

The gall midge *Obolodiplosis robiniae* Haldemann (Diptera Cecidomyiidae) new invasive alien species in Europa – Review

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Abstract

The most important pests of acacia black locust (*Robinia pseudoacacia* L.) (Fabaceae) are: *Appendisetia robiniae* Gillette, *Phyllonorycter robiniella* Clemens, *Parectopa robiniella* Clemens, *Nematus tibialis* Newman and *Obolodiplosis robiniae* Haldemann, species from North America, where it also originates the acacia, which is the host plant. In Europe these species were introduced accidentally, being considered invasive species. In Europe, *O. robiniae* Haldemann was first reported in July 2003 in northeastern Italy, in Paese near Padua, from where it spread to almost the entire European continent, especially through trade in infested biological material from nurseries. The attack occurs in the species *R. pseudoacacia* L., *R. viscosa* L. and *R. hispida* L., mainly in urban areas, where it mainly affects their aesthetic value. The larvae develop characteristic leaf margin roll galls on the infested leaves. There are up to 6 galls on a leaflet, and on average there are up to 5-6 larvae. In Europe it develops two, three and, in optimal conditions, even four generations a year. Zoophagous entomofauna can cause population decline, especially parasitoid species. For *Platygaster robiniae* Buhl & Duso (Hymenoptera: Platygastridae) the percentage of parasites reported was up to 40%. A low percentage of parasitism can be produced by other species, such as: *Aprostocetus* sp., *Chrysocharis* sp. (Hymenoptera: Eulophidae), *Eupelmus urozonus* Dalman (Hymenoptera: Eupelmidae), *Eurytoma verticillata* F. (Hymenoptera: Eurytomidae), *Mesopolobus mediterraneus* Mayr. (Hymenoptera: Pteromalidae) and *Torymus* sp. (Hymenoptera: Torymidae).

Keywords: bioecology; morphology; *Obolodiplosis robiniae* Haldemann; *Robinia pseudoacacia* L.; zoophagous entomofauna

Introduction

Black locust (*Robinia pseudoacacia* L.) (Fabaceae) is native to the eastern part of North America. It was introduced to Europe in the early 17th century, first in France around 1601 and in Italy in 1622, then expanded throughout Europe. Growing demand for timber during the early industrialization (late 18th and early 19th centuries) led to extensive acacia planting in Europe (Cierjacks *et al.*, 2013). The wood is tough, rot-resistant and durable, and wood flavonoids make it possible to last more than 100 years in the soil (Vidano, 1983; Rusňák

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et al., 2022). This plant species is now considered invasive in several European countries (Glavendekić *et al.*, 2009; Matos *et al.*, 2023). This is because the leaves contain substances which affect their decomposition and prevent the formation of humus (Mařová *et al.*, 2018). In addition, three other leaf compounds (robinetin, myricetin and quercetin) inhibit the growth of other plant roots in acacia habitats, enhancing the invasive nature of acacia (Nasir *et al.*, 2005).

It is used for afforestation as an ornamental plant in parks and gardens, and it is also planted along roadsides. Acacia is a drought-tolerant and fast-growing tree (De Gomez and Wagner, 2001). It is also a plant used to combat soil erosion on rugged terrain (Cierjacks *et al.*, 2013) and an important honey plant (Mocreaç and Timuș, 2014; Tokuda *et al.*, 2019). In addition, wood extracts contain substances with antimicrobial action for some human pathogens (Nicolescu *et al.*, 2020).

Black locust is a host plant for some pest species. These include *Appendiseta robiniae* Gillette, *Parthenolecanicum corni* Bouché, *Aphis craccivora* Koch, *Phyllonorycter robiniella* Clemens, *Parectopa robiniella* Clemens, *Nematus tibialis* Newman and *Obolodiplosis robiniae* Haldemann, species that originate in North America (Vidano, 1970; Serini and Trematerra, 1989; Šefrová, 2003; Bella, 2013; Csóka *et al.*, 2009; Bakay and Kollár, 2010, 2014; Martynov and Nikulina, 2016; Bacal *et al.*, 2020; Kolyada *et al.*, 2022). In addition, in urbanized sites, the vitality of acacia trees is affected by some destabilizing factors (air pollution, compacted soil, soil contamination), which accentuate the sensitivity of trees to pest species (Bakay and Kollár, 2014).

Invasive alien species

Invasive alien species are organisms introduced by humans (deliberately or accidentally) out of their natural environment and have multiplied and begun to have adverse effects on the new ecosystem. Invasive species go through three essential stages in the new area: introduction, stabilization, and spread (Roy *et al.*, 2011). These species critically affect ecosystems and are a direct factor in biodiversity loss (Bellard *et al.*, 2016; Paine *et al.*, 2016; Bălăcenoiu *et al.*, 2020; Gubin, 2020; Vicente *et al.*, 2022). In Europe, most invasive alien insects have been introduced accidentally, and a small number of species have been introduced intentionally, most of them for biological control (Roques, 2015).

