous beetles were found inhabiting raw wood material harvested from western and southern Poland (Mazur et al. 2018). In 2018, *G. materiarius* was found on only 3% of experimental stumps in the National Park Bohemian Switzerland (Northern Bohemia) (Resnerová unpublished, see Supp. S1), while in 2020 and only 50 km to the south, the beetle infested more than 70% of experimental logs (Resnerová unpublished). Similarly, in the area surrounding Sopron in Hungary laying on the edge of *G. materiarius* recent range in Central Europe, the number of *G. materiarius* captured in pheromone traps increased significantly from 2016 to 2019 (Fig. 6).

The spread of *G. materiarius* can also be supported by the wilting of spruce due to drought and subsequent infestation by honey fungus (*Armillaria* ssp.) and bark beetles (Holuša et al. 2018). The presence of withering trees, an outbreak dominated by *Ips typographus* Linnaeus, 1758 (Hlásny et al. 2021), and the subsequent transport of wood could accelerate the spread of the beetle. This corresponds to the rapid spread of the beetle in Poland following the *I. typographus* outbreak that began in 2015. Whereas the beetle required only 5 years to spread widely in Poland, it required 20 years to spread widely in the Czech Republic.

It is possible that *G. materiarius* was imported with timber to Austria, the Czech Republic, and Poland. One of the materials used by bark beetles is wood packaging material (Meurisse et al. 2019), the treatment of which in international trade is based on phytosanitary measure ISPM 15. According to this measure, all wood imported from outside of Europe must be debarked and then heated or fumigated. However, about 0.1 to 0.5% of wood treated according to the ISPM 15 standard was found to contain live quarantine insects under the bark (Haack and Petrice 2009, Haack and Brockerhoff 2011). Thus, even the ISPM 15 standard will not protect the importing country from the spread of invasive species (Evans 2007). As evidence of that problem, *G. materiarius* was found in Sweden in debarked wood that was imported from France (Gillerfors 1988, Schroeder 1990).

Precipitation of the driest quarter of the year is the most important predictor of G. *materiarius* distributions. In Europe, highly suitable areas are concentrated in the Balkans, the Black Sea and Caucasus region, the Baltic countries, the Scandinavian Peninsula and Ukraine. The model indicated that *G. materiarius* can find suitable climate conditions for its occurrence across 13.1% of Europe recently. The most pessimistic predictions suggest that as the climate changes, the species' range will expand mainly eastwards to the Ural Mountains and northwards almost to the Arctic Circle on the Scandinavian Peninsula. Only slight shifts in the western and southern parts of the species' range are predicted (Witkowski et al. 2022).

Monitoring Options

We have summarized the results of many local surveys (published as well as unpublished) and found data from 78 localities in Central Europe (Austria, the Czech Republic, Hungary, and Poland). For almost 95% of the detections, we know the method of detection (Supp. S1). Of the detected beetles, almost 40% were captured in pheromone traps and > 30% were captured with artificially produced pheromone evaporators.

Half of the data were obtained by systematic and targeted searches of stumps or experimental logs (Supp. S1). However, this method is time and technically demanding. The stumps or logs must be debarked. If boreholes are detected in the wood, smaller sections of wood must be cut with a chainsaw and stored in the laboratory in emergence traps. OOnly a small portion of the data (6%) represents accidental findings or hobby collecting activities of amateur entomologists (captured in flight, sweeping, or debarking, or found on firewood or in a pool) (Table 4).

Most of the published data concerning *G. materiarius* detection in Europe come from pheromone traps lured for bark beetles of the genus *Ips* (Schneider 1985, Valkama et al. 1998, Knížek 2009, Mazur et al. 2018. Similarly, our data were obtained by trapping beetles on artificial lures containing ethanol or ethanol combined with other substances were used (Supp. S1). The attraction of ambrosia beetles to ethanol is related to their preference for woody material that has sufficiently aged to allow anaerobic respiration to generate ethanol (Moeck 1970).

When comparing the capture of *G. materiarius* in Wood Stainers Lure, containing the pheromone sulcatol (Flechtmann and Berisford 2003) with other lure treatments, no beetles were captured with α-pinene (Fiala et al. 2023), which was used due to the preference of bark beetles for conifers (Schroeder and Lindelöw 1989). Fewer beetles were captured with Cembräwit (Fiala et al. 2023) since *G. materiarius* has shown a positive response to this lure (Schneider 1985), but more beetles were captured with ethanol (Fiala et al. 2023). The attractiveness of sulcatol in a mixture of α-pinene and ethanol as a major aggregation pheromone was therefore not confirmed and the universal bait for the detection of this species remains ethanol (Fiala et al. 2023).

Pest's impact

Not every invasive species becomes a pest. Whether an invasive species becomes a pest depends on ecological variables, the number of introductions, and the origin of the species. Only about 10% of invasive species become significant pests in the new territory (Williamson and Fitter 1996, Smith et al. 1999), and only a small percentage of ambrosia beetle species introduced into non-native habitats cause significant damage to living trees (e.g., Francardi et al. 2017). Most cases of newly established ambrosia beetles are still assumed to be non-damaging. The most common factor in bark beetle damage is the host's physiological stress (Netherer et al. 2021). Although introduced ambrosia beetles are not pests in Europe, they can easily detect the ethanol emitted by stressed trees and can thereby locate and colonize those trees (Ranger et al. 2015). Another factor that could affect the impact of invasive species is the evolutionary relationships of new hosts to the pest's original host (Mech et al. 2019).

Most of the major damage, e.g. the killing of healthy trees or the infestation of stressed trees, that are consequently killed, caused by invasive bark beetles is reported from southern Europe (Pennacchio et al. 2012, Montecchio and Faccoli 2014, Daubree 2016, Faccoli et al. 2016, Francardi et al. 2017, Leza et al. 2020). Only trees infested by invasive bark beetles have been reported from Central Europe, and this includes only colonizing downed wood by the ambrosia bark beetle *Xylosandrus germanus* in Switzerland and Slovakia (Maksymov 1987, Galko et al. 2019). On the other hand, no trees killed by *X. germanus* has been reported in the Czech Republic (Fiala et al. 2020).

Many authors have warned that *G. materiarius* could potentially cause damage (Table 1). However, the species has been present in Europe for almost 100 years, and no killed trees has been identified or reported, even though spruce and pine, its host trees, are the most numerous species in the commercial forests of Central Europe (Table 2). In Switzerland, *G. materiarius* was first found in the northern part of the country in the 1980s. Bovey (1987) warned of the serious damage that *G. materiarius* could cause to Swiss forests, but so far (i.e., after >35 years), no major problems caused by this species have been reported (Sanchez et al. 2020).

In Poland, *G. materiarius* infestation was recently observed for the first time in Scots pine wood in managed forests in south-west Poland. The scale of the infestation was not economically important (Mazur et al. 2018). *Gnathotrichus materiarius* infestation of standing trees was found in southern Bohemia, but the infestation was not quantified (Knížek et al. 2020). In locations where we conducted more intensive surveys, the surrounding trees were not attacked; no signs of tree infestation by *G. materiarius* were observed in the Bohemian Switzerland National Park (see above), in the Landscape protected Area Kokořínsko – Máchův kraj (Resnerová unpublished), or in the area around Sopron (Fig. 6).

Gnathotrichus materiarius may be a typical secondary pest when propagated on withering trees or trees infested and killed by other bark beetle species. *Gnathotrichus materiarius* inhabits dead and weakened trees, there is no information about cases of attacks on healthy trees. It most often inhabits the butt of standing trees and the bedding, both with and without bark (Bussler and Immler 2007), but in the case of logs without bark, the beetles dig only in the parts with the bark. *Gnathotrichus materiarius* inhabits both thick- and thin-barked fragments (Witkowski 2020).

Gnathotrichus materiarius is a technical pest that causes damage to the sapwood of coniferous wood. Despite the small diameter of the holes and sidewalks, measuring approximately 1 mm, inhabited wood is a much less valuable raw material (Faccoli 1998, Lindgren and Fraser 1994; Witkowski 2020). Galleries of *G. materiarius* reach quite deep, which reduces the size of valuable assortments and causes financial losses. The density of enter holes can reach almost 500 per m^2 (Witkowski 2020). The fungal mycelium can cause wood staining, which is considered an aesthetic defect (Mazur et al. 2018). The mechanical properties of the wood are not impaired by the fungal infestation, as has been found in *I. typographus* (Hýsek et al. 2021).

Conclusions

Gnathotrichus materiarius is an invasive species that has been repeatedly introduced into Europe with the transport of wood and wood packaging materials. Once in Europe, it has probably spread naturally with the transport wood to the east and currently occurs throughout Western and Central Europe. Considering the biology and ecology, as well as the dynamic spread of the species, *G. materiarius* can become one of the most important technical pests of conifer trees in the future.

Detection of the species can be expected in other countries to the east of its current known distribution. *Gnathotrichus materiarius* adults can be found in traps deployed for the capture of bark beetles in the genus *Ips*. Because trap deployment is a common method of monitoring economically important species in many countries, we recommend that, in those countries to the east of the current known occurrence of *G. materiarius*, the insects caught in these traps be examined for the presence *G. materiarius* and other non-target species. In the case of targeted monitoring, it is best to use ethanol in traps for the detection of this species.

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Availability of data and material The data on the occurrence of species are included in Supplementary material. Small datasets dealing with numbers of beetles sampled at Sopron and sharing of two bark beetle species logs are available from the corresponding author on reasonable request.

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Fig. 1 Adult of *Gnathotrichus materiarius* in dorsal view (a) and lateral view (b).

Fig. 2 Log of spruce (a), pine stump (b), broken spruce stump (c), and a cut on a pine stump (d) heavily occupied by *Gnathotrichus materiarius* with obvious piles of sawdust indicating that there are enter holes of bark beetles.

Fig. 3 Young larvae in larval galleries, which emerge from the maternal gallery, pass diagonally through the image (a) and larva before pupation (top) and pupae (bottom) of *Gnathotrichus materiarius*.

Fig. 4. Distribution of *Gnathotrichus materiarius* throughout Europe with the years and locations (stars) of its discovery.

Fig. 5. Distribution of *Gnathotrichus materiarius* in Austria, Germany, Hungary, northern Italy, Poland, Slovenia, and Switzerland with directions of expected spread (full arrows are very likely directions of spread, and dashed arrows are hypothetical directions of spread).

Fig. 6. Numbers (means ± SE) of *Gnathotrichus materiarius* beetles caught per trap near Sopron (western Hungary). The abundance of beetles over the years was tested by GLM with a negative binomial distribution (logarithmic link) and with individual sampling plots as a covariate. The number of beetles differed among years (df = 11, F = 5.76, P = 0.016) (six Econex crosstrap mini® pheromone traps were lured with ethanol, alpha pinene, and ipsdienol).

Table 1. Authors who suggested that *Gnathotrichus materiarius* could (or could not) potentially cause substantial damage to forerst trees in Europe.

Reference	Comments
Doom 1967	Isolated damage to Douglas fir.
Gauss 1970	It could cause great damage.
Gladitsch 1969	It could be the most important wood pest.
Harde 1967	It could be the most important wood pest.
Hirschheydt 1992	There was damage higher than *Trypodendron lineatum* in the Rottenburg/Neckar area estimated, but no damage was reported in Switzerland.
Kamp 1979	There is no damage in Germany.
Knížek et al. 2020	It could be a major wood pest.
Mazur et al. 2018	In Poland, no important damage is known, but it could be a major pest of woody plants.
Moucheron and Warzée 2006	It can cause damage.
Schedl 1966	It could be a primary pest.

Table 2. Percentage of tree species in forests of Austria, Czech Republic, Hungary, and Poland.

Country	Percentage of all forest trees represented by Scotch pine and Norway spruce		
	Scotch pine	Norway spruce	source
Austria	6.2	59.8	Fao.org
Czech Republic	9.8	43.0	Fao.org
Hungary	9.0	1.0	Fao.org
Poland	58.5	6.0	Fao.org

Table 3. Numbers of localities in Central Europe where *G. materiarius* was detected by the indicated method.

Method of detection	Numbers of localities
Artificial pheromone	23
Blended volatile	5
From tree/log/stump	39
Window traps	1
In flight/taping	5
Unspecified	5

Table 3. First year and locality of *Gnathotrichus materiarius* detection in European countries

Country	Year	Locality	Latitude	Longitude	Comments	Reference
Austria	2012	Rote Wand	46.5865N	13.7391E	60 km from the discovery place in Slovenia	Aurenhammer et al. 2015
Belgium	1979	Brasschaat	51.2830N	4.4902E	port	Boosten 1982
Czech Republic	2005	Bělá nad Radbuzou	49.5985N	12.6882E	near boundary with Bavaria	Knížek 2009

Finland	1996	Vantaa	60.2421N	25.1701E	port	Valkama et al. 1997
France	1933	Rouen	49.3866N	0.9904E	port	Balachowsky 1949
Germany	1964	Forchheim	49.7155N	11.0272E	near international airport at Nurnberg	Gladitsch 1969
Hungary	2016	Sopron	47.6682N	16.5767E	near boundary with Austria	Lakatos 2019
Italy	1993	Pombia	45.6461N	8.6293E	international airport	Bernabò 2000
Netherlands*	1965	Vaassen	52.2925N	5.9257E	near port	Schedl 1966
Poland	2015	Krzeszów	50.7304N	16.0338E	near boundary with the Czech Republic	Witkowski et al. 2016
Schwitzerland	1984	Buchenegg	47.2941N	8.5096E	near international airport at Zurich	Hirschheydt 1992
Slovenia	2007	Brdo pri Kranju	46.2754N	14.3821E	80 km far from port of Terst and Italian discovery	Jurc et al. 2012
Spain	2003	Inama	43.2882N	2.6912W	near port and international airport, near France	López et al. 2007
Sweeden	1986	Varberg	57.1057N	12.2502E	port, probably imported from France or Spain	Gillerfors 1988
United Kingdom	2013	Ringwood Forest	50.8800N	1.8300W	near port	Inward 2020

*Based on the presence of galleries, researchers assumed that the species occurred in the Netherlands as of 1954, but adults were not caught until 1965 (Doom 1967).

10.3 Occurence of the invasive bark beetle *Phloeosinus aubei* on common juniper trees in the Czech Republic.

forests

Article

Occurrence of the Invasive Bark Beetle *Phloeosinus aubei* on Common Juniper Trees in the Czech Republic

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Abstract: The small cypress bark beetle *Phloeosinus aubei* is considered an invasive pest in several central European countries, and we have determined its current distribution on common juniper trees (*Juniperus communis*) in the Czech Republic. The results indicated that *P. aubei* is widely distributed in the country but is more common in the east than in the west. The beetle was mainly detected on older, damaged trees and on stems with diameters > 3 cm. The apparently widespread and abundant populations of *P. aubei* could explain infestations of the beetle on *Thuja* spp. and *Juniperus* spp. in gardens (three confirmed cases during the last 10 years). We consider *P. aubei* to be a potential pest on older, naturally occurring *J. communis* in protected areas where its population density could increase on weakened and damaged trees. We suggest that *P. aubei* can be monitored via simple inspection of dying and dead *J. communis* trees in the field.

Keywords: scolytid; small cypress bark beetle; *Juniperus communis*; longitude; monitoring; biological invasions

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1. Introduction

Bark beetles (Coleoptera: Curculionidae, Scolytinae) are among the most damaging pests of trees, because the adults and larvae tunnel beneath the bark; such tunnelling can eventually girdle and kill hosts. Most bark beetles breed in recently felled trees and especially in trees weakened by drought, defoliation by other insects, or diseases [1–3]. Many bark beetles are invasive and are easily transported with wood products, wooden packaging materials, nursery stock, and bonsai trees [4,5].

The small cypress bark beetle (sometimes referred to as the eastern juniper bark beetle or the cedar bark beetle) *Phloeosinus aubei* (Perris, 1855) (syn. *P. bicolor* Brullé) is a Mediterranean species that occurs mainly in northern Africa, the Near and Far East, and in southern Europe [6–11]. The main hosts of this phloeo- and xylophagous beetle are cypresses (*Cupressus* spp.), junipers (*Juniperus* spp.), and thujas (*Thuja* spp.) [9,12,13]. The number of generations per year ranges from two to four in the Mediterranean and Pontic areas [14–16], where the beetle overwinters as adults [14] and larvae [16] and begins to swarm when the temperature increases to 12 °C in the spring [15]. In Central Europe, in contrast, *P. aubei* has only one generation per year [17,18], overwinters mainly in the adult stage [18,19], and begins to swarm when temperatures exceed 20 °C [20]. In the Mediterranean area, *P. aubei* is an important pest, because it attacks and can kill host trees [21].

In the 1980s, the northern edge of the *P. aubei* range was in Central Europe, i.e., in northern Austria, southern Moravia (Czech Republic), southern Slovakia, and southwestern Hungary [7,9,17,22,23]. This invasive beetle was subsequently detected further north, i.e., in Brandenburg in 2001 [22,24], in the Netherlands in 2004 [25], in eastern Slovakia in 2007 [26], and in Poland in 2014 [20].

In Mediterranean countries, where *P. aubei* has been responsible for large areas of deforestation, its primary native host is the Italian cypress *Cupressus sempervirens* L., 1753 (Cupressaceae). The primary symptoms of infestation by this pest are dry branches or dry stems. The tunnelling activity of *P. aubei* in the woody parts of trees facilitates infection by the fungus that causes cypress canker, *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson, 1972, and it is cypress canker that eventually kills the host [14,27]. As vectors, bark beetles primarily transfer ophiostomatoid fungi [28–30].

In addition to attacking forest trees, *P. aubei* can damage trees in gardens [20,25,31–34]. These cases mainly involved *Thuja* trees and other exotic species. The occurrence of *P. aubei* on the common juniper *Juniperus communis* L., 1753, which is native to Central Europe, has not been reported. Given the rapid spread of *P. aubei*, its effects on *J. communis* should be assessed.

Juniperus communis is of conservation concern in that part of its range where it is failing to regenerate [35], and is considered to be a "near-threatened" species in the Czech Republic [36]. Although *J. communis* is not threatened with extinction globally in any of its forms (subspecies or varieties) [37], the species is struggling to survive in those areas where changes in land-use and site management have reduced plant survival and recruitment [35].

Many *J. communis* populations are aging in the Czech Republic our study area, and this is thought to reduce reproductive vigor [38]. Moreover, diffuse pollution has been shown to interrupt pollination, fertilization, and embryo development [39]. Nitrogen deposition, sulphur deposition, and increased temperatures can have similar effects [40–43]. These factors, together with a wide array of non-bark beetle arthropods that attack *J. communis*, including the mite *Trisetacus quadrisetus* Thomas, 1889, and the chalcid wasp *Megastigmus bipunctatus* Swederus, 1795 [38,40], are weakening this highly valued tree species. Because *P. aubei* prefers weakened hosts, we hypothesized that its frequency of occurrence is increasing on *J. communis* in the Czech Republic.

The goals of the current study were to determine the distribution of *P. aubei* on *J. communis* in the Czech Republic, and to identify factors associated with its occurrence.

2. Materials and Methods

Field data were collected throughout the Czech Republic from January to October 2018 in 27 localities in protected areas and forests with >10% of one or more of the following *J. communis* habitats (Figure 1): T3.4A (broad-leaved dry grasslands with orchids and *J. communis*), T3.4B (broad-leaved dry grasslands with few or no orchids and with *J. communis*), T8.1A (dry lowland and colline heaths with *J. communis*), and T8.2A (secondary submontane and montane heaths with *J. communis*). The areas and forests with these habitats were selected based on the updated mapping of Natura 2000 [44] from the years 2007–2018. Each plot (one plot per locality) was surveyed only one time.

In each of the 27 plots, which ranged in area from 0.1 to 103.5 ha (habitat boundaries), we checked all *J. communis* trees with dry twigs (Figure 2). We debarked the trunk of such trees and checked the exposed wood and bark for galleries and beetles typical of *Phloeosinus* spp. The removed bark was 50 cm long on the vertical plane and included the entire circumference (Figure 2). Any bark beetles present were removed with tweezers, stored in alcohol, and identified to species by examination with a dissecting microscope (Bresser Advanced ICD Microscope 10x – 160x; Bresser GmbH, Rhede, Germany). The detection of a specimen of *P. aubei* was considered to indicate that *P. aubei* was present in the locality; the detection of galleries alone was not considered evidence of *P. aubei*. We also recorded the time required to detect the first *P. aubei* specimen (the 'finding time') in a plot; the finding time was considered an indicator of *P. aubei* abundance.

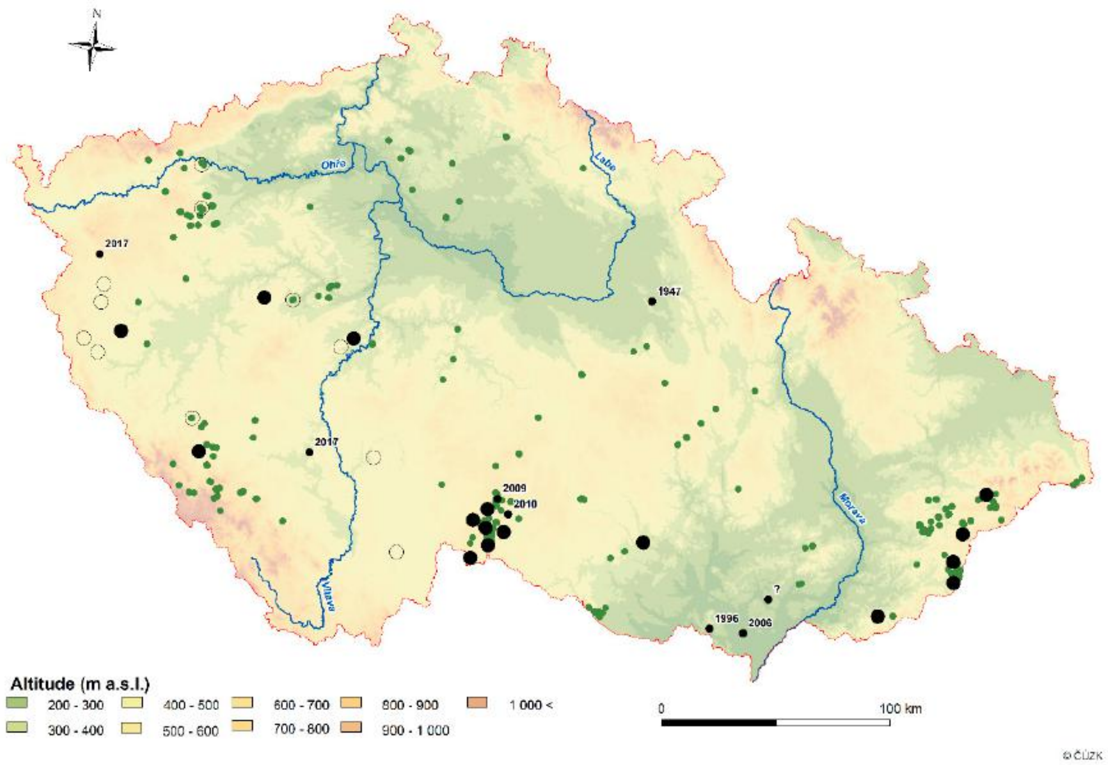

Figure 1. Occurrence of *Phloeosinus aubei* in the Czech Republic. Positive detections as documented in records before 2017 are indicated by small black circles (with detection year), and positive detections in 2018 are indicated by large black circles. Negative detections in 2018 are indicated by empty circles. Small green circles indicate juniper habitats (T3.4A, T3.4B, T8.1A, and T8.2A) according to Natura 2000 mapping in the Czech Republic (see Materials and Methods for details).

Figure 2. Dying junipers (*Juniperus communis*) in the Rajchéřov locality (**a**, the dying trees are red), galleries of *Phloeosinus aubei* on *J. communis* in the Mariánské Lázně locality (**b**), and a juniper tree damaged by antler rubbing in the Vrbička locality (**c**).

Data from private collections and museum collections were also summarized. Data from "Finding Database of Agency of Protection Nature and Landscape ČR" [45] are included as well as data from unpublished entomological reports.

The relationship between the presence/absence of *P. aubei* and longitude was analysed using a logistic model (Quassi-Newton method of estimation). Mann–Whitney U-tests were used to compare the following variables between localities with and without *P. aubei*: altitude, locality area, distance of the locality from the nearest *J. communis* locality, and population density of junipers. GLM analyses (Poisson distribution, LN link function) were used to determine the relationships between the 'finding time' and the locality characteristics indicated in the previous sentence plus locality management (managed vs. unmanaged). As noted earlier, finding time served as a surrogate measure of *P. aubei* population density. All tests were performed with Statistica 12.0 sofware (StatSoft CR, s.r.o.; Prague, Czech Republic).

3. Results

P. aubei was found in 16 of the 27 localities studied in 2018; the localities with *P. aubei* in 2018 were scattered throughout the Czech Republic and over a wide range of altitudes (160 to 720 m) (Figure 1). All localities with *P. aubei* in 2018 also contained *Phloeosinus thujae* (Perris, 1855). *Phloeosinus aubei* was found in all localities in the eastern part of the Czech Republic but in only about half of the localities in the western part of the country (Figure 3); this trend was statistically significant ($\chi^2 = 14.89$; $p < 0.001$). The habitats were also different in eastern vs. western parts of the country: protected areas were regularly maintained by grazing or cutting in the eastern localities but were overgrown with *Frangula alnus* Mill., 1768, *Prunus spinosa* L., 1753, *Rosa* spp., and other species in the western localities (Figure 4, Appendix A). The probability of *P. aubei* detection was not related to locality altitude ($z = 0.87$; $p > 0.05$), locality area ($z = 1.66$; $p > 0.10$), distance of the locality to the nearest juniper ($z = -0.30$; $p > 0.05$), or juniper population density in the locality ($z = 1.73$; $p > 0.10$) (Figure 5). Across all localities, the mean (±standard deviation) finding time was 14 (±16.6) min. Finding time was unrelated to locality altitude, locality area, distance of the locality to the nearest juniper, juniper population density in the locality, or locality management (F = 0.76; $p > 0.10$).

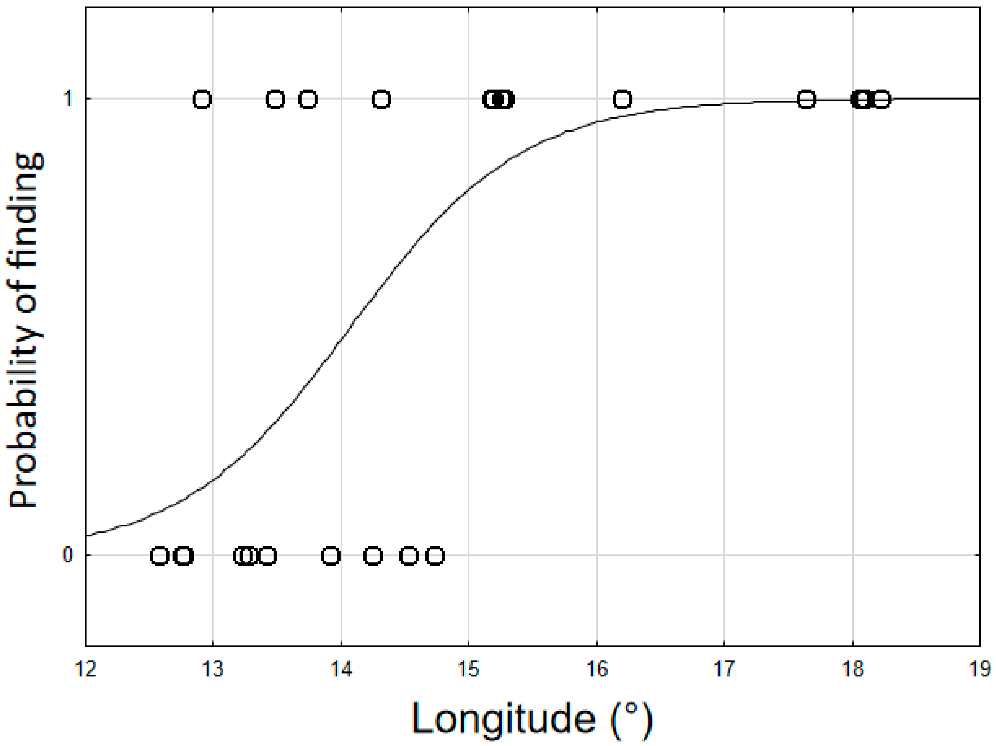

Figure 3. Logistic model for the probability of detecting *Phloeosinus aubei* in the Czech Republic as related to longitude. On the y-axis, 0 and 1 indicate that *P. aubei* was not detected or was detected, respectively.

Figure 4. Junipers overgrown by *Prunus spinosa* and *Rosa* spp. (**a**), and a juniper habitat regularly maintained by grazing at the Vrbička locality (**b**).

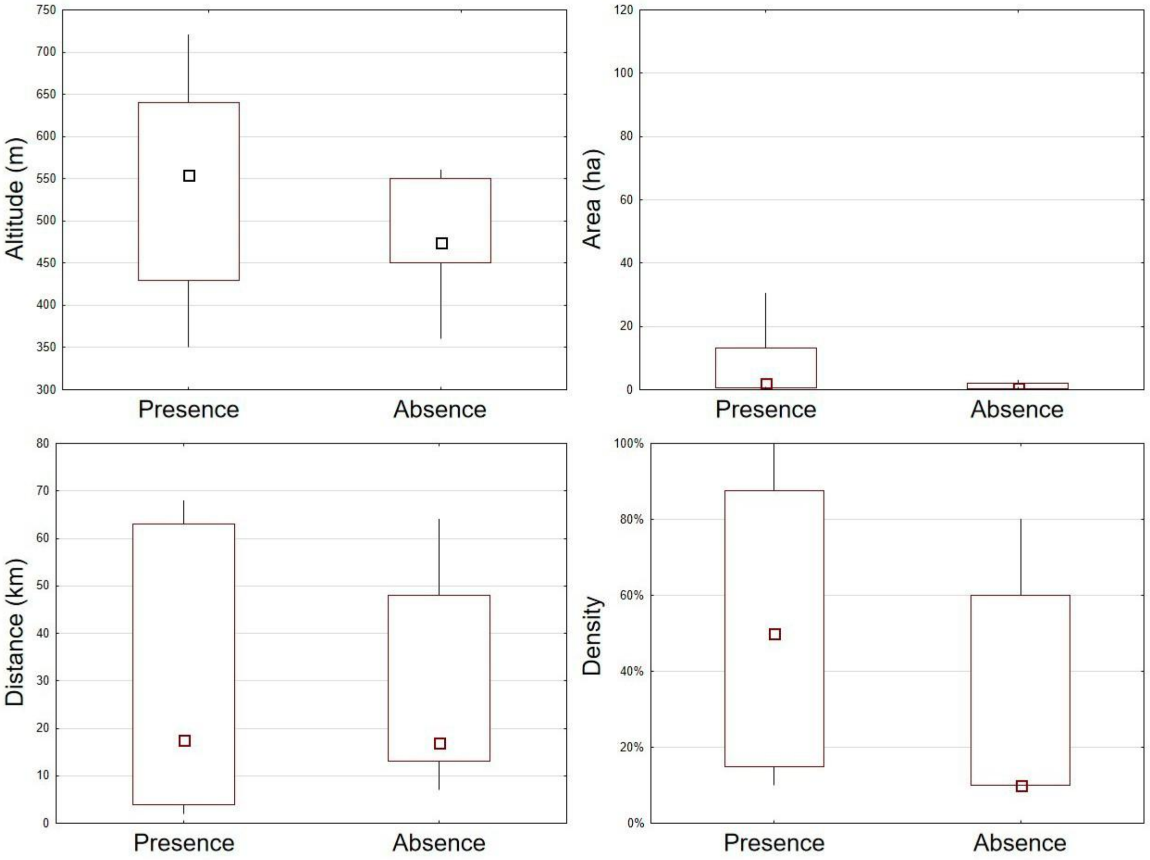

Figure 5. Relationships between the presence and absence of *Phloeosinus aubei* and the following characteristics of the localities: altitude, area, distance from the nearest juniper, and juniper population density. Small squares indicate medians, boxplots indicate the 25% and 75% quartiles, and lines indicate minimum and maximum values.

In addition to being found in 16 localities in 2018, *P. aubei* was previously found in 8 other localities (Figure 1). In all 24 localities where *P. aubei* has been detected, the host tree was usually *J. communis*, although the host tree was not indicated in some earlier reports and was *Thuja* spp. in one earlier report (Appendix A). Across all 24 localities, *P. aubei* was mainly found on *J. communis* stems thicker than 3 cm (Appendix A). In the one case in which *P. aubei* was found on *P. thujae*, the beetle was also found on branches thinner than 1 cm. In all localities where *P. aubei* was detected on *J. communis*, the trees had been damaged by cattle or game (Figure 2).

4. Discussion

The small cypress bark beetle *P. aubei* is native to the Caucasus, Asia Minor, and the Mediterranean area [6] from Israel [27] to southwest France [46]. Over the last 100 years or so, *P. aubei* has been spreading north from the Mediterranean area into Central Europe, where it has been reported as an alien pest [24,25,32,47–52].

The presence of *P. aubei* in the Czech Republic was first mentioned in the literature by Pfeffer [17], who referred to a single specimen captured by J. Picka. Although the details regarding that specimens are few, we do know that it was collected in the southeastern part of the country (i.e., in the historical country of Moravia) and probably in the village of Čejč. Its year of collection is unknown, but it was probably collected in the 1960s. The first dated finding of *P. aubei* in the Czech Republic was in 1947, and that specimen was found in the east-central part of the country (i.e., in the historical country of Bohemia) (current study). Previous studies had reported the detection of *P. thujae* [53,54] in the Czech Republic but not *P. aubei*. In the 1990s, *P. aubei* was reported from southern Moravia [55] and from Bohemia [56]; the latter report concerned outbreaks in several places in Prague and central Bohemia on *Juniperus* spp., *Chamaecyparis* spp., *Thuja* spp., and *Cupressus* x *leylandii* A.B. Jacks & Dallim., 1926.

In the Czech Republic, *P. aubei* has perhaps been spreading west (Figure 1), because *P. aubei* detection decreased from east to west (Figure 3). All studied variables (altitude, locality area, distance of the locality to the nearest juniper, and juniper population density) had large ranges in values in localities with and without *P. aubei* occurrence (Figure 5). Accordingly, these variables cannot be used to predict where the beetle will spread to, and only the presence of host trees appears to be important. A strong influence of host is typical for bark beetles [57]. That *P. aubei* is able to detect its host is confirmed by the fact that the 'finding time' was unrelated to host variables (area, distance of the nearest juniper, and density). *Phloeosinus* bark beetles have strong dispersal capabilities and can fly over 24 km [58,59]. The abilities to disperse and to detect hosts promote the spread of invading populations in a landscape matrix [60].

