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EGG PARASITOIDS OF *HALYOMORPHA HALYS* AND OTHER STINK BUGS (HEMIPTERA: PENTATOMIDAE) ALONG WITH THEIR ASSOCIATED PLANT SPECIES IN AN URBAN ENVIRONMENT

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Abstract

Stink bugs (Hemiptera: Pentatomidae) occur in both agricultural and urban environments in Serbia. Among them, the invasive brown marmorated stink bug (*Halyomorpha halys* Stål, 1855) is of particular importance due to its considerable economic impact. This study investigated the occurrence and species composition of egg parasitoids associated with *H. halys* and other stink bugs, as well as their host plant species, in urban parks of Novi Sad, Serbia. During the 2024 growing season, a total of 319 egg masses were collected, of which 69.3% were parasitized. Between 2022 and 2024, 12 parasitoid wasp species (Hymenoptera) were recorded, with *Trissolcus japonicus* (Ashmead) and *Trissolcus mitsukurii* (Ashmead) being the dominant species. Parasitized egg masses were found on plants belonging to 10 families, with Fabaceae representing the most common host family. These findings highlight the important role of egg parasitoids in the natural regulation of stink bug populations in urban ecosystems.

KEYWORDS: Pentatomidae, egg masses, parasitoids, urban parks, host plants, Fabaceae

Introduction

The brown marmorated stink bug (*Halyomorpha halys* (Stål, 1855)) is one of the most important invasive and highly polyphagous species in the family Pentatomidae. Native to East Asia (Hoebeke & Carter, 2003), it has rapidly expanded its range over the past decades, establishing populations throughout the United States and Europe. In Serbia, its presence was first recorded in 2015 near Vršac (Šeat, 2015).

Owing to its broad host range, *H. halys* readily adapts to diverse environmental conditions and is considered a major pest in both agricultural production and urban ecosystems, where suitable resources for feeding and oviposition are widely available (Gariepy *et al.*, 2014). The species has been recorded on more than 300

plant species, with oviposition site selection influenced by host plant availability and microclimatic conditions (Nielsen *et al.*, 2009). Its rapid spread into new areas has been facilitated primarily by international trade, which enables passive transport, as well as by increasing human mobility and tourism (Lee *et al.*, 2013).

Egg masses of stink bugs (Pentatomidae) are attacked by a range of natural enemies, primarily parasitoids, which contribute to the natural regulation of their populations. Monitoring of parasitoid communities is particularly important in the context of the biological control of *H. halys*, as it allows the assessment of their impact on pest populations and the identification of species with potential for effective natural suppression. The principal parasitoids of *H. halys* belong to the families Scelionidae (*Trissolcus* spp., *Telenomus* spp.), Eupelmidae (*Anastatus* spp.), and Encyrtidae (*Ooencyrtus* spp.) (Haye *et al.*, 2015; Dieckhoff *et al.*, 2017; Costi *et al.*, 2019). Among them, *Trissolcus japonicus* (Ashmead) and *Tr. mitsukurii* (Ashmead) are considered the most effective parasitoids of *H. halys* eggs (Yang *et al.*, 2009; Bergmann *et al.*, 2016; Zhang *et al.*, 2017). The presence of *Tr. japonicus* in Europe was first recorded in 2017 (Stahl *et al.*, 2019), while *Tr. mitsukurii* was first detected in Italy in 2016 and subsequently in France (Scaccini *et al.*, 2020; Bout *et al.*, 2021). In the urban environment of Novi Sad, the first parasitized egg masses of *H. halys* were recorded in 2022, when both *Tr. japonicus* and *Tr. mitsukurii* were identified for the first time in Serbia (Konjević *et al.*, 2024).

The main aim of this study was to establish a baseline assessment of the spatial distribution of stink bug egg masses, with particular emphasis on the invasive *H. halys*, and their associated egg parasitoids across urban parks in Novi Sad, Serbia, while also identifying the host plant species on which they occur.

