

Invasive alien forest insect species in south-eastern Romania

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Abstract

Biological invasions in forest ecosystems are recognised as a global scale challenge. However, our current knowledge of invasive alien forest insect species (IAFIS) in Romania is still lacking and rather insufficient to support clear policies. The aim of this study is to contribute to a better understanding of the distribution of IAFIS in the Muntenia and Dobrogea provinces of Romania. For this purpose, direct observations and insect collections were made two consecutive years, in 76 locations. Of these, 67 sampling locations forming a long field observation route were visited only once, and nine sampling locations were fixed monitoring points with primed traps used in first year for regular collection of insects at 1-2-week intervals. Seventeen non-native forest insect species from five orders and 11 families were detected: *Aproceros leucopoda*, *Cameraria ohridella*, *Corythucha arcuata*, *Cydalima perspectalis*, *Dasineura gleditchiae*, *Eopineus strobus*, *Euura tibialis*, *Gilletteella cooleyi*, *Hyphantria cunea*, *Ips duplicatus*, *Macrosaccus robinella*, *Neoclytus acuminatus*, *Obolodiplosis robiniae*, *Parectopa robinella*, *Phyllonorycter issikii*, *Prociophilus fraxinifolii* and *Xylosandrus germanus*. Eleven IAFIS species originate from North America, four from Asia and two from Europe. All detected species were previously reported from Romania, but eight species had not been recorded in these two provinces. Seven species live on native tree species, while 10 thrive on exotic trees or shrubs. The highest number of IAFIS was recorded in urban areas. Except for *E. tibialis*, all found IAFIS have a significant ecological or economic impact across invaded habitats. The risks posed by biological invasions need more thorough consideration that deserves greater attention and scientific support.

Keywords: alien insect species; biological invasions; forest; new pests

Introduction

Humanity is currently facing a growing number of global problems, such as explosive population growth, overexploitation of natural resources, climate change and loss of biodiversity. Closely related to these issues is the increased number of exotic pest species and the damage caused by them in the territories where they have

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been introduced due to the expansion of international tourism, air transport, and trade in agricultural and forest products (Kiritani and Morimoto, 2004; Kiritani, 2014). When individuals of a non-native species arrive with human assistance, whether deliberate or accidental, and establish a population that afterwards spreads and has a negative impact within the new territory, the phenomenon is called biological invasion and the species is regarded as invasive, at least in the new territory (Simberloff *et al.*, 2013). This phenomenon, which leads to so-called 'biological globalisation' (van der Weijden *et al.*, 2007), is rooted in the very distant past, but is becoming stronger. It affects a worrying proportion of agricultural crops and the health of humans and domestic animals. It also endangers the existence of many species in all ecosystems and, through this, the normal functioning of the ecosystems.

However, no checklist of alien terrestrial arthropods was available at the level of European countries until the early 2000s because the problems caused by them were considered minor and insignificant (Roques, 2010). From the 2000s onwards, the impacts of alien terrestrial arthropods began to be increasingly noticeable in Europe; the gradual expansion of *Ips duplicatus* (Sahlberg, 1836) (Knížek and Beaver, 2004), the growing damage caused by *Cameraria ohridella* Deschka & Dimic, 1986 to horse chestnut, and the introduction of *Corythucha arcuata* (Say, 1832) (Bernardinelli and Zandigiacomo, 2000) for which at least 30 million hectares of oak forest is suitable European habitat (Paulin *et al.*, 2020) are worth noting. After 2000, European researchers' concern about invasive species began to be more frequently expressed, with intense efforts to report on and study new invasive species, both at the national level through numerous individual projects for inventorying in various countries, and at the European level through network projects coordinated by international teams of experts in the field of biological invasions (e.g., DAISIE, 2009). According to the inventory of invasive alien species in Europe, 1517 terrestrial invertebrates have been identified, including 1424 arthropods (DAISIE, 2009). Moreover, 87% of all arthropods already established in Europe are invasive alien insect species, and the rate of reporting of these species has almost doubled in recent decades, rising from an average of 10.9 (1950-1974) to 19.6 (2000-2008) species per year (Roques, 2010).

As in Europe, the trend in the study of invasive invertebrate species in Romania became more intense after the 2000s. A review of the time dynamics of studies on alien terrestrial invertebrates (Adam *et al.*, 2020) shows that before the 2000s, a maximum of 38 studies were published every decade, while in 2001-2010, 125 studies were published, and from 2011 to date, 216 studies focused on invasive alien invertebrate species. In close connection with the European project DAISIE (2009), the first inventory of invasive insect species in Romania, which was based on bibliographic research, was carried out in 2006-2008 (Olenici *et al.*, unpublished). Likewise, against the background of the continuous increase in the number of invasive species and their negative effects on forest ecosystems, another project, entitled "Detection of invasive alien forest insect species (IAFIS) in Romania and establishing their distribution area", was carried out in 2016–2017. This project resulted in four scientific publications, two focusing on the inventory of invasive alien insect species in the Moldova Province (Olenici and Duduman, 2016; Olenici *et al.*, 2018) and one on the Oltenia Province (Netoiu *et al.*, 2018), while the fourth paper deals with the invasion of the species *Corythucha arcuata* in Romania (Tomescu *et al.*, 2018). Using data collected in the same project for two other historical provinces, Muntenia and Dobrogea, this study aims to contribute to a better knowledge and understanding of the distribution of IAFIS in Romania.

Materials and Methods

Study area

Muntenia and Dobrogea are two historical provinces in the south and south-east of Romania (Figure 1). We considered these provinces together because, on the one hand, Bucharest, the country's capital and the intersection of many roads for transport of goods and people, is located in Muntenia, and on the other, the Constanța Seaport, which benefits from an advantageous geographical position due to being located on the

pan-European Rhine–Danube transport corridor, is located in Dobrogea. In addition, this territory is worthy of study from the point of view of invasive alien species because of the important border crossing points, i.e., Giurgiu-Ruse and Constanța, and Romania’s largest airport, Henri Coandă International Airport, located there. For these reasons, this territory is home to many important factories, such as the Dacia car factory, which is relied on throughout the European continent for transport of many goods.

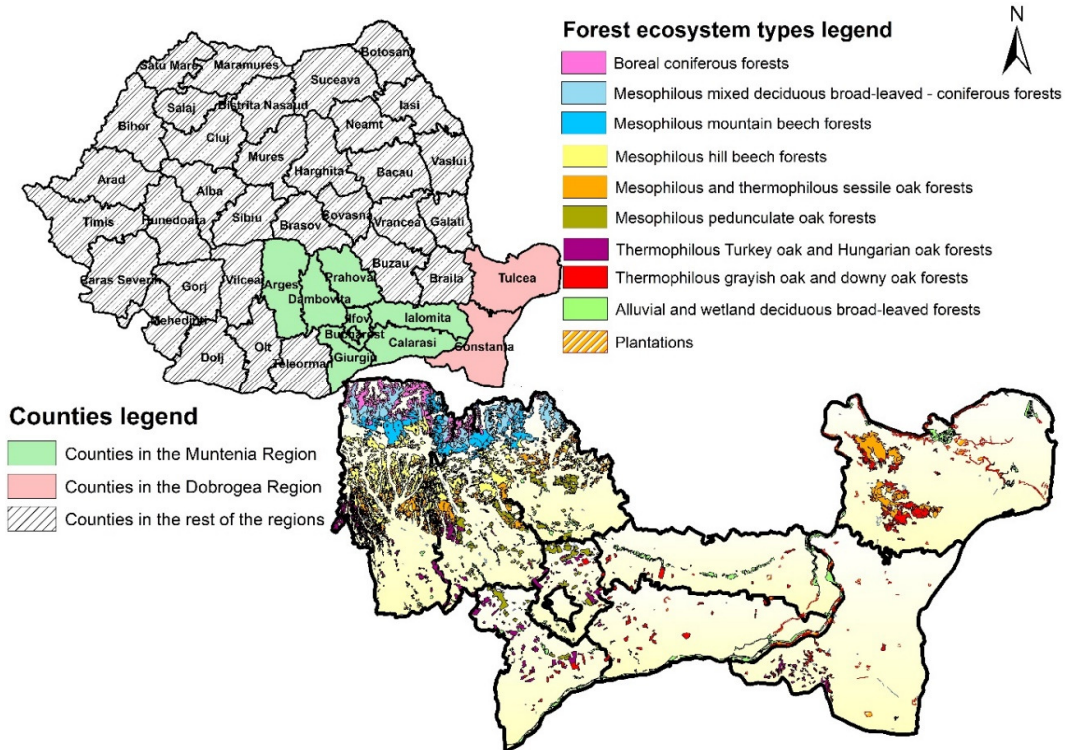


Figure 1. Study area and its location in Romania

Muntenia and Dobrogea together form a complex territory in terms of forest ecosystems. Alluvial and wetland deciduous broadleaved forests with alder (*Alnus* spp.), poplar (*Populus* spp.), willow (*Salix* spp.), ash (*Fraxinus* spp.) and common oak (*Quercus robur* L.) occur in the south, while ecosystems ranging from sessile oak forests to boreal coniferous forests are found in the north. The study area includes the counties of Argeș, Dâmbovița, Prahova, Giurgiu, Bucharest - Ilfov, Călărași and Ialomița in Muntenia and Tulcea and Constanța in Dobrogea.

