Research Article

Amblycerus robiniae (Fabricius, 1781) (Chrysomelidae: Bruchinae), an alien species established in Europe

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Abstract

After more than 30 years from the first and only record of *Amblycerus robiniae* in Europe (Kecskemét, Hungary), two new established populations are reported. The new records came from Romania, 210–250 km away from the initial record, suggesting the possibility that the species was already established in Hungary and that it spread slowly to the south-east. *Gleditsia triacanthos* Linnaeus, 1753 seed pods were collected in order to detect the species and assess its abundance and frequency. The emerging adults were reared in laboratory conditions to collect information regarding its parasitoids and pest potential. Information regarding rearing, life cycle and biology of the species are also provided.

Key words: allochthonous, Coleoptera, distribution, pest potential, Romania, seed beetles

Introduction

In recent years a significant proliferation of species developing on ornamental plants and crops has been observed. Due to globalization and climate change, numerous species have been accidentally introduced and even become invasive. At the moment there are 42 allochthonous seed beetles species recorded in Europe (Yus-Ramos et al. 2014). Of these, 15 species are considered to be established (i.e. they can complete their life cycles in outdoor conditions), 5 are non-established (i.e. they cannot complete their life cycles in outdoor conditions, so are only found inside storehouses) and 22 are only occasionally recorded (Yus-Ramos et al. 2014). *Amblycerus robiniae* (Fabricius, 1781) is one of the most common bruchines in the eastern half of the United States, generally following the distribution of its main host plants, *Gleditsia triacanthos* L. (the "honey locust") and *Gleditsia aquatica* (Marshall) Raf. (Kingsolver 2004). It was observed in Europe for the first time in 1986 in Hungary (Merkl 2001), reared from infested seeds.

The seeds were provided to the Hungarian entomologist Ottó Merkl by a person working in forestry, with the information that the beetles emerged from seeds of *Gleditsia inermis* (probably *Gleditsia triacanthos* var. *inermis* Willd.) imported from the USA (O. Merkl *personal communication*). No other observations of the species were made until now, the specimens from Hungary being the only evidence of the presence of *Amblycerus robiniae* in Europe; thus, it was considered just an accidental occurrence here. However, its main host plant is an alien and invasive species in Europe (Drake 2009) and therefore it could be more widely spread. Thus, the occurrence of *A. robiniae* in a single location after its emergence from some *Gleditsia* seeds is an intriguing possibility as most probably a big part of the infested seeds were planted. The aim of the present study was to investigate the possibility of establishment of *A. robiniae* in Europe and to provide information regarding its biology and pest potential (i.e. its ability to shift to plants that are outside its native range).

Materials and methods

In a survey started in 2015, seed pods of *Gleditsia triacanthos* were collected from different places in Romania, between 2015 and 2020. The seeds were contained in 10 L plastic containers, kept at room conditions and checked regularly for any emerging adults. After emergence, the adults were transferred in 3 L plastic containers with seeds and a food source, for further observations. We used a slice of apple about 1 cm thick and a 10 ml vial containing a solution 3:1 of water and sugar with a cotton wool plug as adult food source. The vial and the slice of apple were renewed once a week. The larval food source was represented mainly by seeds and seed pods of *Gleditsia triacanthos* but we also tested the development of the species on seeds from other legume plants (Fabaceae): *Albizia julibrissin* Durazz. (Mimosoideae, Ingeae), *Ceratonia siliqua* L. (Caesalpionoideae, Cassieae), *Cercis siliquastrum* L. (Caesalpionoideae, Cercideae), *Cicer arietinum* L. (Faboideae, Cicereae), *Glycine max* (L.) Merr. (Faboideae, Phaseoleae), *Lens culinaris* Medik. (Faboideae, Fabeae), *Phaseolus vulgaris* L. (Faboideae, Phaseoleae), *Robinia pseudoacacia* L. (Faboideae, Robinieae), *Styphnolobium japonicum* (L.) Schott (Faboideae, Sophoreae), *Wisteria sinensis* Sims (Sweet) (Faboideae, Milletieae) and *Wisteria floribunda* (Willd.) DC. (Faboideae, Milletieae).

In order to mount specimens, adults were killed using ethyl acetate or directly in ethanol, and mounted them on glue boards. Identifications were made after Yus-Ramos et al. (2014) and Kingsolver (2004).