Invasion is a typical ecological process, but human activities can stimulate it through trade in various plant materials (Kenis *et al.*, 2009; Liebhold *et al.*, 2012; Eschen *et al.*, 2015; Ormsby, 2022). In addition, it often allows pest species to cross natural barriers that limit their spread (Levine and D'Antonio, 2003; Kollár, 2014; Mormul *et al.*, 2022). Of the approximately 1500 invasive arthropods introduced and stabilized on the European continent, about 87% are taxonomically represented by insects (Roques, 2010 a).

In Europe, there has been a continuous increase in the number of newly introduced invasive species, most associated with woody plants (over 75%) and less with agricultural plants (Roques *et al.*, 2009; Roques, 2015). In addition, many wood species are brought for ornamental purposes or various afforestation, and insects can be in different stages of development on the imported material, often the infestation being difficult to detect (Blyummer, 2020). By 2007, 109 invasive phytophagous insect species had been introduced into Europe's forest ecosystems, significantly affecting the trees (Mattson *et al.*, 2007; Matošević and Pernek, 2011). The initial spread of species that arrived in Europe after 1990 is 3-4 times higher than that of previously introduced species (Roques *et al.*, 2016). The annual average of introduced invasive species was about 4 to 11 new species per year in 1950-1974, respectively between 8 to about 15 new species per year after 2000 (Roques, 2008, 2015; Bălăcenoiu *et al.*, 2020).

Introduction pathways play a pivotal role in the success of invasive alien species (Turbelin *et al.*, 2022). The spread of invasive species in the EU is also facilitated by the fact that freight transport is constantly increasing, but also due to the decrease of customs controls at border crossing points (Tomov and Trencheva,

2014; Olenici and Duduman, 2016; Nețoiu *et al.*, 2018; Bălăcenoiu *et al.*, 2020). As a result, new species entered in different European countries are registered every year.

Initially, in the invasive species, the population is small, often being difficult to signal its first presence in the new area, followed by a slow period of increasing population density and, in the end, by a fast spread. The first two phases can extend over 3-4 years (Roques *et al.*, 2016).

The penetration of invasive alien species and especially their acclimatization (many of them being slightly thermophilic) on the European continent is also facilitated by climate change, especially by the phenomenon of global warming, which directly affects their survival, fecundity, development and dispersal (Hrubík and Kollár, 2007; Roques, 2010 b; Zhao *et al.*, 2023). There have been increases in average annual temperatures at some weather stations by about 1 °C. Temperature rise simulation models predict an increase from 1.6 to 6.4 °C by 2100 (Parmesan and Yohe, 2003; Parmesan, 2006). Due to the absence of native enemies and altered ecological conditions, accidentally introduced pest species may suddenly increase their population density in the new area (Hrubík and Kollár, 2007). Italy is at high risk of introducing exotic insect species, mainly due to its climatic conditions, favourable for insects in tropical and subtropical areas and by positioning it in the Mediterranean area, with intense trade in American goods to Europe and Asia, being a country where tourism is highly developed (Bella, 2013). As a result, Italy has the most significant foreign entomofauna in Europe. Between 1945 and 2019, over 300 species of invasive insects were found in this country (Blyummer, 2020).

Another phenomenon reported in invasive species is their distribution in the new area. At insect species that attack trees, it has been found that in urban and suburban areas, their population is much higher than in rural areas (Gaertner *et al.*, 2016, 2017). Also, the first records in a European country of invasive species were primarily found in cities or suburban areas and very few in sites far from cities (Branco *et al.*, 2019). Cities can offer better thermal conditions for establishing non-native species due to the heat island effect and the different sites, like public parks, street trees, university campuses, botanical gardens (Dale and Frank, 2014; Branco *et al.*, 2019). In these locations, there is also a higher degree of attack of trees, determined both by the numerical density of the pest population and by the fact that the trees are exposed to many factors of stress (lack of water, poor soil conditions and pollution) they sensitize the trees (Sjöman and Nielsen, 2010; Kollár, 2014).