Data collection

To achieve the main objective of this study, as a first step, we compiled a list of IAFIS expected to be present in Muntenia and Dobrogea Provinces (Appendix 1). In drawing up the list of species to be searched for, both IAFIS whose presence had already been reported in Romania and those reported in Europe, but not yet reported in Romania, were taken into account. For each insect species, we established certain factors to consider when choosing the sites where observations were to be made or where traps were to be installed. Furthermore, to facilitate fieldwork, for each of these insect species, an identification sheet based on insect morphology and type of attack (Appendix 2) was developed. In the case of some of the species, we used the field guides already available (see Appendix 1).

As a second step, a network of monitoring points, depending on the detection method, was established. Direct observation at points along a field observation route was used to detect the presence of species whose

attack characteristics are easily observable, while trapping was used to find species that live under bark or in wood.

IAFIS detected by direct observation at points along a field observation route

In order to establish the spatial distribution of insect species whose presence could be easily detected by attack characteristics or by the presence of individuals at various developmental stages, the easiest and most effectively applied method of observation at points along a field route was chosen. Therefore, a network of 67 observation points directly related to the presence of host tree species was established (Appendix 3; Figure 2). The observation points were located inside forests of the National Forest Fund (forest stands [FS]), and outside in parks (P) and street tree alignments (STA) and on isolated trees (IT), where the presence of invasive species was most likely to be detected.

For easier identification of each location on the map (Figure 2), each observation point was assigned a unique ID consisting of the county name abbreviation and an identification number (for example, the location in Argeş/Mioveni has the unique ID AG1). For each observation point and each species detected, we recorded the date of observation, the type of habitat and damage level, separately estimated for each insect species (Appendix 1), and the frequency of attacked trees. The damage level was established visually, not on the basis of detailed sampling and sample analysis, thus being a rather subjective estimate. In addition, we collected samples of biological material (various developmental stages of the insects detected and plant material with traces of attack) from the 67 observation points for confirmation of fieldwork with laboratory investigations. Insect species detected by direct observation were identified taking into account the host species, the characteristics of the attack and the appearance of the insects, without using specific keys.

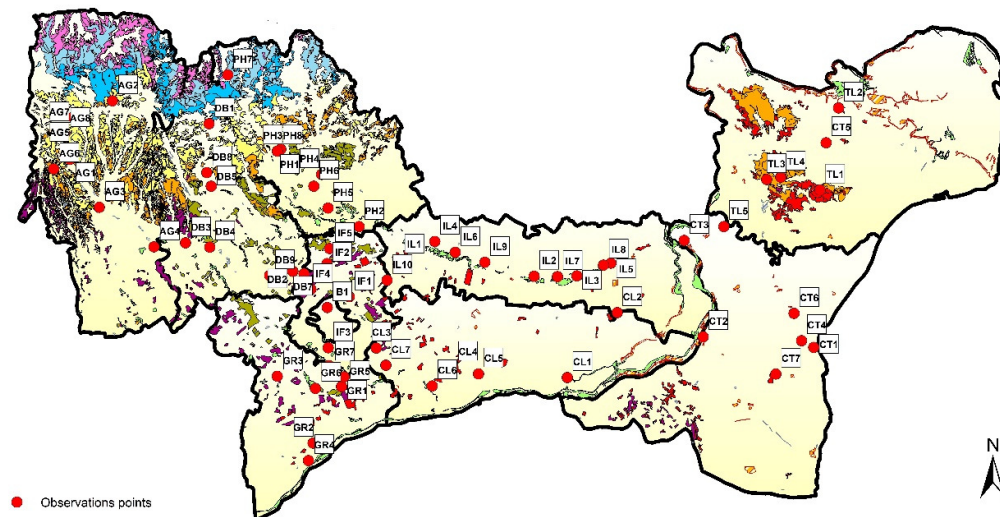


Figure 2. The network of observation points for detecting IAFIS by direct observation

IAFIS collected with primed traps

To detect xylophagous and xylomyetophagous species, whose presence was difficult to observe due to their very small size and hidden lifestyle (under the bark or in the wood of trees), we set up a network of nine monitoring points (Appendix 4; Figure 3). In May 2016, at each of these points, we placed traps with various pheromone or kairomone attractants (atraTYP, atraDUP and atraLINEA, generally used to detect *Ips typographus* (Linnaeus, 1758), *Ips duplicatus* and *Trypodendron lineatum* (Olivier, 1795), respectively, and ethanol and alpha-pinene). The nine monitoring points were established in areas with stored timber, either imported or domestically produced, as well as in tree stands. Two trap types were chosen as efficient for capturing the insects of interest: traps for intercepting insects in flight (Intercept Traps – IT) (Figure 4A) and

traps made of polyethylene bottles (Bottle Traps – BT) (Figure 4B). Two to four ITs, each baited with a different lure, and one BT baited with ethanol were used in each location (Appendix 4), depending on the species expected to be found there. Salty water was used as a preservative in the collecting jar. During the 2016 growing season (May–September), periodic visits were made to each point at 7-14 days to collect the captured biological material and to refresh the attractant lures. Nine to ten samples were collected from each location. All biological material captured, including the non-target species, was preserved in 96% ethanol and sent to the Forest Protection Laboratory of the National Institute for Research and Development in Forestry, Campulung Moldovenesc Station, for sorting and identification.

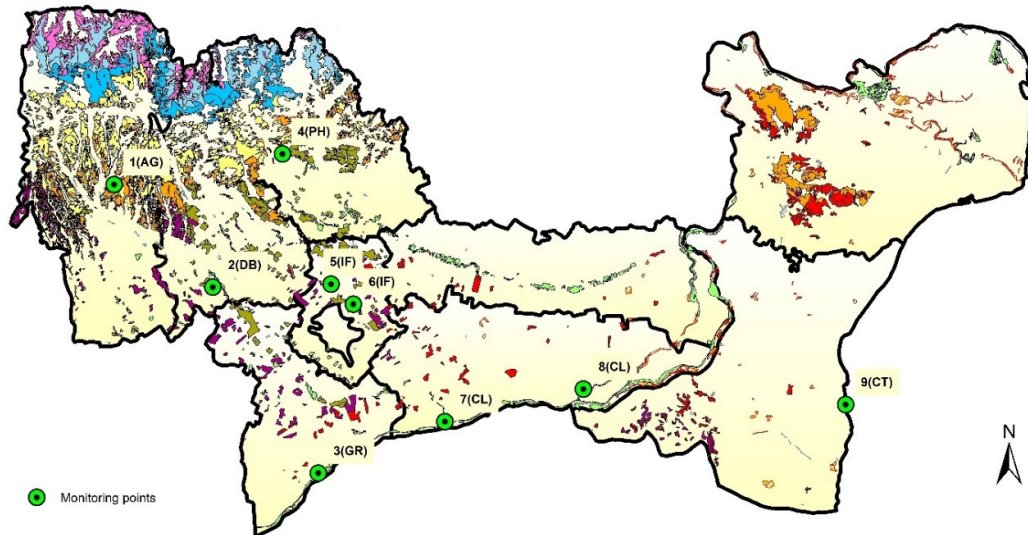


Figure 3. The network of monitoring points for detecting IAFIS captured with primed traps



(A)



(B)

Figure 4. Traps primed with attractants used for capturing bark beetles, ambrosia beetles and xylophagous beetles: (A) Trap for intercepting insects in flight (IT); (B) Trap made with a polyethylene bottle (BT)

Identification of Coleoptera species and taxonomic classification

The first author identified Coleoptera species using Pfeiffer (1995) for the Scolytinae and Bense's (1995) keys for the Cerambycidae. Beetles from other families were identified using keys by Lompe (2002).

We adopted the classification system in the BioLiB database (Zicha, 1999-2021) for taxonomic classification of insect species, and Flora Europaea for plant species.

Statistical analyses

The sample of invasive insect species obtained by direct observation was large enough to allow the analysis of detection frequency of a certain number of species per location, the mean and median number of species observed in each of the four habitat types (IT, P, STA, and FS) and the association of each species with one or more habitat types. The distribution of the number of species occupying different numbers of areas (occupancy frequency distribution) was also analysed, but without computing any statistics because the frequency values were too low in this case.

To determine whether the frequency of detecting a certain number of invasive species at the observation points followed a non-random pattern, the multinomial goodness of fit test was used to compare the distributions of observed and expected (found by chance) frequencies, the null hypothesis being that the two distributions did not differ. Because the Chi-square test required that the observed and expected frequencies were higher than 5, the observed frequencies were grouped into four classes (1-2 species, 3-4 species, 5-6 species and 7+ species). The observed distribution was also analysed to identify the theoretical distribution that best fit the data.

To compare the number of species detected in the four habitats, the average and the median number of species were calculated for each distribution as the distributions were not normal (Shapiro–Wilk test) and could not be normalised by various transformations. Grubbs' test was used to identify outliers. Finally, the distributions were compared using the non-parametric Kruskal–Wallis test, and the Dwass, Steel, Critchlow–Fligner procedure was used to identify differences between distributions.

The alpha was 0.05 for the above tests which were conducted using the XLSTAT 2019.1.1 programme (Addinsoft, 2021). The presence of the detected species in one or more of the four habitats was illustrated using Venn diagrams (VIB/UGent, 2021).