The increased spread of *P. aubei* in the Czech Republic corresponds with recent findings of increased spread of *P. aubei* in Germany [22,24], the Netherlands [25], and Poland [20]. This increase in *P. aubei* spread is probably a result of climate change [61–65] and increases in global trade [66–71].

The severe drought of 2003 possibly increased the susceptibility of juniper trees in Central Europe to bark beetles. For Western Europe, climate change models predict increasing summer drought and heat waves, which will increase the susceptibility of trees to secondary insect pests such as *Phloeosinus* spp. [25,72]. The finding of *P. aubei* in 1996 in the United Kingdom can be attributed to global trade, because *P. aubei* was found on *Thuja* spp. imported from Italy [52].

The main host of *P. aubei* in the Czech Republic is the common juniper, *J. communis*, which is listed in the ICUN Red List as near-threatened in the country [36]. Given the fragmented and small populations of *J. communis* (see Figure 1), gene flow is probably limited, with potential implications for the tree's long-term fitness and survival even where viable seed production occurs [73–75]. In such places, *P. aubei* can be considered a threat to the tree. This beetle kills *J. communis* trees that are weakened by drought or damaged by cattle grazing or antler rubbing by game [76,77]. Older stands of *J. communis* in the Czech Republic are in poor condition, and in many places they are overgrown (Figure 4). Factors that weaken the trees increase the spread of *P. aubei* and *P. thujae*, but precise quantification of both beetles is difficult because their galleries are very similar.

The frequent occurrence of *P. aubei* in many regions of the Czech Republic could lead to attack on thujas and junipers in ornamental gardens and cities, as has already occurred in the towns of Písek and Mariánské Lázně (Appendix A) and in Prague and other places in central Bohemia [56]. In contrast to *P. aubei*, *P. thujae* has not been reported on thujas and junipers in ornamental gardens and cities. In addition, *P. aubei* is a vector of pathogens and especially of the causal agent of cypress canker, *S. cardinale* ([18,51,78].

The monitoring of invasive bark beetle is necessary [79]. The use of pheromone trapping for monitoring *P. aubei* is not currently possible, because *P. aubei* pheromones have yet to be identified [23]. At present, *P. aubei* populations can be monitored by the debarking of symptomatic juniper and thuja trees in the field. Such field monitoring can be conducted throughout the year, because *P. aubei* adults and larvae are present under the bark throughout the year [19,34].

Expansion of either the true range or the outbreak range is observed in several model species/groups of major insect guilds in boreal and temperate biomes. Effects of climate change on forest insects are demonstrated for a number of species and guilds, although generalizations of results available so far are difficult because of species-specific responses to climate change. There is evidence that recent warmer temperatures have permitted the expansion of bark beetle outbreaks to higher latitudes and elevations than in the past [80].

Climate and weather can have direct effects on trees, as drought and storms can weaken trees and predispose them to attack by bark beetles and pathogens. Climate can also have direct effects on insects as they are small poikilotherms with limited thermoregulation ability [81]. *P. aubei* is a typical case of this pattern and may be followed by other species from Mediterranean countries. Climate change could also result in the spread of insect species from the opposite direction. *Ips duplicatus* (C.R. Sahlberg, 1836), a native bark beetle on Norway spruce in Scandinavia, currently is spreading to Central, Eastern, and Southern Europe. The outbreak is supported by the planting of spruce out of its original distribution, physiological weakness of the tree, and the consequent occurrence of fungal pathogens on host trees [82].

On the contrary, the spread direction of alien bark beetles in Europe could be different. Bark beetles mainly travel in wood and in wooden packing materials such as crating, dunnage, and pallets [83], so the places of introduction can be different. A North American species, *Gnathotrichus materiarius* (Fitch, 1858), and an East Asian species, *Xylosandrus germanus* (Blandford, 1894), currently occur in the Czech Republic, but they have been spreading from west to east from Germany [84].

5. Conclusions

The small cypress bark beetle has probably been present in the Czech Republic since the 1950s, and we have determined its current distribution on common juniper trees. The results indicate that *P. aubei* is widely distributed in the country but is more common in the east than in the west. The apparently widespread and abundant populations of *P. aubei* could explain infestations of the beetle on *Thuja* spp. and *Juniperus* spp. in gardens. We consider *P. aubei* to be a potential pest on older, naturally occurring *J. communis* in protected areas where its population density could increase on weakened and damaged trees.

Author Contributions: Data curation, T.F. and J.H.; Formal analysis, J.H.; Methodology, T.F.; Writing—original draft, T.F. and J.H.; Writing—review and editing, T.F. and J.H.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Details of localities in the Czech Republic where *P. aubei* was detected in the current study (2018) and in earlier studies.

Locality	Coordinates	Year of Detection/ Collection	Source	Host Tree	Detail of Collections (Dimension of Trees and Management of Localities)
Čejč	48.9443903N, 16.9784694E	unknown	National Museum Prague	Unknown	collection of Jaroslav Picka
Drahoňův Újezd	49.8775892N, 13.7380344E	2018	T.Fiala	*Juniperus communis*	on 3-cm-d stems in a managed locality, 6 adults
Horní Jelení	50.0603294N, 16.0870206E	1947	V.Týr	Unknown	
Horní Němčí	48.9209667N, 17.6396400E	2018	T.Fiala	*J. communis*	on 5-cm-d stems in a managed locality, 3 adults
Horní Pole	49.2055956N, 15.2880703E	2018	L.Skořepa	*J. communis*	
Hutisko-Solanec	49.4359167N, 18.2234289E	2018	T.Fiala	*J. communis*	on 3-cm-d stems in a managed locality, 1 adult
Klatovec	49.2176503N, 15.3003911E	2009	[85]	*J. communis*	
Kunžak	49.1225056N, 15.1698425E	2009, 2018	[86], T.Fiala	*J. communis*	on 5-cm-d stems in an overgrown locality, 2 adults
Lednice	48.8028108N, 16.8508247E	2006	[45]	unknown	window trap on *Ulmus* spp.
Mariánské Lázně	49.9489864N, 12.7076350E	2017	T.Fiala	*J. communis*	on 4-cm-d stems of dead trees in a garden
Matějovec	49.0640136N, 15.2587572E	2018	L.Skořepa	*J. communis*	
Mikulov	48.8063058N, 16.6477686E	1996	[55]	unknown	
Mohelno	49.1153328N, 16.1991564E	2018	[45]	*J. communis*	
Nedašov	49.1015664N, 18.0897692E	2018	T.Fiala	*J. communis*	on 5-cm-d stems in a managed locality, 2 adults
Nový Svět	49.0820736N, 15.2574772E	2011,2018	[45], L.Skořepa	*J. communis*	
Odolenov	49.2413858N, 13.4837028E	2018	T.Fiala	*J. communis*	on 5-cm-d stems in a managed locality, 1 adult
Olší	49.1620167N, 15.3748231E	2010	[87]	*J. communis*	
Písek	49.3011936N, 14.1422844E	2017	D.Čudan	*Thuja* spp.	abundantly infested dead trees in a garden
Prostiboř	49.6638825N, 12.9095417E	2018	T.Fiala	*J. communis*	on 5-cm-d stems in a managed locality, 2 adults
Rajchéřov	48.9623267N, 15.1974642E	2009,2018	[88], T.Fiala	*J. communis*	on 3-cm-d stems in an overgrown locality, 1 adult
Valašské Klobouky	49.1395381N, 18.0633761E	2018	T.Fiala	*J. communis*	on 4-cm-d stems in a managed locality, 1 adult
Valtínov	49.0978514N, 15.2517989E	2009,2018	[89], T.Fiala	*J. communis*	on 3-cm-d stems in an overgrown locality, 2 adults
Záborná Lhota	49.7680669N, 14.3120808E	2018	T.Fiala	*J. communis*	on 3-cm-d stems in an overgrown locality, 5 adults
Zděchov	49.2726797N, 18.1021503E	2018	T.Fiala	*J. communis*	on 5-cm-d stems in a managed locality, 2 adults

Coordinates of localities without *P. aubei* occurrence: Bezděkov (49.7843667N, 12.7571769E), Doubravka (49.5852244N, 12.7634050E), Čečín (49.5915397N, 12.7508308E), Černýšovice (49.3151389N, 14.5270231E), Domanín (48.9584072N, 14.7393708E), Nechalov (49.7270689N, 14.2398522E), Vrbička (50.1882300N, 13.2770272E), Úhošť (50.3576158N, 13.2384503E), Ústí (49.8126472N, 12.7664183E), Zdebořice (49.3679767N, 13.4133169E), Žebrák (49.8847200N, 13.9121225E).

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10.4 Distribution of the invasive ambrosia beetle *Xyleborinus attenuatus* Blandford, 1894 (Coleoptera: Curculionidae: Scolytinae) in the Czech Republic (Central Europe).

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REVIEW PAPER

Distribution of the invasive ambrosia beetle *Xyleborinus attenuatus* Blandford, 1894 (Coleoptera: Curculionidae: Scolytinae) in the Czech Republic (Central Europe)

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Abstract

In Europe, *Xyleborinus attenuatus* Blandford is considered an invasive bark beetle native to East Asia. We used the results of many local surveys and data from private and museum collections to get information on the distribution and abundance of *X. attenuatus* Blandford in the Czech Republic. The ambrosia beetle *X. attenuatus* Blandford is probably widespread throughout the territory of the Czech Republic, from the lowlands to the mountains, and has one generation per year in Central Europe. The flight activity lasts from mid-March to May, F1 adults emerge in August and September and overwinter in wood or in the ground under an infested tree. *X. attenuatus* Blandford predominantly occupies soft deciduous trees and so far there economic damage have not been reported yet. However, *X. attenuatus* Blandford is a vector of ambrosia fungi *Ambrosiella* spp. and *Raffaelea* spp., and therefore this ambrosia beetle should be monitored.

Key words: ethanol; first record; flight actvity; window trap; *Xyleborinus saxesenii* Ratzeburg

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1. Introduction

Bark beetles are important economic pests of forest stands. They play an important ecological role in the disturbance of forests (Hlásny et al. 2021). They also introduce various types of fungi, bacteria, and yeast into the wood (Kirisits 2004; Kolařík et al. 2008; García-Fraile 2018), which slow down the decomposition of dead matter (Skelton et al. 2019). Bark beetles as pests can be divided into primary pests such as *Ips typographus* Linnaeus, 1758, which have the potential to attack and kill even healthy trees (Hlásny et al. 2021), or secondary pests such as ambrosia beetles (Peng et al. 2022), reproduce rapidly under exceptional conditions such as the presence of sufficient suitable material (presence of felled, dying or weakened trees). During outbreaks, populations can cause local mortality of host trees (Holuša et al. 2019).

Xyleborinus attenuatus Blandford, 1894 (Fig. 1) is a non-native bark beetle in Europe, considered native to East Asia (Smith et al. 2020). In Europe, *X.attenuatus* Blandford was first reported in the Czech Republic and Slovakia in 1987 (Knížek 1988), but in fact, the first evidence came from Spain in 1960 (Lombardero 1998). This discrepancy is because *X. attenuatus* Blandford is very similar to *Xyleborinus saxesenii* Ratzeburg, 1837, and is often misidentified (Lombardero 1998; Mokrzycki et al. 2011; Kvamme et al. 2020). The difference between the females of *X. attenuatus* Blandford and *X. saxesenii* Ratzeburg, recorded more frequently in both species, is the size and shape of the tubercles on the elytral declivity. *X. attenuatus* Blandford is the larger species at 2.5–2.8 mm and has sharper and more curved tubercles, whereas *X. saxesenii* Ratzeburg is smaller at 2.0–2.5 mm and the tubercles are smaller and blunter. Males are flightless and rarely leave galleries and hence are exceptionally encountered. After the determination was clarified, *X. attenuatus* Blandford was gradually found in almost all countries in Europe, in addition to the USA and Canada, and findings from five countries came before 1987 (Tab. 1, Fig. 2). *X. attenuatus* Blandford is a vector of *Ambrosiella* spp. (Nakashima et al. 1992) and Raffaelea spp. (Gharabigloozare 2015), but the economic damages have not yet been identified.

The aim of the work was to summarize the distribution of *X. attenuatus* Blandford in the Czech Republic. Due to its distribution throughout Europe (Tab. 1) and development in a wide range of tree species (Kvamme et al. 2020), we assume that it occurs throughout the studied area.

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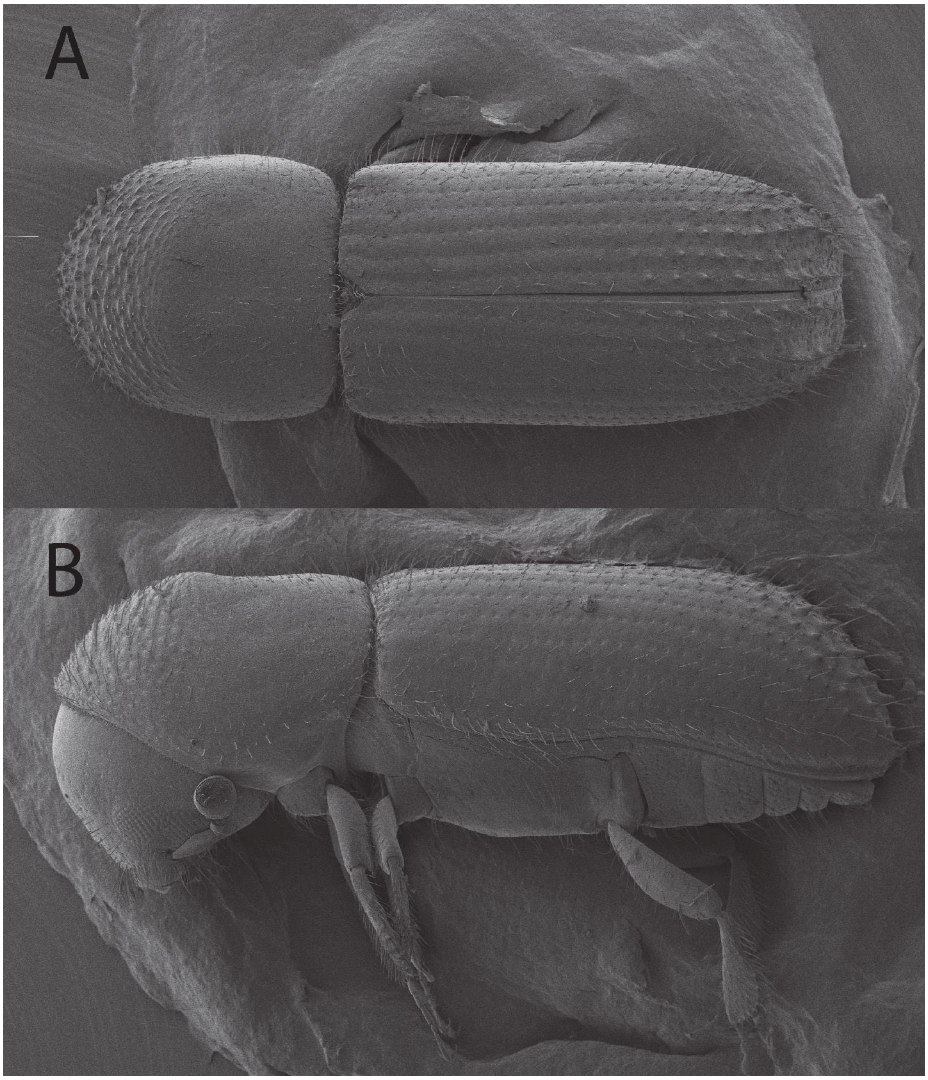

Fig. 1. Female of *Xyleborinus attenuatus* Blandford in dorsal view (a) and lateral view (b).

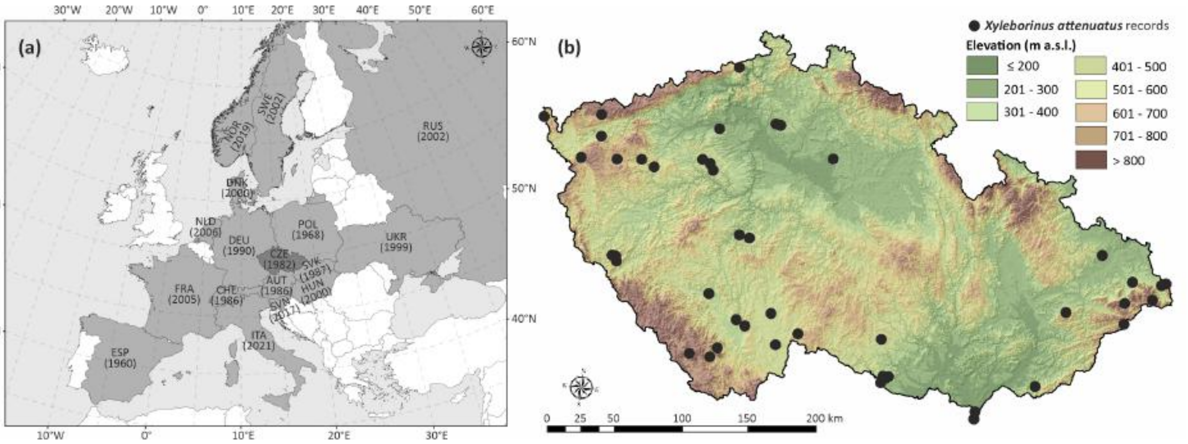

Fig. 2. Known distribution of *Xyleborinus attenuatus* Blandford in European countries with the year of detection and localities in the Czech Republic.

Table 1. First detection of *Xyleborinus attenuatus* Blandford in European and North American countries. Changes since the last list of first detection published by Kvamme et al. (2020) are highlighted in red.

Year	Country	Source
1960	Spain	Lombardero 1998
1968	Poland	Mokrzycki et al. 2011
1982	Czech Republic	this study
1986	Austria	Holzschuh 1995
1986	Switzerland	Sanchez et al. 2020
1987	Slovakia	Knížek 1988
1990	Germany	Gebhardt 2002
1995	Canada	Mudge et al. 2001
1996	USA	Haack 2006
1999	Ukraine	Martynov & Nikulina 2016
2000	Denmark	Pedersen et al. 2010
2000	Hungary	Podlussány 2001
2002	Russia (EU part)	Nikulina et al. 2007
2002	Sweden	Kvamme et al. 2020
2005–2011	France	Freeman & Grancher 2014
2006	Netherlands	Vorst et al. 2008
2017	Slovenia	Hauptman et al. 2019
2019	Norway	Kvamme et al. 2020
2021	Italy	Marchioro et al. 2022

2. Material and methods

As no nationwide monitoring has been carried out yet, we used the results of many local surveys and data from private and museum collections (Tab. 2) to gain information on the distribution and abundance of *X. attenuatus* Blandford in the Czech Republic. For the overview, we have selected finds that are reliably determined, i.e., we caught them ourselves, we revised the material, or the authors determined specimens according by the Pfeffer's key (1989).

We present sampling methods, although they are not known in all cases. Especially for data from literature and museum specimens, we do not have details about collection methods. Non-baited traps (window trap and light trap) or traps baited with ethanol or specific lures for economically important bark beetles were used. Some entomologists extract beetles directly from the galleries in the wood of the host trees. A few individuals were caught directly in flight using an entomological net or were found by sifting the litter. Material sampled by T. Fiala was preserved as dry. Samples were mounted on specimen stubs, sputter-coated with gold in the Sputter Coater JFC-1300 (JEOL, Tokyo, Japan) under argon atmosphere, and examined by scanning electron microscopy using a JEOL JSM-IT500HR instrument (JEOL, Tokyo, Japan) operating at 3 kV.

3. Results

In the Czech Republic, the occurrence of *X. attenuatus* Blandford has been confirmed in 46 localities (Fig. 2), in all cases found beetles were females (Tab. 2). The known localities are mainly located in the southern and central parts of the Czech Republic. The species was found at altitudes from 150 to 1,200 m above sea level, but most findings come from altitudes between 400 and 600 m above sea level. Although we do not know sampling methods in published data, the analysis of known trapping methods shows that most individuals were caught in traps lured by ethanol (7 cases), window trap (7 cases), and entomological nets in flight (6 cases). Catching in a light trap and using a study of litter is unusual (Tab. 2).

In total, *X. attenuatus* Blandford was recorded in seven tree species (*Alnus glutinosa* [L.] Gaertn, *Alnus incana* [L.] Moench, *Betula pendula* Roth, *Populus tremulae* L., *Quercus* sp., *Salix caprea* L., *Salix viminalis* L.) if the individuals were obtained by extracting from the host tree. Flying beetles captured in the traps or by entomological net were found from mid-March to mid-May, with exceptional captures in June (Tab. 2).

4. Discussion

Although *X. attenuatus* Blandford was first reported in the Czech Republic in 1987 (Knížek 1988) we have found one beetle collected in 1982 in Drahov in southern Bohemia. Similarly, beetles collected earlier than this species was reported were also found in Poland and Switzerland (Kvamme et al. 2020). Up to now occurrence in Hungary, Slovenia, and Italy (Tab. 1) has not been listed (see Kvamme et al. 2020).

Known localities of *X. attenuatus* Blandford are predominantly distributed in the southern and central parts of the Czech Republic (Fig. 2), but the ecological requirements of the species meet a wide range of habitats throughout the territory of the Czech Republic. Also, the altitude of the sites with *X. attenuatus* Blandford presence represents almost the entire altitudinal gradient of the Czech Republic; so, *X. attenuatus* Blandford occurs from the lowest positions in the Pannonian basin to the highest sites of the Carpathian Mountains. It can be assumed that the species is distributed throughout the Czech Republic, but its abundance is probably low because in all cases only several beetles were collected.

We have confirmed almost all known host tree species of *X. attenuatus* Blandford in the Czech Republic. This confirms, that *Xyleborinus attenuatus* Blandford attacks mainly deciduous trees and prefers lightweight hardwood trees such as *Salix*, *Betula*, *Alnus*, *Populus*, less often it is also found on *Quercus* or *Fagus* (Kvamme et al. 2020). Among conifers, this ambrosia beetle was found on *Pinus resinosa* Aiton (Dodds et al. 2017) and *Pinus armandi* Franch. (Smith et al. 2020) but we have no evidence of the occurrence of *X. attenuatus* Blandford on conifers in the Czech Republic.

Table 2. Records of *Xyleborinus attenuatus* Blandford in the Czech Republic with a localities, date and sampling methods. Localities are villages or protected areas. Data were compiled from published literature, as well from private and museum collections (JCM – Jihočeské muzeum, České Budějovice; NNR – National Nature Reserve; NR – Nature Reserve).

Locality	Coordinates	Altitude [m a.s.l.]	Date of sampling	Number of specimens	Sampling method	Host tree	Source
Bystřice nad Olší	49.6400N, 18.7358E	400	30.III.2002	1	In flight		R. Szopa
Bystřice nad Olší	49.6400N, 18.7358E	400	11.IV.2008	1	In flight		R. Szopa
Cahnov NNR	48.6551N, 16.9413E	150	?? 2007		Window trap		Prochazka 2011
Chotýčany	49.0683N, 14.5134E	500	31.VI.1992	1	Unknown		JCM
Chramosty	49.6696N, 14.3294E	500	23.III.2005	4	Unknown		A. Trmal
Chramosty	49.6696N, 14.3294E	500	28.IV.2005	3	Unknown		A. Trmal
Číměř	49.0627N, 15.0612E	550	30.VIII.1987	8	Dissection	*Salix viminalis* L., *S. caprea* L.	Knížek 1988
Číměř	49.0627N, 15.0612E	550	5.IX.1987	44	Dissection	*Salix viminalis* L., *S. caprea* L.	Knížek 1988
Drahov	49.1774N, 14.7638E	440	13.VI.1982	1	Unknown		JCM
Dymokury	50.2568N, 15.2034E	200	23.V.1988	5	Dissection	*Quercus* sp.	Knížek 1988
Hluboká nad Vltavou	49.1005N, 14.4180E	430	20.III.2010	1	Sowing	willow chips	F. Houška
Hnanice	48.8040N, 15.9633E	350	?	1	Unknown		R. Stejskal
Jaroměřice nad Rokytnou	49.0933N, 15.9157E	450	1997		Unknown		Knížek & Zahradník 1999
Jílové – Sněžník	50.7835N, 14.0838E	600	5.V.2022	1	Light trap		E. Kula
Kelčský Javorník NR	49.4039N, 17.7714E	700	10.V.2021	1	Window trap		O. Konvička
Klimkovice	49.8082N, 18.0963E	370	15.IV.2013	1	In flight		J. Vávra
Lány – Amerika	50.1154N, 13.9264E	450	16.VI.2015	1	Unknown	*Quercus* sp.	Januš 2016
Lány – Běleč	50.0731N, 13.9657E	450	12.V.2002	2	Unknown		Rébl 2010
Lhota u Kestřan	49.2527N, 14.0979E	380	27.III.2011	1	Leaf litter		F. Houška
Loket	50.1875N, 12.7592E	400	1.V.2019	1	Lure trap	Ethanol	Holuša et al. 2021
Loučej	48.9013N, 14.2657E	580	10.IV.2018	2	In flight		D. Čudan
Loučim	49.3769N, 13.1123E	650	7.V.2021	1	Trap lured with ethanol + peace of spruce bark		M. Kacerovský
Majdalena	48.9700N, 14.8487E	450	22.IV.1993	3	Unknown		JCM
Mašovice	48.8465N, 15.9773E	370	3.IV.2018	1	Dissection	*Populus tremulae* L.	R. Stejskal
Mionší NNR	49.5335N, 18.6511E	800	2008	1	Window trap		Procházka et al. 2014
Nejdek	50.3322N, 12.7252E	620	7.IV.2020	1	Unknown		Benedikt et al. 2021
Nižní Lhoty	49.6462N, 18.4309E	350	19.III.2004	6	In flight		R. Szopa
Nový Herštejn	49.4089N, 13.0636E	650	7.V.2021	1	Trap lured with ethanol + peace of spruce bark		M. Kacerovský
Nýdek	49.6551N, 18.7808E	500	20.IV.2004	1	In flight		R. Szopa
Peruc	50.3549N, 13.9686E	310	2018	2	Trap lured with Trypowit®		Fiala & Holuša 2020
Podvoří	48.8323N, 14.2051E	580	2019	More ex.	Dissection	*Betula pendula* Roth	D. Čudan
Popice	48.8361N, 16.0078E	340	?	1	Unknown		R. Stejskal
Ranšpurk NNR	48.6786N, 16.9468E	150	2007	22	Window trap		Procházka 2011
Razula NNR	49.3599N, 18.3824E	750	2008	1	Window trap		Procházka et al. 2014
Ruda – Dolní Kracle	50.1373N, 13.8522E	400	21.IV.2002	More ex.	Dissection	*Alnus glutinosa* [L.] Gaertn	Januš 2016
Sedlčany	49.6544N, 14.4399E	350	24.III.2001	1	Unknown		Trmal 2008
Senec u Mšena	50.4360N, 14.6070E	350	9.IV.2021	1	Trap lured with XL Ecolure®		K. Resnerová
Slunečná	48.8327N, 13.9885E	850	29.IV.2018	1	Dissection	*Alnus incana* [L.] Moench	T. Fiala
Smrk NR	49.5000N, 18.3666E	1200	2008	1	Window trap		Procházka et al. 2014
Soutok	48.6352N, 16.9411E	150	IV.-V.2012	1	Unknown		J. Procházka
Štítary	50.2555N, 12.1286E	680	6.VI.2010	1	Unknown		S. Benedikt
Toužim	50.0518N, 12.9620E	640	26.V.2018	1	Dissection	*Alnus incana* [L.] Moench	Tájková et al. 2018
Truskavna	50.4390N, 14.5550E	250	9.IV.2021	1	Trap lured with XL Ecolure®		K. Resnerová
Úbočí u Dolního Žandova	50.0260N, 12.5860E	750	6.V.2022	3	Trap lured with Ethanol		T. Fiala
Úsilov	49.4045N, 13.0987E	600	6.V.2022	1	Trap lured with Ethanol		T. Fiala
Vladař NR	50.0756N, 13.2081E	610	2.V.2020	9	Trap lured with Ethanol		Holuša et al. 2021
Zahrady pod Hájem NNR	48.8861N, 17.5351E	400	2015	1	Window trap		J. Procházka
Znojmo	48.8436N, 16.0231E	340	2.IV.2009	1	Unknown		R. Stejskal
Žihle	50.0398N, 13.3520E	520	6.V.2022	3	Trap lured with Ethanol		T. Fiala

Flight activity of *X. attenuatus* Blandford begins early in spring. Based on our data it lasts from mid-March to May, and the single peak of the seasonal distribution of our data is the result of only one generation per year. This corresponds to the ecology of other species of the tribe Xyleborini in Central Europe. And in North America (Missouri), the flight period of *X. saxesenii* begins at the end of April or the beginning of May (Roling & Kearby 1975); although probably depending on the temperatures, *X. saxesenii* can have two periods of flight activity per year (Rauleder 2003). But other species like the ambrosia beetles *Xyleborus monographus* Fabricius, 1792, and *Xyleborus dryographus* Ratzeburg, 1837 also have one generation per year. *Anisandrus dispar* Fabricius, 1792 also has one period of flight activity within a year, but it is longer and does not have such a clear peak (Markalas & Kalapanida 1997; Salmane et al. 2015; Fiala 2019). Adults of *X. attenuatus* Blandford emerge probably in August and September because at that time they are cut from the wood; they overwinter in wood or in the ground under an infested tree, as evidenced by findings in the litter.

Since only a few individuals were caught at each locality, even if they were caught during the whole season, it is not possible to conclude which trapping method or bait is the most suitable for *X. attenuatus* Blandford detection. It is logical that many ambrosia species is attracted to ethanol (Kelsey 1994), but it is also accidentally caught in a window trap. This also complicates the creation and use of any systematic monitoring of *X. attenuates* Blandford.

We have found only females because the ratio between the sexes is biased. Females are plenty, but the males are sparse. Usually, 1 to 3 males and up to approximately 50 females emerged in one breeding gallery (Kvamme et al. 2020). Males of *X. attenuatus* Blandford are flightless, dwarf, and mostly remain in galleries throughout their lives, while females fly well (Kirkendall 1983). Therefore, only females are caught in traps.

The distribution of *X. attenuatus* Blandford in Europe is poorly defined, due to misidentification with the ambrosia beetle *X. saxesenii* (Lombardero 1998; Mokrzycki et al. 2011; Kvamme et al. 2020). Already the author of the description Blandford (1894), who described the species as *X. attenuatus* Blandford and described it from a single female from Japan, writes that the species is very similar to *X. saxesenii* Ratzeburg. The first finding in Europe comes from Spain in 1960 (Lombardero 1998) and another from Poland in 1968 (Mokrzycki et al. 2011). The first beetles in the Czech Republic were detected in 1982 (this study). These findings in different states that are thousands of kilometres apart may indicate that *X. attenuatus* Blandford is a native species with a wide Palaearctic distribution and is only invasive in North America. Similar distances in distribution between sites (hundreds of kilometres) are known for the long-horned beetle *Gerania bosci bosci* Fabricius (Hawkeswood et al. 2021). Of course, we cannot rule out that *X. attenuatus* Blandford was introduced into Europe repeatedly within a short time and spread rapidly from several sources, as demonstrated in *Xylosandrus germanus* Blandford, 1894 in the USA (Dzurenko et al. 2021). These questions can only be solved by genetic analysis of European and Asian individuals, but obtaining more material is almost difficult, as there is no simple trapping method.

5. Conclusions

The ambrosia beetle *X. attenuatus* Blandford is probably widespread throughout the territory of the Czech Republic, from the lowlands to the mountains. We assumed this because the Czech Republic lies within the entire area of this ambrosia beetle. The available data suggest that this extensive area proves the origin of the species in wide Palaearctic region, rather than the fact that it is an invasive species on the territory of Europe. It is unlikely that it would occupy the entire continent evenly within 40 years from the first detection in Europe. Any impact traps and/or ethanol-baited traps can be used to detect this species, but it cannot be expected that the number of beetles caught will be high due to an apparently low population density.

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10.5 *Xylosandrus germanus* in Central Europe: Spread into and within the Czech Republic.

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REVIEW ARTICLE

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Xylosandrus germanus in Central Europe: Spread into and within the Czech Republic

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Abstract

Invasive organisms represent great threats to ecosystems and great challenges to forest management. In Europe, the black timber bark beetle (*Xylosandrus germanus*) is an invasive secondary pest that mostly attacks the logs of felled trees. We showed the invasion history for Europe and using many local surveys, we summarize the current distribution and other available information on *X. germanus* in the Czech Republic. We report that this species is distributed from the lowlands to the mountains in the Czech Republic; it is widespread in the eastern half of the country, where it is more abundant in the warmer south and southeast areas than in the cooler areas. Most (78%) of the known localities are at elevation below 400 m a.s.l. Although an ice storm greatly increased *X. germanus* abundance near the border with Austria, its high abundance did not result in damage to standing trees. Presence of *X. germanus* in the Czech Republic for over 10 years has not led to heavy tree infestation.

KEYWORDS

biological invasion, black timber bark beetle, Central Europe, spread

1 | INTRODUCTION

Invasive organisms represent significant threats to ecosystems and substantial challenges to forest management (Evans & Oszako, 2007; Pimentel, Zuniga, & Morrison, 2005). Invasive species of bark beetles are especially important in forest ecosystems (Kirkendall & Faccoli, 2010). Although 28 non-native bark beetles are registered in Europe (Alonso-Zarazaga et al., 2017), we know relatively little about their spread and recent distribution. Among the invasive species, the black timber bark beetle *Xylosandrus germanus* (Blandford, 1894) may have a potential for causing damage (Björklund & Boberg, 2017; Galko et al., 2019).

Xylosandrus germanus is native in China, Japan, Korea, Taiwan, Thailand and Vietnam (Beaver & Liu, 2010). The species has been introduced multiple times to Europe and North America with the import of timber from eastern Asia (Alonso-Zarazaga et al., 2017; Wood & Bright, 1992). It is a widely polyphagous ambrosia beetle that develops in the wood of both broadleaf and coniferous trees (Weber & McPherson, 1983a); the beetle mainly attacks beech (*Fagus* spp.) and oak (*Quercus* spp.) (Maksymov, 1987), but also spruce (*Picea* spp.), pine (*Pinus* spp.) and fir (*Abies* spp.) (Graf & Manser, 2000). This bark beetle acts as an important vector of ophiostomatoid fungal pathogens of trees such as *Fusarium*, *Ambrosiella* and *Ophiostoma* (Batra, 1967; Buchanan, 1940; Ito & Kajimura, 2017; Weber & McPherson, 1985).