Materials and Methods

During three consecutive years (2022-2024), sites with diverse plant species were selected in urban areas of Novi Sad, Serbia (Futoški, Limanski, Železnički, and Novo Naselje parks) to investigate the presence of stink bug egg masses and their parasitoids. Neither the study sites nor the sampled plant species were treated with pesticides, enabling the monitoring of natural interactions between stink bugs and their egg parasitoids.

From May to September of each study year, weekly visual surveys were conducted in the selected parks to collect stink bug eggs from randomly chosen flowering or fruit-bearing trees and shrubs. Surveys were conducted weekly in each park by one or two observers using a standardized visual search method, with a sampling effort of 60 minutes per site. Sampling was performed in the morning, between 08:00 and 12:00. Surveys included examinations of the abaxial leaf surfaces, branches, and trunks following the protocol described by Konjević *et al.* (2024), with particular attention given to egg masses of pentatomid stink bugs, especially *H. halys*.

Egg masses were collected manually and placed separately in sterile containers. Each sample was labeled with the date of collection, site/park, and host plant species. Samples were stored in the entomology laboratory at the Faculty of Agriculture, University of Novi Sad, until nymphs hatched or parasitoid wasps eclosed. Stink bug species were identified based on egg morphological characteristics (Reeve & Haye, 2021) prior to nymph emergence. Collected egg masses were counted and classified as either (1) parasitized or (2) viable, unhatched, or damaged by predators. Following hatching, nymphs were reared to adulthood and identified morphologically (Winiger & Kment, 2010); these eggs were classified as viable. If parasitoid wasps emerged, the eggs were classified as parasitized. Egg masses showing neither nymph nor parasitoid emergence were classified as unhatched. Parasitoids that emerged from the egg masses were identified to species level at the University of Turin (Italy). Data on egg masses and parasitoids were analyzed using descriptive statistics, focusing on the abundance and distribution of parasitized and non-parasitized egg masses across plant families and sampling sites. No inferential statistical analyses were performed, as the

primary objective was to describe the spatial and host plant distribution of stink bug egg masses and provide a basic assessment of parasitoid occurrence in urban parks of Novi Sad.

Results

Results of egg sampling in 2024 revealed a total of 319 *H. halys* egg masses collected, of which 221 (69.3%) were parasitized, while 98 egg masses were classified as viable, unhatched, or damaged (Fig. 1). Only egg masses from which parasitoids emerged were considered parasitized. In addition to parasitized egg masses, those from which stink bug nymphs emerged (viable), those showing no emergence (unhatched), and those damaged by predators (damaged) were also recorded.

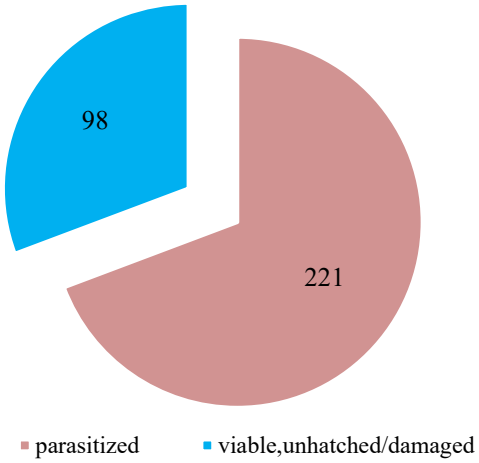

Figure 1. Distribution of parasitized, viable, and unhatched/damaged egg masses in the total number of collected *H. halys* egg masses collected in 2024.

The relatively high proportion of parasitized egg masses (69.3%) indicates a substantial presence and activity of stink bug egg parasitoids in the urban environment of Novi Sad. The predominance of parasitized egg masses suggests that these natural enemies may play an important role in the regulation of *H. halys* populations.

Egg parasitoids of stink bugs (Pentatomidae) have been monitored in Serbia since 2022. The results of this three-year survey are presented in Table I and illustrate the spatial distribution of *H. halys* egg parasitoids in the period 2022-2024. Adult parasitoids that emerged in the lab were identified at the University of Turin based on morphological characteristics. To date, 12 parasitoid wasp species (Hymenoptera) have been recorded in Serbia.