Map used to represent species distribution

To highlight the possible relationships between the conditions that determine the natural vegetation in the area studied and the spatial distribution of the alien forest insect species detected, the distribution was graphically illustrated using the map of forest ecosystems of Romania (Gancz *et al.*, 2008). The maps were obtained through the ArcGIS 10.3. software by ESRI.

Results

IAFIS detected by direct observations at points along the field observation route

Species spectrum, spatial distribution and association with habitat types

Of the 23 IAFIS sought by direct observation, 14 species were detected in south-eastern Muntenia and Dobrogea during the 2017 growing season. Taxonomically, they belonged to four orders and nine families (Table 1). Most were monophagous or oligophagous species that lived on hosts from their natural range introduced to Romania. However, other species, either oligophagous or polyphagous like *C. arcuata*, *Aproceros leucopoda* Takeuchi, 1939, *Phyllonorycter issikii* (Kumata, 1963), *Hyphantria cunea* (Drury, 1773), colonised native woody species, but from the same genera found in their natural range. According to the way they fed and

the damage they caused to plants, they were categorised into four distinct groups: defoliators (leaf chewers), galling insects, leaf miners and sap-sucking insects.

Table 1. The invasive alien forest insect species detected via direct observation, their hosts and the types of damage they caused

Order	Family	Insect species	Host species	Damage type
Diptera	Cecidomyiidae	<i>Dasineura gleditchiae</i> (Osten Sacken, 1866)	<i>Gleditsia triacanthos</i> L.	Galls on leaves
		<i>Obolodiplosis robiniae</i> (Haldeman, 1847)	<i>Robinia pseudoacacia</i> L.	Galls on leaves
Hemiptera	Adelgidae	<i>Eopineus strobilus</i> (Hartig, 1837)	<i>Pinus strobus</i> L.	Sap sucking
		<i>Gilletteella cooleyi</i> (Gillette, 1907)	<i>Pseudotsuga menziesii</i> (Mirb.) Franco	Sap sucking
	Aphididae	<i>Prociphilus fraxinifolii</i> (Riley, 1879)	<i>Fraxinus pennsylvanica</i> Marshall	Sap sucking
	Tingidae	<i>Corythucha arcuata</i> (Say, 1832)	<i>Quercus robur</i> L., <i>Q. cerris</i> L., <i>Tilia tomentosa</i> Moench	Sap sucking
Hymenoptera	Argidae	<i>Aproceros leucopoda</i> Takeuchi, 1939	<i>Ulmus minor</i> Mill.	Defoliation
	Tenthredinidae	<i>Euura tibialis</i> (Newman, 1837)	<i>Robinia pseudoacacia</i> L.	Defoliation
Lepidoptera	Crambidae	<i>Cydalima perspectalis</i> (Walker, 1859)	<i>Buxus sempervirens</i> L.	Defoliation
	Erebidae	<i>Hyphantria cunea</i> (Drury, 1773)	<i>Acer negundo</i> L. <i>Morus</i> spp. <i>Prunus domestica</i> L.	Defoliation
	Gracillariidae	<i>Cameraria ohridella</i> Deschka & Dimic, 1986	<i>Aesculus hippocastanum</i> L.	Mines on leaves
		<i>Macrosaccus robiniella</i> (Clemens, 1859)	<i>Robinia pseudoacacia</i> L.	Mines on leaves
		<i>Parectopa robiniella</i> Clemens, 1863	<i>Robinia pseudoacacia</i> L.	Mines on leaves
		<i>Phyllonorycter issikii</i> (Kumata, 1963)	<i>Tilia</i> spp.	Mines on leaves

At least one species was found in each observation point, but the number of detected species varied between one and 11 per point (Figure 5A). The highest number of species, 11, was detected at Voluntari, near Bucharest. On the second place, with nine species, were Bucharest and Călărași, followed by Ștefan cel Mare and Tulcea with eight species. The frequency of points with different numbers of detected species followed a non-random pattern (Chi-square = 18.941, DF = 3, $p = 0.00028$). The negative binomial (2) distribution best fit the data in the goodness of fit test (Figure 5B), with the parameters $k = 4.490$ and $p = 0.748$ ($p = 0.595$). However, the Chi-square test revealed that the sample only approached the negative binomial (2) distribution, but did not really follow this type of distribution (Chi-square = 22.236, DF = 8, $p = 0.004$).

The detected species in a certain number of observation sites could be categorised into three groups (Figure 6). One group was represented by four species recorded at over 20 sites (representing nearly a third of the observation points). These species were *O. robiniae* (detected at 34 sites), *P. robiniella* (26 sites), *E. tibialis* (22 sites) and *C. ohridella* (24 sites); the first three species thrived on black locust and the last on horse chestnut. The second group consisted of seven species detected at 11-20 sites. These were *C. arcuata* (detected at 19 sites), *D. gleditchiae* (18 sites), *H. cunea* (18 sites), *P. fraxinifolii* (15 sites), *A. leucopoda* (14 sites), *M. robiniella* (12 sites) and *C. perspectalis* (11 sites). The third group had only three species: *G. cooleyi*, *E. strobilus* and *P. issikii*, which were observed at less than 10 sites.

The number of species found in each of the four habitat types varied considerably; a higher number of species were usually found in parks and in forest stands than on isolated trees or street tree alignments (Table 2 and Figure 7). The observed difference between IT and FS and between STA and FS was statistically significant ($p = 0.009$ and $p = 0.043$, respectively). However, only two species (*G. cooleyi* and *P. issikii*) were found in a single habitat, while half were found in all four habitats (Figure 8 and Table 3).

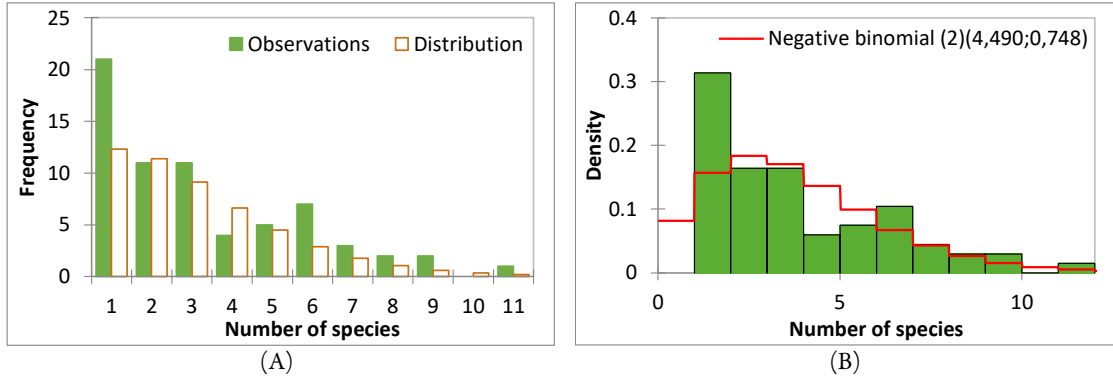


Figure 5. The number of observation sites in which a certain number of invasive alien insect species were detected; (A) Comparison between the observed and theoretical frequencies corresponding to the negative binomial (2) distribution; (B) The probability density function

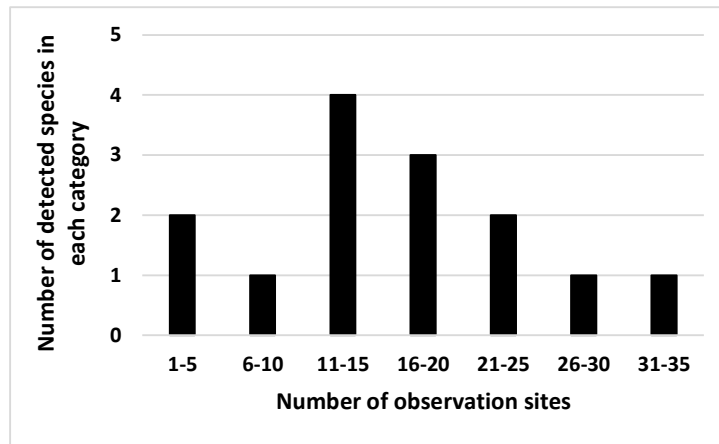


Figure 6. The variation in the number of detected invasive alien insect species according to the number of sites at which each species was recorded

Table 2. The number of invasive alien insect species detected in different habitat types

Habitat type	Mean* ± S.D.	Median*	Rank mean*
Isolated trees (IT)	1.7 ± 0.8	2.0	21.5
Parks (P)	3.9 ± 2.7	3.0	37.7
Street tree alignments (STA)	1.9 ± 1.1	1.0	22.9
Forest stands (FS)	4.0 ± 2.2	3.5	40.1

*Values were computed after removing outliers (IT: 5; P: 11; FS: 9).