In Europe, *X. germanus* is a secondary pest that mostly colonizes the logs of felled trees (Bouget & Noblecourt, 2005; Heidenreich, 1964). It occasionally attacks healthy and newly transplanted trees and shrubs (Rabaglia, Dole, & Cognato, 2006). It is a successful invader in North America, where it has become a serious pest in tree nurseries and orchards (Oliver & Mannion, 2001; Ranger, Reding, Persad, & Herms, 2010; Ranger, Schultz, Frank, & Reding, 2019; Reding et al., 2015; Reding, Schultz, Ranger, & Oliver, 2011; Werle et al., 2019). In Turkey, it is one of the most significant pests in hazelnut orchards (Tuncer, Kushiyev, Erper, Ozdemir, & Saruhan, 2019), but it damaged also the kiwifruit trees (Ak, Saruhan, Tuncer, Akyol, & Kiliç, 2011). In Europe, in contrast, most published records concerning this species come from forests (Björklund & Boberg, 2017; Bussler et al., 2011; Gossner, Falck, & Weisser, 2019; Mokrzycki & Grodzki, 2014).

In Europe, *X. germanus* was first reported from Germany in 1951 (Groschke, 1952; Kamp, 1968; Wichmann, 1955). According to Wichmann (1957), this bark beetles was most probably introduced into Germany with oak timber from Japan during the years 1907–1914 and 1919–1929. In the 1950s, it had established in two large areas in the warmer part of Germany, the Rhine floristic province (Wichmann, 1957). The next record from another European country was in 1984 from Switzerland (Maksymov, 1987). During the last two decades of the 20th century, the beetle invaded most of the continent (Björklund & Boberg, 2017; Galko et al., 2019).

In the 1990s, *X. germanus* was recorded in Austria (Holzschuh, 1993) and Poland (Mokrzycki et al., 2011), which neighbour the Czech Republic (Figure 1). Locally, it became the dominant species in assemblages of bark and ambrosia beetles (Henin & Versteirt, 2004; Müller, Bußler, & Kneib, 2008; Wermelinger, Flückiger, Obrist, & Duelli, 2007; Zach, Topp, Kulfan, & Simon, 2001). It has become a serious forest pest in many countries regardless of whether it is native or exotic in those countries (e.g. Kaneko, 1965; Nobuchi, 1981; Weber & McPherson, 1983b, 1984; Grégoire, Piel, de Proft, & Gilbert, 2001). Many states in the United States, and many countries in Europe, and in Russia have therefore been monitoring its spread with the use of ethanol-baited traps or flight intercept traps (Bussler et al., 2011; Galko et al., 2014, 2019; Gossner et al., 2019; Hauptman, Pavlin, Grošelj, & Jurc, 2019; Henin & Versteirt, 2004; Oliver & Mannion, 2001; Ranger et al., 2010; Reding et al., 2011; Sweeney, Silk, Grebennikov, & Mandelshtam, 2016; Werle et al., 2019).

Although no countrywide monitoring of this insect has been performed, we used the results of many local surveys to gain information on the distribution and abundance of *X. germanus* in the Czech Republic and some neighbouring countries in Central Europe.

2 | OCCURRENCE IN THE CZECH REPUBLIC

Xylosandrus germanus was first recorded in the Czech Republic in 2007; the individuals were caught in flight in the central part of the country by another collector. No detail is known (Knížek, 2009). There were no further records until 2011, when several individuals were found in the Theysohn® traps baited with the pheromone lure ID Ecolure® for capture of *Ips duplicatus* (Sahlberg, 1836) in the eastern part of the Czech Republic (Knížek & Foit, 2012; Appendix 1). In the same year, this bark beetle was also recorded from the southern part of the country, near the border with Austria (Figure 1). Between 2012 and 2018, the species was recorded mainly in samples from various traps (mostly flight intercept traps) at more than 50 localities in closed-canopy and open forests scattered across the country; a total of 3,435 beetles were collected (Appendix 1).

In the Czech Republic, *X. germanus* occurs from the lowlands (150 m a.s.l.) to the mountains (935 m a.s.l.) (Figure 2). The elevation of the majority of known localities (78%) is <400 m a.s.l. (Figure 2). In most of the country, its abundance has been low, with fewer than one individual per trap and trapping season on average. In the lowlands of the southern and southeastern part of the country, however, more than 10 beetles per trap were collected (Figure 1).

The frequent detection of *X. germanus* at elevations below 400 m a. s. l. suggests that populations of the beetle have established at low elevations (Bruge, 1995; Henin & Versteirt, 2004; Holzschuh, 1993). The abundance of the beetle in higher elevation will probably increase because the optimum conditions for the species are at approximately 500 and 700 m a.s.l. (Galko et al., 2019). The relatively high elevations where it was detected on Stožec Mt. in the Czech Republic (935 m a.s.l.), in the Eastern Carpathians of Romania (760–900 m a.s.l.) (Olenici, Knížek, Olenici, Duduman, & Birş, 2014) and in Slovenia (941 m a.s.l.) (Hauptman et al., 2019) appear to be lower than those with the highest known occurrence of the species from the West Carpathians (1,020 m a.s.l.) (Galko et al., 2019).

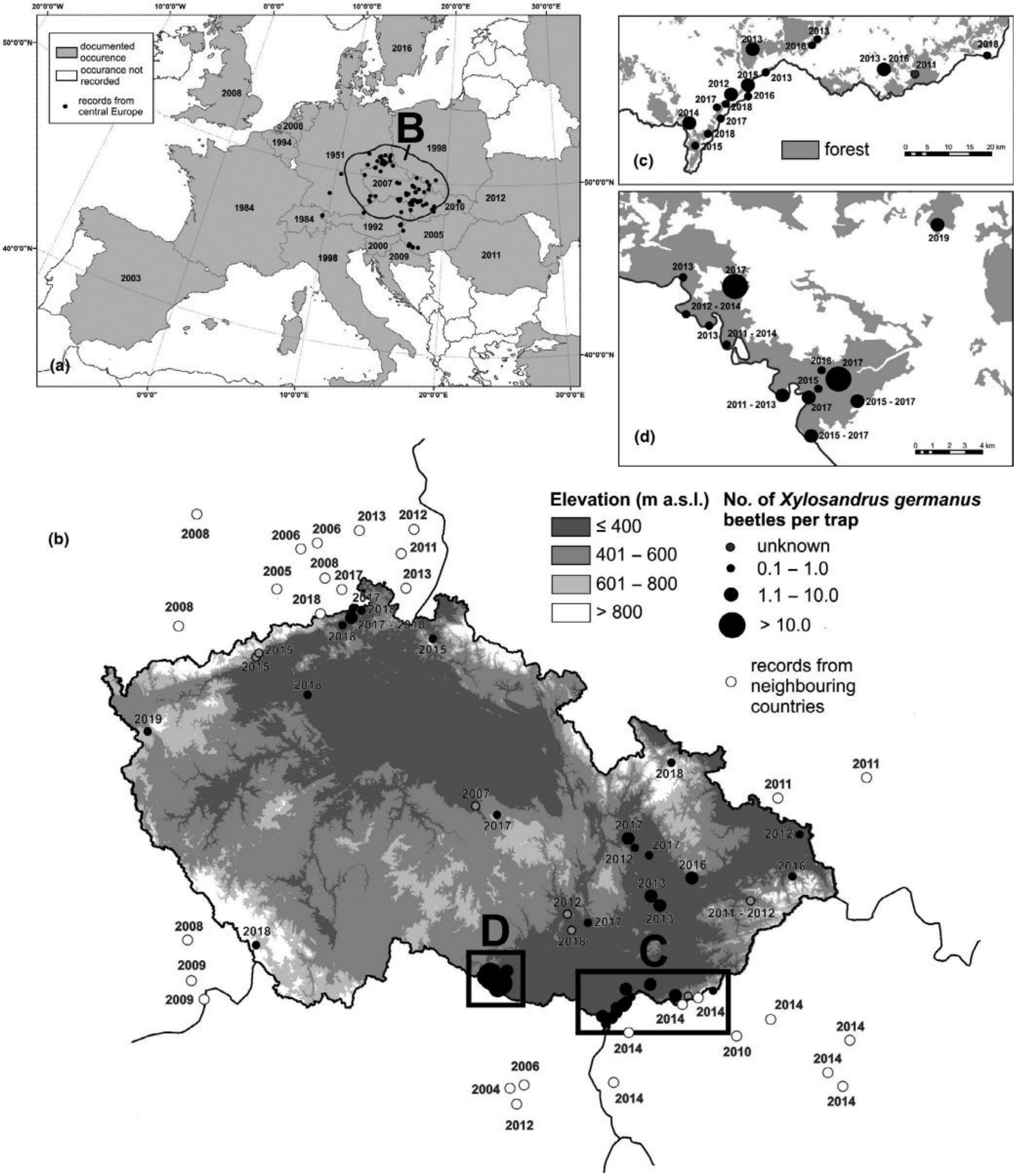

FIGURE 1 Known occurrence of *Xylosandrus germanus* (a) in Europe with year of first record and known localities in Central Europe (Line—buffer of 100 km from the Czech borders), and occurrence in the Czech Republic and adjacent areas with collection year (b–d) (grey colour in c and d map details indicate forests)

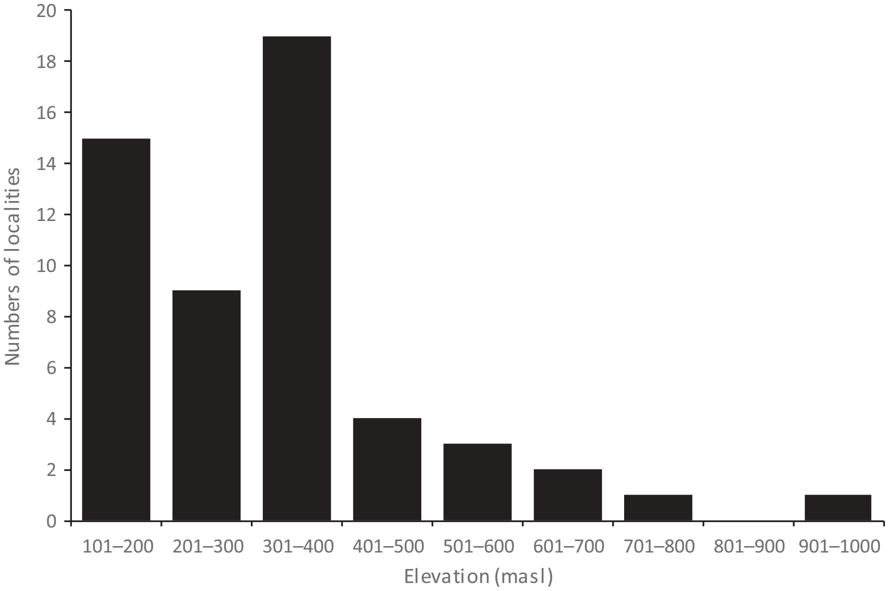

FIGURE 2 Elevational distribution of *Xylosandrus germanus* in the Czech Republic from 2007 to 2019
Our data show that *X. germanus* is distributed from the lowlands to the mountains in the Czech Republic, that it is widespread in the eastern half of the country (Moravia), and that it is more abundant in warmer areas (in South and Southeast) than in cooler ones. Available information on the distribution of this species in the Czech Republic, however, is not based on surveys from all regions. Records of the species are much scarcer from the western half (Bohemia) than from the eastern half of the Czech Republic (Moravia), even though the first records in the country were from the western half.

3 | SPREAD INTO THE CZECH REPUBLIC

The recent spreading of *X. germanus* in Central Europe might be facilitated by environmental changes and especially by increasing temperatures, but also by timber trade, which has been increasing (Galko et al., 2019). Its presence in Bohemia may have been overlooked for several years. We cannot exclude the possibility that the species was introduced to the first recorded locality in Bohemia in 2007 with timber or wood products.

The higher abundance of *X. germanus* in the southeast than in the other parts of the Czech Republic suggests that it may be spreading from the south, possibly from Austria, where it has been known from the Vienna area since at least the 2000s (Figure 1; Appendix 2). In addition, it had not been detected in southern Moravia (near Austria and Slovakia) as of 2007 (Appendix 1: locality of Ranšpurk). The first record of the species in southern Moravia could correspond with its first detection in Slovakia in 2010 (Galko, 2013; Galko et al., 2019). In Slovakia, it was found to be spreading from the south-southwest from Hungary (Lakatos & Kajimura, 2007; see Figure 1; Appendix 2) to the north-northeast and also from a low to high elevations over a period of 5–10 years (Galko et al., 2019).

The first record of *X. germanus* in the Czech Republic, however, came from the central part of the country in 2007 (Knížek, 2009). Hence, the species might also spread into the Czech Republic via Germany and northern Bohemia. *X. germanus* has been present in the Saxony region of Germany from the 2000s (Reike & Sobczyk, 2007). Since 2010, it has been reported from additional localities in Saxony and also from the adjacent area of the Czech Republic, that is northern Bohemia since the 2010s (Figure 1; Appendix 2).

Similarly, the detection of the species in the southern Bohemian locality of Stožec in 2018 could result from it spreads from Bavaria (southeastern Germany), where it was recorded in the late 2000s (Blaschke & Bussler, 2012; Appendix 2; Figure 1).

In southern Poland near its border with the Czech Republic, this bark beetle was not found until 2011 (Mokrzycki et al., 2011). In the following year, however, the species was found in northern Moravia, near the border with Poland (Appendix 1; Figure 1).

Although *X. germanus* may have been initially introduced into the Czech Republic with importation of timber, since 2010 the species has spread to the Czech Republic from all surrounding countries.

4 | ABUNDANCE IN THE CZECH REPUBLIC

Thousands of *X. germanus* individuals were recently detected in ethanol-baited traps in Slovakia (Galko et al., 2019), Austria (Holzinger, Frieß, Holzer, & Mehlmauer, 2014) and Hungary (Lakatos unpublished). Either this insect is indeed more abundant in the neighbouring countries than in the Czech Republic, or the difference is to be attributed to the widespread use of unbaited traps in the Czech Republic. Some of our records, however, come also from bark beetle pheromone traps that also contained alcohols (ID Ecolure contains 96% alcohols; Fytofarm, 2018).

We also collected much fewer *X. germanus* individuals in the southeast of the Czech Republic, even in traps with 85% ethanol (localities Strážnice, Ochoz u Brna), than in Slovakia where hundreds to thousands of individuals were collected using equal numbers of identical flight interception traps baited with 85% ethanol of the same concentration (E. Kula, J. Kulfan and P. Zach, unpublished data). It is possible that its abundance has yet to increase in the Czech Republic, because the captures have been increasing steadily in Slovakia (West Carpathians) since its first record in 2010 (Galko et al., 2019). High numbers in Slovakia might indicate high long-term availability of suitable breeding substrates as well as the ability of the beetle to colonize a variety of forest habitats. In southern Moravia, the presence of numerous broken trees also increased the abundance of the beetle (see also Hauptman et al., 2019).

Tunnelling females produce a distinctive symptom resulting from the production of defensive sap by the tree. This symptom, which consists of boring dust “toothpicks” that project from the stems and branches of trees, has not been observed in the Czech Republic. Several old branches with galleries and dead specimens of *X. germanus* were found at one locality (Zahrady pod Hájem, Appendix 1). Only 11 individuals of *X. germanus* were reared from experimental trap logs (*Juglans nigra* L.) (Jeřábek, 2015, Appendix 1). Near the border with Austria, hundreds of individuals were collected in a hornbeam-lime forest that had been damaged by an ice storm (localities Čížov and Podmolí, Appendix 1).

It is difficult to find signs of its presence even in forests where the species is abundant (Hauptman et al., 2019), but using thin tree parts to record the beetle can help (Zach et al., 2001). The high abundance of the species in the ice-storm damaged area as a result of many broken trees did not lead to damage to standing trees (Hauptman et al., 2019). Presence of *X. germanus* in the Czech Republic for over 10 years has not led to heavy tree infestation. A high abundance does not cause substantial damage because the species exploits thinner dead branches and dead trunks that are approximately 10 cm in diameter (Maksymov, 1987; Ranger et al., 2016; Weber & McPherson, 1983b); it rarely attacks trunks with diameters greater than 10 cm (Reed, Juzwik, English, & Ginzel, 2015).

5 | CONCLUSION

Xylosandrus germanus has been introduced multiple times to Europe and North America with the import of timber from eastern Asia. In the 1950s, it established in Germany. During the last two decades of the 20th century, the beetle invaded most of the continent. In the 1990s, *X. germanus* was recorded in Austria and Poland, which neighbour the Czech Republic. *X. germanus* was first recorded in the Czech Republic in 2000s but the origin of this introduction is unknown. We cannot exclude the possibility that the species was introduced with timber or wood products.

In the 2010s, the species spread into the Czech Republic from all surrounding countries, from Saxony to northern Bohemia, from Bavaria to southern Bohemia, from southern Poland to northern Moravia, and from Austria and Slovakia to southern Moravia. Nowadays, this species has been distributed from the lowlands to the mountains in the Czech Republic. It is very likely that its abundance will increase.

The presence of *X. germanus* for over 10 years has not led to heavy tree infestation in forests. Because the species exploits thinner branches and trunks, we cannot exclude future infestation of young trees in plantations.

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CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

AUTHOR CONTRIBUTIONS

TF and JH conceived the research. TF, JH, JP, LČ, MD, JF, JG, JKašak, JKulfan, FL, ON, JS, PZach and PZábranský provided insect material. MS and JT produced the map. JH, TF and JP wrote the manuscript and all authors read, edited, and approved the manuscript.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in Appendix 1 and Appendix 2.

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APPENDIX 1

Records of *Xylosandrus germanus* in the Czech Republic from 2007 to 2019. Localities disturbed by ice storms are in bold and italicized, and localities with repeated sampling are in bold without italics. NR = Nature Reserve, NM = Natural Monument, NNR = National Nature Reserve. Most records came from flight intercept traps that were deployed for most of the growing season, usually from April or May to August or September. All individuals of *X. germanus* reported in this research were identified by (or identifications were confirmed by) at least one of the following experts: J. Procházka, J. Foit, T. Fiala, J. Kadlec, E. Kula, P. Zach, J. Kašák and M. Knížek. Data also include published and unpublished documents that were not peer-reviewed such as such as monitoring reports, research reports and diploma theses.

Locality	Coordinates	Year of Detection/ Collection	Source	Lure/trap	Adults	Cumulative number of traps
Běstvina	49.8333333N, 15.5800000E	2007	Knížek (2009)	In flight	1	
Bynovec	50.8204253N, 14.2480478E	2017–2018	J. Kadlec, L. Blažej	Ground trap/ Malaise trap	16	5
Červený Hrádek NM	50.5139719N, 13.4430733E	2015	J. Kašák, J. Foit	On beech log	1	
Čížov	48.8758333N, 15.8861111E	2017	J. Procházka	Window trap	1,424	20
Děčín	50.7804931N, 14.1914931E	2018	J. Kadlec, L. Blažej	Ground trap	1	1
Doubrava NR	49.762013N, 16.986296E	2017	O. Nakládal, J. Procházka	Window trap	13	7
Gáliš	48.8428714N, 15.8857858E	2011–2014	L. Čížek, F. Trnka, M. Plátek, P. Šebek, P. Kozel, Š. Vodka, J. Procházka	Window trap	23	14
Hardegg	48.8541394N, 15.8699714E	2013	L. Čížek, M. Plátek, P. Šebek, P. Kozel, J. Procházka	Window trap	1	1
Hlubocká louka	48.8233653N, 15.9403956E	2011–2013	L. Čížek, F. Trnka, M. Plátek, P. Šebek, P. Kozel, J. Procházka	Window trap	6	3
Hluboké Mašůvky	48.9248700N, 16.0479689E	2019	D. Kopr, J. Kašák, J. Foit	Window trap	5	2
Hnanice	***48.8021650N, 15.9657231E***	***2015–2017***	***D. Kopr, J. Procházka, J. Schlaghamerský***	***Window trap***	***147***	***30***
Hodonín	48.847802N, 17.148252E	2013	L. Čížek, J. Procházka	Window trap	4	4
Hodonínská dúbrava NNM	48894248N, 17.099774E	2013	L. Čížek, J. Procházka	Window trap	9	3
Jasenový hrúd	48.7719231N, 17.0307936E	2018	J. Foit	Window trap	6	11
Jetřichovice	50.8808722N, 14.3455133E	2018	J. Kadlec, L. Blažej	Ground trap	1	1
Kamenný vrch NR	49.1831614N, 16.5517583E	2018	J. Procházka	In flight	1	
Kenický NR	49.6720928N, 17.1782225E	2017	Židů (2018)	Window trap	1	5
Kozinec	49.8714114N, 18.5004489E	2012	J. Vávra, J. Procházka	Window trap	1	1
Kuřim	49.2733333N, 16.4991667E	2012	Knížek and Foit (2012)	ID-Ecolure	7	Unknown

(Continues)

APPENDIX 1 (Continued)

Locality	Coordinates	Year of Detection/ Collection	Source	Lure/trap	Adults	Cumulative number of traps
Lanžhot	48.7249167N, 16.9211667E	2014	Jeřábek (2015)	TRAP log from *Juglans nigra*	11	2
Lázně Kynžvart	50.0052786N, 12.5966586E	2019	T.Fiala	ethanol	1	1
Liberec	50.7717753N, 15.0247606E	2015	Čtvrtečka (2015)	Window trap	2	4
Lipina	48.8242300N, 15.9622100E	2015	J. Horák, J. Procházka	Window trap	1	12
Lipina	48.8242300N, 15.9622100E	2017	T. Sitek, J. Procházka	Light trap	5	1
Malý Bojek	48.7049239N, 16.9830717E	2018	J. Foit	Window trap	1	11
Mašovice	48.8364992N, 15.9662219E	2018	T. Sitek, J. Procházka	Light trap	1	1
Obírka	49.5767511N, 17.5891339E	2016	J. Kašák, V. Holec	Window trap	37	2
Ochoz u Brna	49.2490778N, 16.7019944E	2017	E. Kula	Ethanol/window trap	13	36
Peruc	50.3549364N, 13.9686869E	2018	J. Holuša	Lineatin	1	10
Podmolí	48.8330556N, 15.9813889E	2017	J. Procházka	Window trap	400	30
Popice	***48.8227819N, 16.0001197E***	***2015–2017***	***D. Kopr, J. Procházka, J. Schlaghamerský***	***Window trap***	***126***	***30***
Ranšpurk NNR	**48.6766700N, 16.9466200E**	**2007**	**J. Procházka, J. Schlaghamerský**	**Window trap**	**0**	**30**
Ranšpurk NNR	**48.6766700N, 16.9466200E**	**2015**	**J. Horák, J. Procházka**	**Window trap**	**3**	**12**
Raškovice	49.6254150N, 18.4671728E	2016	J. Vávra, J. Procházka	Window trap	1	1
Rejvíz NNR	50.2180367N, 17.2950200E	2018	J. Procházka	Window trap	2	15
Rohozná	49.80846N, 15.79403E	2017	O. Nakládal, J. Procházka	Window trap	1	1
Rožnov pod Radhoštěm	49.4544444N, 18.1008333E	2011–2012	Knížek and Foit (2012)	ID-Ecolure	62	Unknown
Skařiny NR	48.8009092N, 17.0921797E	2012	L. Čížek, D. Hauck, Š. Vodka, J. Procházka	Window trap	3	3
Skařiny NR	48.8012197N, 17.0936431E	2016	J. Vávra, J. Procházka	Window trap	2	1
Skařiny NR	48.8038878N, 17.0985356E	2015	J. Foit	Window trap	17	12
Stožec	48.8799444N, 13.8356389E	2018	J. Procházka	Window trap	1	20
Strážnice	48.9220283N, 17.2921600E	2018	E. Kula, P. Zach, J. Kulfan	Ethanol/window trap	171	30
Strážnice - Muchárov	48.915472N, 17.288682E	2013	L. Čížek, J. Procházka	Window trap	1	1

(Continues)

APPENDIX 1 (Continued)

Locality	Coordinates	Year of Detection/ Collection	Source	Lure/trap	Adults	Cumulative number of traps
Suchá Bělá	50.8888800N, 14.2655311E	2017	J. Vávra, J. Procházka	Window trap	1	1
Široké pole	48.8603089N, 15.8517539E	2012–2014	L. Čížek, F. Trnka, M. Plátek, P. Šebek, P. Kozel, J. Procházka	Window trap	8	5
Tvrdonice	48.7398108N, 17.0193353E	2017	J. Foit	Window trap	3	8
Týnec	48.7659267N, 17.0175756E	2017	J. Foit	Window trap	1	8
Vápenky	48.8744444N, 17.6294444E	2011	Knížek and Foit (2012)	ID-Ecolure	1	Unknown
Ve Vlčí NR	48.9291667N, 17.8570000E	2018	E. Ezer, J. Procházka	Window trap	1	1
Vranov nad Dyjí	48.8787758N, 15.8438081E	2013	J. Vávra, J. Procházka	Window trap	1	1
Vrapač NR	49.7080556N, 17.0469444E	2012	Knížek and Foit (2012)	Window trap	1	18
Vysoká Pec	50.5406817N, 13.4650333E	2015	J. Foit	In flight	3	
Zahrady pod Hájem NNR	48.8850625N, 17.5368217E	2013–2016	J. Procházka, J. Schlaghamerský, Z. Račanský, J. Budka	Window trap	868	48
Zářičí	49.394767N, 17.324334E	2013	L. Čížek, J. Procházka	Window trap	12	2
Zástudánčí NNR	49.397861N, 17.311125E	2013	L. Čížek, J. Procházka	Window trap	4	2

APPENDIX 2

Records of *Xylosandrus germanus* in the Czech Republic and within 100 km of its border with neighbouring countries and in Hungary (Coordinates in bold were estimated according to source maps.)

Country	Locality	Coordinates	Year of collection	Source
Austria	Salzburg	47.822N, 13.032E	1994	Geiser and Geiser (2000)
Austria	Wien	48.1467N, 16.254E	2012	Holzinger et al. (2014)
Austria	Wien	48.191N, 16.215E	2004	Petr Zábranský
Austria	Wien	48.223N, 16.230E	2006	Petr Zábranský
Germany	Spießberg	51.219N, 13.837E	2006	Reike and Sobczyk (2007)
Germany	Alberoda	50.624N, 12.708E	2008	https://www.gbif.org/species/8469824
Germany	Altliebel	51.374N, 14.708E	2012	https://www.gbif.org/species/8469824
Germany	Baruth	51.225N, 14.625E	2011	https://www.gbif.org/species/8469824
Germany	Frauenberg NWR	**48.846N, 13.381E**	2008	Blaschke and Bussler (2012)

(Continues)

APPENDIX 1 (Continued)

Country	Locality	Coordinates	Year of collection	Source
Germany	Friedensthal	51.025N, 14.708E	2013	https://www.gbif.org/species/8469824
Germany	Hecke NWR	**48.526N, 13.428E**	2009	Blaschke and Bussler (2012)
Germany	Hellendorf	50.825N, 13.958E	2009	https://www.gbif.org/species/8469824
Germany	Hohnstein	50.975N, 14.125E	2005	Reike and Sobczyk (2007)
Germany	Klingenberg	50.925N, 13.541E	2005	https://www.gbif.org/species/8469824
Germany	Leitenwies NWR	**48.596N, 13.324E**	2009	Blaschke and Bussler (2012)
Germany	Moritzburg	51.175N, 13.708E	2006	https://www.gbif.org/species/8469824
Germany	Seelingstädt	51.275N, 12.708E	2008	https://www.gbif.org/species/8469824
Germany	Schönau	51.325N, 14.208E	2013	https://www.gbif.org/species/8469824
Germany	Steigerwald	**49.833N, 10.483E**	2004	Müller et al. (2008)
Germany	Wünschendorf	51.025N, 13.958E	2008	https://www.gbif.org/species/8469824
Hungary	Bakoca	46.186N, 17.976E	2005	Lakatos and Kajimura (2007)
Hungary	Böhönye	46.234N, 17.190E	2019	Ferenc Lakatos
Hungary	Cserénfa	46.173N, 17.520E	2019	Ferenc Lakatos
Hungary	Káld	47.085N, 16.594E	2018	Ferenc Lakatos
Hungary	Kaposhomok	46.191N, 17.560E	2019	Ferenc Lakatos
Hungary	Marcali	46.350N, 17.251E	2019	Ferenc Lakatos
Hungary	Mesztegnyő	46.290N, 17.300E	2019	Ferenc Lakatos
Hungary	Nagymáté	46.186N, 17.976E	2005	Lakatos and Kajimura (2007)
Hungary	Somogyzsitfa	46.325N, 17.202E	2019	Ferenc Lakatos
Hungary	Sopron	47.385N, 16.331E	2016	Ferenc Lakatos
Hungary	Sopron	47.391N, 16.272E	2010	Ferenc Lakatos
Hungary	Sopron	47.450N, 16.350E	2016	Ferenc Lakatos
Poland	Murcki	**50.185N, 19.052E**	2011	Mokrzycki and Grodzki (2014)
Poland	Racibórz	**50.058N, 18.262E**	2011	Mokrzycki and Grodzki (2014)
Slovakia	Banská Štiavnica	48.463N, 18.905E	2014	Galko et al. (2019)
Slovakia	Duchonka	48.666N, 18.083E	2010	Galko (2013)
Slovakia	Krupina	48.346N, 19.069E	2014	Galko et al. (2019)
Slovakia	Lozorno	48.334N, 17.061E	2014	Galko et al. (2019)
Slovakia	Sielnica	48.659N, 19.073E	2014	Galko et al. (2019)
Slovakia	Stará Myjava	48.825N, 17.592E	2014	Galko et al. (2019)
Slovakia	Šance	48.868N, 17.719E	2014	Galko et al. (2019)
Slovakia	Šaštín	48.633N, 17.144E	2014	Galko et al. (2019)
Slovakia	Uhrovské Podhradie	48.779N, 18.371E	2014	Galko et al. (2019)
Slovakia	Utekáč	48.606N, 19.797E	2015	Galko et al. (2019)
Slovakia	Veľký Šariš	49.058N, 21.243E	2016	Galko et al. (2019)

10.6 Trapping ambrosia beetles by artificially produced lures in an oak forest.

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Trapping ambrosia beetles by artificially produced lures in an oak forest

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Abstract: Ambrosia beetles are among the most damaging forest pests. They are frequently moved intercontinentally and are therefore subject to quarantines. The objective of the current research was to determine whether two commercially produced lures for *Trypodendron* species also attract central European ambrosia beetles. In 2018, Theysohn® traps were deployed in an oak forest that also contained hornbeam and linden trees. Five pair of traps was baited with the standard synthetic pheromone lures, Trypowit® or Lineatin Kombi®. The 201 adults of ambrosia bark beetles that were trapped were identified to eight species, which represents almost the entire spectrum of oak ambrosia scolytids in the region. *Trypodendron domesticum*, *Xyleborinus saxeseni*, and *Xyleborus monographus* were the most abundant species and exhibited a slight preference for the lure with a higher content of alcohols (Lineatin Kombi®). Both lures attracted *T. lineatum* because both contain lineatin. The number of beetles trapped was low probably because food sources (damaged or wilting oaks) were rare and because the forest was surrounded by agricultural land and therefore isolated from other oak forests.

Keywords: *Xyleborinus saxeseni; Xyleborus monographus*; Xyleborini; Trypowit®; Lineatin Kombi®; Czech Republic

Ambrosia beetles, which include *Anisandrus* sp., *Trypodendron* sp., *Gnathotrichus* sp., *Xyleborus* sp., *Xylosandrus* sp. (Curculionidae: Scolytinae) and *Platypus* sp. (Curculionidae: Platypodinae), are among the most important pests of timber. For example, ambrosia beetles were estimated to damage an average of 6 000 m^3 of timber per year in Slovakia (Vakula et al. 2016), and damaged 250 000 m^3 of timber during a 15-month period in 2000–2001 in Belgium (Huart & Rondeux 2001). In 1980–1981, ambrosia beetles degraded C$63.7 million worth of sawlogs in British Columbia, Canada (McLean 1985). Large sums are also spent in protecting wood from ambrosia beetles (Dobie 1978; Wylie et al. 1999).

Pheromone lures have been used to monitor the ambrosia beetle *Trypodendron lineatum* (Olivier, 1795) for many years. All of these lures contain lineatin (the pheromone produced by *Trypodendron* females) and sometimes other substances (Kvamme 1986). New lures that have higher alcohol contents and that are attractive to other *Trypodendron* species, including *Trypodendron domesticum* (Linnaeus, 1758) and *Trypodendron signatum* (Fabricius, 1792), have been recently produced (Trypowit®) (http://www.witasek.com; Holuša & Lukášová 2017).

Ambrosia beetles are generally attracted to ethanol, which is consistent with their preference for aged woody material in which anaerobic respiration generates ethanol (Moeck 1970). Several studies have suggested that ethanol is produced from the fermentative processes in trees in response to various environmental stresses including flooding, drought, or high levels of pollutant gases (Montgomery & Wargo 1983).

The Lineatin Kombi® (Witasek PflanzenSchutz GmbH, Austria) lure contains quaiacol, nonyl aldehyde, and 3-hydroxy-2-methyl-2-butanone (http://www.witasek.com/). These substances were added

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because the isomers of 2-methoxy-phenol and methyl-butanol can attract *T. domesticum* (Holighaus & Schütz 2006) and cause the lure to be slightly more effective than the Trypowit® (Witasek PflanzenSchutz GmbH, Austria) lure for monitoring the *Trypodendron* species that attack broadleaf trees in forests dominated by coniferous trees (Holuša & Lukášová 2017).

The use of pheromone traps to monitor ambrosia beetles in deciduous forests has been studied several times (Grégoire et al. 2001; Petercord 2006; Franjević 2013; Franjević et al. 2019; Galko et al. 2019; Gossner et al. 2019). The aim of the current study was to determine whether two commercially produced lures for *Trypodendron* species also attract other species of central European ambrosia beetles like *Xyleborus* sp. and *Xyleborinus* sp.

MATERIAL AND METHODS

In 2018, five pairs of Theysohn traps were deployed in an oak forest near the village of Peruc (50.3549364N, 13.9686869E; 280–360 m a.s.l.); the forests were dominated by oak trees, but also contained hornbeam and linden trees. Pairs were 20 m apart, and pairs were ca 200 m apart. The traps were deployed in small clearings (< 0.5 ha) > 10 m from the forest edge.

One trap of each pair was baited with Trypowit®, and the other was baited with Lineatin Kombi®. The main active ingredients in the Trypowit® lure are alfa-pinen (2,6,6-trimethyl-bicyclo-[3,1,1] hept-3-en) and lineatin (3,3,7-trimethyl-2,9-dioxatricyclo-[4.2.10 ^{4,7}] nonane), and those in the Lineatin Kombi® lure are lineatin (3,3,7-trimethyl-2, 9-dioxatricyclo-[4.2.10 ^{4,7}] nonane), quaiacol (2-methoxyphenol), nonyl aldehyde, and 3-hydroxy-2-methyl-2-butanone (http://www.witasek.com/). The traps were checked weekly from the 20th of March 2018 to the 31st of July 2018, and the lures were replaced after 8 weeks.