The gall midge species presents the primary importance of the large group of invasive insects. Many were introduced from where the attacked host plant originated (Kollár, 2011). Plague-inducing insects are the most specialized phytophagous species (Shorthouse *et al.*, 2005; Oates *et al.*, 2016). These, together with some species of mites, are the only phytophagous arthropods capable of modifying the structures of the host plant at the histological and cellular level to induce galls (Stone and Schönrogge, 2003; Giron *et al.*, 2016). However, most gallic species systematically belong to only four families of insects of three orders: Hemiptera (Adelgidae), Diptera (Cecidomyiidae) and Hymenoptera (Cynipidae and Eulophidae) (Csóka *et al.*, 2017; Mirumian and Skuhrová, 2022). Galls are irregular growths of attacked plants caused by the plant's reaction to hormones and chemicals secreted by the larvae of these species (Molnár *et al.*, 2009; Oates *et al.*, 2016; De Araújo *et al.*, 2017; Staszak *et al.*, 2023). The galls provide feeding conditions for the larvae and protection against insecticides used in chemical treatments and against the attack of many zoophagous species (Molnár *et al.*, 2009; Csóka *et al.*, 2017). Most biliary species are strictly monophagous or oligophagous, and very few attack host plants from different families (Carneiro *et al.*, 2009).

***Obolodiplosis robinae* Haldemann (Diptera Cecidomyiidae) new invasive alien species in Europa**

The aim of this paper is to review the recent status of the pest *O. robinae* Haldemann. The paper summarises the current state of knowledge on the its spread, emphasizing the timing and the geographical area where the pest was notified, morphology and biology aspects and biological control methods.

Origin and spread. The species *O. robiniae* Haldemann has its origin on the American continent, where it also originates the acacia, which is the host plant. Haldeman first described this species in 1847, who reported the first gall in eastern Pennsylvania and was named *Cecidomyia robiniae* (Skuhrová and Skuhrový, 2004; Buhl and Duso, 2008).

From there, the species spread to almost the entire world, where the host plant is. In 1999 it was reported in Canada, far from its native area (Csóka *et al.*, 2017). In July 2002, the species was detected on acacia trees near Fukuoka Prefecture, Japan (Kodoi *et al.*, 2003) and was reported in the same year in South Korea (Woo *et al.*, 2003). The first discovery in China was in 2005 (Yang *et al.*, 2006; Lin *et al.*, 2007), where the level of infestation became extremely strong (Lu *et al.*, 2010). In New Zealand, the species was reported in 2018 (Martin, 2019).

It was first reported in Europe in July 2003 in north-eastern Italy, at Paese near Padova, Veneto region (Duso and Skuhrová, 2003; Navone and Tavella, 2004). However, it soon spread throughout Italy. In 2004 and 2005, the pest was found in five Italian regions (Veneto, Friuli-Venezia Giulia, Trentino-Alto Adige, Lombardy and Emilia Romagna) and at higher population densities than in the previous year (Calvi and Tantardini, 2005; Duso *et al.*, 2005). In South Tyrol (northern Italy), the species is also present at altitudes above 1160 m (Hellrigl, 2006; Hellrigl and Minerbi, 2012).

Annually, the species has increased its range, currently being reported in almost all of Europe. In 2004 it was reported in Slovenia (Duso *et al.*, 2005; Skuhrová *et al.*, 2007). In the same year, it was found in the Czech Republic, in Prague in the Bohemian area (Skuhrová and Skuhrový, 2004), and in the period 2005-2006, the level of acacia infestation in the central part was very high (Skuhrová *et al.*, 2007).

In 2005, Skuhrová *et al.* (2007) found acacia galls on Samos, Greece.

During 2006-2007, the species spread rapidly over many states, with the broadest dispersal. In 2006 it was reported in Croatia, near Rabac (Skuhrová *et al.*, 2007); in Germany in the Winterbach locality from where it spread rapidly along the Rhine (Wehrmaker, 2007; Hoffmann *et al.*, 2007); Luxembourg (Schneider and Walisch, 2009); Montenegro (Glavendekić, 2007); Ukraine (Berest and Titar, 2007). In October 2006, it was reported in western Serbia, at Šabac (Glavendekić and Mihajlović, 2007), and in June 2007, the larvae were found in the Belgrade area (Glavendekić *et al.*, 2008). Also in October 2006, the species was found in Nitra and Bratislava, areas in western Slovakia (Hrubík and Kollár, 2007; Zúbrik *et al.*, 2007), and after a year, it was found throughout the country (Tóth *et al.*, 2009). In Hungary, it was reported in the western part of the country in 2006 (Csóka, 2006), and a year later, it spread throughout the country (Skuhrová *et al.*, 2007).