Table I. Records of *H. halys* egg parasitoid species in Serbia during 2022–2024

PARASITOID SPECIES	YEAR 2022	YEAR 2023	YEAR 2024
Anastatus bifasciatus	Novi Sad, Sombor, Bački Vinogradi	+Zaječar	Novi Sad
Ooencyrtus spp.	Sombor		
Telenomus turesis	Ribari, Bački Petrovac	+Sombor	
Trissolcus basalis	Novi Sad, Sombor, Bački Vinogradi, Ribari	+Padina	Novi Sad
Trissolcus belenus	Novi Sad	+Zaječar	
Trissolcus colemanii	Ribari	+Miokus, Mala Remeta	
Trissolcus semistriatus	Srpski Miletić	+Novi Sad	
*Trissolcus japonicus**	Novi Sad*	+Miokus, Bački Petrovac, Ljukovo, Nova Crvenka	+Zaječar, Ribari, Rumenka
*Trissolcus mitsukurii**	Novi Sad*		
Telenomus truncatus		Novi Sad, Starčevo, Boljevac	
Hadronotus leptocorisae		Novi Sad, Ilinci	
*Trissolcus cultratus**			Novi Sad*

* - First record in Serbia; + – additional locality record relative to the previous year

During 2022, a total of nine egg parasitoid species associated with the brown marmorated stink bug were recorded. The most frequently detected species were *Trissolcus basalis* and *Anastatus bifasciatus*, predominantly at localities in Vojvodina (Novi Sad, Sombor, Bački Vinogradi, and Ribari). The remaining species were recorded only sporadically and had a more restricted distribution, including *Tr. japonicus*, *Tr. semistriatus*, *Tr. colemanii*, *Tr. belenus*, *Ooencyrtus spp*., *Tr. mitsukurii*, and *Telenomus turesis*. Of particular importance is the first record of two non-native species, *Tr. japonicus* and *Tr. mitsukurii*, in 2022 in Novi Sad and Serbia (Konjević *et al.*, 2024). In contrast, all other recorded parasitoid species are native to the European fauna.

In 2023, an increase in the number of localities with recorded parasitoids was observed, along with the occurrence of two additional species, *Telenomus truncatus* and *Hadronotus leptocorisae*. Particularly noteworthy was the spread of *Tr. japonicus*, which was recorded in several new localities (Miokus, Bački Petrovac, Ljukovo, and Nova Crvenka), indicating its successful establishment, expansion, and adaptation to local environmental conditions.

In 2024, *Trissolcus cultratus* was recorded for the first time in Serbia (Novi Sad), representing a significant addition to the known egg parasitoid fauna associated with *H. halys*. Further expansion of *Tr. japonicus* was also confirmed beyond the Vojvodina region (Zaječar), suggesting a potentially important role in the natural regulation of *H. halys* populations across a broader geographical area of Serbia.

The increasing species richness and number of recorded localities during the study period suggest that *H. halys* egg parasitoid communities in Serbia are undergoing dynamic development. The establishment and spread of species, particularly *Tr. japonicus* and *Tr. mitsukurii*, indicate a growing potential for the natural biological control of this invasive pest (Konjević *et al.*, 2024; Stahl *et al.*, 2019). However, further studies are needed to assess their abundance, seasonal dynamics, and actual impact on *H. halys* populations.

Table II presents the parasitoid species recorded from the egg masses of different pentatomid stink bugs. The results indicate a high diversity of parasitoids associated with stink bug egg masses, with most recorded species belonging to the genus *Trissolcus*, a group recognized as one of the most important egg parasitoids of Pentatomidae (Tillman, 2016; Stahl *et al.*, 2019).