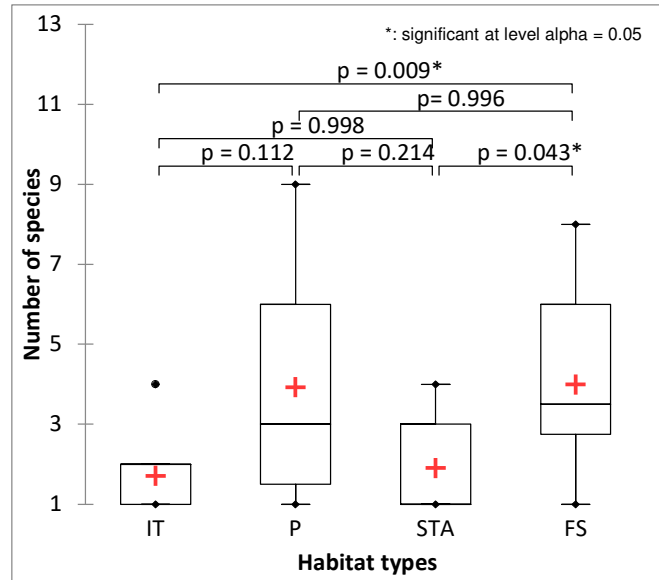


Figure 7. Box-and-whisker plots representing the number of invasive alien insect species detected in the four investigated habitat types (isolated trees [IT], parks [P], street tree alignments [STA], and forest stands [FS])

The midline of the box plot indicates the median; the red cross is the mean; the upper and lower limits of the box indicate the third and first quartiles; whiskers extend up to 1.5 times the interquartile range from the top/bottom of the respective box, and dots represent data beyond the whiskers. The distributions were compared using the Kruskal–Wallis test and the Dwass, Steel, Critchlow–Fligner procedure, after removing outliers.

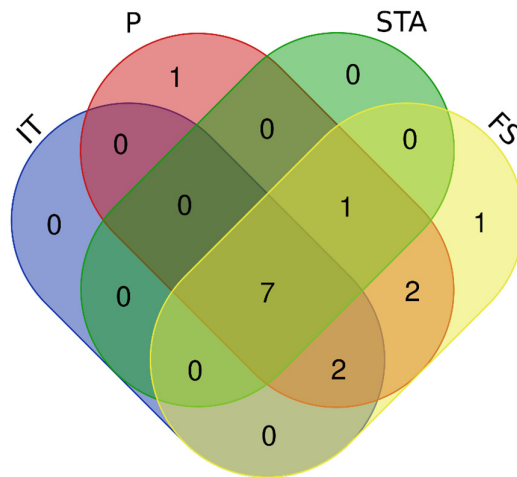


Figure 8. Occurrence of 14 invasive alien insect species detected by direct observation in the four habitat types (IT = isolated trees; P = parks, STA = street tree alignments, and FS = forest stands)

Defoliators

Four of the IAFIS detected by direct observation, the elm zigzag sawfly (*A. leucopoda*), box tree pyralid (*C. perspectalis*), fall webworm (*H. cunea*) and false acacia sawfly (*E. tibialis*), feed on the foliage of their host trees and cause defoliation.

Aproceros leucopoda was found in 14 of the study sites, on elm trees growing in parks or tree stands, generally located in areas of mixed thermophilous forest dominated by the common oak and Hungarian oak (*Quercus frainetto* Ten.) (Figure 9). The damage level was between 5% and 15%, with the frequency of attacked trees ranging from 5% to 100% (Appendix 5).

Table 3. The distribution of invasive alien insect species detected in different habitat types (IT = isolated trees; P = parks, STA = street tree alignments, and FS = forest stands)

Habitat types	Total number of species	Insect species
IT, P, STA, FS	7	<i>Dasineura gleditchiae</i> , <i>Euura tibialis</i> , <i>Cameraria ohridella</i> , <i>Hyphantria cunea</i> , <i>Parectopa robinella</i> , <i>Obolodiplosis robiniae</i> , <i>Prociophilus fraxinifolii</i>
IT, P, FS	2	<i>Eopineus strobilus</i> , <i>Corythucha arcuata</i>
P, STA, FS	1	<i>Macrosaccus robinella</i>
P, FS	2	<i>Aproceros leucopoda</i> , <i>Cydalima perspectalis</i>
P	1	<i>Gilletteella cooleyi</i>
FS	1	<i>Phyllonorycter issikii</i>

Cydalima perspectalis was observed on its host species, the common box (*Buxus sempervirens* L.), at 11 observation points, most of them located in parks. Thus, it was found in the area of mixed thermophilous forest of grayish oak (*Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky) and downy oak (*Quercus pubescens* Willd.), as well as in the area of mixed mesophilous forest of common beech (*Fagus sylvatica* L.) and conifers (Figure 9). The damage level varied between 5% and 100%, with the frequency of attacked shrubs ranging from 20% to 100% (Appendix 5).

Webb nests of *H. cunea* were noted at 18 observation points on mulberry (*Morus* spp.), boxelder maple (*Acer negundo* L.) or European plum (*Prunus domestica* L.), mainly on isolated trees or in street tree alignments, especially in the area of mixed thermophilous forest of greyish oak and downy oak (Figure 9). Between 50% and 100% of the investigated trees were attacked and the defoliation intensity was 5–90% (Appendix 5).

The presence of the false acacia sawfly was documented at 22 observation points, generally located in the southern part of the study area, where black locust has been introduced as a plantation tree in the areas of mixed thermophilous forest of common oak and Hungarian oak and of greyish oak and downy oak (Figure 9). In general, the defoliation intensity was very low (less than 5%) and the frequency of attacked trees varied between 5% and 60% (Appendix 5).

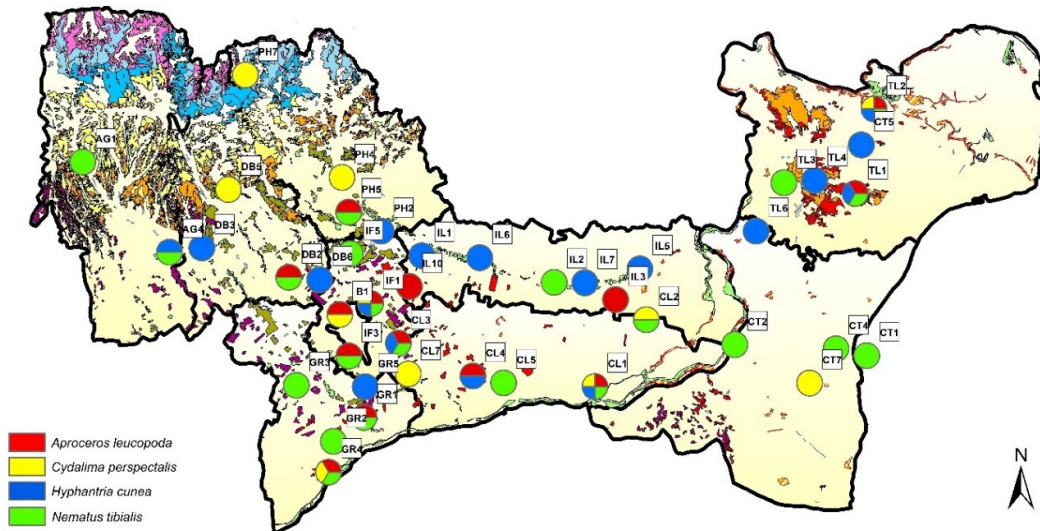


Figure 9. The distribution of defoliator IAFIS

Sap-sucking insects

The detected species belonging to this feeding guild were the pine bark adelgid (*E. strobus*), the Douglas fir adelgid (*G. cooleyi*), the leaf curl ash aphid (*P. fraxinifolii*) and the oak lace bug (*C. arcuata*).

The pine bark adelgid was found on eastern white pine, *P. strobus*, at six observation points, mainly in parks, but also on isolated trees and in a forest stand. These points were located in the areas of hilly mesophilic beech forest and mixed thermophilic forest of common oak and Hungarian oak (Figure 10). The host is a tree species introduced to Romania. The damage level was estimated at 5–70% and the frequency of attacked trees was between 30% and 100% (Appendix 6).

The Douglas fir adelgid, *G. cooleyi*, was found only at two observation points (Figure 10), both located in parks, where the host species, *P. menziesii*, is cultivated as an exotic ornamental tree. The damage level was very low (5-10%), but the frequency of attacked trees was quite high (50-80%) (Appendix 6).

The leaf curl ash aphid was detected on green ash, *F. pennsylvanica*, in 15 locations (Figure 10) in all four habitat types. The damage level was between 1% and 50%, with the frequency of attacked trees between 10% and 100% (Appendix 6).

The oak lace bug was observed in 19 locations, mainly on the leaves of oak (*Q. robur* and *Q. cerris*) and sometimes on the leaves of silver lime (*T. tomentosa*). It was found in the southern part of Muntenia, from the alluvial forest area (where the oaks are found sporadically) to the sessile oak forest area (Figure 10). The damage level was between 5% and 90%, with the frequency of the attacked trees between 50% and 100% (Appendix 6).