In 2007 the species was reported in: Albania (Mihajlović *et al.*, 2008); Austria (Skuhrová *et al.*, 2007); Switzerland at Birmensdorf, Canton of Zurich (Wermelinger and Skuhrová, 2007); the Netherlands in Amsterdam and Rotterdam (Roskam *et al.*, 2008); France (Laguerre and Dauphin, 2007); Belgium (Mihajlović *et al.*, 2008); England, at Oxford University Botanic Gardens and then Kent in south-east England (Skuhrová *et al.*, 2007; Badmin, 2011); Bosnia and Herzegovina (Mihajlović *et al.*, 2008); Russia, in the Voronezh area (Mihajlović *et al.*, 2008) in 2008 the species was recorded in the Russian Far East, in Vladivostok and Sakhalin Island (Gninenko, 2013) and 2010 it reaches the Volgograd region. (Gribust *et al.*, 2021). In Poland, the first report was in Krakow in 2007 (Skrzypczyńska, 2008). One year later, the species was found in the country's southwest in the Opole region (Olszanowska-Kuńka, 2008), and Kostro-Ambroziak and Mieczkowska (2017) made the first report in northeastern Poland. In Romania, the species was reported in Bucharest in 2007 (Bálint *et al.*, 2010), then in 2009 in Ploiești (Bălăcenoiu *et al.*, 2020), in 2010 in Arad (Don *et al.*, 2016), but also in the northern part of Romania (Olenici and Duduman, 2016). In 2020, it was also reported in the Cluj area (Ilea *et al.*, 2021).

The presence of the species was also reported in Macedonia in 2008 (Glavendekić *et al.*, 2009); Sweden, in the province of Skåne, is the first report in northern Europe (Molnár *et al.*, 2009). In 2009 it was reported in Bulgaria (Tomov *et al.*, 2009) and Denmark at Holte, which is by far the northernmost attitude (55°48'N)

in Europe (Jørgensen, 2009). In 2010 it was reported in the Republic of Moldova, in the Cahul district (Mocreac and Timuş, 2014); in 2011 in Portugal (Bella, 2013); in 2012 in Latvia (Rupais *et al.*, 2014).

Since 2013, it has been reported in Georgia at Avchala, a suburb from the north of the capital Tbilisi (Skuhrová *et al.*, 2013); in Lithuania (Stalažs, 2014) and Spain, Barcelona area (Sánchez and Umaran, 2013). The latest European report is from Armenia, where the species was detected in 2019 (Gubin, 2021). *Obolodiplosis robiniae* is within the range of 21°34' and 65°39' N in the Eurasian continent (Zhao *et al.*, 2023).

After the accidental introduction of the species in Europe, it spread rapidly throughout most of the continent, most likely due to trade with infested biological material from nurseries. Young *R. pseudoacacia* seedlings may contain eggs and larvae that are difficult to detect without strict determinations (Mihajlović *et al.*, 2008; Glavendekić *et al.*, 2009; Toth *et al.*, 2009). On the other hand, infected and fallen leaves can be spread by road vehicles, attaching to the wheels and other parts of vehicles and then scattered over long distances, as was the case with the acacia leaf moths *P. robiniella* Clemens and *Ph. robiniella* Clemens (Skuhrová *et al.*, 2007; Duso *et al.*, 2011; Pap *et al.*, 2015). The widespread was also favoured by an extensive distribution of its host plant along the main routes (Duso *et al.*, 2011; Mally *et al.*, 2021). Therefore, it can be concluded that the anthropogenic factor is the most important in spreading the species (Kohútová and Oboňa, 2016). Also, adults' small size and weight can facilitate long-distance dispersal by air currents (Gilbert *et al.*, 2005; Glavendekić *et al.*, 2009; Cusimano *et al.*, 2016).

Morphology. The adult is a dipterous with a body size between 2.6-3.2 mm (Pernek and Matošević, 2009; Straker *et al.*, 2015). It has a slight sexual dimorphism. While males are 2.6-2.8 mm, females are longer (3-3.2 mm) (Bálint *et al.*, 2010; Mocreac and Timuş, 2014). In addition, the female has a non-retractable ovipositor (Roskam *et al.*, 2008). The body colour is yellowish-brown, and the head is black. Antennae are filiform with a flagellum consisting of 12 articles (Kodoi *et al.*, 2003; Roskam *et al.*, 2008). The wing is transparent slightly shiny (Mocreac and Timuş, 2014). The legs are long (characteristic of the suborder Nematocera) with a simple tarsal claw (Kodoi *et al.*, 2003). Genitals have been described by Kodoi *et al.* (2003) and Mihajlović *et al.* (2008).

Because there may be differences between populations in different areas, genetic studies are performed at the mitochondrial cytochrome level to identify differences between species genotypes (Shang *et al.*, 2015; Yao *et al.*, 2015, 2020). In addition, genetic variation is considered an essential factor in the colonization of invasive species (Horst and Lau, 2015).