Table II. Parasitoid species recorded from the egg masses of stink bugs during the three-year study

PARASITOID SPECIES	STINK BUG SPECIES
Anastatus bifasciatus	*Halyomorpha halys, Nezara viridula, Rhaphigaster nebulosa, Eurydema ventralis*
Ooencyrtus spp.	*Halyomorpha halys, Nezara viridula, Podisus maculiventris*
Telenomus turesis	*Halyomorpha halys, Nezara viridula*
Trissolcus basalis	*Halyomorpha halys, Nezara viridula*
Trissolcus belenus	*Podisus maculiventris, Palomena prasina, Halyomorpha halys*
Trissolcus colemanii	*Halyomorpha halys, Nezara viridula*
Trissolcus semistriatus	*Nezara viridula, Peribalus strictus*
Trissolcus japonicus	*Halyomorpha halys, Nezara viridula, Palomena prasina*
Trissolcus mitsukurii	*Halyomorpha halys*
Telenomus truncatus	*Halyomorpha halys, Palomena prasina, Rhaphigaster nebulosa*
Hadronotus leptocorisae	*Halyomorpha halys*
Trissolcus cultratus	*Halyomorpha halys*

The highest parasitoid diversity was recorded in egg masses of the invasive species *H. halys*, indicating that it serves as an important host for a wide range of parasitoid species. Parasitoids were also found in the egg masses of other stink bug species, including *Nezara viridula* and *Palomena prasina*, suggesting that some parasitoid species have a relatively broad host range.

The distribution of viable and parasitized *H. halys* egg masses across plant families revealed differences in parasitism levels among host plants (Fig. 2). Parasitized egg masses were found on plants belonging to 10 families. The highest number was found on Fabaceae, with 124 egg masses (38.87%), highlighting the importance of this plant family as a key host for both *H. halys* and its egg parasitoid.

A relatively high number of parasitized egg masses was also recorded on plants from the families Sapindaceae (34 egg masses; 10.66%), Oleaceae (25; 7.83%), and Bignoniaceae (17; 5.33%), suggesting that these plant families are frequently used for both *H. halys* oviposition and parasitoid activity. In contrast, plants from the families Rosaceae, Malvaceae, Cannabaceae, Fagaceae, Betulaceae, and Magnoliaceae hosted fewer egg masses and exhibited lower levels of parasitism, which may reflect less frequent use of these plants by *H. halys* or reduced accessibility of egg masses to parasitoids. These results are consistent with studies from Switzerland and Slovenia (Haye *et al.*, 2014; Rot *et al.*, 2021), which reported *H. halys* on host plants from the genus *Prunus* (Rosaceae), the genus *Acer* (Sapindaceae), and species belonging to the families Oleaceae, Bignoniaceae, and Malvaceae. The observed patterns suggest that vegetation structure and host plant selection play important roles in the population dynamics of *H. halys* and the effectiveness of its natural regulation by egg parasitoids (Rice *et al.*, 2014; Tillman, 2016).

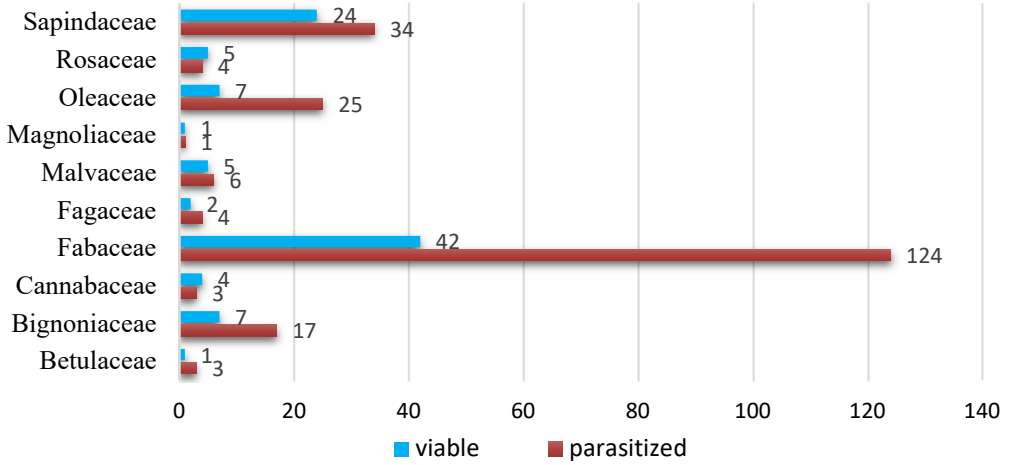

Figure 2. Number of viable and parasitized *H. halys* egg masses on different plant families in 2024