The *Trypodendron* species were identified by the authors, and the other species were identified by Miloš Knížek (Forestry and Game Management Research Institute, Jíloviště). The mean number of beetles trapped by each kind of lure was compared with a Wilcoxon pair test in Statistica software (version 12.0.).

RESULTS AND DISCUSSION

A total of 201 adult ambrosia beetles representing eight species were detected (Table 1). The most abundant species, *T. domesticum*, *Xyleborinus saxeseni* (Ratzeburg, 1837), *T. lineatum,* and *Xyleborus monographus* (Fabricius, 1792), are common polyphagous species, and the first three are recognised as serious pests (Tuncer et al. 2017).

Fewer than ten specimens in total of other ambrosia beetle species were trapped; these included the invasive *Xylosandrus germanus* (Blandford, 1894) (Fiala et al. in press.) and *Xyleborinus attenuatus* (Blandford, 1894) (Knížek 1988) (Table 1). The seven species represent almost all of the known oak ambrosia scolytids in Bohemia (Pfeffer 1989); the rare *Trypodendron signatum* (Fabricius, 1792) was not detected. *Anisandrus dispar* (Fabricius, 1792) is a secondary pest that attacks almost all of the deciduous tree species in forests and trees that have been weakened by other biotic and/or abiotic factors (Tanasković et al. 2016). *Xyleborus dryographus* (Ratzeburg, 1837) is a common polyphagous beetle at low elevations and feeds mainly on oaks (Pfeffer 1989).

The number of ambrosia beetles trapped was much lower than previously reported in central Europe (Galko et al. 2014), perhaps because bark and ambrosia beetles depend on ephemeral and usually scattered substrates for breeding (Forsse & Solbreck 1985). Few food sources (damaged or wilting oaks) may have been available in the study locality. The number of trapped beetles may also have been small because the traps were deployed in forests that are surrounded by agricultural land and, therefore, separated from other forests (Rukke 2000; Ryall & Fahrig 2005). Low numbers of beetles were also trapped in an isolated deciduous forest in western Bohemia (Fiala 2019). The population density of ambrosia beetles in fragmented forests is influenced by the flight capability of the beetles. Ambrosia beetles are weak fliers. Whereas other bark beetles can fly tens of kilometres (Nilssen 1984), *T. lineatum* and *T. domesticum* have a maximum range of 0.5 km, and *X. germanus* have a maximum range of 2 km (Salom & McLean 1989; Grégoire et al. 2001).

The number of trapped beetles may also be influenced by the height of the trap placement (Hanula et al. 2011); forest openness (Dodds 2011); trap type, colour, and shape, and the volatiles used as lures (Hanula et al. 2011). Theysohn traps are preferred by some, but not all, species of ambrosia beetles (Flechtmann et al. 2000).

Both of the tested lures attracted *T. lineatum* because both contain lineatin. A higher content of alcohols probably explained the higher numbers of *A. dispar*, *T. domesticum*, and *X. saxeseni* in the traps

Table 1. The total numbers of ambrosia beetles captured in the Theysohn traps, the mean numbers (± SE) captured per trap containing Trypowit® or Lineatin Kombi®, and a statistical comparison of trapping by Trypowit® vs. Lineatin Kombi®

Species	Total No. of beetles trapped	Mean No. of beetles per trap^1	Mean No. of beetles per trap^2	Wilcoxon pair test (z; P)
Anisandrus dispar (Fabricius, 1792)	8	0.2 ± 0.2	1.4 ± 0.5	1.83; 0.06
Trypodendron domesticum (Linnaeus, 1758)	95	6.6 ± 1.4	12.4 ± 3.0	1.35; 0.18
Trypodendron lineatum (Olivier, 1795)	35	3.8 ± 1.6	3.2 ± 1.1	0.73; 0.46
Xyleborinus attenuatus (Blandford, 1894)	2	0.2 ± 0.2	–	
Xyleborinus saxeseni (Ratzeburg, 1837)	46	1.2 ± 1.2	8.0 ± 3.8	1.83; 0.06
Xyleborus dryographus (Ratzeburg, 1837)	2	0.4 ± 0.4	–	
Xyleborus monographus (Fabricius, 1792)	12	0.4 ± 0.4	2.0 ± 1.0	1.60; 0.11
Xylosandrus germanus (Blandford, 1894)	1	–	0.2 ± 0.2	

^{1}containing Trypowit®; ^{2}containing Lineatin Kombi®; z – Wilcoxon pair test

containing Lineatin Kombi® than in the traps containing Trypowit®, although the differences were not statistically significant (Table 1). However, ambrosia beetles could be also caught in high numbers in lineatin-baited traps (Franjević et al. 2019), although the reason is not known. The attraction of ambrosia beetles to ethanol is generally recognised, even in the very common *X. saxeseni* species (Markalas & Kalapanida 1997). Some semiochemicals are known to increase or reduce the attraction of ambrosia beetles to pheromone traps; conophthorin, for example, increases the attraction of *X. germanus* (Ranger et al. 2014), and terpinolene and verbenone reduce the attraction of *Xyleborus fornicatus* (Eichhoff, 1868) (Karunaratne et al. 2008). These compounds, however, probably represent only a few of the components of the complex mixtures of volatiles that affect beetle behaviour. In addition, different ratios of volatiles can elicit different reactions from insects.

Information on ambrosia beetle numbers and on the spread of pathogens by ambrosia beetles is generally based on randomly collected data that are not readily accessible to forest managers. This unfortunate situation could be improved to some extent by the permanent monitoring of the most important forest pests with ethanol lures. Also, however, the lures investigated in the current research could be useful for the monitoring of ambrosia beetles in oak forests, which have been in decline for many years in Europe (Oszako 1998).

Ambrosia beetles are among the pests that most damage forests and warrant being quarantined because they are frequently moved intercontinentally (Haack 2001; Westphal et al. 2008). Early detection of these potentially invasive species is critical for their effective management; although the current surveillance methods have intercepted many species, they failed to detect others that subsequently became significant pests (Bashford 2012; Rassati et al. 2015). Adding pheromones to host volatile-baited traps increased the number of species detected (Flaherty et al. 2018). We suggest further research using the lures in the current study for detection of pests in large wood-storage areas, because the mass trapping of ambrosia beetles in such areas could substantially reduce the damage to the wood (Lindgren & Fraser 1994).

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10.7 Efficacy of different lures for *Phloeosinus aubei* and other native and exotic bark and ambrosia beetles.

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Efficacy of different lures for *Phloeosinus aubei* and other native and exotic bark and ambrosia beetles

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Abstract

Phloeosinus aubei is invasive in Central Europe, spreading northward from the Mediterranean region because of climate change. *P. aubei* has the potential to become a pest of mature, naturally occurring juniper trees (*Juniperus communis*) in protected areas, as well as junipers, thujas and cypresses in nurseries and urban environments. In 2020 and 2021, we conducted two experiments to compare various lures for trapping *P. aubei*. In the first experiment, we compared four lures: α-pinene; turpentine; a mixture of cade oil, juniper berry oil and ethanol; and a juniper branch (8–10 cm long, 3–5 cm wide) deployed with ethanol. In the second experiment, we compared Hostowit® with a juniper branch (8–10 cm long, 3–5 cm wide) deployed with ethanol. In both experiments, juniper branches deployed with ethanol attracted a greater number of *P. aubei* beetles compared to the other lures. In total, we captured 1056 individuals belonging to 36 species of Scolytinae. Among the most abundant species, *Xyleborinus saxesenii*, *Xyleborus monographus* and *Anisandrus dispar* were lured by ethanol in combination with other substances or deployed with juniper branches. *Xyleborus dryographus* showed a positive association with turpentine. *Ips typographus* was positively associated with α-pinene, while *Orthotomicus laricis*, *Hylastes attenuatus* and *Gnathotrichus materiarius* were positively associated with Hostowit® lure.

KEYWORDS
ambrosia beetles, bark beetles, invasive beetles, *Juniperus*, lures, small cypress bark beetle, trapping, turpentine

1 | INTRODUCTION

Early detection of invasive bark and ambrosia beetles is crucial for mitigating the potential damage they can cause to trees, habitats, biodiversity and the economy (Aukema et al., 2011). Bark beetles primarily feed on phloem, disrupting the transportation of substances produced by photosynthesis, while ambrosia beetles feed on fungi that they carry in their mycangia into tunnels in the wood (Hulcr et al., 2007). In addition to direct damage, both, bark and ambrosia beetles can also vector pathogenic fungi, leading to further detrimental impacts (Hulcr & Stelinski, 2017; Masood & Saeed, 2012; Montecchio & Faccoli, 2014). With climate change projected to increase the establishment of new nonnative species (Lovett et al., 2013; Pureswaran et al., 2022), the availability of reliable tools for early interception of incoming species becomes paramount.

The bark beetle *Phleosinus aubei* Perris, 1855 is native to the Mediterranean region (Balachowsky, 1949; Beaver et al., 2016; Schedl, 1969) and is known to cause extensive tree mortality in that area. Its primary native host is the Italian cypress *Cupressus sempervirens* L. Infestation by this pest leads to the drying of its branches and stems. In Tunisia, *P. aubei* exhibits two generations per year and three sister broods, with a fecundity rate of 10–25 eggs per gallery and a nearly equal sex ratio (Bel Habib et al., 2007, 2009). The tunnelling activity of *P. aubei* within trees provides an entry point for the fungus *Seiridium cardinalce* (W.W. Wagener) B. Sutton & I.A.S. Gibson, which causes cypress canker—a disease that ultimately results in the death of the host tree (Danti & Rocca, 2017; Ponchet, 1981; Verdú, 1991). Both the fungus and its beetle vector have been introduced to Central Europe from Mediterranean regions likely because of climate change (Bozsik & Szöcs, 2017; Fiala & Holuša, 2019; Sobczyk & Lehmann, 2007). Additionally, the spread of the fungus is associated with global trade (Mertelík & Šindelková, 2003; Urbasch, 1993).

In Central Europe *P. aubei* has the potential to become a pest of mature, naturally occurring juniper trees (*Juniperus communis* L.) in protected areas, particularly on weakened and damaged trees (Fiala & Holuša, 2019). It can also infest other host trees such as thujas and cypresses, commonly found in urban gardens, parks and commercial horticulture. Incidents of *P. aubei* damage have already been observed in ornamental nurseries and parks (see Bozsik et al., 2022; Fiala et al., 2022; Fiala & Holuša, 2019). Thus, the development of effective monitoring strategies for the early detection of *P. aubei* is of utmost importance and should be considered a research priority.

The use of attractive lures, including pheromones, is essential in trapping protocols for monitoring beetles of the Scolytinae subfamily (Coleoptera: Curculionidae) (Seybold et al., 2018; Zhang et al., 2007). However, the current unavailability of identified pheromones for *P. aubei* prevents the use of pheromone traps (Bozsik et al., 2016). Previous studies have collected volatiles (juniper berry oil, turpentine) from common hosts of *P. aubei*, such as the American arborvitae, *Thuja occidentalis* L. cultivar 'Smaragd' (Bozsik et al., 2016, 2022) and *Platycladus orientalis* (L.) (Yang et al., 2006). Electroantennography demonstrated the attraction of adult beetles to the volatiles from the latter. However, other volatiles collected from *P. orientalis* (α-pinene, 3-carene, sabinene and widdrene) failed to attract the adult beetles in the field (Sun et al., 2013), despite some, particularly α-pinene, being known as primary attractants for bark beetles associated with conifer trees (Schroeder, 1988). The commercially produced lure Hostowit® (Witasek GmbH, Austria) has also shown attractiveness to *P. aubei* (Sun et al., 2013), although its specific components are unknown. In addition to baited traps, another common approach for attracting bark and ambrosia beetles is using host tree parts soaked or filled with ethanol (Cavaletto et al., 2023; Ranger et al., 2021). Ethanol serves as a key olfactory cue for several wood-boring beetles (Miller & Rabaglia, 2009) and can enhance the attractiveness of other host volatiles emitted by the logs.

To enhance trapping strategies for *P. aubei*, our study aimed to investigate (i) the attractiveness of juniper branches deployed with ethanol compared to traps baited with previously identified efficient volatiles for *P. aubei*, and (ii) the attractiveness of juniper branches deployed with ethanol compared to traps baited with a commercially produced lure known to attract *P. aubei*. We hypothesised that the number of beetles captured would be higher using juniper branches deployed with ethanol as compared to the other, simpler lures. Additionally, (iii) we took the opportunity to assess whether other Scolytinae species trapped during the survey exhibited any preference for the tested lures, considering that the trapping strategies tested might also attract other bark and ambrosia beetle species.

2 | MATERIALS AND METHODS

2.1 | Study localities

The experiments were conducted in 2020 and 2021 at five selected localities in central and western Bohemia, where host trees were present. The presence of *P. aubei* had been previously confirmed at all the study sites (Fiala & Holuša, 2019; Fiala observ.). Three of the localities consisted of open forests or abandoned pastures with juniper trees: Záborná Lhota (49.7679° N, 14.3120° E, 1.2 ha in area, 10% juniper representation, 400 m asl); Nature Monument Jalovce na Světovině (49.8774° N, 13.7380° E, 0.9 ha in area, 50% juniper representation, 405 m asl); and Nature Monument Jalovcové stráně nad Vrbičkou (50.1878° N, 13.2772° E, 48 ha in area, 30% juniper representation, 520 m asl). The remaining two localities were in the village of Hudlice (Hudlice 1 and Hudlice 2, 49.9624° N, 13.9732° E and 49.9618° N, 13.9616° E, respectively, 400 m asl), where damage to thujas and cypress trees in the gardens of residents had been observed (Fiala observ.).

2.1.1 | Experiment 1

In 2020, we compared the attractiveness and trapping efficacy of four treatments for *P. aubei*. The treatments included α-pinene (as a control); turpentine; a mixture of oils (cade oil, juniper berry oil and ethanol) (see Appendix 1) (all provided by Fytofarm s.r.o., Slovakia), and a juniper branch with ethanol. We used α-pinene as a control because it is a basic compound emitted by juniper trees (Foudil-Cherif et al., 2009) and we hypothesised that *P. aubei* would be primarily attracted to α-pinene. The three chemical lures were placed in evaporators (polypropylene tubes; diameter = 23 mm, height = 49 mm, volume = 12.5 mL, weight without attractant = 3.5 to 4.0 g); each evaporator contained 6 ml of lure. The juniper branches used were 8–10 cm long and 3–5 cm wide, and were deployed with UHR ethanol (Alpha Scents, Inc., USA), hung side by side. The branches were cut from a single juniper tree immediately before the experiment. The branches were checked for *P. aubei* and other bark beetles' presence before experiment, small twigs were removed, and branches were cut into shorter pieces. All four lures were deployed at traps at all five localities. At each locality, we placed 20 Ecotrap® black traps with dry cups (Fytofarm spol. s r.o., Slovakia; five replicates for each treatment); each trap had an intercept area of 5550 cm^2. The traps with lures were positioned 130 cm above the ground using sticks, along an east–west transect, with one evaporator or branch per trap and 10 m spacing between traps. The height of the traps corresponded to the height of the host trees.

2.1.2 | Experiment 2

In 2021, we conducted the subsequent experiment at two localities (Hudlice 1 and Jalovce na Světovině). Two lines of 10 Ecotrap® black traps were set up in each locality, running in an east–west direction. The traps within a line were 10 m apart, and the lines themselves were also 10 m apart. In each trap, we placed Hostowit® tube dispensers (Witasek GmbH, Austria) and juniper branches with UHR ethanol.

The traps with lures were deployed at the end of April and were checked every 14 days until the end of June in both experiments. Collected beetles were frozen and subsequently identified to species by the first author of the study. Even if the beetles were damaged by predators, we were able to determine the remains and ascertain the number of specimens and species present.

2.2 | Statistical analyses

We analysed the data in the R 4.2.1 programme (R Core Team, 2022). In order to determine the factors influencing the trapping results, we built a series of generalised linear mixed models (GLMM) based on the Akaike information criterion (AIC) from the 'lme4' library (Bates et al., 2015). Locality was included as a random term, while lures, sampling dates and their interaction were considered as explanatory variables. The significance of individual explanatory variables in the GLMM was determined by comparing the models with the corresponding simplified models (excluding the focal variable) or the null model. To address algorithm convergence issues, models were rescaled to 100,000 iterations, with the tolerance for declaring convergence in the penalised iteratively weighted residual sum-of-squares set on 1×10^{-3}, and with the 'nloptwrap' optimization algorithm.

Initially, we analysed the variation in the occurrence of *P. aubei* among the four types of lures in Experiment 1. As a result of the limited capture of *P. aubei* beetles (rarely two specimens per replication), we opted to treat the data as presence/absence rather than abundance data (following a binomial distribution, logistic link). The abundance was considered as a weight, where a weight of 1 was assigned for traps with 0 or 1 beetle and a weight of two for traps with two beetles. To build the model, we focused on data from the first three sampling dates, as no *P. aubei* specimens were captured on the fourth date. Including the fourth date would not substantially alter the results but could hinder model algorithm convergence.

Subsequently, we conducted a similar analysis to investigate the factors influencing the overall abundance of all beetles. For this analysis, we employed a GLMM with a negative binomial distribution and a logarithmic link from the 'MASS' library (Venables & Ripley, 2002). Furthermore, we examined the species richness (GLMM with Poisson distribution, logarithmic link), and a ratio of *P. aubei* specimens to all beetles (GLMM with binomial distribution, logistic link). Corresponding models were built and analysed for Experiment 2 as well. Finally, visualisations of the data were generated using the 'sciplot' library (Morales, 2020).

To examine the factors influencing community composition, we conducted permutational multivariate analysis of variance using distance matrices (PERMANOVA) with the Bray-Curtis distance matrix. This analysis was performed using the 'vegan' library (Oksanen et al., 2022), using a permutation test with 999 permutations. We employed stepwise forward selection based on AIC for model construction. Complementing the PERMANOVA, we conducted constrained ordinations: partial canonical correspondence analysis (p-CCA) in the case of the first, and partial redundancy analysis (p-RDA) in the case of the second experiment. In these ordinations, locality and sampling date were treated as covariates, with lure considered as an explanatory variable. The statistical significance of the results was assessed using the Monte-Carlo permutation test with 999 permutations. Additionally, we employed multi-level pattern analysis from the 'indicspecies' library (De Caceres & Legendre, 2009) to identify bark beetle species that exhibited significant associations with specific lures.

3 | RESULTS

A total of 1056 bark and ambrosia beetles (Scolytinae) representing 36 species were detected in both experiments (Appendices 2 and 3). Among these, four species were detected in all localities and in both experiments: *Hylastes attenuatus*, *Pityogenes chalcographus*, *Xyleborinus saxesenii* and *P. aubei*. *P. chalcographus* was the most abundant species, representing almost 40% of all individuals detected, followed by *Ips typographus* and *H. attenuatus*, both representing >10% of all bark and ambrosia beetles detected. *X. saxesenii* accounted for 8% of the detected bark beetles. The remaining species represented less than 5% of the total number of bark beetles detected. Only 36 specimens of *P. aubei* were detected, representing 3.6% of all individuals observed (Appendices 2 and 3). No bark beetles were observed actively digging the branches.

3.1 | Experiment 1

3.1.1 | *Phloeosinus aubei* presence

After accounting for the effect of locality, the presence of *P. aubei* varied among sampling dates (df = 290, $\chi^2 = 25.66$, $p < .001$), lures (df = 292, $\chi^2 = 17.14$, $p < .001$), and showed a significant interaction between sampling date and lure (df = 289, $\chi^2 = 9.92$, $p = .019$). Throughout the sampling period, the number of *P. aubei* beetles gradually decreased. The highest capture rate of the *P. aubei* beetles was observed in trap baited with a juniper branch with ethanol, but its effectiveness decreased rapidly compared to other lures (Figure 1a). Juniper branch with ethanol attracted significantly more *P. aubei* beetles compared to traps baited with α-pinene ($z = 2.70$, $p = .007$) but similar to mixture of oils ($z = -4.40$, $p = .661$) and turpentine ($z = -4.28$, $p = .719$).

3.1.2 | Abundances of all bark and ambrosia beetles

After accounting for the effect of locality, the total bark beetle abundance varied among sampling dates (df = 389, $\chi^2 = 28.20$, $p < .001$) and lures (df = 391, $\chi^2 = 14.54$, $p = .002$), with significant interaction between sampling date and lure (df = 386, $\chi^2 = 20.83$, $p < .001$). Throughout the sampling period, the number of beetles detected

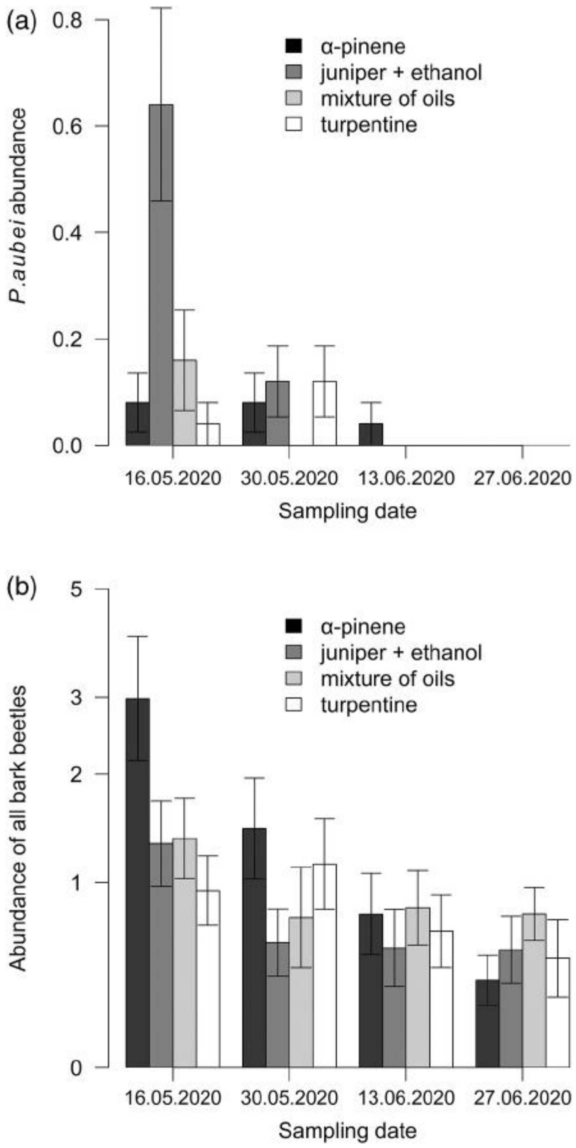

FIGURE 1 (a) Abundance of *Phloeosinus aubei*. (b) Abundance of all bark beetles in traps, affected by sampling dates and lures in Experiment 1 (mean ± SE).

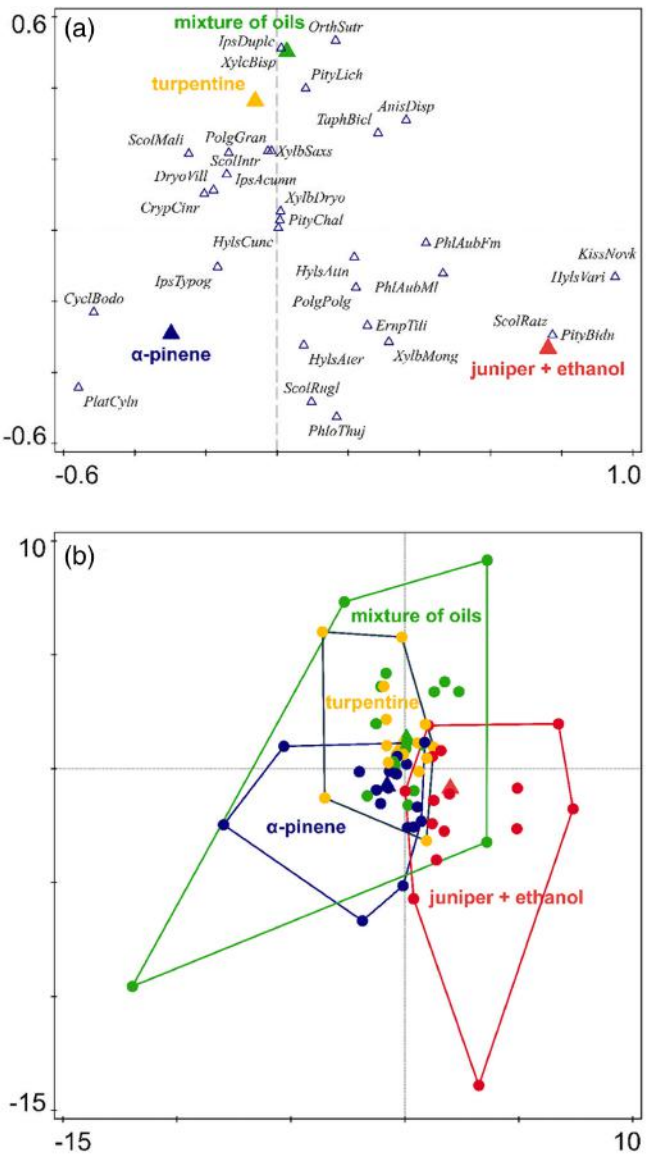

FIGURE 2 (a) Diagram of partial canonical correspondence analysis (p-CCA) analysis showing the species composition of beetles in relation to individual lures. (b) PCoA based on p-CCA showing differences between samples in relation to individual lures.

gradually decreased. Most of them were captured using α-pinene lure. However, the effectiveness of a juniper branch with ethanol, unlike other lures, rapidly decreased over time (Figure 1b).

3.1.3 | Community and species affiliation to lures

The community composition was primarily affected by locality ($df = 196$, $F = 12.72$, $p = .001$, $R^2 = .198$), followed by sampling date ($df = 195$, $F = 5.73$, $p = .001$, $R^2 = .022$), and then by lure ($df = 192$, $F = 2.78$, $p = .001$, $R^2 = .032$). Partial CCA considering the effect of locality and sampling date as covariates, revealed a significant effect of lure ($df = 192$, $F = 1.70$, $p = .002$) (Figure 2a,b).

Multi-level pattern analysis revealed associations between certain bark beetle species and specific lures. *X. saxesenii* was associated with the mixture of oils (stat = 0.429, $p = .001$), *I. typographus* with α-pinene (stat = 0.606, $p = .001$), *P. aubei* and *Xyleborus monographus* with juniper branch deployed with ethanol (stat = 0.325, $p = .007$; stat = 0.323, $p = .028$; stat = 0.273, $p = .046$), and *Xyleborus dryographus* with turpentine (stat = 0.411, $p = .011$).

3.2 | Experiment 2

3.2.1 | *Phloeosinus aubei* presence

After accounting for the effect of locality, the presence of *P. aubei* did not significantly differ among sampling dates ($df = 35$, $\chi^2 = 0.28$, $p = .599$) or between lures ($df = 35$, $\chi^2 = 0.28$, $p = .599$) (Figure 3a).

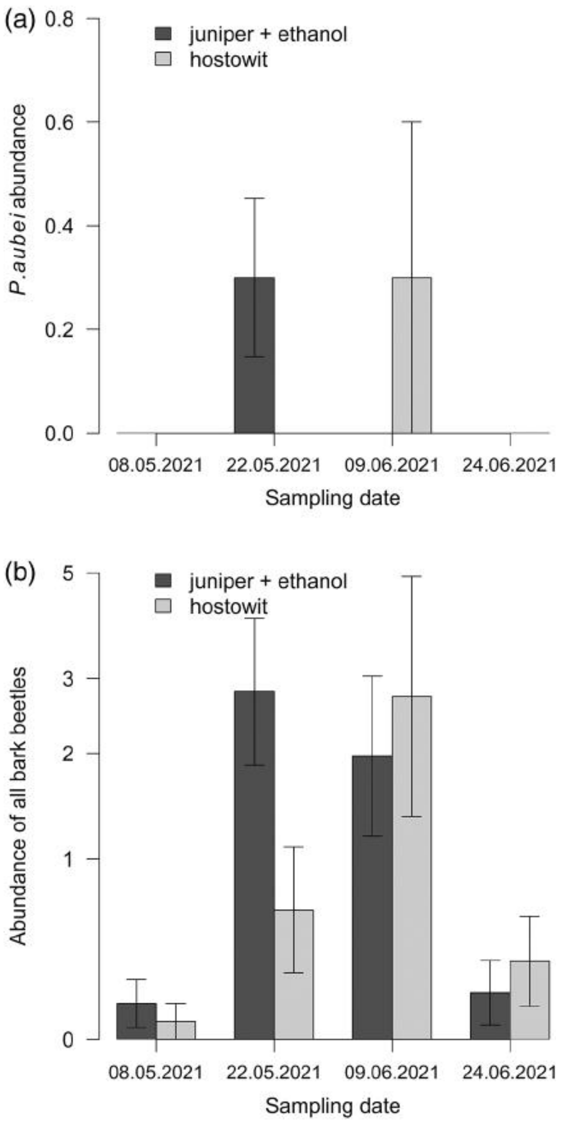

FIGURE 3 (a) Abundance of *Phloeosinus aubei*. (b) Abundance of all bark beetles, in traps, affected by sampling dates and lures in Experiment 2 (mean ± SE).

3.2.2 | Abundances of all bark beetle species

After accounting for the effect of locality, the abundance differed among sampling dates (df = 71, $\chi^2 = 78.02$, $p < .001$) but not between lures (df = 70, $\chi^2 = 0.39$, $p = .531$). However, there was a significant interaction between the sampling date and the lure (df = 70, $\chi^2 = 7.53$, $p = .023$). The number of beetles detected increased over time and then decreased, but the effectiveness of a juniper branch with ethanol decreased earlier compared to Hostowit® (Figure 3b).

3.2.3 | Community and species affiliation to lures

The community composition was primarily affected by lure (df = 31, $F = 4.76$, $p = .001$, $R^2 = .133$). Partial CCA analysis, considering the effect of locality and sampling date as covariates, confirmed the significant effect of lure (df = 31, $F = 1.30$, $p = .001$) (Figure 4a,b).

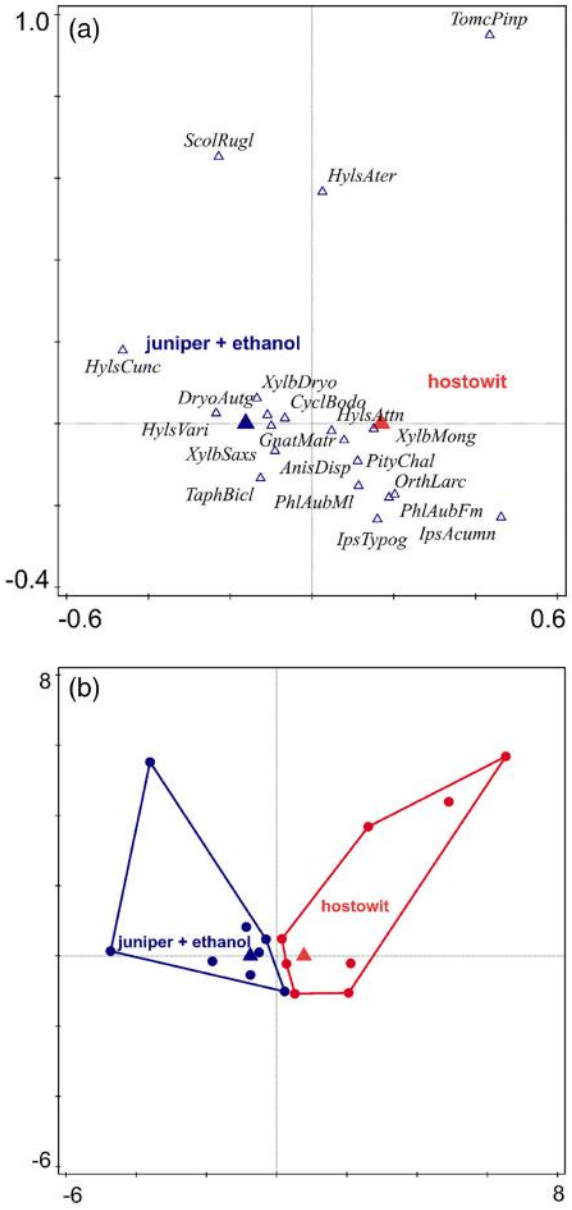

FIGURE 4 (a) Diagram of partial canonical correspondence analysis (p-CCA) analysis showing the species composition of beetles in relation to individual lures. (b) PCoA based on p-CCA showing differences between samples in relation to individual lures.

Multi-level pattern analysis diagnosed that *Anisandrus dispar* was associated with juniper branch deployed with ethanol (stat = 0.784, $p = .002$), while *H. attenuatus*, *Orthotomicus laricis* and *Gnathotrichus materiarius* were associated with Hostowit® (stat = 0.799, $p = .001$; stat = 0.598, $p = .015$; stat = 0.586, $p = .016$, respectively). *P. aubei* did not show a clear association with any specific lure.

4 | DISCUSSION

Our finding indicate that juniper branches deployed with ethanol were the most effective lure in attracting *P. aubei*. In Experiment 1, both male and female *P. aubei* beetles were more attracted to juniper branches with ethanol compared to other lures. The volatiles released by host tissues (e.g., freshly felled trees, trunk segments, branches, wood fragments, or bark) are known to attract bark and ambrosia beetles (Addesso et al., 2019; Chen et al., 2021; Holuša et al., 2017; Lieutier et al., 2004; Mezei et al., 2022; Pfeffer, 1955; Resnerová et al., 2020). Juniper branches likely provide a more attractive combination of multiple substances, including minor compounds that may play a role in host finding but have not been fully recognised yet (Kalinová et al., 2014), compared to simple substances like turpentine, cade oil and juniper berry oil. The content of monoterpenes in volatile emissions differs qualitatively (Loreto et al., 2000) and quantitatively (Nault & Alfaro, 2001) between intact and wounded conifer branches. Cut branches typically emit a greater variety of volatile compounds compared to intact ones, although some compounds are specific to intact plants (Fall et al., 1999). Despite our observation of catching *P. aubei* on branches lacking small twigs and needles, we did not find evidence of the beetles chewing on the cut branches. This highlights the advantage of using cut branches in monitoring, as it eliminates the need to search for beetles within the branches.