The egg has a size of 0.1 mm (Park *et al.*, 2009).

The larva is apodous. It goes through three larval ages (Pernek and Matošević, 2009). The body colour is white in the first two ages, and the last larvae have a yellow-orange colour (Bálint *et al.*, 2010). At hatching, they are less than 1 mm, and in the last age, they can reach up to 4 mm (Hoffmann *et al.*, 2007). The larvae of the last age have a long sternal spatula on the ventral side of the prothoracic segment (Mocreac and Timuş, 2014).

The pupae is 3.2 mm (Park *et al.*, 2009). At first, they are white, then they become red-yellow (Uechi *et al.*, 2005; Mocreac and Timuş, 2014).

Biology. The species is multivoltine. In Europe, it develops two, three and, in optimal conditions, even four generations per year (Duso *et al.*, 2005; Bella, 2007; Mihajlović *et al.*, 2008). These generations may overlap (Skuhrová *et al.*, 2007). In Korea, the species has had a maximum of three generations per year (Lee *et al.*, 2009), in Japan up to four generations (Uechi *et al.*, 2005), and Chinese populations can develop between four and six generations per year (Lu *et al.*, 2009; Mu *et al.*, 2010; Shao *et al.*, 2010; Liu, 2014).

In the last larval stage, the species winters in the soil under the attacked acacia's crown projection. In the spring, the pupae appear, and soon the adults of the hibernating generation. Their appearance corresponds to the leafing phenophase of the host plant, most frequently in early May, but in the years with warm springs, adults can also appear in April (Duso *et al.*, 2011). After mating, females lay eggs on the underside of the leaf,

most often on the top of the shoots (Glavendekić and Mihajlović, 2007; Tóth *et al.*, 2009; Duso *et al.*, 2011). For oviposition, females detect the host plant based on the olfactory indices emitted by acacia (Molnár *et al.*, 2018). The average prolificacy of the species is around 190 eggs (Park *et al.*, 2009). The hatched larvae cause the appearance of galls on the leaflet. The larval development is spread over 2 to 4 weeks, depending on the climatic conditions (Mirumian and Skuhrová, 2022). The larvae of this generation turn into pupae in the feeding place, in the galls they formed (Glavendekić and Mihajlović, 2007). The new adults fly from the beginning of July to August and begin the development of the second generation. They mate near their place of appearance (Molnár *et al.*, 2018) and then lay their eggs on the shoot leaves where they developed, or even on previously infested leaves to be found simultaneously young larvae, last age larvae and pupae. This generation is evolving like the previous generation, the pupae development also taking place in the galls. However, the development of this generation is often dependent on climatic conditions. Thus, hot and dry periods can reduce the density of the larval population, prolong the developmental stages, and sometimes can induce the appearance of larvae that will enter diapause until next spring (Duso *et al.*, 2011). In the last generation, the autumn larvae, the young larvae leave the galls and retreat into the soil for the hiemal diapause (Glavendekić and Mihajlović, 2007).

The timing of generations depends on the climatic characteristics of the year. In Slovakia, the first generation was from mid-May to early July, the second generation in July and August, and the third generation in September to mid-November (Tóth *et al.*, 2009). Three generations are developing in the Czech Republic: the first generation from mid-May to the end of June or early July, the second generation in July and August, and the third generation in September and October until mid-November. Under optimal conditions, even the fourth generation can develop, at least partially (Skuhrová *et al.*, 2007). In Serbia, the generations followed one another in the colder years: the first generation develops from May to early June, the second generation from the second half of June to the start of August, and the third generation in September and October. (Glavendekić and Mihajlović, 2007). In the warmer years, the species developed four generations. The first generation develops in April-May, the second generation in June and until the beginning of July, the third generation from July to August, and the fourth generation from September to the end of October (Mihajlović *et al.*, 2008; Glavendekić *et al.*, 2009). Lu *et al.*, (2009) established the thermal constants of the species. The lower thermal threshold is 8.01 °C, and a total of 506.93 active degrees are required to go through the developmental stages of a generation, of which: 62.20 active degrees for incubation; 281.42 active degrees for larval development, 124.45 active degrees for the pupae stage, respectively, 55.48 active degrees for the adult stage.

Host plants and damages. *O. robiniae* Haldeman (Diptera: Cecidomyiidae) is a monophagous species, with host plants being species of the genus *Robinia*. The white-flowered acacia *R. pseudoacacia* L. is the most heavily attacked (Uechi *et al.*, 2005; Csoka, 2006; Sheppard *et al.*, 2006; Yang *et al.*, 2006). Molnár *et al.* (2009) reported a strong attack on *R. pseudoacacia* "Umbraculifera", a globular clone typical in Swedish gardens and plantations. On the golden-leaf acacia, *R. pseudoacacia* f. *frisia*, the attack is lower (Badmin, 2011, 2016).