Among host plants from the Fabaceae family, the most frequently used species were *Styphnolobium japonicum*, *Cercis siliquastrum*, *C. canadensis*, and *Robinia pseudoacacia* (Lee *et al.*, 2013). The preference of *Halyomorpha halys* for Fabaceae observed in this study is consistent with previous reports and highlights the importance of these plants for the development and persistence of its populations (Owens *et al.*, 2013). *S. japonicum* was the most important host plant, with 162 recorded egg masses, of which 123 were parasitized. Plant species hosting larger numbers of egg masses, particularly those belonging to Fabaceae and Sapindaceae, also exhibited higher numbers of parasitized egg masses, suggesting a positive relationship between *H. halys* abundance and parasitoid activity (Nielsen *et al.*, 2009; Gariepy *et al.*, 2014; Costi *et al.*, 2019).

Conclusion

Research conducted in the urban parks of Novi Sad in 2024 revealed a high level of parasitism of *H. halys* egg masses, suggesting that egg parasitoids contribute to the natural mortality of this invasive species in urban habitats. The relatively high diversity of egg parasitoids recorded during the three-year study, dominated by representatives of the genus *Trissolcus*, indicates that stable interactions between stink bugs and their natural enemies have become established in the urban environments of Novi Sad and, potentially, more broadly across Serbia.

The presence of parasitoids, including the non-native species *Tr. japonicus* and *Tr. mitsukurii*, confirms their successful establishment and spread in urban environments and suggests a potential role in the natural suppression of *H. halys* populations. Several parasitoid species were also found in the egg masses of other stink bugs, such as *Nezara viridula* and *Palomena prasina*, indicating a broader host range in urban areas of Serbia and further highlighting their ecological significance.

Host plants belonging to the Fabaceae, particularly *Styphnolobium japonicum*, appear to play an important role in the oviposition and development of the brown marmorated stink bug and its egg parasitoids. These findings highlight the importance of urban green areas as habitats that support interactions between stink bugs and their natural enemies. At the same time, the presence of several plant species, particularly *Styphnolobium japonicum*, *Cercis siliquastrum*, *C. canadensis*, and *Robinia pseudoacacia*, in areas adjacent to agricultural landscapes may facilitate the persistence and spread of stink bug populations, indicating a need for an integrated approach to the management of urban and agricultural ecosystems. The results of this study provide a foundation for further research and may contribute to the development of biological control strategies for *H. halys* in Serbia.

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ПАРАЗИТОИДИ ЈАЈА ВРСТЕ *HALYOMORPHA HALYS* И ДРУГИХ СТЕНИЦА (HEMIPTERA: PENTATOMIDAE) И БИЉНЕ ВРСТЕ НА КОЈИМА СУ НАЛАЖЕНИ У УРБАНОЈ СРЕДИНИ

Милана Човић, Војин Пузић и Александра Коњевић

Извод

Стенице из породице Pentatomidae присутне су и у агроекосистемима и урбаним срединама Србије. Међу њима, инвазивна браон мрамораста стеница (*Halyomorpha halys* Stål, 1855) је посебно значајна као економски важна штетна врста. Циљ овог истраживања био је да се утврди појава и састав јајних паразитоида повезаних са овом врстом, као и да се идентификују врсте биљака домаћина у градским парковима Новог Сада (Србија). Током вегетационе сезоне 2024. године прикупљено је укупно 319 јајних легала, од којих је 69,3% било паразитирано. Од 2022. до 2024. године забележено је присуство 12 врста паразитских оса (Hymenoptera), међу којима се *Trissolcus japonicus* (Ashmead) и *Trissolcus mitsukurii* (Ashmead) издвајају као најзначајније. Паразитирана јајна легла регистрована су на биљкама из 10 различитих биљних фамилија, при чему се фамилија Fabaceae истиче као доминантан домаћин. Добијени резултати указују на важну улогу јајних паразитоида у природној регулацији популација стеница унутар урбаних екосистема.

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