As described in the literature, drying gradually reduces the attractiveness of trees to bark beetles (Långström, 1983). Therefore, it was not surprising that the number of *P. aubei* beetles caught in traps baited with juniper branches with ethanol decreased over time as the cut branches dried. Additionally, the attraction of branches can decline as beetles feed on them (Tilles et al., 1986; Zumr & Starý, 1992). In Central Europe, *P. aubei* exhibits two flight periods. The first period, which occurs from April to June with a peak in mid-May, is associated with the construction of nuptial chambers (Nowak et al., 2017). Only adults from the F1 generation overwinter, as larvae develop slowly (Pfeffer, 1955). The second flight period, taking place from August to October, is related to the preparation of overwintering galleries where maturation feeding occurs (Bozsik & Szöcs, 2017; Tiberi et al., 2016). Therefore, installing traps with juniper branches and ethanol in early May is sufficient to detect *P. aubei* during May and the branches will remain attractive throughout their flight activity.

In Experiment 2, we compared Hostowit®, a lure previously found to be attractive to *P. aubei* (Sun et al., 2013), and juniper branches with ethanol. The numbers of beetles caught did not differ among sampling dates or between the two lure types. This finding suggests that using host branches can effectively attract and detect *P. aubei*, eliminating the need for purchasing commercially produced lures.

Despite the probable presence of *P. aubei* in the Czech Republic since the 1950s, this species was found in small numbers during the current study. *P. aubei* is more common in the eastern part of the country compared to the western part (Fiala & Holuša, 2019), which could explain its low abundance at localities in west Bohemia. However, even with low population densities *P. aubei* has the potential to multiply rapidly in suitable environments, such as ornamental nurseries (Fiala et al., 2022; Fiala & Holuša, 2019; Mertelík et al., 2007). The effectiveness of the trap strategies used in this study may generally be low, as evidenced by the lack of attacks or deaths of juniper trees in most of the studied localities. However, in the Hudlice locality (Hudlice 1), *P. aubei* caused significant damage by destroying an entire cypress plantation, despite only capturing one male in the area. This highlights the need to consider the mere presence of *P. aubei* as a potential threat to trees. *Phloeosinus* bark beetles, including *P. aubei*, have strong dispersal capabilities and can cover distances of over 24 km during flight (Furniss & Furniss, 1972). Consequently, they can quickly appear in locations where they were previously unknown. Increased population densities of *P. aubei* can occur on weakened and damaged juniper trees found naturally in protected areas (Fiala & Holuša, 2019), and the beetle are then likely to spread to surrounding areas.

Besides *P. aubei*, we captured a total of 34 other bark and ambrosia beetle species, which represents more than one-fourth of the known species in Central Europe (Alonso-Zarazaga et al., 2021). Among these species, the most frequent and/or most abundant ones in both years of the study were *H. attenuatus*, *X. saxesenii*, *I. typographus* and *P. chalcographus*. These species are commonly found in central Europe (Pfeffer, 1955). The latter two species are currently experiencing outbreaks in the coniferous and urban forests throughout Central Europe (Fiala et al., 2022; Hlásny et al., 2021). Consistent with a previous report, which found that the flight activity of the most abundant beetles was highest in May (Pfeffer, 1955), we observed that the number of captured beetles was highest in May in both experiments of the current study. The pattern may be attributed to the fact that α-pinene, the main attractive substance of conifers for many abundant bark beetles (Gallego et al., 2008; Schroeder, 1988; Schroeder & Lindelöw, 1989; Zumr, 1989), was used as the lure during the period. Thus, the number of captured bark beetles, particularly *I. typographus*, was highest when α-pinene was used. The species richness varied greatly among localities because of differences in tree species composition among those areas.

In our study, we observed that *X. saxesenii*, *X. monographus* and *A. dispar*, were attracted to ethanol in combination with other substances or deployed with branches. This response is consistent with the general preference of ambrosia beetles for ethanol, which is produced in aged woody material through anaerobic respiration (Lindelöw et al., 1993; Miller & Rabaglia, 2009; Moeck, 1970). Ethanol is known to be produced in trees in response to various environmental stresses including flooding, drought, or high levels of pollutant gases (MacDonald & Kimmerer, 1991; Montgomery & Wargo, 1983). Interestingly, the effectiveness of ethanol as an attractant varied among different species. Higher concentrations were found to enhance the attraction of *X. saxesenii* and *A. dispar*, while they decreased the ability of *Xylosandrus germanus* or *Xylosandrus crassiusculus* to attack the tree. The addition of α-pinene to ethanol has been shown to affect the capture of ambrosia bark beetles, increasing the attraction of *X. saxesenii* and decreasing the attraction of *Xyloterinus politus* (Addesso et al., 2019; Cavaletto et al., 2021; Ranger et al., 2011, 2018; Rassati et al., 2020; Reding et al., 2011).

This suggests that the response to ethanol and combination of different volatile compounds can be species-specific for ambrosia beetles.

X. dryographus, a common polyphagous ambrosia beetle, is known to feed primarily on oak trees, particularly at lower elevations (Pfeffer, 1955). Its positive attraction to turpentine can be attributed to the fact that oak trees emit terpenoids (Loreto, 2002; Staudt et al., 2004); attraction of *X. dryographus* to turpentine, in addition to ethanol, has been observed in previous studies (Fiala, 2019; Galko et al., 2014), as this species was captured on traps baited with turpentine in Italy (Francardi et al., 2009).

Hostowit® is presented as a universal attractant for pests on coniferous wood, intended to enhance the effectiveness of other attractants. We observed positive attraction of three species, *H. attenuatus*, *O. laricis*, *G. materiarius*, whose host plants are conifers, to this lure. However, it is important to note that this observation is based solely on a comparison of number of beetles caught on juniper branch deployed with ethanol in Experiment 2. The higher number of captured beetles from these three species was only observed at one site in the experiment, probably because of the influx of species emerging from coniferous firewood stored in a neighbouring plot near the traps with Hostowit®. Thus, it can be inferred that beetle of these species exhibited a preference for traps with Hostowit® over bait with ethanol. However, conclusive statements cannot be made based on this finding alone.

H. attenuatus and *O. laricis* are common species in Central Europe (Holuša et al., 2019; Pfeffer, 1955), but little is known about their pheromones, except that they are typically attracted to ethanol and α-pinene (Faccoli et al., 2020).

G. materiarius is polyphagous invasive bark beetle species that infests conifers and has successfully spread in Europe. Most of the published data in Europe regarding this species come from pheromone traps targeting bark beetles of the *Ips* genus (Knížek, 2009; Mazur et al., 2018; Schneider, 1985; Valkama et al., 1998). The primary attractant for capturing *G. materiarius* is sulcatol (Flechtmann & Berisford, 2003), an alcohol compound. This is why *G. materiarius* is also found in traps intended for other species of bark beetles, including those using Hostowit® as lure, which contains other alcohol-based pheromones.

5 | CONCLUSION

Flight intercept traps, including those made from PET bottles (Pajek et al., 2020; Sanguansub et al., 2020; Steininger et al., 2015; Tarno et al., 2021) can effectively be used for monitoring *P. aubei* by baiting them with a juniper or thuja branch (*c.* 10 cm long and *c.* 3 cm thick) deployed with ethanol. Our study demonstrates that even at low population densities, the detection of this bark beetle is possible. Traps and baits should be placed in central Europe at the end of April and be exposed throughout May. However, traps baited with branches deployed with ethanol may also capture other species of bark beetles, providing an opportunity to monitor multiple species simultaneously.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX 1: Descriptions of the lures used for catching *Phleosinus aubei*.

Lure	Abbreviations	Daily vapour (g)	Ratio of compounds	CAS number
Alpha-pinene[a]	α-pinene	0.070		7785-70-8
Turpentine[a]	Turpentine	0.241		8006-64-2
Cade oil + juniper berry oil + ethanol[a]	Mixture of oils	0.210	1:1:1	Cade oil: 8013-10-3 Juniper berry oil: 8002-68-4 Ethanol: 64-17-5
Juniper branch plus ethanol UHR	Juniper + ethanol	0.300		64-17-5

[a] The evaporators for these attractants were polypropylene tubes (diameter = 23 mm, height = 49 mm, volume = 12.5 mL, weight without attractant = 3.5 to 4.0 g). Each tube contained 6 mL of attractant.

APPENDIX 2: Numbers of bark beetles caught with all lures (α-pinene, turpentine, mixture of cade oil, juniper berry oil with ethanol and juniper branch with ethanol) in all localities of western and central Bohemia in Experiment 1 (2020).

Species/locality	Hudlice No1 2020	Hudlice No2 2020	Jalovce na Světovině 2020	Jalovcová stráň u Vrbičky 2020	Záborná Lhota 2020	Total
Anisandrus dispar Fabricius, 1792	1		1		1	3
Crypturgus cinereus Herbst, 1793		3				3
Cyclorhipidion bodoanum Reitter, 1913			3			3
Dryocoetes villosus Fabricius, 1792			1	3	1	5
Ernoporus tiliae Panzer, 1793	2					2
Hylastes ater Paykull, 1800		2		1		3
Hylastes attenuatus Erichson, 1836	1	10	2	1	3	17
Hylastes cunicularius Erichson, 1836	4	2	4		1	11
Hylesinus varius Fabricius, 1775	1					1
Ips acuminatus Gyllenhal, 1827		1	1			2
Ips duplicatus C.R. Sahlberg, 1836		1				1
Ips typographus Linnaeus, 1758	110	33	4	2	5	154
Kissophagus novaki Reitter, 1894	1					1
Orthotomicus suturalis Gyllenhal, 1827				1		1
Phloeosinus aubei Perris, 1855 (female)	3	2	7	4	2	18
Phloeosinus aubei Perris, 1855 (male)	5	3	6			14
Phloeosinus thujae Perris, 1855					2	2
Pityogenes bidentatus Herbst, 1783	1					1
Pityogenes chalcographus Linnaeus, 1760	89	284	5	32	1	411
Pityophthorus lichtensteinii Ratzeburg, 1837				1		1
Platypus cylindrus Fabricius, 1792			1			1
Polygraphus grandiclava C.G. Thomson, 1886	8					8
Polygraphus poligraphus Linnaeus, 1758	1	2	1			4
Scolytus intricatus Ratzeburg, 1837					1	1
Scolytus mali Bechstein, 1805		1				1
Scolytus ratzeburgii E.W. Janson, 1856	1					1
Scolytus rugulosus P.W.J. Müller, 1818	1			1		2
Taphrorychus bicolor Herbst, 1793	3					3
Xyleborinus saxesenii Ratzeburg, 1837	1	4	7	2	20	34

(Continues)

Species/locality	Hudlice No1 2020	Hudlice No2 2020	Jalovce na Světovině 2020	Jalovcová stráň u Vrbičky 2020	Záborná Lhota 2020	Total
Xyleborus dryographus Ratzeburg, 1837		3		46		49
Xyleborus monographus Fabricius, 1792		8		3		11
Xylocleptes bispinus Duftschmid, 1825		1				1
Total	233	360	43	97	37	770

APPENDIX 3: Numbers of bark beetles caught with two lures (Hostowit®, and juniper branch with ethanol) in two localities of western and central Bohemia in Experiment 2 (2021).

Species/locality	Hudlice No1 2021	Jalovce na Světovině 2021	Total
Anisandrus dispar Fabricius, 1792	5	44	49
Cyclorhipidion bodoanum Reitter, 1913		11	11
Dryocoetes autographus Ratzeburg, 1837		1	1
Gnathotrichus materiarius Fitch, 1858	19		19
Hylastes ater Paykull, 1800	1	1	2
Hylastes attenuatus Erichson, 1836	96	5	101
Hylastes cunicularius Erichson, 1836	2		2
Hylesinus varius Fabricius, 1775	1	1	2
Ips acuminatus Gyllenhal, 1827		1	1
Ips typographus Linnaeus, 1758	1		1
Orthotomicus laricis Fabricius, 1792	6	1	7
Phloeosinus aubei Perris, 1855 (female)		2	2
Phloeosinus aubei Perris, 1855 (male)	1	3	4
Pityogenes chalcographus Linnaeus, 1760	4	3	7
Scolytus rugulosus P.W.J. Müller, 1818	2		2
Taphrorychus bicolor Herbst, 1793	2		2
Tomicus piniperda Linnaeus, 1758	2	5	7
Xyleborinus saxesenii Ratzeburg, 1837	6	54	60
Xyleborus dryographus Ratzeburg, 1837		2	2
Xyleborus monographus Fabricius, 1792		4	4
Total	148	138	286

10.8 Using ethanol and other lures to monitor invasive ambrosia beetles in endemic populations: case study from the Czech Republic.

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Using ethanol and other lures to monitor invasive ambrosia beetles in endemic populations: case study from the Czech Republic

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Bark and ambrosia beetles pose significant threats to the stability of forest stands worldwide, making their control crucial. Among these pests, *Gnathotrichus materiarius*, a polyphagous invasive ambrosia beetle living on conifers, has successfully established itself in Europe. Early identification of these pests plays a fundamental role in designing effective pest control strategies. The work aimed to assess the efficacy of different lures in Ecotrap® for capturing of invasive ambrosia bark beetles. The lures tested included Wood Stainers Lure (containing the potential pheromone sulcatol for capturing *Gnathotrichus materiarius* adults), α-pinene, ethanol UHR, and Cembräwit. The objective was to determine the most suitable lure for use in traps. In four locations in western Bohemia, a total of 7,410 individuals from 46 species of ambrosia and bark beetleswere captured. The abundance of invasive ambrosia beetles (*Gnathotrichus materiarius*, *Cyclorhipidion bodoanum*, *Xyleborinus attenuatus*, and *Xylosandrus germanus*) primarily depended on the day of the season and secondarily on the lure used. Although their population density was low, more beetles were caught using ethanol as the lure. Notably, these invasive ambrosia beetles accounted for less than 3 % of the total number of ambrosia and bark beetles detected (187 individuals). Ethanol was found to be a universal lure for attracting ambrosia beetles, with the majority of Scolytinae species being captured in traps baited with ethanol.

KEYWORDS

α-pinene, Cembräwit, *Cyclorhipidion bodoanum*, *Gnathotrichus materiarius*, sulcatol, *Xylosandrus germanus*, *Xyleborinus attenuatus*

1. Introduction

Bark and ambrosia beetles are among the most serious pests that threaten the stability of forest stands worldwide (Hulcr and Dunn, 2011; Hlásny et al., 2021). While bark beetles feed on the phloem, interrupting the transport of substances produced by photosynthesis and utilizing transferred fungi to overcome the defensive reactions of the host tree, ambrosia beetles feed on fungi carried in their mycangia and actively cultivate them on gallery walls within the wood (Hulcr et al., 2007). Invasive bark and ambrosia beetles pose threats not only to native biodiversity and functional ecosystems but also to the economic productivity of forest management (Brockerhoff et al., 2006a; Aukema et al., 2011; Gohli et al., 2016). Early identification of pests is a fundamental step in invasive bark and ambrosia beetle control, as it aids in the design of pest management strategies (Douglas et al., 2009).

Implementing integrated control measures proves more cost-effective than the loss of timber (Franjević et al., 2016). For example, failure to monitor the invasive ambrosia beetle in *Pinus taeda* L. forests in the southern USA has resulted in an estimated economic loss amounting to several hundred dollars per hectare for landowners (Susaeta 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).

Bark and ambrosia beetles use a complex chemical communication system (utilizing pheromones, allomones, kairomones, synomones) to locate and infest new host trees for feeding, mating, and reproduction (Wood, 1982; Hulcr et al., 2007; Seybold et al., 2018). Trees play a crucial role as producers of kairomones. When trees experience stress due to factors like drought, frost, floods, fire, or human-induced damage, they release ethanol, which is the most important volatile compound attractive to ambrosia beetles (Kühnholz et al., 2001; Kelsey and Joseph, 2003; Ranger et al., 2013, 2019, 2021). Different combinations of volatile compounds have been employed to monitor and detect bark and ambrosia beetles at an early stage. One of the most used approaches involves the simultaneous use of ethanol and α-pinene (Borden et al., 1980; Schroeder and Lindelöw, 1989; Miller and Rabaglia, 2009; Burbano et al., 2012; Flaherty et al., 2019).

In Europe, most invasive ambrosia beetles originate from temperate or subtropical regions. When these invasive beetles colonize new territories with suitable conditions, their population numbers can increase dramatically over time (Galko et al., 2014; Boland, 2016). Conversely, under unsuitable conditions, the abundance of ambrosia beetles remains low, or the population will even disappear (Fiala et al., 2020). *Gnathotrichus materiarius* Fitch, 1858 a polyphagous invasive ambrosia beetle that primarily infests conifers, has successfully spread throughout Europe (Kamp, 1970). Despite its presence in European forests for nearly a century, no significant damage has been reported from *G. materiarius* infestations (Mazur et al., 2018). Although we can lure this ambrosia beetle to host tree logs (Fiala et al. in prep.), it is often caught in traps baited with lures designed for capturing bark beetles of the *Ips* genus (Valkama et al., 1997; Fiala, 2019). Commonly used lures for this purpose include ID Ecolure®, IT Ecolure®, Cembräwit®, Amitinuswit®, Hostowit®, and ethanol combined with other lures (Schneider, 1985; Knížek, 2009; Mazur et al., 2018). It is worth noting that sulcatol, a potential aggregation pheromone, has also been identified (Flechtmann and Berisford, 2003) and for other member of Corthylini ambrosia beetles, *Monarthrum mali* Fitch, 1856 (Miller and Crowe, 2020). All mentioned lures are alcohol-based, aligning with the use of ethanol for capturing ambrosia beetles (Lingren and Fraser, 1994; Rassati et al., 2015; Rabaglia et al., 2019).

We aimed to: (i) Analyze the effectiveness of various lure types on capturing invasive ambrosia beetles in endemic populations in central Europe; (ii) Analyze the impact of various lure types on species richness and abundance of both bark and ambrosia beetles, identifying their distinct responses to lure treatments; (iii) Examine the proportions of invasive ambrosia beetles and native coniferous bark beetles in relation to lure attractiveness, emphasizing the lure preferences of each group; (iv) Assess the potential interaction effects between season and lure type, elucidating how these factors jointly influence beetle behavior and their distribution across the study period.

This work aimed to compare the attractiveness of different lures and determine which one is most effective in attracting *G. materiarius*. The lures tested included Wood Stainers Lure, containing the pheromone sulcatol (Flechtmann and Berisford, 2003); α-pinene due to the preference of coniferous bark beetles (Schroeder and Lindelöw, 1989); ethanol as *G. materiarius* is an ambrosia beetle known to respond to ethanol (Kelsey and Joseph, 2003), and Cembräwit since *G. materiarius* has shown a positive response also to this lure (Schneider, 1985). We predicted greater catches of invasive ambrosia beetles, coniferous bark beetles, and *Ips* spp., in traps baited, respectively, with ethanol, α-pinene, and the Cembräwit lure.

2. Materials and methods

2.1. Study localities

The experiments were conducted in four localities in western Bohemia (Czech Republic), in 2022. The invasive ambrosia beetle species, *G. materiarius*, *Xylosandrus germanus*, *Cyclorhipidion bodoanum* Reitter, 1913, and *Xyleborinus attenuatus* Blandford, 1894, were found to occur in the studied areas. All the localities are coniferous forests, namely Úbočí (50.0259°N, 12.5859°E, 750 m asl), Kladská (50.0116°N, 12.6746°E, 865 m asl), Žihle (50.0391°N, 13.3520°E, 510 m asl), and Kdyně (GPS 49.4024°N, 13.0995°E, 600 m asl) (Figure 1).

2.2. Trapping

In 2022, four lures were compared for their ability to attract and trap *G. materiarius*: Cembräwit (as the control, Witasek GmbH, Austria), α-pinene, ethanol UHR (all from Synergy Semiochemicals Corp., United States), and Wood Stainers Lure (a mixture of α-pinene, ethanol, and sulcatol) (abbr. as WSL) (Alpha Scents, Inc., United States) (Table 1). All four lures were deployed in traps across all four localities. Each locality had 20 Ecotrap® traps (4 treatments × 5 replicates per treatment) (Fytofarm spol. s r.o., Slovakia) arranged in a linear transect at 10-meter intervals. This interval is sufficient because the effectiveness of traps on flying bark beetles can be calculated in units of meters (Duelli et al., 1997). The traps were placed 130 cm above the ground using wooden stakes along an east-west transect, with one lure assigned per trap. Randomly placed in the trap row, there were five replicates per lure treatment per locality.

The traps, baited with the lures, were deployed at the end of April, and checked every 14 days until mid-August. The collected beetles were frozen and subsequently identified by the first author according to Pfeffer (1989). Even if the beetles exceptionally had been damaged by predators, it was possible to study the remains and determine the number of specimens and species.

2.3. Statistical analysis

The data were processed using R 4.2.2 (R Core Team, 2022). Negative Binomial Zero-Inflated Mixed Models from the NBZIMM library (Yi, 2020) were fitted to analyze the richness and abundance of bark and ambrosia beetles. The response variables included the abundance of all beetles, the richness of all beetles, the overall abundance of invasive ambrosia beetles (*C. bodoanum*, *G. materiarius*,

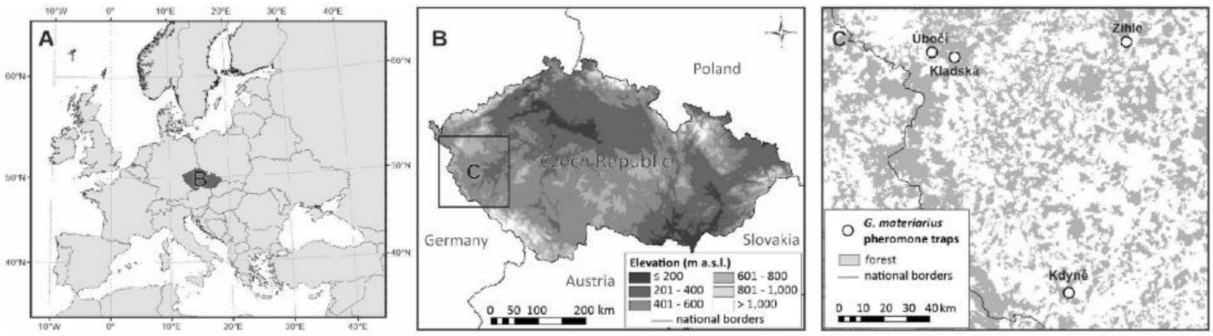

FIGURE 1
The location of the Czech Republic in Europe **(A)**, the geography of the Czech Republic **(B)**, and the localities where lures for their ability to attract *Gnathotrichus materiarius* were compared **(C)**.

TABLE 1 Descriptions of the lures used for catching *Gnathotrichus materiarius.*

Lure	Abbreviations	Release rate (g/day)/type of package	Compounds	CAS number
Alpha-pinene	α-pinene	0.070/bubble cap		80-56-8
Cembräwit	Cembräwit	0.060/pouch	Ipsenol, ipsdienol, methyl butenol and amitinol	
Wood Stainers lure	WSL	0.362/bubble cap	Sulcatol, alpha-pinene, ethanol; 1:1:1	Sulcatol: 1569-60-4 alpha-pinene: 80-56-8 ethanol: 64-17-5
Ethanol UHR	Ethanol	0.300/pouch		64-17-5

X. germanus, and *X. attenuatus*), their richness, and their individual abundances (except for *X. attenuatus*, where the model was omitted due to limited data). As potential explanatory variables, we considered the type of lure, the day of the season (dos), the quadratic polynomial of the dos, and the interaction between dos and the type of lure as this analysis focused on investigating the season-part-dependent effect of individual lures. The model incorporated sampling location, individual plots nested within the sampling location, and individual traps nested in plots as random terms. These components simultaneously defined the autocorrelation structure as "ar1" in the model.

For the second part of the analysis, Generalized Linear Mixed Models with binomial distribution from the lme4 library (Bates et al., 2015) were employed. Since the focus shifted away from the season, the catches from individual traps were aggregated across the season. The explanatory variable considered was the type of substance, while the random terms included sampling location and individual plots nested within the sampling location. The response variables were the proportions of individuals of invasive ambrosia beetles in all ambrosia beetles, the genus *Ips*, and native coniferous bark beetles (excluding *Ips*) in all individuals. The resulting models were compared with the respective null models to assess their fit. To monitor potential autocorrelation, auto- and cross-covariance and -correlation function estimation was performed on the residuals of the model. Given the interdependence of proportions, the resulting *p*-values were adjusted based on the false discovery rate. Potential convergence issues in the algorithm were addressed by setting the maximum number of iterations for the model to 10^5, establishing a tolPwrss tolerance level of 10^{-3} for declaring convergence in the penalized iteratively weighted residual sum-of-squares, and utilizing the Gauss-Hermite algorithm. Furthermore, in the model examining the ratio of individuals of invasive ambrosia beetles to all individuals, the sampling location Kladská and α-pinene lure were excluded from the analysis. This decision was made due to the absence of recorded invasive ambrosia beetles. In the analyses, WSL was chosen as the control group due to the absence of invasive ambrosia beetles on α-pinene and their limited abundance on Cembräwit.

Partial Canonical Correspondence Analysis (p-CCA) from Canoco 5.01 (ter Braak and Šmilauer, 2012) was utilized to investigate whether the dos and lure had a significant impact on the composition of beetle communities, with sampling locations included as a covariate. Before the analysis, rare species were down-weighted, and the results were assessed using the Monte-Carlo permutation test with 999 permutations. To identify indicator species for each lure, the IndVal method was employed, which considers the frequency and relative abundance of the beetles (Dufrêne and Legendre, 1997). This was followed by multilevel pattern analysis using "indicspecies" (De Cáceres and Legendre, 2009) and "labdsv" (Roberts, 2019) libraries. Most of the plots were generated using the sciplot library (Morales, 2020).

3. Results

In total, 46 species of bark and ambrosia beetleswere caught amounting to a total of 7,410 individuals (Supplementary Table S1).

3.1. Invasive ambrosia beetles

The numbers of *G. materiarius* captured were primarily dependent on the day of the season ($\mathrm{df}_{\mathrm{num}} = 1$, $\mathrm{df}_{\mathrm{den}} = 556$, $F = 119.65$, $p < 0.001$), with higher numbers observed at the beginning of the season. The lure type also had an effect ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 57$, $F = 4.95$, $p = 0.004$), where most beetles were caught using ethanol (Figure 2A). The interaction between the day of the season and the lure was not found to be significant ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 556$, $F = 1.69$, $p = 0.168$). When comparing the capture of *G. materiarius* in WSL-baited traps with other lure treatments, no beetles were captured with α-pinene, fewer beetles were captured with Cembräwit, and more beetles were captured with ethanol.

The numbers of *C. bodoanum* captured were significantly influenced by the day of the season ($\mathrm{df}_{\mathrm{num}} = 1$, $\mathrm{df}_{\mathrm{den}} = 556$, $F = 405.82$, $p < 0.001$) with higher numbers observed at the beginning of the season. The lure type, on the other hand, had an insignificant effect ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 57$, $F = 0.80$, $p = 0.497$). However, there was a strong interaction between the day of the season and the type of lure ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 556$, $F = 35.80$, $p < 0.001$). The highest number of beetles was found in traps baited with ethanol at the beginning of the season (Figure 2B).

The numbers of *X. germanus* were significantly influenced by the day of the season ($\mathrm{df}_{\mathrm{num}} = 1$, $\mathrm{df}_{\mathrm{den}} = 556$, $F = 633.56$, $p < 0.001$) with higher numbers observed at the beginning of the season. The lure type, however, had an insignificant effect ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 57$, $F = 0.81$, $p = 0.491$). Nevertheless, there was a strong interaction between the day of the season and the type of lure ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 556$, $F = 8.00$, $p < 0.001$). When comparing the catch with WSL-baited traps, no beetles were found on α-pinene or Cembräwit, while a higher number of beetles were found on ethanol at the beginning of the season (Figure 2C).

3.2. Richness and abundance of bark and ambrosia beetles

The average species richness of ambrosia beetles per trap depended on the day of the season ($\mathrm{df}_{\mathrm{num}} = 2$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 115.26$, $p < 0.001$) with higher richness at the beginning of the season around the end of the May, and on the type of lure ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 57$, $F = 39.96$, $p < 0.001$), the interaction between them was not significant ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 1.36$, $p = 0.228$, Figure 3A). In comparison to WSL, lower species richness was captured on α-pinene and Cembräwit, but higher species richness was captured on ethanol UHR.

The average abundance of ambrosia beetles per trap depended on the day of the season ($\mathrm{df}_{\mathrm{num}} = 2$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 274.99$, $p < 0.001$) with higher abundance at the beginning of the season around the end of the May, and on the type of lure ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 57$, $F = 46.98$, $p < 0.001$) with their interaction ($\mathrm{df}_{\mathrm{num}} = 6$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 2.20$, $p = 0.041$, Figure 3B). In comparison to WSL, lower abundance was captured on α-pinene and Cembräwit, and similar abundance was captured on ethanol UHR with except of period from end of May till end of June, where ethanol UHR was most effective lure.

The average species richness of bark beetles per trap depended on the day of the season ($\mathrm{df}_{\mathrm{num}} = 2$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 156.16$, $p < 0.001$) with higher richness at the beginning of the season around the end of the May, and tended to depend on the type of lure ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 57$, $F = 2.76$, $p = 0.050$), the interaction between them tended to be significant ($\mathrm{df}_{\mathrm{num}} = 3$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 1.87$, $p = 0.084$, Figure 3C). WSL was the most effective lure at the beginning of the season, later in the season, its efficacy became comparable to other compounds, leading to a diminishing distinction in attractant preferences among bark beetles.

The average abundance of bark beetles per trap depended on the day of the season ($\mathrm{df}_{\mathrm{num}} = 2$, $\mathrm{df}_{\mathrm{den}} = 552$, $F = 187.65$, $p < 0.001$) with higher abundance at the beginning of the season around the end of

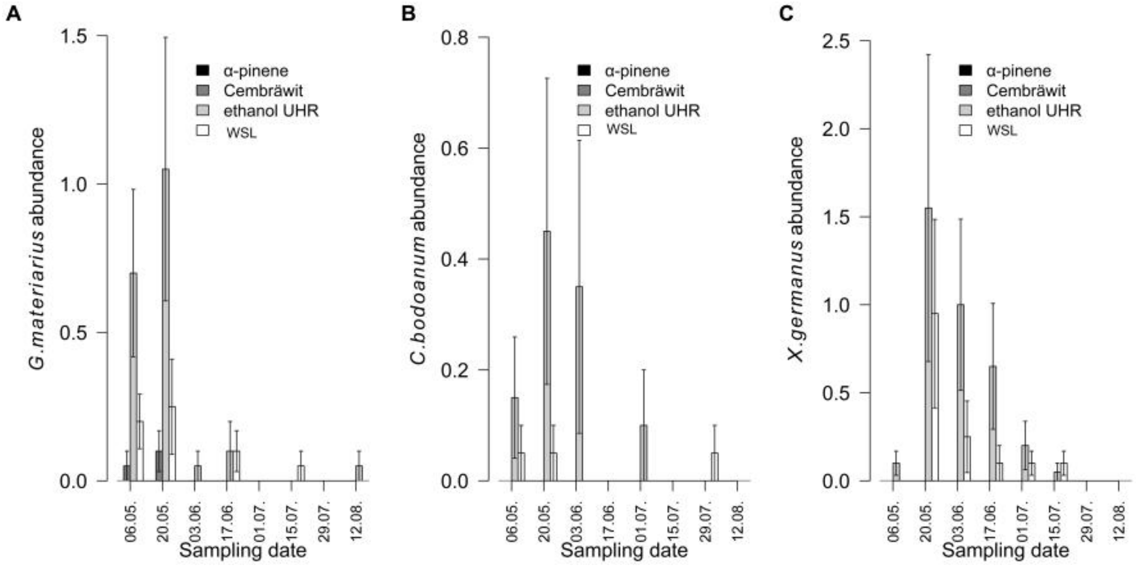

FIGURE 2
The numbers of ambrosia beetles caught per trap, including *Gnathotrichus materiarius* **(A)**, *Cyclorhipidion bodoanum* **(B)**, and *Xylosandrus germanus* **(C)**, varied depending on the type of lure and the date of checking in year 2022 (mean ± SE) (WSL, Wood Stainers lure).

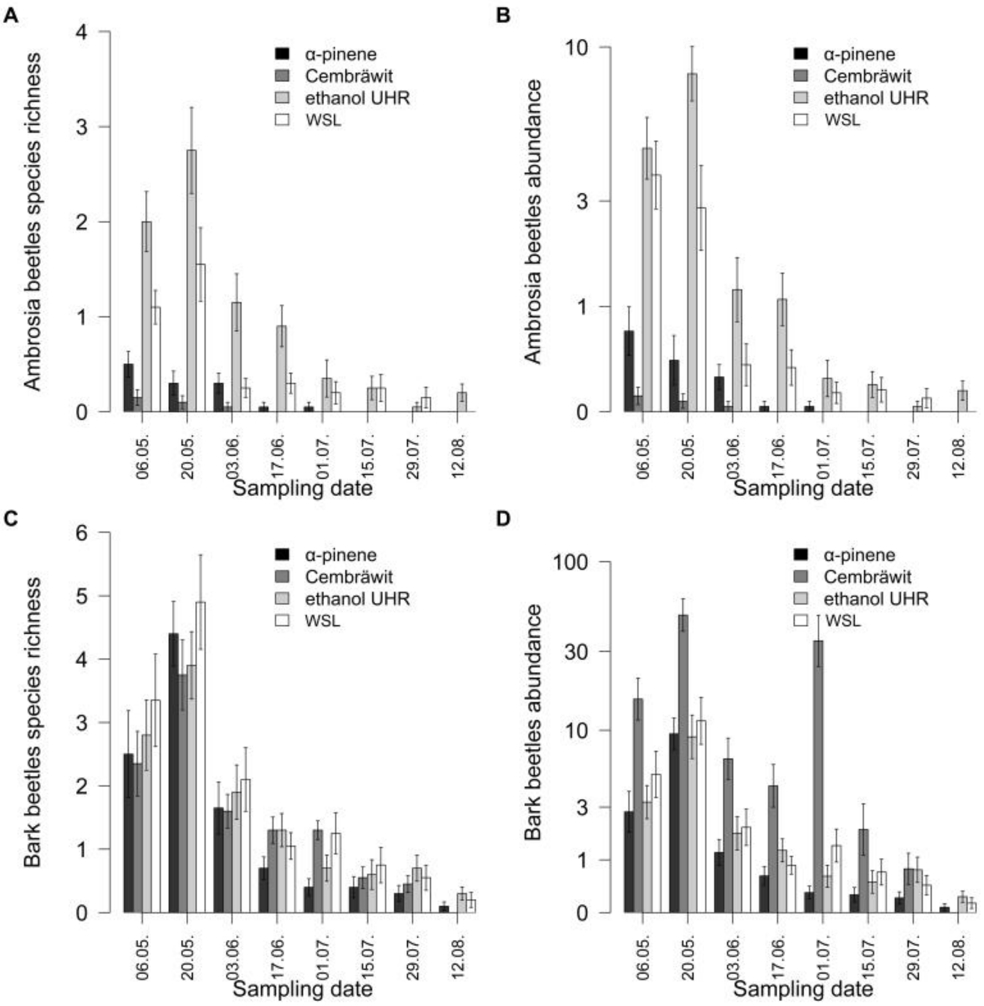

FIGURE 3
The richness **(A,C)** and abundance **(B,D)** of ambrosia and bark beetles varied depending on the type of lure and the date of checking in year 2022 (mean ± SE) (WSL, Wood Stainers lure).

the May, and on the type of lure ($\mathrm{df_{num}} = 3$, $\mathrm{df_{den}} = 57$, $F = 52.86$, $p < 0.001$) with their interaction ($\mathrm{df_{num}} = 6$, $\mathrm{df_{den}} = 552$, $F = 12.55$, $p < 0.001$, Figure 3D). In comparison to WSL, lower abundance was captured on α-pinene, higher abundance was captured on Cembräwit, and similar abundance was captured on ethanol UHR. Cembräwit had a different progression of the season than the other types of lure—a second peak during the season and a much faster decline after that.