The pest is also commonly reported on the red-flowered acacia *R. viscosa* L., another ornamental tree commonly found in parks and landscaping (Skuhrová *et al.*, 2007; Tóth *et al.*, 2009; Csoka *et al.*, 2017). Damage has also been reported to *R. hispida* L., which grows in urban green spaces, a red flower species (Glavendekić and Mihajlović, 2007; Hrubík and Kollár, 2007).

The attack of this species is produced by the larval stage and is manifested at the level of the leaf. After hatching, the endophytic and gregarious larvae cause changes in the structure of the parenchyma that led to the appearance of characteristic galls located at the edge of the leaflet and rolled towards the bottom (Skuhrová and Skuhrová, 2005; Csoka, 2006; Hoffmann *et al.*, 2007; Pernek and Matošević, 2009). The length of a scab is between 7-16 mm (Mocrea and Timuş, 2014). When the larvae attack very young leaves or at high larval densities, the leaflets are rolled along the central vein, they become linear (Duso and Skuhrová, 2004; Duso *et*

al., 2011). Thus, the attack of the species is unmistakable. At first, the galls are green, then they turn yellow to pink (Figure 1a), and finally, they turn brown (Figure 1b).



Figure 1. Leaflets attack by *O. robiniae* Haldeman (photo I. Hlujjan)

The attack's impact on the host plant depends on the frequency of the attacked leaves and the number of galls produced on one leaf. The percentage of leaflets attacked on a leaf can reach up to about 45% (Glavendekić and Mihajlović, 2007; Olszanowska-Kuńka, 2008). The damage is significant when the frequency of the attacked leaves is between 25-75% and the number of galls on a leaf is between one and six (Glavendekić and Mihajlović, 2007; Bakay and Kollár, 2014). On the same leaflet are galls of different generations that the pest develops (Skuhrová *et al.*, 2007). The distribution of the attacked leaves depends on the evolution of the generations of the pest, on the phenophase of the development of the host plant, on the exposure of the branches, on the section of the crown, and others. In the first generation, the attack frequency is higher at the base of the crown, followed by the middle and then at the top of the crown (Jingshuang *et al.*, 2013). The leaves on the tree branches located to the east and north had the highest degree of attack (Glavendekić *et al.*, 2008). Leaves located on the terminal parts of the shoots are also preferred, more often on the apical shoots (Duso *et al.*, 2005), so the largest number of larvae on the leaves is on the outside of the crown compared to the inside of the crown (Lee *et al.*, 2009).

The new leaves continue to grow during the summer, and their growth stops in late autumn with the first frost (Skuhrová *et al.*, 2007). The damage of the leaves also depends on the number of larvae that live in the galls (Duso and Skuhrová, 2003). One or more larvae are found in the same gall (Figure 2), perhaps because several females laid their eggs in the same place (Molnár *et al.*, 2009). The average number of larvae in a gall is up to 5-6 larvae (Skuhrová *et al.*, 2007; Tóth *et al.*, 2011), and the maximum number can reach 9-11 larvae (Pernek and Matošević, 2009; Mocreac and Timuş, 2014; Csóka *et al.*, 2017). 3-8 larvae have been reported in China (Yang *et al.*, 2006; Zhongqi *et al.*, 2006). In Korea, up to 9 larvae have been reported in a gall (Lee *et al.*, 2009).



Figure 2. One-larva gall (left) and three-larva gall (right) (photo I. Hulujan)

A large number of galls on the leaf causes a decrease in the assimilation function of the leaf and a premature fall of the leaves, which affects the vitality of the acacia (Duso *et al.*, 2005; Sheppard *et al.*, 2006). Young trees are most vulnerable to attack (Csóka *et al.*, 2017), but vigorous trees can compensate for this loss of leaves by new growths (Duso *et al.*, 2005; Tóth *et al.*, 2009). In the case of ornamental plants, their aesthetic value is also affected (Glavendekić and Mihajlović, 2007; Pernek and Matošević, 2009; Straker *et al.*, 2015). It is also a dangerous risk factor for bee production (Glavendekić *et al.*, 2008; Mihajlović *et al.*, 2008).

On the other hand, it has been found that the attack's impact is more pronounced in urban areas, often with the highest numerical density of the pest population, as well as the fact that trees can be affected by other destabilizing factors sensitize the tree to attack. (Tóth *et al.*, 2009; Bakay and Kollár, 2010, 2014; Bella, 2013). In addition, in urban areas, there is generally an increased level of toxic metals (Fe, Zn, Pb, Mn and Cd) in plant tissues that conjugated with pest attack affect the acacia vegetation (Wilkaniec *et al.*, 2021).