The photos of live specimens were taken in the studio using a Canon EOS 6D camera equipped with a Canon MP-E 65mm f/2. 1–5x lens and two studio flashes. The RAW files were edited in Adobe Photoshop.

Voucher specimens are stored in the private collection of the last author and in the collection of Grigore Antipa National Museum of Natural History.

Figure 1. Adult of *Amblycerus robiniae* in dorsal (a), side (b) and front view (c). Photographs by Cosmin Ovidiu Manci.

Results and discussion

Distribution and establishment

The only European record of the species was in Kecskemét (Hungary), individuals of *A. robiniae* were obtained from *Gleditsia triacanthos* var. *inermis* (Merkl 2001). Since then, it has not been recorded again in Europe and for this reason it was not considered an established species (Yus-Ramos et al. 2014).

In Romania, although over 60 samples of honey locust seedpods (*Gleditsia triacanthos*) were analyzed in a previous study during 2015–2017, no individuals of *Amblycerus robiniae* were obtained (Pintilioaie et al. 2018). Misidentification or overlooked specimens can be excluded due to the unique appearance compared with other seed beetles present in Europe (Figure 1). We only detected the species for the first time in Romania on February 2018 from seedpods of *Gleditsia triacanthos* collected in the south-west of Romania, in Răcășdia village (44.989083°N; 21.605531°E). In order to eliminate the uncertainty of the establishment, a year later we collected another set of seedpods from the same locality but from a different location (44.996971°N; 21.633978°E). We managed to reconfirm its presence, confirming the existence of an established population in the area. Additionally, individuals of *A. robiniae* were also obtained from Moravița (45.266145°N; 21.333248°E), around 40 km north-west from the first Romanian record and closer from Kecskemét (the first European record),

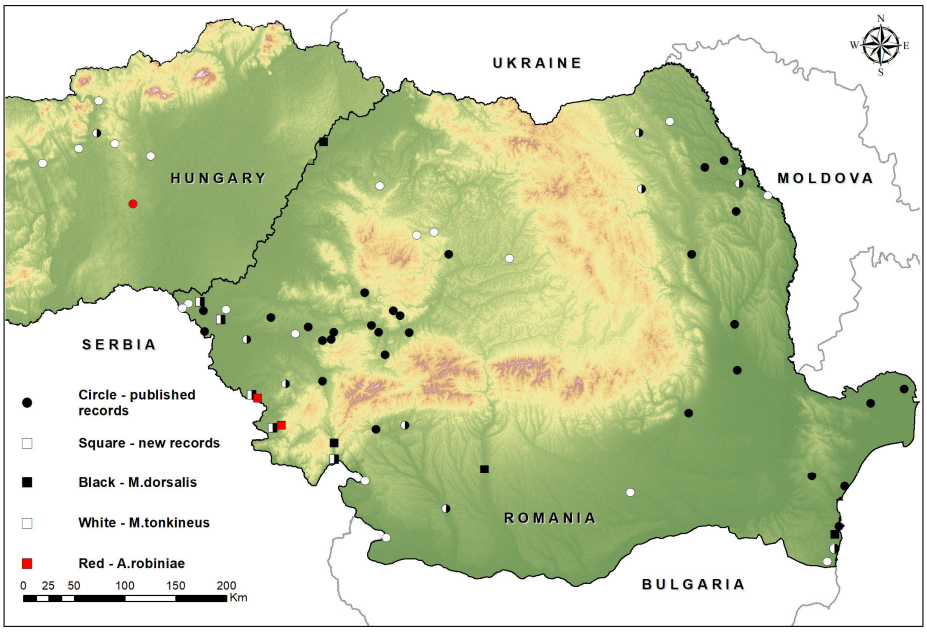

Figure 2. Distribution of *Amblycerus robiniae, Megabruchidius dorsalis* and *M. tonkineus* in Romania and Hungary (after Merkl 2001; György 2007; Bodor 2012; Pintilioaie et al. 2018 and this study).

suggesting the possibility that the species was already established in Hungary in 1986 and it spread slowly to south-east.