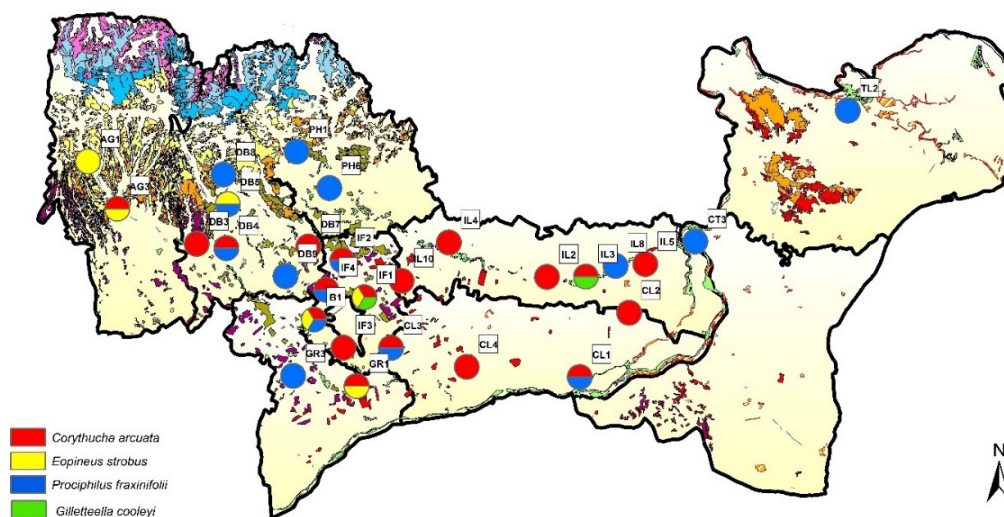


Figure 10. The distribution of sap-sucking IAFIS

Galling insects

Two of the invasive insect species detected by direct observations were gall makers: the honeylocust podgall midge, *D. gleditchiae* and the black locust gall midge, *O. robiniae*.

Dasineura gleditchiae was detected at 18 observation points on its host species, *G. triacanthos*, a tree often planted at the edges of tree stands for their protection or as an ornamental in parks and street tree alignments. It was found from the alluvial and wetland deciduous broad-leaved forest areas up to the common oak forest area (Figure 11). The damage level was low (1-20%), and the frequency of attacked trees varied between 10% and 50% (Appendix 7).

Obolodiplosis robiniae was the most common invasive species found during this study, being detected at 34 observation points in various habitat types (forest stands, parks and isolated trees), where its host species, the black locust, has been introduced either for production or for ornamental purposes. Therefore, this insect species was found in all types of forest ecosystems, from alluvial and wetland deciduous broadleaved forests up

to hilly mesophilous beech forest (Figure 11). The damage level was low to moderate (1-40%) and the frequency of attacked trees ranged between 5% and 100% (Appendix 7).

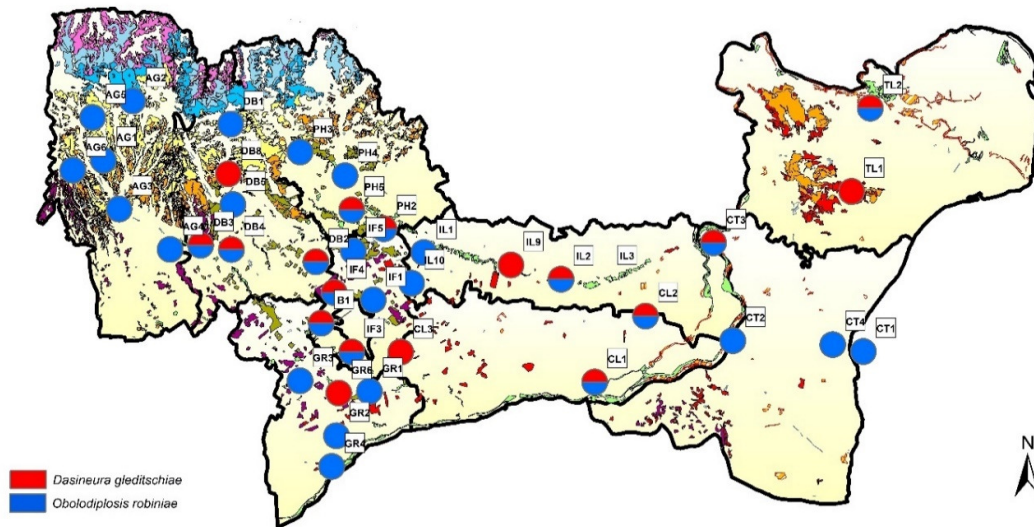


Figure 11. The distribution of galling IAFIS

Leaf miners

Four leaf mining invasive moth species were detected: the horse-chestnut leaf miner (*C. ohridella*), the locust leafminer (*M. robinella*), the locust digitate leafminer (*P. robinella*) and the lime leaf miner (*P. issikii*).

Cameraria ohridella was found at 24 observation points, most of them located in parks or street tree alignments where host trees have been introduced as ornamental species, as well as within tree stands where the horse chestnut has been planted or dispersed. Its spatial distribution extended from the area of mixed thermophilous forest of grayish oak and downy oak to the area of mixed mesophilous forest of European beech and conifers (Figure 12). The damage level was between 5% and 90%, with the frequency of attacked trees between 70% and 100% (Appendix 8).

The two invasive alien moth species which produce mines on the leaves of *R. pseudoacacia*, *M. robinella* and *P. robinella*, were detected mainly in forest stands, but were also found in other habitat types (parks and isolated trees), where their host tree has been introduced either for wood production or for ornamental purposes. *M. robinella* was observed at only 12 observation points, while *P. robinella* was found at 26 points. Both species were present, from the forested plains to higher altitudes, in the hilly mesophilous beech forest area (Figure 12). The damage level was very low (less than 5%) at all observation points, with the frequency of attacked trees between 5% and 90% (Appendix 8).

The lime leaf miner, *P. issikii*, was found in only two forest stands (Figure 12) and the two observation points were located in the hilly mesophilous beech forest area, where its host trees, of the genus *Tilia*, grow naturally. About 60-70% of the observed trees were attacked and 10-15% of the leaves had mines (Appendix 8).

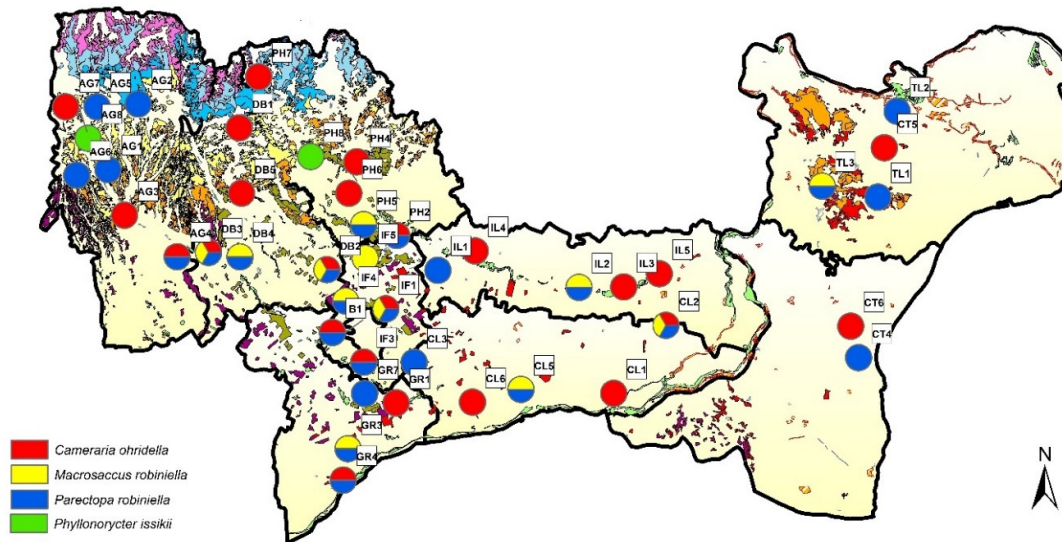


Figure 12. The distribution of leaf miner IAFIS

Invasive forest insect species collected with primed traps

Three invasive insect species were found at the monitoring points during the growing season of 2016: the double-spined bark beetle (*Ips duplicatus* (Sahlberg, 1836); Coleoptera, Curculionidae), the black stem borer (*Xylosandrus germanus* (Blandford, 1894); Coleoptera, Curculionidae), and the red-headed ash borer (*Neoclytus acuminatus* (Fabricius, 1775); Coleoptera, Cerambycidae).

Ips duplicatus was collected at three of the nine monitoring points (Figure 12), with the highest captures in the whole sampling period (85 specimens) recorded at Point 4 (PH), where the traps were installed within a Norway spruce stand; the other two points, 5(IF) and 9(CT), which were near Otopeni International Airport and Constanța Seaport, respectively, were far from any spruce trees. In the last two sites, only five and nine specimens, respectively, were collected. Overall, most specimens (77.3%) were caught in ITs baited with pheromone lures for *I. duplicatus*. The other specimens were caught in ITs with pheromone lures for *I. typographus*.

Xylosandrus germanus was captured at five of the nine monitoring points (Figure 12), most captures in the whole sampling period (497 specimens) being recorded at Point 1 (AG), which is located near the Dacia car factory, in a 35-year-old mixed tree stand of Scots pine and sessile oak. Between 1 and 21 specimens were captured at the other four points. Most specimens (92.3%) were captured with BTs with ethanol and 7.5% with ITs with AtrALINEA lure.

Neoclytus acuminatus was found at only one location, Point 8 (CL), which was a wood processing centre (Figure 12). Ten specimens were caught throughout the capture period, nine in IT with AtrALINEA lure and one in IT with alpha-pinene and ethanol.