Control measures. Control of this pest, especially by applying chemical treatments, is relatively complex due to the particulars of the sites where it attacks and the vast area of spread of the host plant. Furthermore, in urban areas, the application of chemical treatments is limited due to the side effects of insecticides, and the range of authorized insecticides is limited (Bella, 2013). At present there is limited literature addressing the issue of chemical control of this species.

An important measure is the control of this pest in nurseries so that the planting material is free of it (Duso *et al.*, 2011).

Repeated cuts and burning of attacked parts of attacked trees is an effective method of protection, especially in landscaping (Hrubík and Kollár, 2007; Maľová *et al.*, 2018).

The zoophagous entomofauna can play an essential role in decreasing the population of this species.

In nature, the dynamics of the numerical density of pest populations is often regulated by the complexity of zoophagous species (parasites and predators). For invasive species, the lack of natural enemies in the new area allows them to continuously increase their population (Torchin *et al.*, 2003). On the other hand, the native zoophagous entomofauna can adapt to the new pest, thus controlling the population for some invasive species. However, it takes time to reach a balance between the pest and the zoophagous (Grabenweger *et al.*, 2005, 2010). One such example is the impact of parasitoid Hymenoptera on the declining populations of two acacia-attacking microlepidoptera, *P. robiniella* Clemens and *Ph. robiniella* Clemens (Lepidoptera: Gracillariidae), which were accidentally introduced to Europe (Vidano, 1970; Serini, 1990; Gibogini *et al.*, 1996; Csóka *et al.*, 2009). At other times, the zoophagous entomofauna may be introduced simultaneously as the invasive species. Therefore, parasites could play a key role in controlling invasive species (Prenter *et al.*, 2004).

For *O. robiniae* the main parasitoid species is the wasp *P. robiniae* Buhl & Duso (Hymenoptera: Platygasteridae), a monophagous parasitoid that is thought to have been introduced to Europe, Asia and North America along with the invasive species. *Platygaster* species mainly parasitise Cecidomyiidae species (Diptera). Most species of this genus are monophagous or, at most oligophagous (Buhl and Duso, 2008), widespread in several ecosystems, contributing to the biocontrol of pests (Prenter *et al.*, 2004; Yang *et al.*, 2021).

In Europe, the species was first reported in Italy, where the host species was also introduced (Buhl and Duso, 2008). Currently, the parasite is found almost all over Europe. In some countries, the parasitoid was reported simultaneously with *O. robiniae*, and in other states, a few years after the host was reported. Almost simultaneous detection of *O. robiniae* and *P. robiniae* in several areas of Europe and Asia strongly suggests that the parasitoid was introduced along with the pest (Jørgensen, 2009; Sviridov and Bazhenova, 2009; Duso *et al.*, 2011).

In Switzerland, the parasitoid appeared in 2007, simultaneously with *O. robiniae* (Wermelinger and Skuhrová, 2007) and in Montenegro (Glavendekić *et al.*, 2008). The parasitoid was reported shortly after the introduction of the invasive species and in the Czech Republic (Wermelinger and Skuhrová, 2007), Croatia (Pernek and Matošević, 2009), Denmark (Jørgensen, 2009), Germany (Bathon, 2007), Greece Corfu Island (Bella, 2014), Serbia (Glavendekić *et al.*, 2008; Mihajlović *et al.*, 2008), Moldova (Mocreac and Timuş, 2014), Romania (Bálint *et al.*, 2010), Slovakia (Tóth *et al.*, 2009), Hungary (Csóka *et al.*, 2017). The same has been reported in Asia: China (Lu *et al.*, 2010), South Korea (Park *et al.*, 2009; Kim *et al.*, 2011), Japan (Skruva *et al.*, 2007).

P. robiniae Buhl & Duso is a gregarious endoparasite with a biological cycle synchronized with its host (Glavendekić *et al.*, 2009; Kim *et al.*, 2011). The parasitoid females lay eggs in the larva of the host, in which the larvae of the parasitoid then develop. The number of larvae reported in an *O. robiniae* larva is quite variable, sometimes depending on the size of the parasitized larva. Skuhrová *et al.* (2007) reported between 3 and 12 larvae; Glavendekić *et al.* (2009) between 3 and 14 larvae; Duso *et al.* (2011) reported between 4 and 28 larvae; Mocreac and Timuş, (2014) between 5 and 25 larvae. The development of the parasitoid, especially the pupae and the adult, can be observed through the translucent skin of the parasitized larvae. The parasitic larvae are white, and as the parasitoid develops, they turn dark grey (Pernek and Matošević, 2009; Mocreac and Timuş, 2014). The parasitized larva dies before the parasitoid leaves it. When the parasitoid is fully developed, it makes an opening through which the adult will emerge (Skuhrová *et al.*, 2007). The development time of the parasite from egg to adult lasts about 28 days (Kim *et al.*, 2011).