The fact that the species was not found between 1986 and 2018 can be explained, in our opinion, by three factors: cryptic behaviour (see biology), small area of distribution and the presence of other seed beetles belonging to the genus *Megabruchidius*. Analysing the Romanian samples from 2015 until now, over 90% had at least one species of *Megabruchidius* and the frequency in samples was as follows: *Megabruchidius dorsalis* (Fåhraeus, 1839) was present in 70% of the samples, *Megabruchidius tonkineus* (Pic, 1904) in 41% of the samples and *Amblycerus robiniae* only in 3% of the samples (Supplementary material Table S1). Additionally, in samples where *Amblycerus robiniae* was detected, its numbers were low comparing with the other species. For example, in Răcăşdia, from 2790 seeds collected in the winter, we obtained 220 individuals of *Megabruchidius* and only 53 of *A. robiniae*. Thus, the availability of larval food is reduced substantially by the prevalence of *Megabruchidius* species which likely reduces the abundance of *A. robiniae*. Despite the low abundance and frequency in samples, we suspect that *A. robiniae* could also be present in other neighbouring countries, in locations with mild winter conditions. For example both Romanian records are under 10 km away from Serbia, but due to the low detectability, it was not yet confirmed there (Figure 2).

Rearing, life cycle and biology

In the laboratory, *Amblycerus robiniae* avoids light during the day, it stands still under the seedpods, in shaded areas or in the holes of the seedpods. At

the slightest vibration, the individuals let go and fall at the bottom of the container and move very fast in an agitated way, until they find a safe place to hide. On natural lighting conditions, we observed adult activity starting from late evening to early morning, in accordance with the observation of Mathwig (1971).

After the emergence from the seedpods, due to the nocturnal behavior we did not observe directly mating or egg laying. However, using the red light for observation during the night, Mathwig (1971) showed that adults are sexually mature and ready to mate as soon as they emerge and oviposition occurs in 1–2 days after mating. In rearing conditions with seedpods, the detected eggs were usually deposited into the exit holes of the already damaged seeds, in areas where the seedpods were damaged by other factors, in areas where the seed pods were folded or underside of the seedpods. When provided seeds removed from the seedpods, the species usually lays the eggs on the ventral side of the seed. In both rearing conditions, the females lay egg clusters in hidden areas and single eggs in exposed areas (on the surface of the seeds or seedpods). Egg laying on artificial substrate was rarely observed.

The incubation period for *A. robiniae* eggs vary from 7 days at 30° C, and increases up to 9 days at 24° C. After the hatching, the larva will usually start burrowing in the seed directly from beneath the membrane that protects the egg, filling the egg chamber with fine frass, but entrance holes made away from the egg location can also be observed. In rearing conditions with seeds, we observed in a few cases larva that used up to three seeds to develop. In some of these cases, the larva builds its pupal chamber by gluing up to 3 seeds with fine frass in such way that all seeds holes communicate. Pfaffenberger (1979) also observed pupal chambers within the pods in several cases, when only small fragments of seed integument remained after larval feeding. By analyzing the pods collected in nature, we found that up to 40% of the larvae of *A. robiniae* will use 2 seeds to complete the development. The larval period can vary greatly, from 35 to 50 days, with larvae developing in green pods taking longer to develop (Mathwig 1971).

In the native range, Mathwig (1971) reported that the seasonal activity has 2 peaks: June–July (when pods are growing rapidly and the seed beetles start ovipositing) and October (adults that emerged in previous generation). Based on emergence data from the seedpods, we assume a similar pattern is also present in Europe, as during the winter we found fresh pods with old and new emergence holes, suggesting two emerging periods.

The emergence of the adults started as soon as 4 days after the seedpods were collected and kept at room temperature, and continued up to 43 days from collection. This suggests that the species overwinters in different stages from ready to emerge adults to pupa and different larval stages.