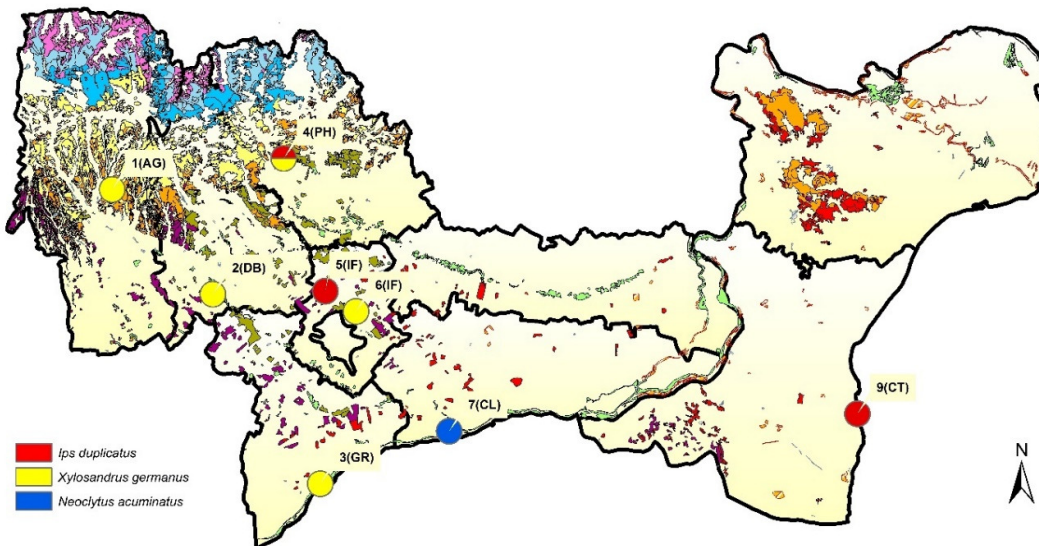


Figure 13. The distribution of invasive forest insect species caught in traps with a lure

Discussion

Invasive forest insect species detected by direct observation

Species spectrum, their spatial distribution and association with habitat types

Although the observations were limited to a few field visits during a single growing season, the presence of 14 alien forest insect species was documented in the study area. The diversity of the species detected is high, from the perspective of both taxonomy and feeding habit. Six species of Lepidoptera, four of Hemiptera, two of Diptera and two of Hymenoptera were found. Lepidopteran species (mainly leaf miners) were better represented, while the hemipteran species, which were sap suckers, were underrepresented among the detected species compared to their share in the total number of non-native insect species associated with woody plants in Europe (Roques *et al.*, 2020). This indicates that only species more easily detectable on the basis of the injuries they cause to woody plants (defoliation, discoloration of leaves, mines and galls) or whose abundance was conspicuous were found, while those whose attack was difficult to detect were not observed. Consequently, at least three species, the lime seed bug (*Oxycarenus lavaterae* (Fabricius, 1787)), the western conifer seed bug (*Leptoglossus occidentalis* (Heidemann, 1910)) and the black locust aphid (*Appendiseta robiniae* (Gillete, 1907)), which had been included in the initial search list, were not detected. The first two species were previously reported in Romania, including in Muntenia (Bărbuceanu and Nicolaescu, 2012; Olenici and Duduman, 2016), and the third was found during an investigation in two other geographical provinces of the country (Nețoiu *et al.*, 2018; Olenici *et al.*, 2018).

At least one invasive insect species was reported from each direct observation point along the route, which means that to the keen eye, these species are a common presence in the landscape of the area. At the same time, this indicates that a closer investigation, over several growing seasons and with more field visits each season, may highlight the presence of a much larger number of non-native insect species, as recent research shows for the parks of Bucharest (Bălăcenoiu *et al.*, 2020). However, the territorial distribution of alien forest insect species is very uneven (Figure 5, $k = 4.49$), the highest number of species being found in the Bucharest area (Bucharest city and its surroundings) and in several other urban areas. Consequently, the pattern of species distribution suggests that large urban agglomerations play an important role in the introduction, establishment and then the spread of invasive species in surrounding areas as suggested by Colunga-Garcia *et al.* (2010), Borden and Flory (2021) and others.

Nevertheless, only two of the species found during this study have been reported from Bucharest as a first record for Romania: *O. robiniae*, discovered in Bucharest in 2007, and only 2-3 years later in other counties (Bálint *et al.*, 2010); and *C. perspectalis*, observed in August 2010 in three different locations in Bucharest (Iamandei, 2010). This may mean that Bucharest does not play an important role in the introduction of new invasive insect species, but contributes to the establishment of those already introduced and then in their spread within the country.

The establishment of alien forest insect species is facilitated by the large number of parks in Bucharest, with their great variety of woody species (Diaconescu *et al.*, 1986; Colesca and Alpopi, 2011; Nagodă *et al.*, 2014; Badea and Enescu, 2016), while transport, in particular, and economic activities facilitate their spread to other areas of the country. An example is *O. robiniae*, which three years after its first detection was present not only in the vicinity of Bucharest but also in Arad (Bálint *et al.*, 2010) and in the north-eastern part of the country (Olenici and Duduman, 2016). The spread of *C. arcuata* in Romania during the first two years after it was first detected in the country was also rapid and was mainly along transport routes (Tomescu *et al.*, 2018). The rapid dissemination of these two species has also been possible due to the fact that their host species are widespread in the country, oak species occupying over 850,000 ha and black locust approximately 260,000 ha (Marin *et al.*, 2019).

Although there are relatively few stands of black locust in the area studied (Ciuvăț *et al.*, 2013), the species is frequently cultivated in parks, along roadsides, in home gardens, on abandoned agricultural land, etc., which explains why three of the four most common alien insect species (detected at more than 20 observation points) were those associated with *R. pseudoacacia*. Recently, Mally *et al.*, (2021) found that the spatial distribution of the black locust is the most consistent predictor of the spread of *P. robinella*, *M. robinella* and *O. robiniae* in Europe. On the other hand, two of the species found at a very small number of observation sites, *G. cooleyi* and *E. strobus*, live on Douglas fir and eastern white pine, respectively. Across the country, the Douglas fir covers about 12,700 ha (Șofletea and Curtu, 2001), while the eastern white pine covers about 1,360 ha (Radu, 2008). Because the Douglas fir is negatively affected by the climatic conditions of the eastern part of Romania and the white pine is damaged by *Cronartium ribicola* J.C. Fisch., the two species are little cultivated, even as ornamental species in urban green spaces, and were, therefore, present only at a few of the observation points.

The fact that most insect species were found in almost all four types of investigated habitats highlights the role played by woody vegetation in parks or located along streets and roads in establishing and spreading these insects. These trees serve as 'bridgeheads' in establishing and spreading non-native insect species, if they are able to serve as hosts for the insects (Paap *et al.*, 2017; Branco *et al.*, 2019).

Defoliators

Aproceros leucopoda is native to East Asia and was reported for the first time in Europe from Hungary and Poland in 2003, and from Romania in 2006 (Blank *et al.*, 2010). Both Blank *et al.* (2010) and Cardaș *et al.* (2011) reported the species only from Moldova and Banat. In 2016-2017, it was also found in Oltenia (Netoiu *et al.*, 2018), and is most probably also present in the other historical provinces of the country. Although in this study the attack intensity was very low at the observation points where the presence of the species was detected, it should be considered an important pest of elms, because moderate and severe defoliation has already been reported in both Romania and other countries (Blank *et al.*, 2010, 2014; Zandigiacomo *et al.*, 2011; Martynov and Nikulina, 2017). Defoliation predisposes elm trees to infestation by bark beetles of the genus *Scolytus*, which are vectors of fungi that cause Dutch elm disease (DED) (Santini and Faccoli, 2015).

It should be borne in mind that elms were severely affected by DED during the twentieth century, a phenomenon that caused a substantial reduction in the share of these tree species in the composition of forests in Romania (Georgescu *et al.*, 1957; Stănescu, 1979).

The box tree moth, *C. perspectalis*, also originates from East Asia. Its first appearance in Europe was recorded in 2006 in Germany (Krüger, 2008). Iamandei (2010) reported the presence of the species for the first

time in Romania based on observations made in Bucharest during August 2010. Over the next few years, it was reported from many other places across the country. It was observed in Timișoara in 2013 (Fora and Poșta, 2015), in Constanța, Mihail Kogălniceanu, Hârșova (Skolka and Zaharia, 2014), Iași (Manci *et al.*, 2014) and Botoșani (Bălan and Corduneanu, 2014) in 2014, in Cluj-Napoca (Bunescu and Florian, 2016) in 2015, and in Macea (Arad County; Don *et al.*, 2016), Jibou (Sălaj County) and Satu-Mare (Katona *et al.*, 2016) in 2016. Consequently, we expected that it would be detected during this study, both in south-eastern Romania and in many places in Oltenia (Nețoiu *et al.* 2018). Because its larvae feed only on boxwood leaves, *C. perspectalis* has been found particularly in parks where its host species is found almost exclusively. It was observed in forest stands only in two cases. However, no boxwood stands were present in these locations, but this ornamental species was planted under the crown of other trees or at the edge of the forest stand. In many of the places where it was observed, this pest caused severe defoliation of boxwood shrubs, its impact being easily noticeable in urban green spaces. As heavily defoliated boxwood plants no longer regain their foliage and die (van der Straten and Muus, 2010; Mitchell *et al.*, 2018), protective measures are needed to avoid such situations (Fora *et al.*, 2016; Somsai *et al.*, 2019).