The level of parasitism is different from one area to another as well as during the development of the host generations, the highest frequency of parasitic larvae being observed in the second and third generation, from the end of July to August (Buhl and Duso, 2008; Glavendekić *et al.*, 2009; Duso *et al.*, 2011). Adult flight of *P. robiniae* may occur as early as October, but some may remain in the parasitized larvae for diapause as early as July (Duso *et al.*, 2011; Mocreac and Timuş, 2014).

In Serbia, the parasitism in 2007, one year after the introduction of the pest, averaged over 6% and increased in 2008 to over 41% (Glavendekić *et al.*, 2008; Mihajlović *et al.*, 2008). In southern Slovakia, one year after the first report of *O. robiniae*, the percentage of parasites was between 10% and 30% (Tóth *et al.*, 2009) and in Croatia about 16% (Pernek and Matošević, 2009). Tóth *et al.* (2011) reported differences in parasitism between urban and rural sites, the rate being higher in urban areas. In England, the proportion of parasitic larvae was up to 40% (Notton and Outlaw, 2021). This species appears to be very effective in regulating the population of this pest (Lu *et al.*, 2010; Tóth *et al.*, 2011; Branco *et al.*, 2019).

Other parasitoid species have been reported for this pest, but with a low percentage of parasitism.

In China, *Systasis obolodiplosis* was reported by Yao (2009) (Hymenoptera: Pteromalidae), which parasitizes the larvae and pupae of *O. robiniae* (Yao *et al.*, 2009). As in the case of the genus *Platygaster*, most species of the genus *Systasis* parasitize, especially pests of the family Cecidomyiidae (Diptera). Bella (2014), on

the island of Corfu, in addition to *P. robiniae*, also reported another parasitoid species, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae).

Most parasitoid species were reported in Slovakia by Tóth *et al.* (2011) in 2007-2008. In addition to *P. robiniae*, which was the dominant species, the acacia fly was parasitized by the following species: *Aprostocetus* sp., *Chrysocharis* sp. (Hymenoptera: Eulophidae), *Eupelmus urozonus* Dalman (Hymenoptera: Eupelmidae), *Eurytoma verticillata* F. (Hymenoptera: Eurytomidae), *Mesopolobus mediterraneus* Mayr. (Hymenoptera: Pteromalidae), *Torymus* sp. (Hymenoptera: Torymidae). Of the total parasitism rate, 98.1% was determined by *P. robiniae*, and the rest of the species contributed a percentage of parasitism between 0.1% and 0.4% of the total. The parasitic galls have been reported since the end of May, with a maximum between August and October, which was on average about 11% in 2007 and 15% in 2008. In some sites, a parasite of over 30% was also reported. *Chrysocharis* sp. is a new species for European wildlife, possibly introduced from North America. In general, *Chrysocharis* species are parasitoids of lepidopterans and dipterans (Yefremova and Erlebach, 2001). Ectoparasitoid *Torymus* sp. is new to Slovak wildlife. Species in the subfamily Toryminae mostly attack insects that form galls (Grissell, 1995).

From the category of predators, the species *Tettigonia viridissima* L. (Orthoptera: Tettigoniidae) is reported, contributing to a slight reduction of *O. robiniae* larvae from May to June, when the parasitoid species have a lower intake. In Slovakia, depending on the monitored location, looted galls ranged from about 2% to over 7% (Tóth *et al.*, 2011). In the field, it has been observed that the species attacks the galls on the fallen leaves on the ground, and the attack frequency is higher in rural sites.

Conclusions

Trade in infested biological material from nurseries as well as climate change have led to the spread of the species *O. robiniae* Haldemann in many European countries. The frequency and intensity of the attack on acacia leaves is higher on trees in urban areas where they are affected by other destabilizing factors. The large number of annual generations and the high prolificacy of females determine the rapid increase of the numerical density of the population. The parasitoid entomofauna has an important contribution and the natural control of this species, especially the wasp *P. robiniae* Buhl & Duso.

Authors' Contributions

I.I. and I.O conceived the idea; I.I., I.B.H., T.F., and I.O collected the literature for this review; I.I and I.O wrote the manuscript; I.B.H. prepared the illustrations; T.F., and V.F., reviewed and improved the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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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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