3.3. Richness and abundance of invasive species and all bark and ambrosia beetles

The average species richness of bark and ambrosia beetles per trap was significantly influenced by the day of the season ($\mathrm{df_{num}} = 2$, $\mathrm{df_{den}} = 552$, $F = 195.32$, $p < 0.001$), with higher richness observed at the beginning of the season, particularly around the end of the May. Additionally, the type of lure also had a significant effect ($\mathrm{df_{num}} = 3$, $\mathrm{df_{den}} = 57$, $F = 15.30$, $p < 0.001$). However, the interaction between them was not found to be significant ($\mathrm{df_{num}} = 3$, $\mathrm{df_{den}} = 552$, $F = 1.72$, $p = 0.114$, Figure 4A). When comparing the species richness captured in WSL-baited traps with other lure treatments, lower species richness was observed on α-pinene and Cembräwit, while higher species richness was observed on ethanol.

The average abundance of bark and ambrosia beetles per trap was significantly influenced by the day of the season ($\mathrm{df_{num}} = 2$, $\mathrm{df_{den}} = 552$, $F = 226.86$, $p < 0.001$), with higher abundance observed at the beginning of the season, particularly around the end of the May. The type of lure also had a significant effect ($\mathrm{df_{num}} = 3$, $\mathrm{df_{den}} = 57$, $F = 63.26$, $p < 0.001$), and there was a significant interaction between them ($\mathrm{df_{num}} = 6$, $\mathrm{df_{den}} = 552$, $F = 14.49$, $p < 0.001$, Figure 4B). When comparing the abundance captured in WSL-baited traps with other lure treatments, lower abundance was observed on α-pinene, higher abundance was observed on Cembräwit, and similar abundance was observed on ethanol. Cembräwit showed a different progression throughout the season compared to the other lure types, with a second peak during the season and a faster decline afterward.

The average species richness of invasive beetles per trap was significantly influenced by the day of the season ($\mathrm{df_{num}} = 2$, $\mathrm{df_{den}} = 552$, $F = 91.80$, $p < 0.001$), with higher richness observed at the beginning of the season, particularly around the end of the May. The type of lure also had a significant effect ($\mathrm{df_{num}} = 3$, $\mathrm{df_{den}} = 57$, $F = 2.99$, $p = 0.038$, Figure 4C).

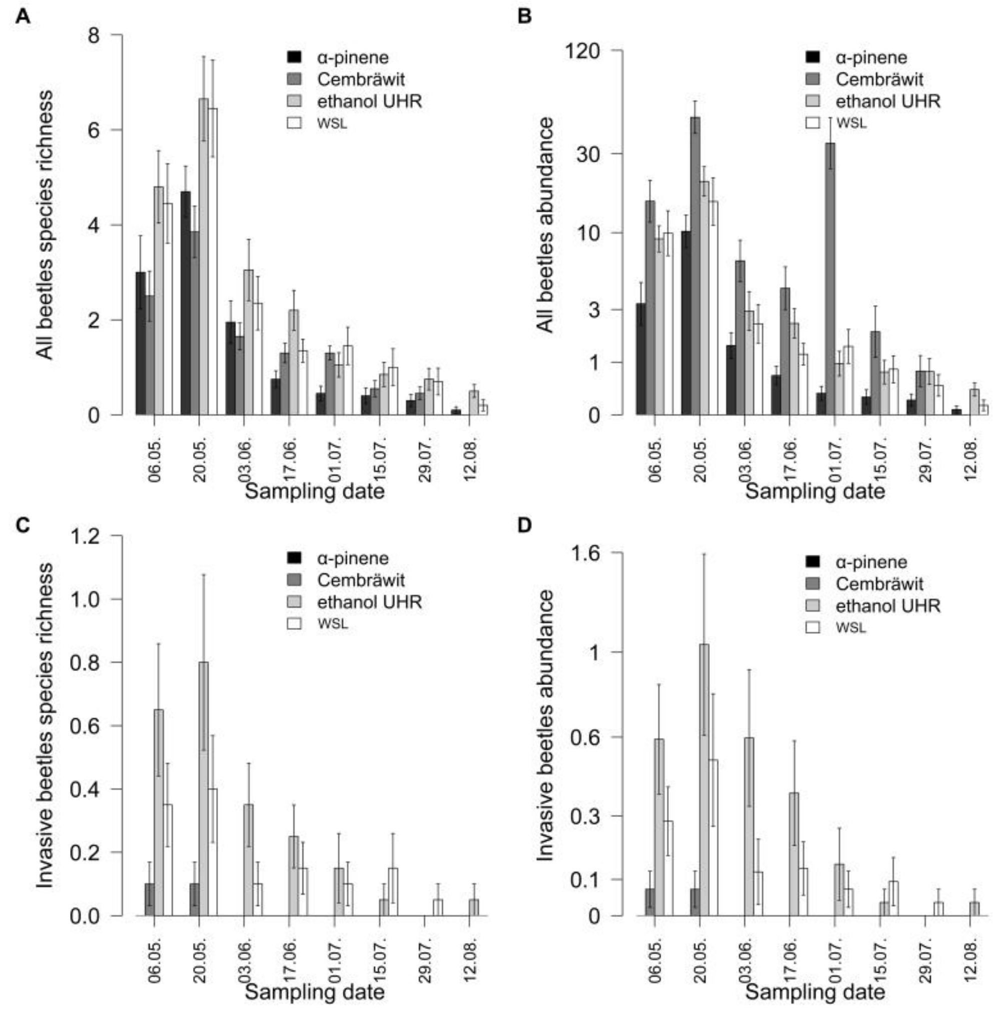

FIGURE 4
The richness **(A,C)** and abundance **(B,D)** of both invasive and all bark and ambrosia beetles varied depending on the type of lure and the date of checking in year 2022 (mean ± SE) (WSL, Wood Stainers lure).

However, the interaction between them was not found to be significant ($df_{num} = 3$, $df_{den} = 552$, $F = 1.13$, $p = 0.339$). When comparing the species richness captured in WSL-baited traps with other lure treatments, no beetles were captured on α-pinene, lower species richness was observed on Cembräwit, and higher species richness was observed on ethanol.

The average abundance of invasive beetles per trap is significantly influenced by the day of the season ($df_{num} = 2$, $df_{den} = 558$, $F = 98.05$, $p < 0.001$), with higher abundance observed at the beginning of the season, particularly around the end of the May. The type of lure also had a significant effect ($df_{num} = 3$, $df_{den} = 57$, $F = 5.89$, $p = 0.001$, Figure 4D). When comparing the abundance captured in WSL-baited traps with other lure treatments, no beetles were captured on α-pinene, lower abundance was observed on Cembräwit, and higher abundance was observed on ethanol.

3.4. Ratio of invasive beetles to all bark and ambrosia beetles

The proportion of invasive ambrosia beetles to all ambrosia beetles depended on the type of the lure ($df = 40$, $\chi^2 = 10.52$, $p = 0.005$, Figure 5A). In comparison to WSL, no beetles were captured on α-pinene, the higher proportion was captured on Cembräwit ($z = 2.96$, $p = 0.003$), and the lower proportion tended to be captured on ethanol UHR ($z = 1.69$, $p = 0.092$). The proportion of *Ips* beetles to all individuals was significantly influenced by the type of the lure ($df = 74$, $\chi^2 = 5267.30$, $p < 0.001$, Figure 5B). When comparing the proportion in WSL-baited traps with other lure treatments, there was a tendency for a higher proportion to be captured on α-pinene ($z = 1.78$, $p = 0.075$), a higher proportion was captured on Cembräwit ($z = 37.51$, $p < 0.001$), and there was a tendency for a lower proportion to be captured on ethanol ($z = -1.73$, $p = 0.083$).

The proportion of native coniferous beetles (excluding *Ips*) to all individuals was significantly influenced by the type of the lure ($df = 74$, $\chi^2 = 33.65$, $p < 0.001$, Figure 5C). When comparing the proportion in WSL-baited traps with other lure treatments, a similar proportion was captured on α-pinene ($z = 0.19$, $p = 0.847$) and Cembräwit ($z = 0.10$, $p = 0.532$), while a lower proportion was captured on ethanol ($z = -5.03$, $p < 0.001$).

3.5. Associated taxa

Of the 46 species of bark and ambrosia beetles identified, 16 were found to significantly respond to one or more lures. Table 2 presents

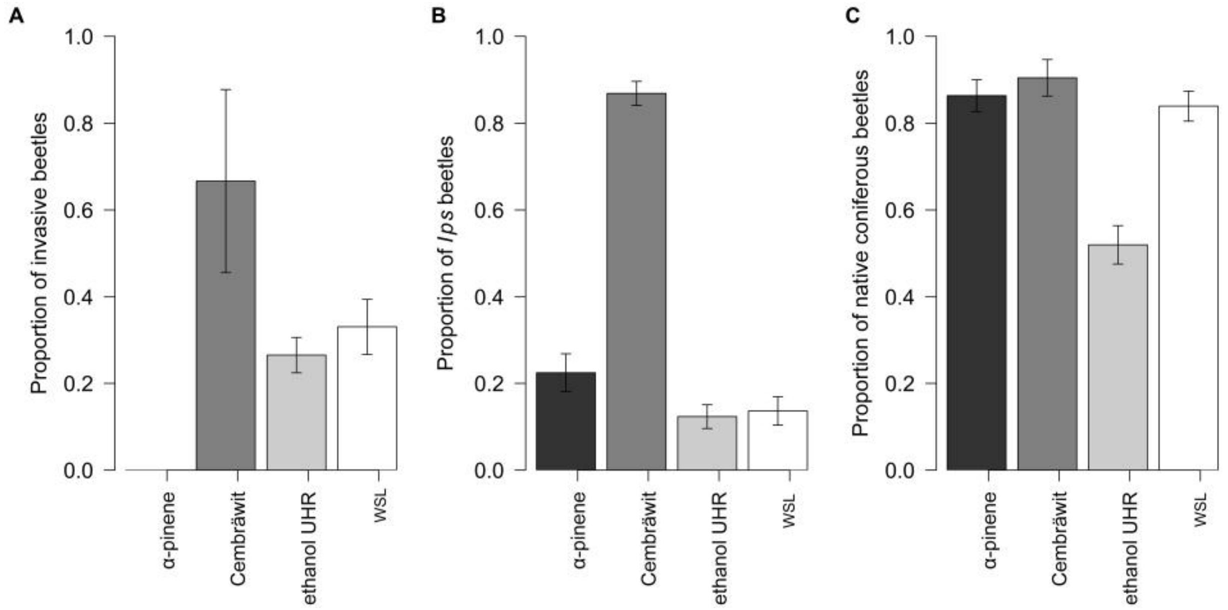

FIGURE 5
The proportion of invasive ambrosia beetles to all ambrosia beetles **(A)**, the proportion of *Ips* beetles to all individuals **(B)**, and the proportion of native coniferous beetles to all individuals (excluding *Ips*) in relation to the type of lure **(C)** (mean ± SE) (WSL, Wood Stainers lure; Dos, day of the season).

TABLE 2 Species of bark and ambrosia beetles significantly associated with studied lures or their associations (WSL, Wood Stainers Lure).

Lure/lures associations	Species	Stat	*p*-value
Cembräwit	*Ips typographus*	0.668	0.001***
	Ips cembrae	0.379	0.001***
	Pityokteines vorontzowi	0.237	0.001***
Ethanol	*Anisandrus dispar*	0.487	0.001***
	Cyclorhipidion bodoanum	0.209	0.002**
α-Pinene and WSL	*Tomicus piniperda*	0.256	0.001***
	Hylastes angustatus	0.254	0.013*
	Hylastes attenuatus	0.241	0.015*
Ethanol and WSL	*Dryocoetes autographus*	0.503	0.001***
	Xyleborinus saxesenii	0.367	0.001***
	Xylosandrus germanus	0.301	0.001***
	Gnathotrichus materiarius	0.282	0.001***
	Hylurgops palliatus	0.244	0.001***
α-Pinene and Ethanol and WSL	*Trypodendron lineatum*	0.387	0.001***
Cembräwit and Ethanol and WSL	*Orthotomicus laricis*	0.25	0.029*

the species that were associated with some lure (i.e., they were caught on this lure at higher frequencies and abundances).

Furthermore, p-CCA analysis (after removing the effect of locality) revealed that the species spectrum of bark and ambrosia beetles was primarily influenced by the lure (df = 381, $F = 20.30$, $p = 0.002$) and secondarily by the day of the season (df = 381, $F = 6.10$, $p = 0.025$; Figures 6A,B).

4. Discussion

Sulcatol has been reported as a potential aggregation pheromone of *G. materiarius* (Flechtmann and Berisford, 2003). This is likely because sulcatol has been detected in the guts of males that initiate attacks on the host tree and ceases production after mating with females (Flechtmann and Berisford, 2003). However, we captured more *G. materiarius* in ethanol-baited traps than in WSL-baited traps (Table 2). The details and chiral ratio of sulcatol in WSL are unknown to us, while the chiral ratio of the pheromone observed from the headspace of *G. materiarius* was 31% (S)-(+)- and 69% (R)-(−)-sulcatol (Flechtmann and Berisford, 2003). No *G. materiarius* has been caught on sulcatol in the USA (Miller and Crowe, 2020).

Ethanol alone significantly attracted all ambrosia beetles. It is well-established that ethanol is the primary volatile lure for ambrosia beetles (Kühnholz et al., 2001; Kelsey and Joseph, 2003; Ranger et al., 2013, 2019, 2021; Supplementary Table S2). Therefore, it was expected that ethanol would have a higher capture ratio of invasive ambrosia beetles compared to the other lures.

Among the 46 bark and ambrosia beetle species detected, *C. bodoanum*, *G. materiarius*, *X. attenuatus*, and *X. germanus* are the invasive ambrosia beetle species that were expected to be found. These

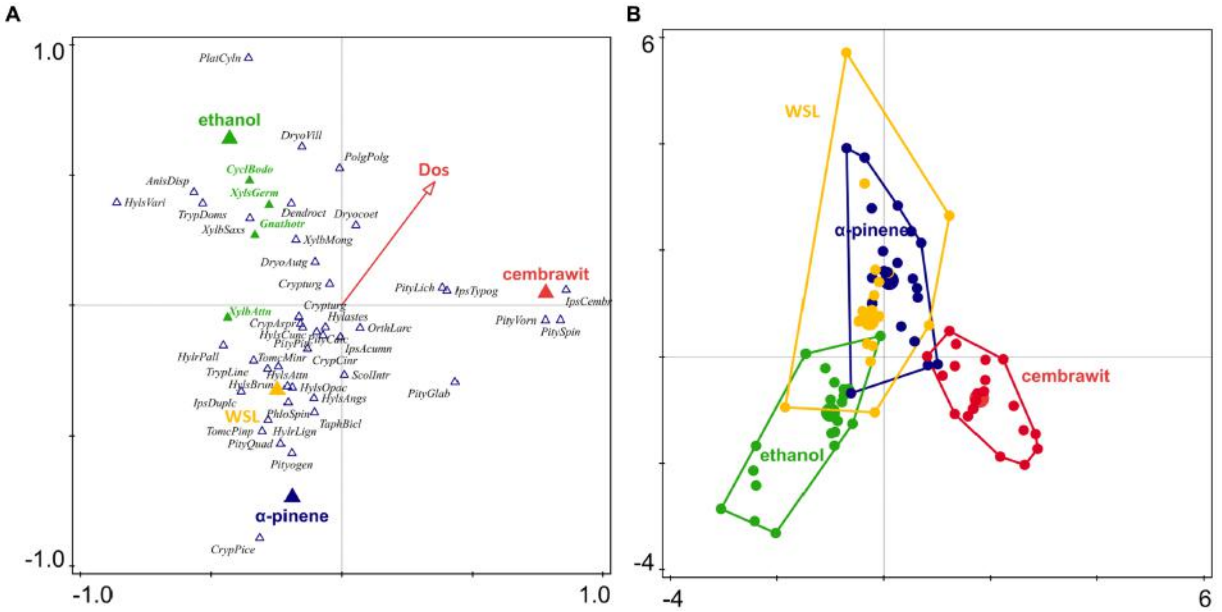

FIGURE 6
Ordination diagram of the p-CCA method showing the different compositions of the bark and ambrosia beetle assemblages depending on the lure **(A)** and PCoA diagram based on the p-CCA **(B)** analysis showing the different communities of bark and ambrosia beetles in individual lures (Dos, day of season; WSL, Wood Stainers Lure).

species were identified in a total of 187 individuals, which accounts for less than 3 % of the total number of bark and ambrosia beetles collected. This suggests that the population densities of these beetles are still relatively low. In comparison, Slovakia and Slovenia have reported higher captures of the invasive ambrosia beetle *X. germanus* (Galko et al., 2018; Franjević et al., 2019), suggesting that the Czech Republic is on the edge of the range of the species' range. Similar observations apply to *C. bodoanum* (Fiala et al., 2021) and *G. materiarius* (Fiala, observ.). *X. attenuatus*, on the other hand, is widespread throughout the Czech Republic but is not abundant (Fiala and Holuša, 2024), which explains its relatively low number of individuals in the current study.

Europe is home to approximately 30 species of invasive Scolytinae, with most of them belonging to the ambrosia beetle group (Marchioro et al., 2022; Alonso-Zarazaga et al., 2023). These ambrosia beetles are predominantly native to temperate or subtropical forests, making them particularly concerning for southern Europe regions due to similar climatic conditions (Francardi et al., 2017; Leza et al., 2020). In the Central and Northern European countries, damage has been recorded only for *X. germanus*, while other species have not caused significant harm (Maksymov, 1987; Galko et al., 2019). *X. attenuatus* is considered a secondary pest (Borowski et al., 2012; Skrylnik et al., 2019). As for *C. bodoanum*, no damage has been documented in Europe; and *G. materiarius* is also not known to cause damage in the region (Mazur et al., 2018). Nevertheless, it is important to note that all these ambrosia beetles can serve as vectors for fungi that can be detrimental to trees, so their occurrence should not be ignored (Batra, 1963; Nakashima et al., 1992; Kawasaki et al., 2010; McPherson et al., 2013; Moore et al., 2019).

The occurrence of all species of invasive ambrosia beetles, as well as most of the other bark and ambrosia beetles, was observed at a single locality (Kdyně). This can be probably attributed to the presence of diverse range of tree species, both deciduous and coniferous, in the surrounding area [e.g., *Quercus robur* L., 1753, *Larix decidua* Mill., 1768, *Fagus sylvatica* L., 1753, *Abies alba* Mill., 1768, *Populus tremulae* L., 1753, *Sorbus aucuparia* L., 1753, *Acer pseudoplatanus* L., 1753, *Pinus sylvestris* L., 1753, *Corylus avellana* L., 1753, *Picea abies* (L.) H. Karst, 1881].

In all conducted analyses, the day of season emerged as a significant factor, with the highest number of captured beetles observed in May during the study (as shown in Figures 2, 3). This pattern can be attributed to the increased flight activity of the most abundant beetle species during the period (Pfeffer, 1989). Notably, traps baited with Cembräwit exhibited a significant peak in summer, which can be attributed to the captures of *Ips cembrae*, with most often two generations per year in Central Europe (Holuša et al., 2014). The observed variation in species richness among the different localities can be attributed to the variations in tree species composition across the study area.

A total of 10 species of bark and ambrosia beetles were captured across all locations (*Anisandrus dispar*, *Crypturgus cinereus*, *Dryocoetes autographus*, *Hylastes attenuatus*, *Hylastes cunicularius*, *Ips typographus*, *Pityogenes chalcographus*, *Pityophthorus pityographus*, *Taphrorychus bicolor*, and *Xyleborinus saxesenii*). The species are commonly found in Central Europe (Pfeffer, 1989). Notably, two of these species, *I. typographus* and *P. chalcographus*, have recently experienced severe outbreaks and have become epidemic in the studied area (Fiala and Holuša, 2022). The occurrence of most of the species was observed in two or three localities, depending on the presence of their respective host trees. For instance, *Pityokteines spinidens* and *Pityokteines vorontzowi* were predominantly found in sites with *Abies alba* Mill. Another group of species was present in several localities but was captured in only a few specimens. An example is *Phloeotribus spinulosus*, which primarily develops on thin dead spruce twigs and is exclusively found on them (Pfeffer, 1989). In our experiment, this bark beetle was detected in three localities, with a total of 5 specimens caught. It can be speculated whether this occurrence is influenced by the *I. typographus* outbreak and the availability of abundant suitable breeding material, as observed in other species (Fiala and Holuša, 2021, 2022), or if it represents a long-term trend.

Relatively low catches of certain bark beetle species can be attributed to specific factors. *Cryphalus piceae* is generally a rare inhabitant of fir forests (Procházka and Schlaghamerský, 2019), and the occurrence of fir trees is limited in the Czech Republic (Kozáková et al., 2011). *Dryocoetes hectographus* primarily occurs in mountain spruce forests (Pfeffer, 1989), where traps were not placed. Only two specimens of the bark beetle *Dendroctonus micans* were caught in the Kladská locality because this species is not attracted to traps (Procházka et al., 2014). Although substances such as E-conophthorin, exo-brevicomin, and ipsdienol elicit a positive response from *D. micans*, they are most likely not its pheromones (Tømmerås et al., 1984; Zhang et al., 2002). Contrarily, the substance exo-brevicomin has shown effectiveness in trapping American and Asian species of the *Dendroctonus* genus (Barclay et al., 1998; Greenwood and Borden, 2000; Zhao et al., 2017). However, the fertilized females of *Dendroctonus* emerge from galleries and primarily rely on visual detection during host tree attacks, suggesting that pheromones may not play a significant role for them, except during the larval stage (Lukášová and Holuša, 2011).

An association between α-pinene and WSL had a significant effect on attracting conifer species of bark beetles that typically inhabit areas where pine bark meets the soil, such as *Hylastes angustatus* and *Hylastes attenuatus* (Schroeder and Lindelöw, 1989; Erasmus and Chown, 1994). While aggregation pheromones cis-verbenol and trans-verbenol have been reported for *T. piniperda* (Kangas et al., 1970), it has been suggested that *T. piniperda* relies on monoterpenes, including kairomones derived from its host, pine, for host and mate location (Lanne et al., 1987). This explains the high attractiveness of α-pinene observed in our experiments, as all the species live on pine trees (Byers et al., 1985; Schroeder and Lindelöw, 1989; Czokajlo and Teale, 1999; Poland et al., 2004).

In Cembräwit, the main component is ipsdienol, which serves as an aggregation pheromone for *Ips typographus* and *Ips cembrae* in low concentrations (Vité et al., 1972; Stoakley et al., 1978). Therefore, it is not surprising that most specimens of both bark beetles were caught in traps baited with Cembräwit (see also Grucmanová et al., 2014; Holuša et al., 2014). *Pityokteines vorontzowi* was also significantly attracted to Cembräwit, as it shares ipsdienol and ipsenol as its aggregation pheromones (Harring, 1978). The highest number of bark beetles caught was observed on Cembräwit® compared to other lures, which can be attributed to the aforementioned outbreak of *I. typographus* in the study area (Fiala et al., 2022).

Ethanol and WSL significantly attracted two species of ambrosia beetles, *Xyleborinus saxesenii* and *X. germanus* as well as two species of bark beetles, *Dryocoetes autographus* and *Hylurgops palliatus*. These species have been frequently observed in traps baited with ethanol (Schroeder and Lindelöw, 1989; Lindelöw et al., 1993). More species of bark and ambrosia beetles responded to these lures compared to any other lure.

The ambrosia beetle *Trypodendron lineatum* was significantly associated with three lures, α-pinene, ethanol, and WSL. This is consistent with previous studies that have shown α-pinene and ethanol to be attractants for this species (McLean and Borden, 1977; Schroeder and Lindelöw, 1989). Conversely, this ambrosia beetle exhibits weak attraction to the substances used in Cembrawit, such as ipsdienol and methylbutenol (Gavyalis et al., 1981). The association of Cembräwit, ethanol, and WSL significantly attracted only the bark beetle *Orthotomicus laricis*. Bark beetles of the entire genus *Orthotomicus* are primarily attracted to ipsdienol, *cis*-verbenol, and 2-methyl-3-buten-2-ol (Giesen et al., 1984; Valkama et al., 1997). Ipsdienol is the main component of Cembräwit®, which also contains ipsenol, methylbutenol, and amitinol (Zuhlke and Mueller, 2008). However, since *O. laricis* is found in the tops of uprooted spruces (Fiala, observ.), on pine stumps, and on felled spruce wood that is shaded (Holuša et al., 2017, 2019), it is evident that this bark beetle is also attracted to ethanol.

Overall, the results demonstrate that accounting for the influence of location and day of the season, the differences among lures were significant (see also Beaver and Löyttyniemi, 1991). Ethanol significantly attracted all ambrosia beetles in our experiments. In the USA and Italy, volatile lures such as ethanol, a combination of α-pinene and ethanol, and other combinations of ipsdienol+*cis*-verbenol+methylbutenol are used for monitoring invasive bark beetles (Rassati et al., 2015; Rabaglia et al., 2019). In Italy, these traps are placed in ports and adjacent forests; with higher capture success observed in deciduous forests compared to coniferous forests or ports (Rassati et al., 2015). Our experiment also shows the higher capture success of invasive ambrosia beetles was in deciduous forests, as evidenced by the capture of 162 specimens (e.g., 87%) at the Kdyně locality, probably due to the prevalence of deciduous trees in that area. A similar approach is employed in New Zealand, where traps are placed in ports, international airports, and adjacent forests using different lure combinations such as α-pinene+ethanol, β-pinene+ethanol, frontalin+ethanol, and ipsdienol. This monitoring model has proven successful in the early detection of invasive bark beetles, increasing the chances of eradication when infestations are still relatively small (Brockerhoff et al., 2006b). In Australia, the monitoring efforts have a broader focus and include the capture of Lepidoptera. Various lures such as ethanol, cineole, α-pinene, phellandrene, and a combination of pinene, phellandrene, cineole, terpene, and cymene as bait are used. Traps are positioned near ports and airports and within a 5 km radius of these areas (Bashford, 2012). In France, monitoring activities targeting invasive cerambycids include testing trapping methods using α-pinene+ethanol in Ecotrap traps. Traps were deployed in natural forests and later extended to ports, airports, and horticulture areas (Fan et al., 2019). In all these cases, the traps successfully captured invasive species.

There are several methods available for monitoring bark and ambrosia beetles, with baited traps being the most effective among them. Baited traps offer several advantages, including their ability to cover large areas, easy application in various locations, and cost-effectiveness. However, they also have some drawbacks, primarily related to the selection of bait types and the physical demands associated with checking numerous traps and subsequent determination in the laboratory (Poland and Rassati, 2019). While citizen science can help alleviate the physical demands of monitoring (Steininger et al., 2015), coordination is still necessary, and the expertise of bark beetle specialists will always be essential for accurate determination.

5. Conclusion

Ethanol serves as a universal lure that effectively attracts both bark and ambrosia beetles, with the majority of Scolytinae species being caught in traps baited with ethanol. Conversely, ethanol's inability to attract economically important species in Central Europe, such as *Ips typographus*, is a significant advantage since these species are typically abundant. By not attracting them, the number of bark and ambrosia beetles captured is not needlessly increased, thus reducing the complexity and cost associated with sorting and determination. For the detection of invasive ambrosia beetles in Central Europe, it is recommended to use one bait of ethanol per year installed in April in any impact trap. Our preference lies with Ecotrap® due to its disassemblability, storage capacity, and ability to catch beetles in a dry state without the need for preservative liquid. Moreover, the material remains intact by predators even after a two-week period.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JH: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. TF: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. PP: Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

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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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1258729/full#supplementary-material

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10.9 Ambrosia beetles prefer closed canopies: a case study in oak forests in Central Europe.

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Ambrosia Beetles Prefer Closed Canopies: A Case Study in Oak Forests in Central Europe

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Abstract: Research Highlights: The percentage of canopy closure was found to be the main factor associated with ambrosia beetle abundance and species richness. The latter two variables increased as canopy closure increased, probably because a high percentage of canopy closure provides a stable and humid environment suitable for the growth of ambrosia fungi. Objectives: Oak is a common host tree for ambrosia beetles (Coleoptera: Curculionidae: Scolytinae), which have independently evolved a nutritional mutualism with fungi. We suspected that ambrosia beetles might have specific habitat preferences that are different from those of other saproxylic beetles and that reflect the specific habitat preferences of their food, i.e., ambrosia fungi. Methods: We assessed ambrosia beetle abundance with ethanol-lured traps in five old-growth oak dominated forests and five managed oak dominated forests (one trap per forest) during the vegetation period in 2020. We determined whether ambrosia beetle abundance and species richness depend on forest type (managed vs. unmanaged), degree of canopy closure, abundance of oak trees, abundance of coarse deadwood, and abundance of dead oak branches. Results: In total, 4137 individuals of six species of ambrosia beetles associated with oaks were captured. The native ambrosia beetle *Anisandrus dispar* represented the majority of trapped ambrosia bark beetles. *A. dispar* along with another ambrosia beetle, *Xyleborinus saxesenii*, represented 99% of all captured beetles. Conclusions: In addition to canopy closure, the abundance of oak trees and the abundance of dead oak branches were significantly associated with ambrosia beetle abundance and species richness. The abundance of *A. dispar* was mainly correlated with dead oak branch abundance and the degree of canopy closure, whereas the abundances of *X. saxesenii* and of the invasive species *Xyleborinus attenuatus* and *Cyclorhipidion bodoanum* were mainly correlated with the net area occupied by oak trees.

Keywords: *Anisandrus dispar*; *Cyclorhipidion bodoanum*; deadwood; invasive species; *Xyleborus saxesenii*; *Xyleborinus attenuatus*; *Xylosandrus germanus*; Scolytinae; Quercus

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1. Introduction

European temperate oak woodlands have a rich and unique biodiversity, which can be mainly attributed to the life history traits and structural characteristics of the oak trees *Quercus robur* and *Q. petraea* [1–3]. In Central Europe, oak trees are components of temperate broadleaf and mixed forests. Oak is a common host tree for ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) [4,5].

The ambrosia beetles have independently evolved a nutritional mutualism with fungi [6]. Most species of ambrosia beetles depend on recently dead or stressed woody plants in which the beetles bore their tunnel systems ("galleries"). In the galleries, ambrosia beetles actively farm one or several fungal mutualists, which serve as their essential food source [6]. Some species of ambrosia beetles are among the most damaging forest pests, and species of quarantine significance are frequently moved intercontinentally [7,8].

Because oak trees attacked by other bark beetles provide suitable breeding substrates and other resources for ambrosia beetles, the volume of oaks and the area occupied by oaks under attack by other bark beetles are likely to affect the abundance of ambrosia beetles in a region [9]. Unlike other bark beetles that can fly tens of kilometers [10], ambrosia beetles can fly only up to about a hundred meters to a few kilometers [11,12]. The short dispersal distance of ambrosia beetles also suggests that the abundance of ambrosia beetles in an oak forest should be affected by the area that is occupied by oak.

Many studies in boreal and temperate forests have indicated that insect diversity increases as stands become more open because of higher temperatures and other changes in the microclimate [13–16]. Sun exposure was found to be the most important factor affecting the composition of buprestids and cerambycids in oak forests [17]. There has been sparse research on the influence of the canopy closure on the occurrence of scolytids, but some research suggests that the effects of canopy closure may differ among scolytid species. For example, *Scolytus intricatus* Ratzeburg, 1837 prefers oaks with a high canopy closure [18], but *Scolytus mali* Bechstein, 1805 prefers orchards with open canopies [19]. Similarly, the position of trap in the forest (edge vs. interior) also generally does not affect the occurrence of bark beetles [20–22], but it does affect the occurrence of some species. The scolytids *Hylurgops palliatus* Gyllenhal, 1813 and *H. glabratus* Zetterstedt, 1828, for example, require the shaded environment of the forest interior for feeding and are found in higher numbers in the forest interior than at the forest edge [23]. Two ambrosia beetles showed opposite patterns patterns: *Xylosandrus crassiusculus* Motschulsky, 1866 is more common at the forest edge, while *Xyleborinus andrewesi* Blandford, 1896 is more common in the forest interior [24]. Similarly, the bark beetle *Hylesinus taranio* Danthoine, 1788 prefers canopy closure at the forest edge [25]. We do not know of research focusing on the effect of canopy closure on ambrosia beetles.

Based on our unpublished observations, however, we suspect that ambrosia beetles are not primarily dependent on the sun exposure provided by an open canopy. On the contrary, as wet and warm conditions are important for the growth of their symbiotic fungi [26], ambrosia beetles are likely to be more abundant in wetter and warmer localities [27,28]. Localities are likely to be wetter and to have a more stable microclimate if the canopy is substantially closed rather than open [29].

The biodiversity of phloexylophagous insects is greater in old-growth oak stands than in many other kinds of forest stands, because old-growth oak stands have more deadwood, including dry branches in treetops [30,31]. For ambrosia species, this dependence was confirmed only in beech stands in that the abundance of ambrosia beetles was found to be higher in unmanaged than in managed stands [32].

In the current study, we tested the hypotheses that ambrosia beetle occurrence will depend on the degree of canopy closure, the abundance of oak trees, the abundance of deadwood, and abundance of dead oak branches. We also tested the hypothesis that ambrosia beetle occurrence is greater in unmanaged oak forests than in commercial forests [31,33,34].

2. Materials and Methods

2.1. Study Plots

The study was conducted in the biogeographically isolated area (the Chebsko-sokolovský bioregion) of "Western European broadleaf forests" in the western Czech Republic (Figure 1) (https://en.wikipedia.org/wiki/Western_European_broadleaf_forests, accessed on 12 August 2021). At lower altitudes in this area, "Western European broadleaf forest" is the main forest type. The natural vegetation of the bioregion consists mainly of acidophilic oaks (*Quercion roboris* Malcuit, 1929), but only 6% of the region is currently occupied by oaks. These are mostly in commercial oak forests, and old-growth oak forests have survived in only a few localities [35].