The fall webworm, *H. cunea*, is native to North America (the continental United States, southern Canada and northern Mexico), where its larvae feed on the foliage of more than 400 species, mainly hardwood trees (Schowalter and Ring, 2017). It was accidentally introduced into Europe (first to Hungary) in the 1940s (Surányi, 1946), and then spread to most countries, becoming a serious pest, especially in orchards (Lopez-Vaamonde *et al.*, 2010). In Romania, it was recorded for the first time in Bihor County in 1949 (Manolache *et al.*, 1957), after which it gradually spread to all areas where environmental factors are favourable and host species are present (Olenici and Duduman, 2016). During this study, severe defoliation was observed mainly in parks, isolated trees or street tree alignments, while forest stands were weakly to moderately affected. These data are in line with the phytosanitary statistics of the forests (Ștefănescu *et al.*, 1980; Simionescu *et al.*, 1992, 2001, 2012), which also show that the largest infested areas in the last five decades were in the south-eastern part of the country. Long-term data also highlight a declining trend in the importance of this species as a forest pest. However, it remains a major pest of orchards, hedgerows, and shade or ornamental trees in urban and suburban areas in Romania (Teodorescu *et al.*, 2003; Pașol *et al.*, 2007), as in other countries (Drooze, 1985; Alford, 2007, 2012).

Euura tibialis is also a North American species whose larvae feed on the leaves of the black locust. In Europe, it was detected for the first time in Germany in 1825 (Rasplus *et al.*, 2010). Much later in 1958, it was collected for the first time in Romania in Budești, Călărași County (Scobiola-Palade, 1968). Taking into account our results, as well as those from previous studies (Scobiola-Palade 1981, Nețoiu *et al.*, 2018, Olenici *et al.*, 2018), it is likely that this species is present in all provinces of the country, where its host species has been introduced, and that its impact on the black locust is negligible. This is consistent with the fact that the species is classified as an unimportant pest of forests (Pschorn-Walcher, 1982) or as a minor pest of ornamental trees and nursery stock (Alford, 2012).

Sap-sucking insects

The pine bark adelgid, *E. strobus*, is native to eastern North America, where it is found throughout the natural range of the eastern white pine (*P. strobus*), its main host (Darr *et al.*, 2018). According to Mifsud *et al.* (2010), the first record of this species in Europa dates from 1900, but Šefrová and Laštůvka (2005) suggest that it was present in the Czech Republic even earlier. It gradually spread wherever the eastern white pine was cultivated (Steffan, 1972). In Romania, the first valid report of this species was from Timișoara (Rogojanu, 1943), but it has also been reported from Baia Mare, Bucharest, Prahova, Câmpulung Moldovenesc and Voluntari (Bolea, 1971; Gusic, 1972; Ceianu and Teodorescu, 1973; Olenici and Duduman, 2016; Bălăcenoiu *et al.*, 2020). In 2017, infestation of trees in the observation points was low to moderate, but severe infestation was reported in previous studies (Bolea, 1971; Fora and Lauer, 2008). Because strong attack can result in the

drying out of tree branches and loss of growth (Steffan, 1972), this species should be regarded as an important pest, especially in parks and other areas where trees are also stressed by other factors.

Gilletteella cooleyi is also from North America, where it develops on *Picea* species as primary hosts and on Douglas fir as the secondary host. It causes cone-shaped galls on spruce twigs, while it settles on new needles and shoots and developing cones of Douglas fir (Furniss and Carolin, 1977). It was reported for the first time in Great Britain in 1913 (Chrystal, 1922) and in the Netherlands in 1928 (Teucher, 1955). In Romania, it was detected for the first time in mature tree stands in Nădrag (Timiș County) in November 1961, as well as on seedlings in Simeria (Hunedoara County) and Dobrești (Bihor County; Blada, 1963) the following year. This suggests that the species was introduced a long time ago. Until 1990, it was reported only from the western part of the country (Nanu, 1971; Boguleanu, 1994), but was reported for the first time from other provinces, including Oltenia, during this study (Nețoiu *et al.*, 2018). Given that attack is of low intensity in general and of moderate or strong intensity less often (Simionescu *et al.*, 2001, 2012), the species can be considered a minor pest of Douglas fir in Romania.

The leaf curl ash aphid, *P. fraxinifolii*, is from North America (Favret *et al.*, 2007) like its main host *F. pennsylvanica*. Although it has been reported in Europe (in Budapest) since 2003 (Remaudière and Ripka, 2003), its first observation in Romania was in Oltenia and Moldova in 2017 (Nețoiu *et al.*, 2018; Olenici *et al.*, 2018). In the same year, it was also found in south-eastern Romania. So far, the infestations of this species in Romania have been weak or moderate, and according to observations from Russia (Orlova-Bienkowskaja and Bieńkowski, 2021), it does not seem to attack the common ash. However, this pest should be treated with caution, especially since green ash was attacked by *Agrilus planipennis* Fairmaire, 1888, a species that has inflicted widespread damage in Russia (Orlova-Bienkowskaja, 2014), has already invaded Ukraine (Orlova-Bienkowskaja *et al.*, 2020), and is expected to reach Romania in a few years.

The oak lace bug, *C. arcuata*, is native to North America and causes chlorotic discolouration of the leaves, especially on oak trees. In Europe, it was first reported in Italy in 2000 (Bernardinelli and Zandigiacomo, 2000). In 2015, it was also discovered in Romania, in the western part of the country, at Macea (Don *et al.*, 2016). It then spread to almost all favourable habitats in just two years and caused significant damage in some cases (Tomescu *et al.*, 2018). Consequently, some insecticides have been tested to control its populations. However, chemical control seems to have limited effectiveness and investigation of classical biological control is necessary (Bălăcenoiu *et al.*, 2021a), especially as results from Croatia (Kovač *et al.*, 2020, 2021) have shown that this species can be controlled by an environmentally friendly method and a pan-European study (Bălăcenoiu *et al.*, 2021b) has shown the circumstances in which the general public and foresters are willing to support such a measure.

Galling insects

Dasineura gleditchiae is a North American species whose host is the honey locust. Although this species was recorded for the first time in Europe in the Netherlands in 1975 (EPPO, 2008), it was not observed in Romania until recently. It is thought to have arrived in the country in the 1990s or early 2000s (Olenici *et al.*, 2018), but is now widespread, being reported from both Oltenia and Moldova (Nețoiu *et al.*, 2018; Olenici *et al.*, 2018). In 2017, it was also found for the first time in the south-eastern part of the country. Because the attack intensity of this species has so far been low to moderate, it has not attracted attention and is not considered a major pest. However, the situation could change, and *D. gleditchiae* could cause severe damage to nurseries and green spaces by significantly diminishing the aesthetic appearance of attacked trees, as is the case in some areas of the US (Thompson *et al.*, 1998) and in Europe (Molnár *et al.*, 2009; Jurc and Jurc, 2010).

Obolodiplosis robiniae, a species native to eastern North America, develops on black locust leaves, causing their margins to fold (Drooze, 1985). In Europe, it was reported for the first time in Italy in 2003 (Duso and Skuhřavá, 2003), and in 2007 was observed for the first time in Bucharest, Romania (Bálint *et al.*, 2010), and has spread throughout the country (Olenici and Duduman, 2016; Nețoiu *et al.*, 2018). So far, the intensity of attack has generally been low in Romania, but in its natural range, it caused severe defoliation in the mid-

19th century (Haldeman, 1847, cited by Bálint *et al.*, 2010). As a result, it is premature to establish the importance of this pest for black locust in Romania.

Leaf miners

Cameraria ohridella was first described as a new species by Deschka and Dimic in 1986; it was discovered in Macedonia, former Yugoslavia, where it was observed attacking ornamental horse chestnut trees in the 1970s (CABI, 2020). Its first report in Romania was based on observations made in 1996 in Lovrin (Șandru, 1998). In the same year, it was observed in the south and west of the country (Šefrová and Laštůvka, 2001). It then rapidly spread through the country, and in 2004 it was present in Suceava (Lupaștean, 2006). In conjunction with the findings of previous studies on the distribution of this species in other areas of the country (Marcu and Simon, 2006, Perju *et al.*, 2004; Olenici *et al.*, 2016; Nețoiu *et al.*, 2018), it may be stated that it covers the entire territory of Romania. Although it can sometimes be found on sycamore leaves (CABI, 2020), in this study, in all 25 observation points where it was recorded, it was observed only on horse chestnut leaves and is the most important pest of this species. According to DAISIE (2009), it is one of the 100 worst invasive species in Europe.

Macrosaccus robiniella is native to North America. It damages black locust leaves by mining the underside of leaflets. Its first report in Europe was from Switzerland in 1983 (Whitebread, 1989), and its first record for Romania was from Dolj county in 2003 (Nețoiu, 2003). So far, the species has also been found in Transylvania (Fodor and Hâruța, 2009) and Moldova (Ureche, 2006; Olenici and Duduman, 2016), but it has not been recorded from Muntenia and Dobrogea. In 2017, in the south-eastern part of the country, both the frequency and intensity of its attack were very low, but much higher infestations have been reported in other cases in Romania (Fodor and Hâruța, 2009) and in other countries (Wojciechowicz-Żytka and Jankowska, 2004). Consequently, this species could be an important pest of the black locust.