Parasitoids and predators

In its native range, the following parasitoid species were obtained from seedpods infested by *A. robiniae*: Braconidae: *Heterospilus bruchi* Viereck, 1910 and *Urosigalphus bruchi* Crawford, 1907 (Mathwig 1971); Eurytomidae: *Eurytoma tylodermatis* Ashmead, 1896 (Mathwig 1971; Pérez-Benavides et al. 2019); Eupelmidae: *Eupelmus cyaniceps* Ashmead, 1886 (Mathwig 1971; Pérez-Benavides et al. 2019) and *Eupelmus pulchriceps* (Cameron, 1904) (Pérez-Benavides et al. 2019); Eulophidae: *Horismenus missouriensis* (Ashmead, 1888) (Mathwig 1971; Hansson et al. 2004; Pérez-Benavides et al. 2019). In the case of *H. missouriensis*, Mathwig (1971) mentions that this species is in fact a hyperparasitoid of *H. bruchi,* but Hansson et al. (2004) and Pérez-Benavides et al. (2019) mention it as a primary parasitoid of *A. robiniae*. However, the authors of the papers from 2004 and 2019 cited only the paper of Peck (1951) regarding the parasitism of this eulophid wasp on *A. robiniae*, while Mathwig (1971) observed frequently black pupae of *Horismenus* within white cocoons of *H. bruchi*. Hence, we consider this host-parasitoid association questionable. All the mentioned species do not occur so far in Europe (Mathwig 1971; Hansson et al. 2004; Pérez-Benavides et al. 2019). In Romania, we obtained the following parasitoids from the pods infested with *A. robiniae*: Pteromalidae: *Dinarmus acutus* (Thomson, 1878); Eupelmidae: *Eupelmus confusus* Al Khatib, 2015. Both parasitoid species were previously recorded developing on other alien seed beetles in Romania (Pintilioaie et al. 2018). *Eupelmus confusus* is a generalist parasitoid species (Al Khatib et al. 2014, 2016) that is using as hosts also Bruchinae larvae (Gibson and Fusu 2016) while *Dinarmus acutus* is a primary parasitoid of Bruchinae (Andriescu and Mitroiu 2004). Although both species emerged from seeds infested with *A. robiniae,* it is not clear if the parasitoids developed on this species or on one of *Megabruchidius* species, as from the same pods *Megabruchidius dorsalis* and *M. tonkineus* were also reared.

In the native range, direct predation of *A. robiniae* by *Dryobates pubescens* (Linnaeus, 1766) was observed (Leatherman 2016). In Romania, *Dendrocopos syriacus* (Hemprich & Ehrenberg, 1833) (Lou Bertalan *personal obs.*), *Dendrocopos major* (Linnaeus, 1758), *Parus major* Linnaeus, 1758 and *Garrulus glandarius* (Linnaeus, 1758) (Daróczi J. Szilárd *personal obs.*) were observed foraging in *Gleditsia triacanthos* trees and even looking for insects inside the seedpods; thus, we consider those species potential predators of the seed beetle. Also, indirect predation by other seed eating animals is probably pretty common, both in native range and in the new areal.

Pest potential

Bruchids are known to infest seeds in the field and continue to multiply during storage, often becoming pests especially in cultures of Fabaceae.

Although *Amblycerus robiniae* larva develops on an arboreal species, its pest potential should not be neglected. In laboratory conditions, *A. robiniae* only developed on *Gleditsia triacanthos.* We tested the development of the species on seeds of *Albizia julibrissin*, *Ceratonia siliqua, Cercis siliquastrum, Cicer arietinum, Glycine max, Lens culinaris, Phaseolus vulgaris, Robinia pseudoacacia*, *Styphnolobium japonicum*, *Wisteria sinensis* and *Wisteria floribunda*. In all this cases, *A. robiniae* laid eggs on the seeds, the larvae penetrated the seed coat and then died, which shows that the species has a very specialized diet. A similar result was also obtained by Mathwig (1971) on *Gymnocladus dioica* (L.) K.Koch. (Caesalpionoideae, Caesalpinieae).

Due to its very specialized diet and the low number of laid eggs per female, we consider that the species does not represent a threat for cultivated legumes. However, switching to other host plant species in the future cannot be completely excluded. For example, *Megabruchidius dorsalis* recently expanded its host range to *Gymnocladus dioica* and *Megabruchidius tonkineus* was already reared from the same plant species in the past (György and Tuda 2020). Thus, we cannot exclude the scenario that with time *Amblycerus robiniae* will be able to develop in the seeds of other species within Caesalpinioideae or Fabaceae.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Geo-referenced record data of the distribution of *Amblycerus robiniae*, *Megabruchidius dorsalis* and *M. tonkineus* in Romania and Hungary.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Radac_etal_SupplementaryMaterial.xlsx