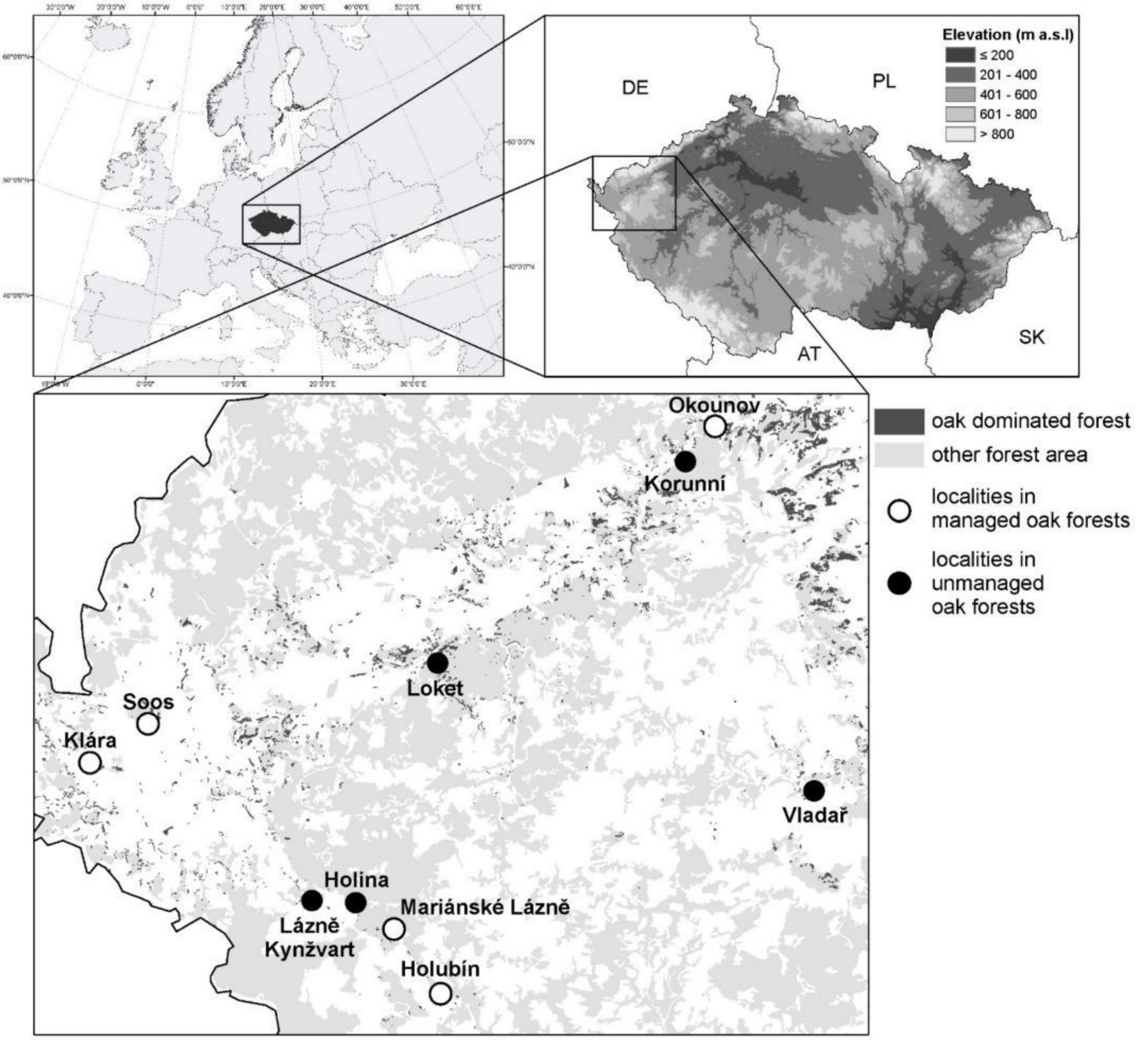

Figure 1. Locations of unmanaged oak dominated forests and managed oak dominated forests in western Bohemia where ambrosia beetle abundance and species richness were determined.

For assessing ambrosia beetle abundance by trapping, we selected oak dominated forests in which oaks represented > 60% of the trees (Only in study plot Soos the oak representation is 40%, and the rest of forest is cover by birch *Betullus* sp.) and that were >80 years old and ≥1 ha. The forests were at altitudes between 400–700 m a.s.l. (Table 1, Figure 1). The study plot is located in the mesophytic zone, which is characterized by an average annual rainfall of around 550–700 mm and an average annual temperature of 7.5 °C. Orographically, the study plots are located on flat land, with only the Korunní and Loket plots (northwest orientation) and the Vladař plot (south orientation) being on a steep slope.

Table 1. Characteristics of the studied oak dominated forests.

Study Plot	Longitude; Latitude	Altitude (m a.s.l.)	Age	Forest Type	DBH (cm)	Net Area Occupied by Oaks (ha)	Volume of Oak Wood (m^3/ha)	Volume of of Coarse Deadwood (m^3/10 m^2)	Abundance of Dead Oak Branches (No. per Tree)	Canopy Closure (%)	Distance from Stand Boundary (m)
Vladař	50°4′31″ N, 13°12′33″ E	605	130	Unmanaged	40	5.2	264	30	3	66	100
Mariánské Lázně	49°58′50″ N, 12°41′40″ E	700	185	Managed	58	0.5	369	0	0.1	48	20
Soos	50°8′51″ N, 12°24′19″ E	440	80	Managed	30	0.3	131	20	0.2	31	20
Okounov	50°21′45″ N, 13°6′28″ E	440	80	Managed	40	0.5	58	5	0	35	10
Korunní	50°20′9″ N, 13°4′11″ E	500	150	Unmanaged	50	1.5	262	20	1	66	50
Klára	50°7′7″ N, 12°19′59″ E	440	90	Managed	40	0.3	215	20	0.2	45	20
Holubín	49°55′44″ N, 12°44′53″ E	615	90	Managed	35	1.8	255	0	0.33	63	20
Loket	50°11′13″ N, 12°45′33″ E	410	110	Unmanaged	20	2.5	123	15	1	64	40
Holina	50°0′8″ N, 12°38′58″ E	700	180	Unmanaged	50	0.5	361	50	5	68	30
Lázně Kynžvart	50°0′19″ N, 12°35′48″ E	565	200	Unmanaged	120	0.7	245	5	1	66	10

Quercus robur was dominant in all localities except Vladař and Loket (see Figure 2), where both *Q. petraea* and *Q. robur* grow but *Q. petraea* dominates.

Figure 2. Views of managed 80-year-old oak dominated forests at Holubín (**a**) and unmanaged oak forests at Kynžvart (**b**) and Vladař (**c**).

2.2. Traps and Lures

To estimate the abundance and species richness of ambrosia beetles [4,36], one trap baited with ethanol was placed in the center of each study plot. Ethanol was released from a plastic-vial dispenser (ca. 250 mg·day^{-1}). These dispensers were made of polyethylene with foam and were 5 cm in diameter and 5 cm high. Each dispenser was placed in a Theysohn® trap that was located ca. 1.3 m above the ground and that faced the main wind direction. The traps were emptied, and the ethanol was replaced every 2 weeks from the beginning of April to the end of August in 2020. All trapped insects were preserved in 70% ethanol.

The insects were identified by the second author, who used Pfeffer's key [37]. Dr. Miloš Knížek (Prague) confirmed the identification of *C. bodoanum*.

2.3. Environmental Variables

Forest type. We recognized two types of forests according to management. Mature-managed forests were oak dominated forests between 80 and 120 years old. All trees in each managed forest were the same age and were very homogenous; cut stumps were abundant. Forests in this category had reached maturity (i.e., had attained their maximum annual increase in volume) and represented typical state-owned forests. The volume of deadwood and dead branches was low (Table 1). These forests were last managed ten years ago.

Most of the oldest trees in unmanaged forests were >120 years old, and the forests had not been managed for the last 70 years. The forests in this category represented the closest-to-natural forests that remain in western Bohemia. The only signs of human interference were a few scattered stumps from past selective cuttings. Unlike the managed forests, the unmanaged forests included trees of all ages including small areas with young trees. The volume of deadwood and dead branches was high (Table 1). These forests were last managed more than fifty years ago. The unmanaged forest at Kynžvart (Figure 2b) had been modified into a park with grasslands, but more than 60% of the area was covered with trees, which grew in large unbroken patches. Because the structure of this stand was otherwise similar to old-growth stands, we included this stand in the unmanaged forest category (Figure 2b). In contrast, the forest at Soos, although located in a protected area, was classified as a managed forest because it was a homogeneous stand that had been planted in a meadow.

Net area occupied by oaks: The net area was calculated as the total area of the stand multiplied by the tree density and the percentage represented by oak. Data were obtained from the regional inventory of forests.

Volume of oak wood: Data for the volume of oak trees (m^3/ha) in the oak dominated forests were obtained from the forest management plan, which contained detailed data for all forest stands.

Volume of coarse deadwood: Deadwood volume was quantified in five areas of 10 m^2/area. The diameters and lengths of the dead trees and dead branches were measured manually.

Abundance of dead oak branches: Numbers of large dead branches were determined on 10 oak trees along a transect running through each study area; the values were subsequently expressed as the mean number of dead branches per tree. The transects were located in the central part of each study area (one transect per study area) and were about 50-m long. Dead oak branches included all standing and lying dead wood with a diameter greater than 7 cm and with a hard consistency based on resistance to finger pressure.

Canopy closure: Canopy closure at each study area was assessed by photographing the sky from the ground straight up. The sky was photographed on ten places with distances of 20 m. The photographed surface was ca 200 m^2. The photographs were analysed for the percentages of white (sky) and black (canopy) using ImageJ software (v.1.47). The percentage of the area of the sky that was black in the photographs was considered equivalent to the percentage of canopy closure.

Distance from stand boundary: The distance of each trap from the nearest boundary of the studied oak dominated forest stand (not the forest edge, stand means a homogeneous unit of the spatial distribution of the forest) was measured to the nearest meter.

Values of all variables are presented in Table 1.

2.4. Statistical Analysis

The importance of forest stand characteristics for ambrosia beetle abundance and species richness was evaluated by implementing a random forest algorithm using conditional inference trees as base learners provided in the party package (function cforest, 10,000 trees generated) in R 4.0.2 software (The R Foundation, Vienna, Austria). This method was used because it is a highly effective for evaluating the importance of explanatory variables, it can manage different types of variables, and is robust with respect to the multicollinearity of variables [38]. Because certain tested explanatory variables exhibited multicollinearity in our dataset, a conditional computation of the importance was performed (option conditional = TRUE). In addition, an unbiased random forest model was constructed (option control = cforest_unbiased), because the tested predictors were both quantitative and categorical variables. The response variable in the models was represented by the number of ambrosia beetle individuals and species in specific samples (sample =

beetles captured in one trap during per 2-week-period deployment as described earlier in the Methods). The statistical significance ($\alpha = 0.05$) of each explanatory variable was evaluated using the permutation-based attribute selection algorithm provided in the Boruta package. Finally, the marginal effect of the selected significant variables on the number of trapped ambrosia beetle individuals and species was visualised with a partial dependence plot using the package pdp (functions partial followed by plotPartial).

Ordination analyses of the relationship between ambrosia beetle abundance (i.e., species composition of their assemblages) and forest stand characteristics were performed in Canoco 5 (Wageningen University & Research, Wageningen, Netherlands). Based on preliminary analysis of the data (gradient length of response data was 2.2 SD units), redundancy analysis (RDA) was used. Data on the abundance of species were log-transformed and centered by species. After a global Monte-Carlo permutation test (10,000 permutations) of a full model (including all of the available explanatory variables) confirmed the overall significance of the relationship between response and explanatory variables (pseudo-$F = 4.2$, $p = 0.001$), a forward selection of explanatory variables was performed to identify the forest stand characteristics most closely associated with ambrosia beetle abundance and species richness.

3. Results

A total of 4179 individuals and 15 species of scolytid beetles were captured in the traps that had been deployed in 10 localities with oak trees in the western Czech Republic. Among the 15 species, six (represented by 4137 individuals) were ambrosia beetle species related to oak (Appendix A, Figure 3). *Anisandrus dispar* was the most abundant beetle trapped with an average of 40.0 individuals per sample. The 3520 specimens of *A. dispar* represented 84% of the trapped beetles (Appendix B). *Xyleborinus saxesenii* Ratzeburg, 1837 was the second most abundant species with an average of 6.5 individuals per sample. The 576 specimens of *X. saxesenii* represented 14% of the trapped beetles. The other four species were represented by fewer than 20 trapped specimens, i.e., they represented less than 1% of all trapped specimens (Appendix A, Figure 3).

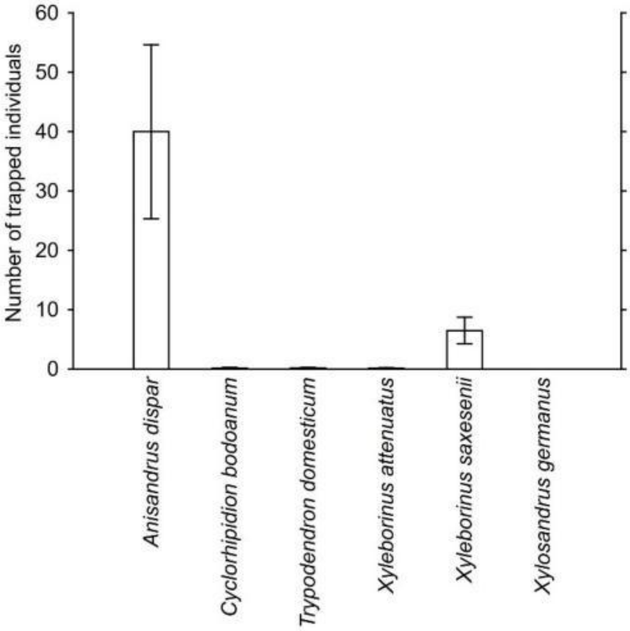

Figure 3. Number (mean ± SE) of individuals of ambrosia beetle species captured per sample. Each sample represented the beetles captured in one trap during 2-week period.

The number of ambrosia beetle individuals caught in the traps was significantly related to date of sampling, the percentage of canopy closure, and the abundance of dead oak branches per tree (Figure 4a). The number of ambrosia beetles trapped increased with the percentage of canopy closure, but the increase was considerable only when canopy closure exceeded 45% (Figure 4b). The number of beetles caught also increased with the number of dead oak branches per tree, but the relationship plateaued with four dead oak branches per tree (Figure 4c).

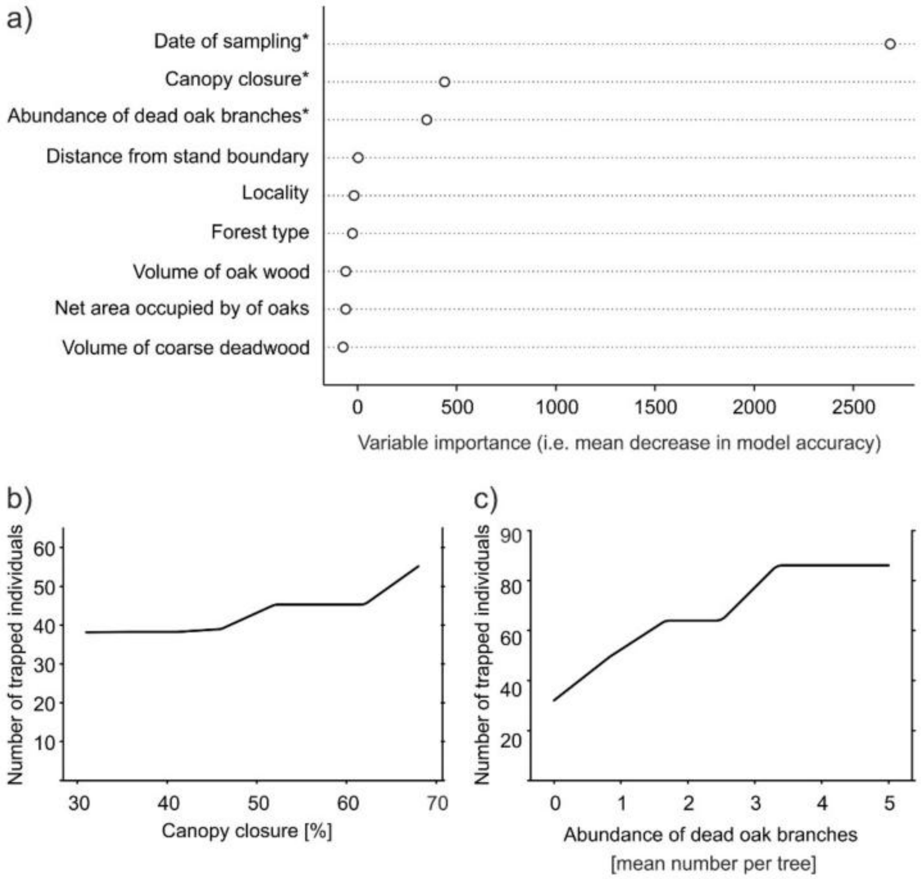

Figure 4. Results of random forest regression of number of ambrosia beetle individuals caught in traps as the response variable: (**a**) variable importance plot (based on the decrease of mean model accuracy with omission of the variable) showing the importance of particular variables for the number of ambrosia beetle individuals caught in the traps. Variables with a significant effect ($p < 0.05$) are denoted with an asterisk (*); (**b**,**c**) partial dependence plots showing the marginal effect of selected significant explanatory variables on the mean number of ambrosia beetle individuals trapped during the eight 2-week periods from April to August.

The number of ambrosia beetle species trapped was most strongly associated with the date of sampling (see Appendix B) and the percentage of canopy closure. Volume of oak wood and abundancy of dead oak branches were also associated with the number of ambrosia beetle species, whereas the associations with study plot and net area occupied by oaks were weak (even if statistically significant) (Figure 5a). The numbers of ambrosia beetle species trapped increased slightly with percentage of canopy closure, volume of oak wood, abundance of dead oak branches, and net area occupied by oaks (Figure 5b–e). The resulting curves were more or less sigmoidal, with most of the increase in the number of trapped species restricted to a narrow interval of explanatory variable values. This interval was between 40% and 50% for canopy closure; 200 and 250 m^3 for volume of oak wood; 2–3 for number of dead oak branches per tree; and 1.0–2.5 ha for the net area occupied by oaks.

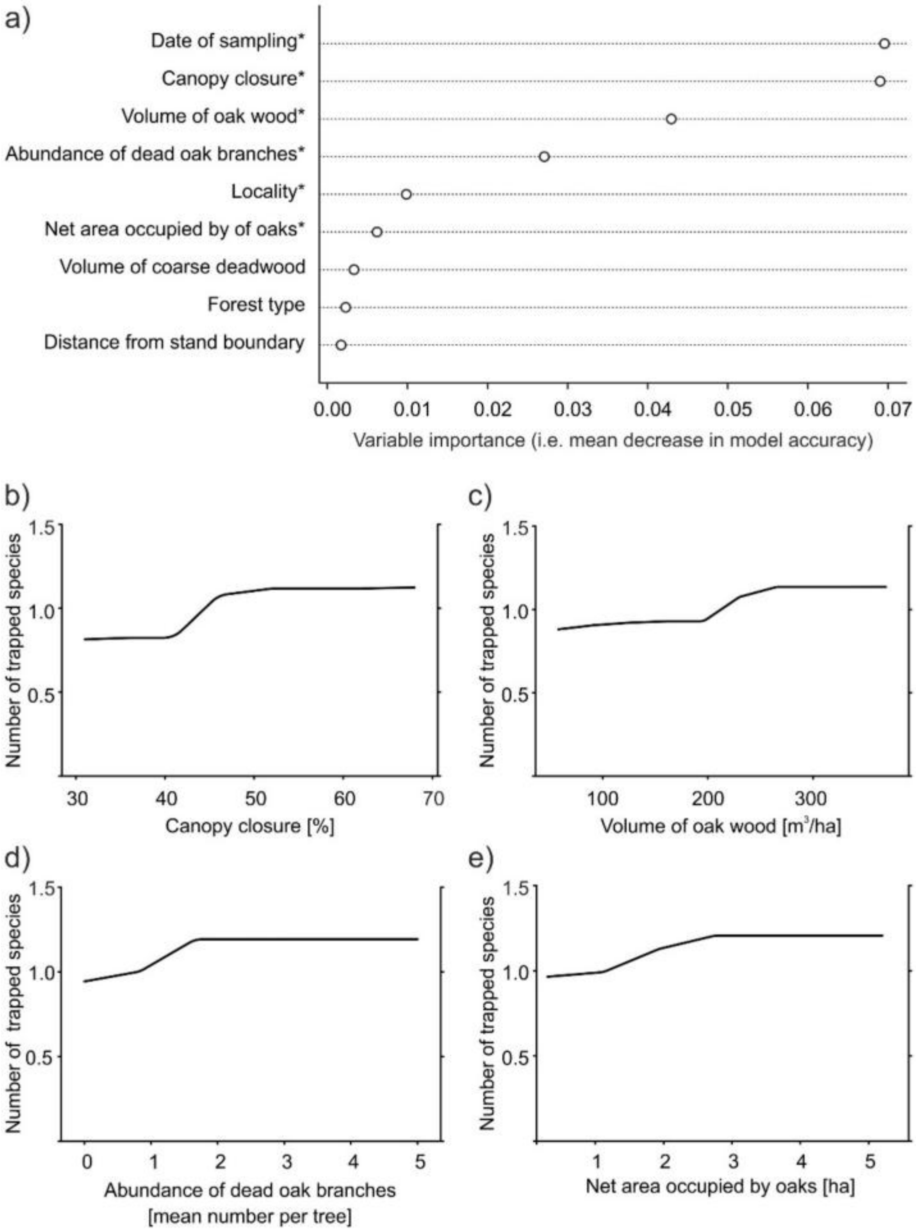

Figure 5. Results of random forest regression of the mean number of ambrosia beetle species captured per trap per 2-week period from April to August as the response variable: (**a**) plot of variable importance (based on the decrease of mean model accuracy with omission of the variable). Variables with a significant effect ($p < 0.05$) are denoted with asterisk (*); (**b**–**e**) partial dependence plots showing the marginal effect of the indicated explanatory variables on the mean number of ambrosia beetle individuals trapped during the eight 2-week periods from April to August.

The partial RDA analysis with the date of sampling treated as a covariable confirmed the significant associations between the studied explanatory variables and the occurrence and the numbers of ambrosia beetle individuals and species. Forward selection of explanatory variables indicated that two explanatory variables had significant effects: canopy closure and net area occupied by oaks (Table 2). Canopy closure had by far the highest explanatory power followed by net area occupied by oaks and the abundance of dead oak branches. The model including these three explanatory variables explained 24.4% of the variability in species occurrence and the numbers of ambrosia beetle individuals and species (Table 2).

Table 2. Results of the partial redundancy analysis forward selection of the percentage of variability in ambrosia species occurrence explained by the indicated forest stand variables. Conditional effects of the explanatory variables are shown. Effect of the date of trap deployment was removed by considering it as a covariable. The upper three explanatory variables highlighted in bold were included in the model based on the results of the forward selection process. Although it was not statistically significant, the abundance of dead oak branches was included in the model because it helped explain the variability in the occurrence of the species.

Forest Stand Variable	Explained Variability in Species Occurrence (%)	Pseudo-*F*	*p*
Canopy closure	**16.5**	**15.6**	**0.002**
Net are occupied by oaks	**6.6**	**6.7**	**0.002**
Abundance of dead oak branches	**1.3**	**1.3**	**0.234**
Volume of coarse deadwood	1.0	1.0	0.360
Distance from forest stand boundary	0.8	1.0	0.368
Forest type	0.4	0.5	0.652
Volume of oak wood	0.2	0.2	0.834

The RDA analysis revealed several associations between the abundance of ambrosia beetles and measured variables. The abundance of *A. dispar* was positively correlated with canopy closure and the abundance of dead oak branches. The abundances of *X. saxesenii* and the invasive species *X. attenuatus* and *C. bodoanum* were positively correlated with the net area occupied by oaks. The abundances of *T. domesticum* and *X. germanus* were positively but weakly correlated with the abundance of dead oak branches (Figure 6).

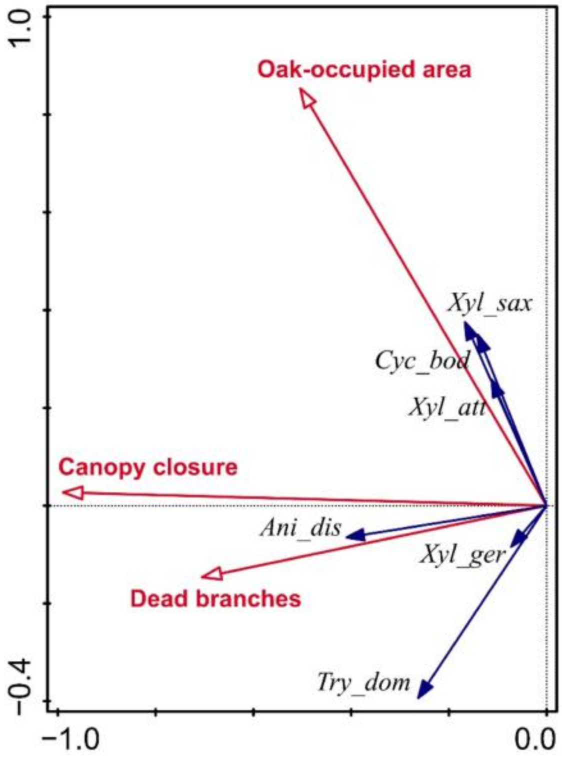

Figure 6. Results of redundancy analysis (RDA) of the relationship between presence of the ambrosia beetle species (blue arrows) and the most important characteristics of forest stand (red arrows). The species and the forest stand characteristics have been abbreviated to simplify the plot. The shown projection of the 1st and the 2nd axes represents 23.8% of the variability in species occurrence. Effect of the date of trap deployment was removed by considering it a covariable (*Ani_dis—Anisandrus dispar*, *Cyc_bod—Cyclorhipidion bodoanum*, *Try_dom—Trypodendron domesticum*, *Xyl_att—Xyleborinus attenuatus*, *Xyl_ger—Xylosandrus germanus*, *Xyl_sax—Xyleborinus saxesenii*).

4. Discussion

Anisandrus dispar and *X. saxesenii* were the most abundant ambrosia beetles detected in this study in western Bohemia (the Czech Republic) and are also very abundant in many other countries [9,39–42]. *Anisandrus dispar* is recognized as a serious pest of fruit and hazelnut trees [43]. It tends to infest trees that have been weakened by biotic and/or abiotic factors [44]. *X. saxesenii* is rarely considered to be a pest [43].

Anisandrus dispar is probably considered to be a pest more often than *X. saxesenii* because it can develop on thinner branches [44]. It is therefore able to attack hazelnut and fruit trees [43,44], which have thinner branches than forest trees. Given the high abundance of *A. dispar* in the current study, one would suppose that this species could cause substantial damage in the region, but such damage occurs only rarely and only on trees with thin stems [45]. Even in regions where *A. dispar* damages fruit trees, the damage it causes to oaks and beeches is insignificant [46], although it has been associated with oak damage [47].

Unlike *A. dispar* females, *X. saxesenii* females directly bore into tree trunks and form a radial entry tunnel [48]. *Xyleborinus saxesenii* females therefore require relatively thick branches and find relatively few resources in forests.

Trypodendron domesticum was the third most abundant species, but it was much less abundant than *A. dispar* and *X. saxesenii*. *Trypodendron* spp. attack the surfaces of tree trunks and thicker branches [37]. A high availability of suitable breeding substrate (e.g., wind-damaged or highly stressed trees) at the forest-stand scale seems to enhance *T. domesticum* population densities and attack rates, e.g., [49] break of bark beetles on spruce has resulted in the increased harvesting of spruce and the suspension of deciduous forest harvesting. As a result, suitable host trees are scarce in oak forests, and the abundance of *T. domesticum* has been low see also [50].

We found only a few individuals of the three species of invasive ambrosia beetles, i.e., *C. bodoanum*, *X. germanus*, and *X. attenuatus*. *Cyclorhipidion bodoanum* and *X. germanus* have recently spread from the west into the Czech Republic, and their abundance remains low [51,52]. In places where both *X. germanus* and *C. bodoanum* have established, however, they are the most abundant Scolytinae species [9,39,40,53].

The introduced ambrosia beetles are considered pests in Europe [54,55]. They can detect stress-induced ethanol emissions from weakened oak trees and can rapidly colonize those trees [56]. Once a forest begins to decline, trees lose vigor, which increases their susceptibility to secondary pests and pathogens [57–61]. If the abundances of invasive species increase overtime, which is likely, these invasive species are likely to contribute to the mortality of trees in weakened oak forests.

The number of ambrosia beetle individuals as well as ambrosia beetle species trapped in the traps was significantly related to date of sampling because of phenology beetles in our study (Figures 4 and 5). The time distribution of the two most abundant species (Appendix B) is in accordance with known seasonal flight activity of these two species [62–64]. The lower numbers of specimens caught in the second half of May are related to the rainy weather.

In this study, we found that the abundance of ambrosia beetles was significantly associated with the percentage of canopy closure and the abundance of dead oak branches (Figure 4). Rather than reflecting the preferences of all species of ambrosia beetles, these associations might mainly reflect the preferences of *A. dispar*, the dominant species in our study (Figure 3). On the other hand, increases in these two variables also increased the number of ambrosia beetle species trapped (Figure 5), suggesting that the percentage of canopy closure and the abundance of dead oak branches may affect species in addition to *A. dispar*. In support of that possibility, the abundance of *T. domesticum* was also positively correlated with the abundance of dead oak branches (there is a positive relationship also in *X. germanus*, but we have trapped only one specimen) (Figure 6). A high percentage of canopy closure is an indication of a stable and humid environment that is suitable for the growth of ambrosia fungi [65,66]. Because ambrosia bark beetles require these fungi as a food source for development, both the fungi and the beetles are more frequent in wetter and warmer localities than in drier and colder localities [26–28]. In addition, ambrosia beetles generally prefer to inhabit the lower parts of tree canopies, such that most bark beetles are caught in traps at a height of 35–200 cm [36,67–71].

The volume of oak wood and the net area occupied by oaks represent the quantity and distribution of ambrosia beetle hosts. Ambrosia and other bark beetles are dependent on ephemeral and generally scattered breeding substrates [72,73], and it is therefore reasonable that the abundance of ambrosia beetle individuals and species at a site will increase with the concentration of host trees (Figures 5 and 6). The abundance of scolytids increases with resource availability [9,74]. In addition, the abundances of the invasive ambrosia beetles *X. attenuatus* and *C. bodoanum* were previously found to be positively correlated with the net area occupied by oaks, because both of these species live in oaks and other deciduous trees [40,49,50,75].

The number of species of ambrosia beetles trapped was significantly affected by study plot (Appendix A). On the other hand, the distance from the stand boundary had no effect on either the species spectrum or the number of captured beetles, which shows that a single trap was sufficient for monitoring the abundance of ambrosia beetle species and individuals at a study plot. This is reasonable because the beetles are lured to the traps by the bait, which was ethanol in the current study. Although not well studied, the population dynamics of ambrosia beetles are probably similar to those of other scolytids. Most individuals that hatched at a given site will probably tend to develop at the same site, but some individuals will disperse to search for new sites with suitable resources [73,76,77].

Although the number of ambrosia individuals trapped was not significantly associated with oak forest type (managed vs. unmanaged; Figure 4a), the abundance of many species was higher in the unmanaged forests than in the managed forests (Appendix A). We therefore cannot draw clear conclusions from these results. The unmanaged forests in the current study were abandoned coppicing forests in three cases (Vladař, Korunní, and Loket), a remnant of an old-growth forest that had been converted into a park in one case (Mariánské Lázně), and a reserve that resembled a virgin forest in only one case (Korunní) (Figure 1). We also captured many beetles at one managed site (Holubín) (Appendix A), which may help explain why the number of beetles captured was not significantly lower in the managed than in the unmanaged sites. Undisturbed, old-growth primary forests are generally considered to support high species richness [78], but species richness for some arthropod assemblages did not differ between primary forests and secondary or degraded (logged) forests in earlier studies, e.g., [79–82]. In a recent study, anthribid species richness did not significantly differ between primary and secondary forests [83], and anthribid species richness was greatly affected by the presence of suitable dead or dying fungus-infested wood, e.g., [84–86].

5. Conclusions

Six species of ambrosia beetles were recorded during the present study. The two most abundant species, *A. dispar* and *X. saxesenii*, represented 98% of the trapped beetles. Both of these ambrosia beetle species were more abundant in oak dominated forests with a high percentage of canopy closure, indicative of a stable and humid environment suitable for the growth of ambrosia fungi, compared to oak forests with a low percentage of canopy closure. Further, a higher abundance of dead oak branches in the canopy was found to be an important factor promoting the occurrence of *A. dispar*. Although the abundance of some species was slightly higher in unmanaged forests, no statistically significant differences in ambrosia beetle abundance in managed vs. unmanaged forests was found. We also have no evidence that particularly high abundances of *A. dispar* in the several studied unmanaged forests would lead to substantial damage to the surrounding forests. In the study area, the abundance of the recorded invasive ambrosia beetles (*C. bodoanum*, *X. attenuated* and *X. germanus*) was low but will probably increase over time. Once the availability of weakened trees increases locally, invasive ambrosia beetles could contribute to oak decline.

Author Contributions: Data curation, T.F.; formal analysis, J.H. and J.F.; methodology, J.H. and T.F.; writing—original draft, J.H., T.F. and J.F.; writing—review and editing, J.H., T.F. and J.F. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in Table 1 and Appendix A.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Total numbers of bark beetles that were trapped at the 10 studied localities in the western Czech Republic (ambrosia beetle species that use oak trees as hosts are in bold).