Parectopa robiniella also originates from eastern North America, where it develops on the leaves of the black locust, causing digitate mines in the upper surface of leaflets (Drooze, 1985). It was first reported in Europe in 1970, before *M. robiniella*, in Italy (Vidano, 1970), and in 1989, it was found in Romania near Drobeta Tr. Severin (Nețoiu, 1994). The invasion of this species occurred relatively quickly in Romania, reaching from the south-west to the east of the country in just a few years, at a speed of about 100 km per year (Nețoiu and Tomescu, 2006). Even if the damage level was only up to 5% in most observation points in this study, careful monitoring of *P. robiniella* is recommended because heavy attacks cause premature fall of leaves and a reduction in biomass accumulation (Nețoiu and Tomescu, 2006). From year to year, it has infested forest areas of varying extent, with a maximum of almost 15,000 hectares between 1991 and 2000, but mostly showing weak or moderate infestation (Simionescu *et al.*, 2001).

Phyllonorycter issikii is native to East Asia (the Russian Far East, Japan, South Korea and China); its larvae damage leaves of lime trees (*Tilia* spp.; Kirichenko *et al.*, 2017). It seems to have been introduced into Europe in the 1970s, to Kiev (Šefrová, 2002), or into the European part of Russia between 1980 and 1984, and was observed at several sites in Moscow in 1985 (Bednova and Belov, 1999, cited by Ermolaev and Rubleva, 2017). In Romania, it was observed for the first time in 2002 in Podu Iloaiei, Iași County and in Dărmănești, Bacău County (Ureche, 2006). Currently, it is spread across the country, being reported from Transylvania (Kovács *et al.*, 2006) as well as Oltenia (Nețoiu *et al.*, 2018). The data collected in 2017 provide the first reports of this species in south-eastern Romania. However, given that the spread of the species in Europe took place from east to west with a speed of at least 110 km/year (Šefrová, 2002) or even 200 km/year (Rodeland, 2007) and that it had already arrived near Sofia, Bulgaria in 2006 (Toshova *et al.*, 2018), it is very likely that it was present in the southern part of Romania for many years without being detected. Even during the search in 2017, it was found at only two observation points because its mines are mostly found on the lower branches or on the undergrowth and in the inner part of the crown (Šefrová, 2002; Ermolaev and Zorin, 2011b) and do not cause a change in colour or premature drying of leaves (Kovács *et al.*, 2006); consequently, the presence of the insect is not apparent. During the 2017 study, the species was only found in forest stands, but it may also

be present in parks or in tree alignments located along streets, as a previous study has shown (Olenici and Duduman, 2016).

So far, the intensity of attacks caused by this species in Romania and in Europe has generally been low (Stolnicu and Ureche, 2007), and the impact on trees has been insignificant, but the situation may change radically if its populations increase (Ermolaev and Zorin, 2011a).

Invasive forest insect species collected with primed traps

The northern bark beetle, *I. duplicatus*, develops mainly on spruce, but in Romania it has also been found on other conifer species (Olenici *et al.*, 2009). Until the end of the nineteenth century, it was found only in the Eurosiberian taiga, from Sweden to the island of Sakhalin, and in the Alps (Pfeffer, 1955 cited in Holusa and Grodzki, 2008), but it gradually spread during the twentieth century into the rest of Europe (Knížek and Beaver, 2004). In Romania, it has been collected since 1948 (Negru and Ceianu, 1957), and is currently widespread in the country (Duduman *et al.*, 2011) and had its first outbreak on Romanian territory (Olenici *et al.*, 2009, 2011). During this study, most captures of *I. duplicatus* were recorded at Băicoi, Prahova County (point 4PH) in a Norway spruce stand growing outside the natural vegetation area of spruce, where some trees had been killed by bark beetles. At two other points (Constanța and Bucharest), some specimens were captured where no Norway spruce occurred in the surroundings. In these cases, the captured specimens most likely came from infested spruce logs brought from other areas, which highlights the role of transport of infested wood in the spread of this species from mountainous areas in other parts of the country.

The black stem borer, *X. germanus*, is native to East Asia. It was accidentally introduced into North America and Europe, where it was observed for the first time in 1932 and 1952, respectively (Groschke, 1952). It was collected in Romania for the first time in 2009 (Olenici *et al.*, 2015) and subsequent investigations have shown that it is widespread in the country at altitudes below 900 m (Olenici *et al.*, 2014; Olenici *et al.*, unpublished). During the current study, most captures were at Mioveni (1AG). However, this is not due to the proximity of the car factory, but to the more favourable environmental conditions for this species in the hilly area (Galko *et al.*, 2019). So far, this species has not been reported to cause damage in Romania, but as populations grow, detrimental impacts are expected, as those occurring in Germany (Heidenreich, 1960), Switzerland (Maksymov, 1987; Graf and Manser, 2000), Turkey (Tuncer *et al.*, 2017), Italy (Dutto *et al.*, 2018) and Slovakia (Galko *et al.*, 2019).

Neoclytus acuminatus is of North American origin (Solomon, 1995), and seems to have been first collected in Europe in the mid-19th century in Rijeka (then Fiume), Croatia (Küster, 1851). It then spread to other countries, both west and east (Keszthelyi, 2021). In Romania, the first specimen was collected in 2002 in Timisoara (Manci, 2005), and our data represent the first record of the species in the south-eastern part of the country. However, in the meantime, the species has been collected or observed in 18 other places (Hănceanu *et al.*, 2021), both in the south-west and the north-east of the country, indicating a much wider spread and possibly multiple introductions to Romania with imported wood. The fact that we found the species only at one observation point and captured only 10 specimens may be due to its rarity in this part of the country and the low attractiveness for adults of the baits used in the traps. The data published by Hănceanu *et al.* (2021) suggest that direct observation of adults during flight, when they aggregate in places with unseasoned logs, and involvement of citizens is a more effective method of detection. At the same time, this aspect and the fact that we detected the presence of the species in a wood processing centre draw attention to the risk of facilitating the spread of this species by the transport of infested wood over long distances. As the populations of this pest are still small, no damage has been reported in Romania, but the situation could change as the species spreads more and more and its population increases. This species is polyphagous and grows in hardwood, preferring ash and oak. It commonly infests weakened, dying or dead trees, but is most destructive to unseasoned logs with bark. In North America, it also attacks seemingly healthy black locust trees (Solomon, 1995).

Conclusions

Investigations conducted in 2016-2017 in south-eastern Romania led to the detection of 17 species of non-native forest insects, which belong to 11 families and five orders. Most of the species found (11) originate from North America and some from Asia (4) and Europe (2).

The first arrival in Europe of all 17 species was in other countries; they then spread to Romania, the gap between the first report in Europe and the first report in Romania being a few years to more than 150 years, depending on the species and the period of its introduction into Europe. This pattern, if maintained in the future due to the gap in Romania's economic development compared to that of many European countries, may be an advantage, provided the country is prepared to prevent the introduction of new invasive species or to implement rapid detection and eradication programmes, with significant involvement of citizens.

All species detected were previously reported in Romania, but eight (*A. leucopoda*, *P. fraxinifolii*, *D. gleditchiae*, *M. robiniella*, *P. issikii*, *I. duplicatus*, *X. germanus* and *N. acuminatus*) had not been found in the south-eastern part of the country. This is due to a lack of sustained effort to detect new species in the country's fauna and a lack of involvement of citizens in observing and reporting the phenomena caused by newly introduced species. Seven of the detected species (*A. leucopoda*, *C. arcuata*, *H. cunea*, *I. duplicatus*, *N. acuminatus*, *P. issikii*, *X. germanus*) live on native tree species, and 10 (*C. ohridella*, *C. perspectalis*, *D. gleditchiae*, *E. strobilus*, *E. tibialis*, *G. cooleyi*, *M. robiniella*, *O. robiniae*, *P. robiniella*, *P. fraxinifolii*) on exotic trees or shrubs. Most of these tree species come from the natural ranges of the alien insect species found during the study and were introduced to Romania a long time ago.

The distribution of insect species detected in the study area is very uneven, and in most cases, depends on the presence of host species. The species associated with the black locust, horse chestnut and oaks are favoured from this point of view. A higher number of alien insect species were found in urban areas, which, through their diversity of tree and shrub species in green spaces (parks, street alignments, etc.) and economic activities (especially transport), facilitate the introduction, establishment and spread of new species. Except for *E. tibialis*, all other species found cause damage, either in their area of origin or in the territories invaded in various countries, which qualifies them as invasive species, i.e., species with significant negative ecological and economic impact.

Authors' Contributions

Conceptualization: NO, FB; Data curation: FB, AB, AA; Formal analysis: NO, FB; Funding acquisition: RT, NO, CN; Investigation: FB, AA; Methodology: NO, CN; Project administration: NO, RT, CN; Resources: NO, FB, RT; Software: NO, FB, AB; Supervision: NO, CN; Visualization: NO, FB; Writing - original draft: NO, FB; Writing - review and editing: NO, FB, RT, CN, AB, AA. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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