	Study Plot										
Species	**Vladař**	**Mariánské Lázně**	**Soos**	**Okounov**	**Korunní**	**Klára**	**Holubín**	**Loket**	**Holina**	**Lázně Kynžvart**	**Total**
Anisandrus dispar **Fabricius, 1792**	**122**	**70**	**2**	**1**	**296**	**2**	**226**	**810**	**1558**	**433**	**3520**
Xyleborinus saxesenii **Ratzeburg, 1837**	**154**	**4**	**49**		**15**	**5**	**57**	**194**	**3**	**95**	**576**
Trypodendron domesticum **Linnaeus, 1758**		**1**							**15**		**16**
Cyclorhipidion bodoanum **Reitter, 1913**	**13**										**13**
Trypodendron lineatum Olivier, 1795		8							1	2	11
Dryocoetes autographus Ratzeburg, 1837		5					1		5		11
Xyleborinus attenuatus **Blandford, 1894**	**9**							**1**			**10**
Hylurgops palliatus Gyllenhal, 1813		6									6
Hylesinus varius Fabricius, 1775		4								1	5
Pityogenes chalcographus Linnaeus, 1761		1			1					1	3
Polygraphus grandiclava C.G. Thomson, 1886					1		1				2
Polygraphus poligraphus Linnaeus, 1758	1						1				2
Hylastes cunicularius Erichson, 1836									1		1
Scolytus intricatus Ratzeburg, 1837								1			1
Xylosandrus germanus **Blandford, 1894**										**1**	**1**
Ips typographus Linnaeus, 1758		1									1
Total	299	100	51	1	313	7	286	1006	1583	533	4179

Appendix B

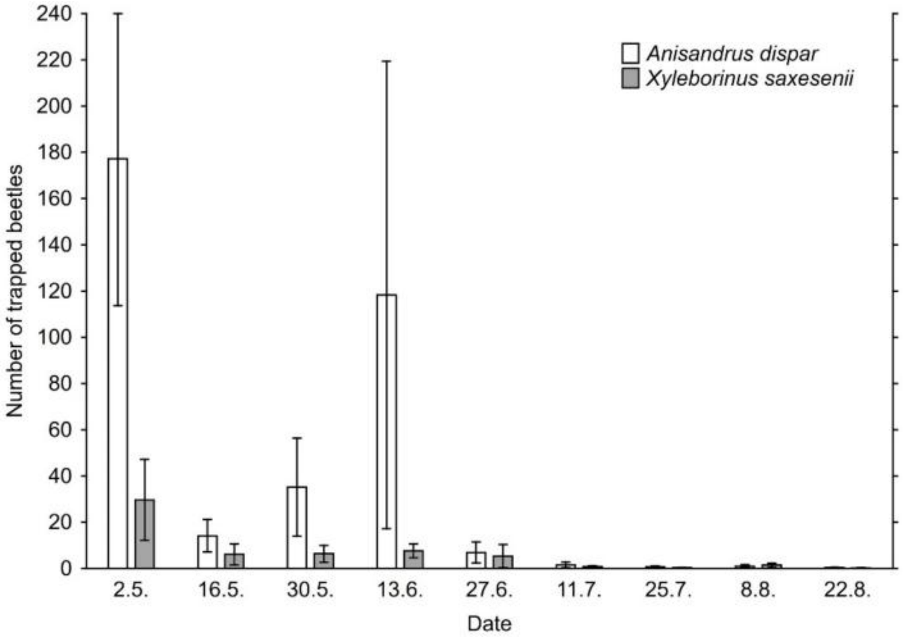

Figure A1. Number (mean ± SE) of individuals of the two most abundant ambrosia beetle species captured per sample in particular sampling periods from April to August 2020. Each sample represented the beetles captured in one trap during 2-week period.

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10.10 Both native and invasive bark beetles threaten exotic conifers within the spa towns in the Czech part of „The Great Spas of Europe“.

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Both native and invasive bark beetles threaten exotic conifers within the spa towns in the Czech part of “The Great Spas of Europe”

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ABSTRACT

Exotic coniferous trees in forests adjacent to spa towns and in parks within spa towns are increasingly being attacked by outbreak populations of native bark beetles in western Bohemia (Central Europe). In the 12 localities in western Czech Republic the current study, we detected a total of 19 native bark beetle species infesting more than 10 % of exotic tree species in the genera *Abies, Picea, Pinus,* and *Pseudotsuga* in 1 year. Several exotic tree species were identified as new hosts for native European bark beetles. We also identified three non-native bark beetles, including *Phloeosinus aubei*, a species that transmits the pathogens that cause cypress canker disease.

The numbers of infested trees in the localities were not correlated with the distance from surrounding bark beetle outbreak sites in commercial forests, because all forests adjacent to spas and all forests in spa towns (i.e., “urban forests”) are close to commercial forests with infested trees. Infestations were greater in spa towns than in the forests adjacent to spa towns. We therefore assume that trees within spa towns will be further attacked due to the increasing abundance of native bark beetles.

1. Introduction

The Great Spas of Europe is a World Heritage Site (https://whc.unesco.org/en/list/1613/documents/) consisting of 11 spa towns located in seven countries: Baden bei Wien (Austria); Spa (Belgium); the ‘Bohemian Spa Triangle’ of Karlovy Vary, Františkovy Lázně, and Mariánské Lázně (Czech Republic); Vichy (France); Bad Ems, Baden-Baden, and Bad Kissingen (Germany); Montecatini Terme (Italy); and City of Bath (United Kingdom). These towns bear witness to the international European spa culture that developed from the early 18th century to the 1930s, leading to the emergence of grand international resorts that affected the landscape around spa “ensembles”. The principal spa ensemble includes springs; pump rooms and drinking halls; bathing and treatment facilities; a ‘kurhaus’; colonnades and galleries; hospitals and sanatoria; assembly rooms; casinos; theatre and concert houses; arcades of shops, hotels, and villas; churches of various denominations, and support infrastructure. These are set within a green environment of promenades, parks and gardens, rides, and woodland walks. The spa ensembles are all integrated into a carefully managed recreational and therapeutic environment in a picturesque landscape.

In the Bohemian Spa Triangle of the western Czech Republic, spa towns include both an inner spa landscape (i.e., parks and “urban forests” within the town) and an outer spa landscape (i.e., parks and forest parks surrounding the spa centers) (https://whc.unesco.org/en/list/1613/documents/). The urban forests in spa towns have a long history in the Czech Republic. As early as the middle of the 16th century, the forests around Karlovy Vary city (“Karlsbad” in German) were used for the leisure enjoyment of spa guests. Because of the aesthetic preferences of guests, parks in spa towns changed during the 19th century with the increased planting of non-native woody plants (Šnajdrová and Pešková, 2017). Very common non-native tree species in parks include those of American origin (*Pinus strobus* L., *Abies grandis* Douglas, *Picea pungens*, and *Pseudotsuga menziesii* (Mirb.)) and the European species *Pinus nigra* J.F. Arnold (Brundu and Richardson, 2016). These exotic trees are currently considered important components of spa town parks and forests and are appreciated by the public (Kunovská, 2019). Parks and forests within and adjacent to spa towns in western Bohemia persist to the present day (Šnajdrová and Pešková, 2017) (Fig. 1). Many of the forests in the area surrounding spa towns are in “natural mineral water protection zones” (Fig. 2). Therefore, they have been recently considered a special type of legally recognized forest (law no. 289/1995 Sb.), i.e., “Forests adjacent to spas” (in Czech original “lázeňské lesy”) (Fig. 1). Forestry management in commercial forests and in forests associated with spas differs mainly in that forest aesthetics are more important than

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economic profit in the latter ones. The selection of trees that compose forests adjacent to spas also reflects safety and hygiene requirements, the inclusion of species that produce wood essential oils (conifers), and the avoidance of large numbers of allergenic trees (Hruban et al., 2020).

In their original range, the exotic woody trees planted in Europe host several species of bark beetles (Kelsey, 1994), and related species of bark beetles of the same genera are present in Europe. In western Bohemia, bark beetles have become very abundant (www.kurovcovamapa.cz). Native bark beetles, such as *Ips typographus* (Linnaeus, 1758), often fly into heavily urbanized areas and attack trees that have been stressed by the urban environment i.e., by pollution (deposition of metals, as well as by the gases SO_2, NO_2, and O_3) but also by heat and drought (Lüttge and Buckeridge, 2020). Trees weakened by these factors are especially sensitive to infestation bark beetles (Marini et al., 2017; Kolb et al., 2019). Although bark beetle abundance decreases towards city centers (Piel et al., 2005) and although the environment for trees is less stressful in spa towns than in cities, it is very likely that non-native spruces in the parks and forests in and around spas will be attacked by breakout populations of the bark beetles *I. typographus* and *Pityogenes chalcographus* (Linnaeus, 1758) (Hedgren, 2004), and that non-native pine species will be attacked by *Ips acuminatus* (Gyllenhal, 1827) (Foit and Čermák, 2014). We already have evidence of the infestation of exotic trees by non-native bark beetles in arboretums, i.e., invasive *Ips amitinus* (Eichhoff, 1871) has recently killed trees of several species of pines and spruce (see Kerchev and Krivets, 2021), and *Ips duplicatus* (C.R. Sahlberg, 1836) has recently attacked *Picea omorika* (Vakula et al., 2021). Other species of economically unimportant bark beetles have also increased in abundance, because large quantities of breeding material is generated by the harvesting of bark beetle-damaged trees (Fiala and Holuša, 2021).

An increased number of host trees represents an increased resource for native bark beetles (Bussler et al., 2011), and urban trees facilitate the establishment of non-native forest pests (Branco et al., 2019). It is therefore possible that non-native species spreading through the Czech Republic (Fiala and Holuša, 2019; Fiala et al., 2021) may attack their original hosts now growing in Europe.

Solitary coniferous trees are especially prone to bark beetle infestation (Netherer et al., 2021), because they are more sunlit and therefore more stressed than trees in commercial forests (Niinemets and Valladares, 2006). We therefore assume that the probability of bark beetle infestation is greater for trees in the parks and urban forests within spa towns than in nearby forests.

In the current study, we tested three hypotheses: (i) the infestation of non-native trees by native bark beetles will be higher in the parks than in the forests; (ii) the numbers of infested trees will increase as the distance from bark beetle infestations in commercial forests decreases; and (iii) the numbers of infested trees will increase with the proportion of native conifers (the primary hosts of the bark beetles of concern) in the area.

2. Materials and methods

The 'Bohemian Spa Triangle' is in western Bohemia in a geographically isolated area by surrounding mountains that prevent beetle flights. We conducted the current study in all 12 parks and forests (referred to as "localities") in western Bohemia (Czech Republic) where exotic tree species are commonly grown (except that *Pseudotsuga mensiesii* is grown in many other places) (Fig. 2, Table 1). While the parks are characterized by typical urban greening with areas of lawns and solitary trees, the forests surrounding the spa towns are semi-commercial and have trees with full canopies (Fig. 1). Most of the 12 localities were represented by either a park or a forest; both habitats occurred together in only one locality (Fig. 2).

Most bark beetle species fly to trees during or before May in Central Europe. They lay eggs and the resulting larvae consume bast (Pfeffer, 1995). The trees wither and turn yellow, and their needles rust and fall off, and have been conspicuous since June (Fig. 1). In all 12 localities, we inspected entire parks and forests every month on foot from June to November 2020 to find all trees that were infested with bark beetles. This is a standard forestry method for searching for bark beetle-infested trees (Schwenke, 1994). Infested trees were debarked over the entire trunk area to a height of 2 m, so that corridors and beetles could be detected. Only the presence of bark beetles was recorded, and the infested trees with living beetles in galleries were counted (Table 2). Bark beetles were also studied on available lower branches or on fallen branches under infested trees (Table 3). Beetles were identified to species according to Pfeffer (1995) by the first author. The assessment of large localities required several hours.

The studied trees ranged in height from 1 m (cypresses) to >30 m (Douglas-fir). The diameters of the trees ranged from a few centimeters (thujas and cypresses) to 150 cm (white pine).

Ordination analysis for the relationship between the relative abundance of bark beetle-infested trees and environmental variables (described below) was performed in Canoco 5. The dependent variable was the relative abundance of bark beetle-infested trunks of trees at the locality. Trees infested with *Phloesinus* spp. that attack only *Juniperus* and *Thuja* spp. were not included in the analysis. We used a semi-quantitative scale to assess the number of bark beetle-infested exotic trees in a locality. The scale was based on our unpublished observation that the probability of a tree being infested by bark beetles is greater if a locality has many potential host trees than if a locality has only one or a few potential host trees. For a given species of bark beetle, the scale ranged from 1 to 3, with 1 indicating that 1 tree was infested, 2 indicating that 2–5 trees were infested, and 3 indicating that > 6 trees were infested at the locality. The following nine environmental variables were assessed: locality type (spa park or spa forest); area of the locality; distance from the nearest bark beetle-infested commercial forest (Table 1); the percentage of potential host trees that were non-native species (*Abies*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga*) among all trees in the

Fig. 1. Infested spruce trees (*Picea pungens*) in the park within the spa town of Mariánské Lázně (left) and a typical "healing landscape" in the forest adjacent to the spa town of Františkovy Lázně (right).

locality; and the percentage of potential host trees that were native coniferous species among all trees in the locality. For non-native species, the mean (± SE), minimum, and maximum percentages were 7 (± 8), 0, and 90, respectively. For native species, these values were 32 (± 32), 5, and 90, respectively. The contribution of predictors was assessed by canonical correspondence analysis (CCA) with forward selection of explanatory variables. The relative abundance of bark beetle-infested trees was square-root transformed.

3. Results

A total of 19 species of bark beetles were detected. At least one species of bark beetle was found in each locality and in each tree species. One to six species of bark beetles were found on individual tree species. The highest number of bark beetle species was detected on *P. pungens* (six species), followed by *P. strobus* and *P. mariana* (five species for both). However, the number of beetle species per tree species per locality was greater for *P. mariana* than for *P. pungens* or *P. strobus* because *P. mariana* occurred in only one locality, while *P. pungens* and *P. strobus* occurred in four and five localities, respectively. The trees *Cupresus*, *Thuja*, and *Picea jezoensis* had only one bark beetle species each (Tables 2 and 3). A total of 123 non-native trees in 11 localities were attacked, which was about 11 % of all non-native trees in 12 localities (Table 1).

The number of host species for each bark beetle species ranged from 1 to 6. *Pityogenes chalcographus* was found on six species of non-native trees, and *P. pityographus* was found on four species of exotic trees in several genera. For beetle species that had two hosts, the hosts were in one genus and were presumably closely related. *Ips typographus* was found on three non-native hosts in two genera. Most bark beetle species were found on one species in the genus *Picea* (Tables 2 and 3).

A total of three species of non-native bark beetles were identified. *Gnathotrichus materarius* was found at one locality on *P. menziesii*. *Phleosinus aubei* was found at one locality but in two species, *Cupressus leylandii* and *Thuja occidentalis*. *Pityogenes bistridentatus* was found on *P. strobus* and *P. nigra* on one locality. The non-native bark beetles, *G. materarius* and *P. aubei*, were found on the trunks of infested trees, but *P. bistridentatus* was found only in the branches (Tables 2 and 3).

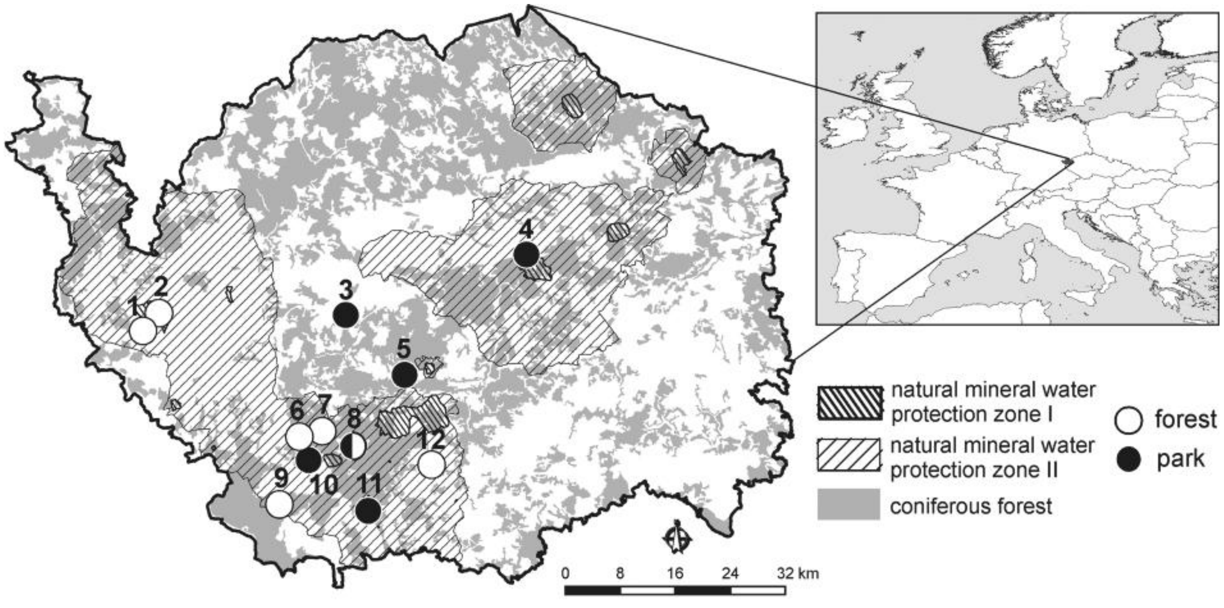
CCA with the forward selection method identified locality type as the only significant predictor ($p = 0.01$) of the abundance of non-native trees infested with bark beetles. Locality type explained 14 % of the variability. Of the species studied, the abundance of *I. typographus* and *P. poligraphus* was higher in parks than in forests while the abundance of *C. pussilus* was higher in forests than in parks (Fig. 3). As predictors of the abundance of non-native trees infested with bark beetles, the following were not statistically significant: the distance from infested commercial forests, the abundance of non-native trees, the proportion of native conifers, and the areas of localities.

4. Discussion

4.1. Bark beetle diversity

In the current study, we found common European species of bark beetles infesting non-native trees in forests and parks within or adjacent to spa towns in western Bohemia; among these species, *I. typographus* and *P. chalcographus* are serious pests. When abundant, they are primary pests, and *I. typographus* is often accompanied by *P. chalcographus* (Pfeffer, 1995; Hedgren, 2004). Other bark beetle species found in forests adjacent to spas and parks in spa towns included the pests *I. acuminatus* and *D. micans* (Lukášová et al., 2014; Siitonen, 2014). The ambrosia beetle *T. lineatum* is also a common species in Central Europe (Pfeffer, 1995). Occasionally, *P. poligraphus, P. pityographus*, and *P. thujae* also cause damage (Kraemer, 1951; Benz, 1985; Viklund et al., 2019).

The three non-native bark beetle species represent three different groups. Among them, *P. aubei* has a Mediterranean distribution with



1 - **Františkovy Lázně**; 50.1190953N, 12.3545719E	7 - **Lazy**; 50.0343778N, 12.6192739E
2 - **Komorní hůrka**; 50.1002836N, 12.3357750E	8 - **Kladská**; 50.0251839N, 12.6677253E
3 - **Sokolov**; 50.1460542N, 12.6260544E	9 - **Jedlová**; 49.9597547N, 12.5744058E
4 - **Karlovy Vary**; 50.2292633N, 12.8745986E	10 - **Lázně Kynžvart**; 50.0054250N, 12.6061417E
5 - **Čistá**; 50.0990861N, 12.7255536E	11 - **Mariánské lázně**; 49.9672636N, 12.7043750E
6 - **Úbočí**; 50.0258042N, 12.5873447E	12 - **Mnichov**; 50.0196556N, 12.7853561E

Fig. 2. Map showing the 12 study localities with forests adjacent to spa towns and with parks in spa towns, and their surrounding forests in western Bohemia. Areas with natural mineral water protections zones containing spa forests visited by spa guests are indicated.

Table 1
Background information on the 12 localities including the numbers of exotic trees and the numbers of exotic trees infested with bark beetles. If trees of more species were infested, the numbers of infested trees of each species are separated by a slash.

Locality	Park/forest	Area (ha)	*Abies*	*Picea*	*Pinus*	*Pseudotsuga*	*Tsuga, Thuja, and Cupressus*	Numbers of infested trees
Čistá	Park	2.5		1				1
Karlovy Vary	Park	18.0			200		10	2/1
Kladská	Park	1.0		10				3
Lázně Kynžvart	Park	3.0	3		20		2	2/7/1
Mariánské Lázně	Park	70.0		250			4	35/2
Sokolov	Park	0.7		200		10		7/3
Františkovy Lázně	Forest	60.0						
Jedlová	Forest	1.5		25				21
Kladská	Forest	2.0				20		10
Komorní hůrka	Forest	2.5			20			1
Lazy	Forest	2.0		100				20
Mnichov	Forest	0.6		40				6
Úbočí	Forest	2.0	200					1

northward range expansion probably due to climate change and is the most significant pest; it can seriously damage juniper and cypress plantations (Fiala and Holuša, 2019). *P. aubei* is a carrier of the pathogen, *Seiridium cardinale* (Wag.) Sutton and Gibson that cause cypress canker disease and is therefore an important pest of cypress trees in southern Europe (Danti and Rocca, 2017). The next most significant non-native bark beetle pest detected in the current study was *G. materiarius*; this species was introduced into Europe from North America almost 90 years ago and has yet to cause economic damage (Mazur et al., 2018). *Pityogenes bistridentatus* might be a South/Central European species with limited previous records in those parts of Europe (Pfeffer and Knížek, 1996; Urban, 2000) but is considered a non-native species in the Czech Republic. It was introduced with host trees and was found infesting the thin branches of non-native pine species in the current study. Other bark beetle species listed in Tables 2 and 3 lack significant economic importance (Pfeffer, 1995).

Not surprisingly, we found that *Picea*- and *Pinus*-associated bark beetles infested other non-native *Picea* and *Pinus* species, and that polyphagous bark beetles that attack conifers infested a range of coniferous species; attack of *P. pungens* by *D. micans* was previously reported (Lukášová et al., 2014). We also found the following new host trees for the following bark beetle species: *P. omorika* for *P. poligraphus*; *P. mariana* for *C. asperatus, C. pusillus,* and *P. pityographus*; *P. pungens* for *X. pilosus*; and *T. heterophylla* for *P. chalcographus* (Tables 2 and 3).

4.2. Effects of environmental variables

The infestation of non-native trees by the native bark beetles *I. typographus* and *P. poligraphus* was higher in parks than in forests. Trees in parks are more endangered than trees in forests in or adjacent to spa towns because the trees in parks grow in more open, sunnier habitats (Tanhuanpää et al., 2019), which facilitates host location by bark beetles (Kašák and Foit, 2018). Generally, bark beetles in outbreak populations of *I. typographus* preferentially attack trees in areas with a high level of sun exposure (Mezei et al., 2019; Ďuračiová et al., 2020).

We expected that the numbers of infested trees would increase as the distance from bark beetle infestations in commercial forests decreased because number of *I. typographus* that penetrate into city centers depends mainly on the distance from the city outskirts and is adversely affected by the built-up area (Piel et al., 2005). In general, scolytins are very good fliers and can fly for several kilometers (Jones et al., 2019). *Ips typographus* is also a good flier, and although 50 % of the population flies less than 500 m, the median of the flying range was >3 km at high population density and was >5 km at low population density when the beetles migrated above the forest canopy (Öhrn et al., 2014) to locate new food sources (Jakuš et al., 2003). Although dispersal success at the level of individual beetles decreases with dispersal distance and is lowest in early flight cohorts, host selectivity increases and colonization density decreases with increasing distance across all environments. The ability to fly long distances is generally important for the spreading of species, but the successful infestation of trees, which was observed in our study, also depends on the density of the host and its susceptibility (Kautz et al., 2016).

There are two main reasons why the number of infested in forests and parks within or adjacent to spa towns was not significantly related to the distance to the nearest *I. typographus*-infested commercial forest. First, the nearest bark beetle-infested commercial forest was always <2 km away, which is less than the median flight range of *I. typographus* (Öhrn et al., 2014); consequently, the distance to bark-beetle infested trees in commercial forests was not a limiting factor. Second, bark beetles dispersing into parks within spa towns probably had a high probability of infesting because the trees in the parks of spa towns grow in open areas and therefore experience increased water loss and drought stress. Host stress helps bark beetles overcome host defenses (Gely et al., 2020). For spruce, another stress is that the trees in forests and parks within or adjacent to spa towns are growing at low altitudes, i.e., at altitudes that are not ideal for spruce (Röder et al., 2010).

Our hypothesis that a larger proportion of native conifers at a locality would support a larger number of infested trees was not supported by the data, although stand density and spruce percentage affect host susceptibility to bark beetle infestation even in outbreaks (Hilszczański et al., 2006; Koreň et al., 2021). The reason is that bark beetles do not distinguish between native and non-native tree species (Tables 2 and 3). However, a high proportion of broadleaved trees, such as birch, poplar, or ash, emit volatiles that repel many bark beetles (Byers et al., 1998; Zhang et al., 1999). If the proportion of broadleaved trees in the parks and urban forests within spa towns in the 12 localities of the current study was high and abundance of bark beetles was low, the broadleaved trees could potentially protect the conifers in the 12 localities. Bark beetle abundance in the forests near spa towns, however, is very high; in the western Czech Republic where the spa towns are located, the recorded volume of spruce wood infested by bark beetles in 2020 was greater than 1 m^3 per ha in most cases and even greater than 5 m^3 per ha in some cases (Knížek and Liška, 2020).

5. Conclusion

The Great Spas of Europe represent a unique urban landscape based on natural mineral springs. Parks and forests within and adjacent to spa towns are currently suffering from infestations by both native and non-native bark beetles. It is alarming that 10 % of non-native coniferous trees within and adjacent to spa towns have been attacked in 1 year. The parks within spa towns are more endangered than the forests surroundings of spa towns, and the level of infestation is evidently not related to the distance to the nearest infestations in commercial forests. These results indicate that a “protective” strip of forest (Jurc et al., 2006) of any width will not prevent the infestation of trees within spa towns. Infested trees with beetles must be rapidly detected and removed to

Table 2
Frequency of bark beetle-infested trunks of exotic tree species in forests adjacent to spa towns or in spa town parks in 12 localities in western Bohemia. The number of localities where the indicated species of bark beetle was found is in parentheses; asterisks indicate the infestation frequency (* = one tree infested; ** = 2 to 5 trees infested; and *** = ≥ 6 trees infested). Tree species that are new hosts for the indicated species of bark beetle are in bold.

Bark beetle species/Tree species	*Abies grandis* (Douglas ex D.Don) Lindl.	*Cupressus leylandii* A. B.Jacks & Dallim.	*Picea mariana* Britton, Sterns & Poggenb.	*Picea omorika* Pančić) Purk.	*Picea pungens* Engelm.	*Pinus nigra* J. F. Arnold	*Pinus strobus* L.	*Pseudotsuga menziesii* (Mirb.) Franco	*Thuja occidentalis* L.	*Tsuga heterophylla* Sarg.	Total exotic tree species colonized
Number of localities with tree occurrence	2	1	1	2	5	1	4	3	2	1	
Cryphalus asperatus Gyllenhal, 1813			****(1)**								1
Cryphalus piceae Ratzeburg, 1837	**(1)										1
Crypturgus cinereus Herbst, 1793					***(1)						1
Crypturgus pusillus Gyllenhal, 1813			****(1)**		***(1)						2
Dendroctonus micans Kugelann, 1794			*(1)								1
Gnathotrichus materiarius Fitch, 1858								**(1)			1
Ips typographus Linnaeus, 1758				***(1)	***(3)		**(2)				3
Phloeosinus aubei Perris, 1855		*(1)							**(1)		2
Phloeosinus thujae Perris, 1855									*(1)		1
Pityogenes chalcographus Linnaeus, 1760	*(1)		**(1)	**(2)				*(3)		***(1)**	5
Pityophthorus pityographus Ratzeburg, 1837	*(1)		****(1)**		**(1)						3
Polygraphus poligraphus Linnaeus, 1758				*****(1)**	*(1)						2
Tomicus minor Hartig, 1834						*(1)					1
Trypodendron lineatum Olivier, 1800								**(1)			1
Xylechinus pilosus Ratzeburg, 1837					****(1)**						1
Cummulative species richness	3	1	5	3	6	1	1	3	2	1	

prevent bark beetle spread. The detection of infested trees can be facilitated using drones (Näsi et al., 2018; Klouček et al., 2019) but also by the participation of citizens who live nearby (Branco et al., 2019).

The protection and management of trees within and around spa towns is the responsibility of the national/regional government but especially of the local authorities. The local authorities can decide to allow the dead trees to stand. Such trees have significant value because they provide habitat and food for birds, mammals, reptiles, insects, and fungi. Such dead trees, however, must be managed, and the public should be educated about the benefits but also the dangers generated by dead trees (Pecenko and Brack, 2021).

Author statement

TF: Conceptualization; Formal analysis; Investigation; Methodology; Validation; Writing - original draft; Writing - review &editing; JH:

Table 3
Presence of bark beetle-infested branches of exotic tree species in forests adjacent to spa townss or in spa town parks in 12 localities in western Bohemia. The number of localities where the indicated species of bark beetles was found is in parentheses.

Bark beetle species/Tree species	*Picea jezoensis* Carrière	*Picea omorika* Pančić) Purk.	*Pinus nigra* J.F. Arnold	*Pinus strobus* L.	*Pseudotsuga menziesii* (Mirb.) Franco	Total exotic tree species colonized
Number of localities with tree occurrence	1	2	1	4	3	
Ips acuminatus Gyllenhal, 1827				(1)		1
Phloeotribus spinulosus Rey, 1883	(1)					1
Pityogenes bistridentatus Eichhoff, 1878			(1)	(1)		2
Pityogenes chalcographus Linnaeus, 1760		(1)			(2)	2
Pityophthorus lichtensteinii Ratzeburg, 1837				(2)		1
Pityophthorus pityographus Ratzeburg, 1837			(1)			1
Cummulative species richness	1	1	2	3	1	

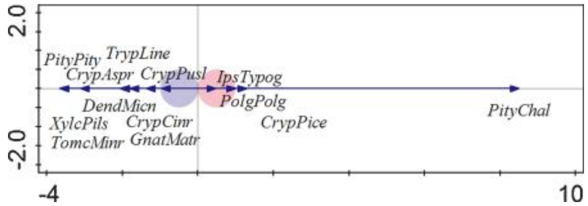


Fig. 3. T-value biplot of the canonical correspondence analysis with results of regression of beetle abundance on the park within the spa town (red circle) and the forest adjacent to the spa town (blue circle) (*Cryphalus asperatus —CrypAspr; Cryphalus piceae —CrypPice; Crypturgus cinereus —CrypCinr; Crypturgus pusillus —CrypPusl; Dendroctonus micans —DendMicn; Gnathotrichus materiarius —GnatMatr; Ips typographus —IpsTypog; Pityogenes bistridentatus —PityBist; Pityogenes chalcographus —PityChal; Pityophthorus pityographus —PityPity; Polygraphus poligraphus —PolgPolg; Tomicus minor —TomcMinr; Trypodendron lineatum —TrypLine; Xylechinus pilosus —XylcPils*).

Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Writing - original draft; Writing - review & editing; AV: Data curation; All authors significantly contributed to this manuscript and have read and approved of the manuscript in its current form.

Data availability

No data was used for the research described in the article.

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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

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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

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[1] www.exoticke-drevo.com

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

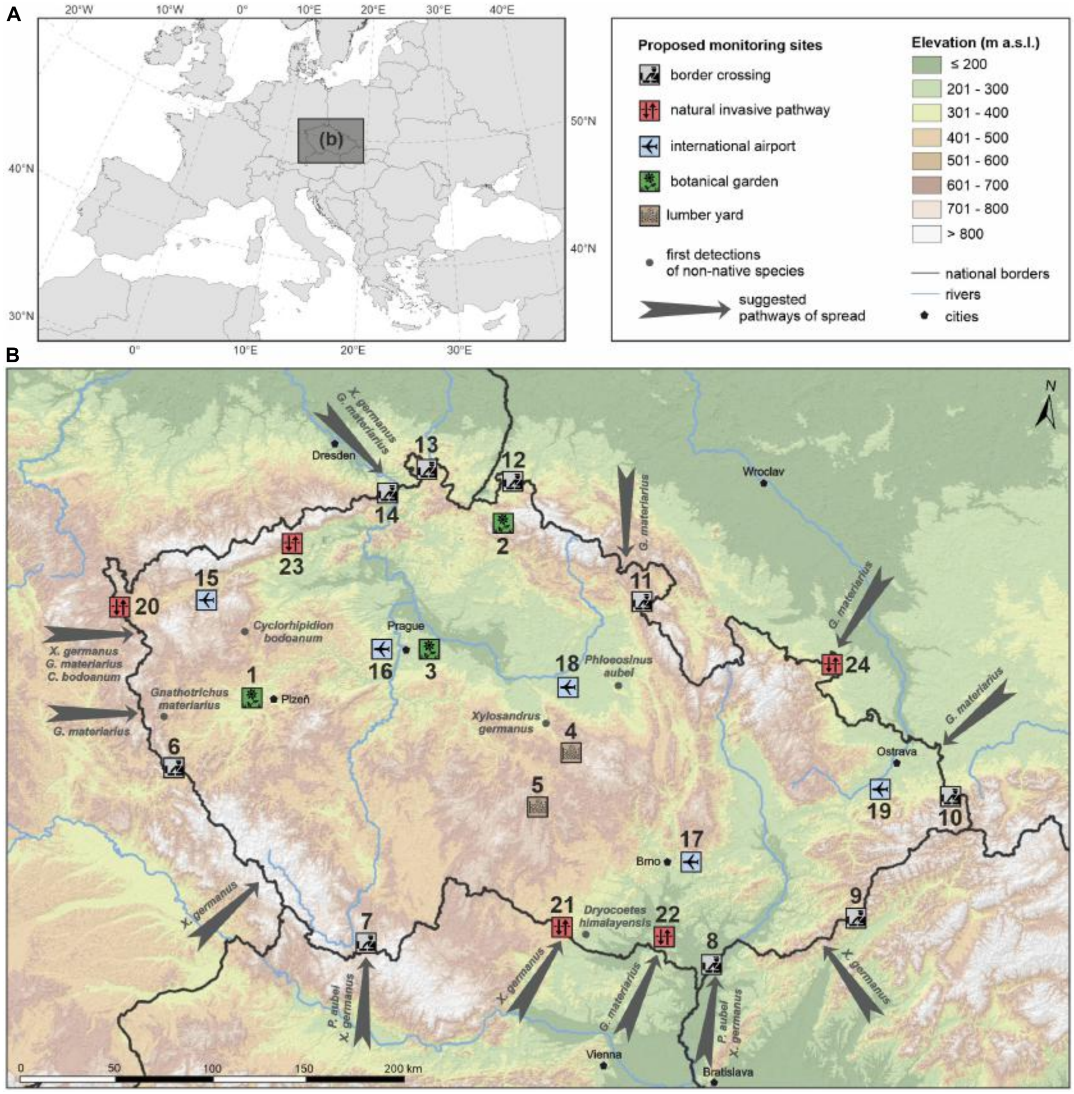

FIGURE 1
The position of the Czech Republic in Europe **(A)** and the possible entry gates, places of first detections, and a proposal for monitoring locations for invasive ambrosia and bark beetles in the Czech Republic **(B)**.

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

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[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.

Publisher's note

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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