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PARASITOIDS OF THE INVASIVE *HALYOMORPHA HALYS* (*HETEROPTERA*: *PENTATOMIDAE*) IN BULGARIA AND THE RATE OF PARASITISM AT FIELD CONDITIONS

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Abstract

The Brown marmorated stink bug (*Halyomorpha halys*) is an invasive species in Bulgaria, first reported in 2016 and since then mass spread all over the country, causing serious damage to many agricultural crops and ornamentals. The aim of this study was to establish which of the native parasitoid species would attack the eggs of *H. halys* and to evaluate the rate of parasitism under field conditions. The egg masses of *H. halys* were collected from several agricultural crops in the region of Plovdiv in surveys during the summer months and reared under laboratory conditions until hatching at the Insectary of the Faculty of Plant Protection and Agroecology, theAgricultural University-Plovdiv. In 2021, egg parasitoids from five species emerged from the collected eggs and were identified as *Trissolcus cultratus*, *Trissolcus basalis*, *Anastatus bifasciatus*, *Ooencyrtus telenomicida*, and *Ooencyrtus sp*. The rate of parasitism in 2021 was 10.57% in June, 21.17% in July and 17.57% August.

Keywords: *Halyomorpha halys*, egg parasitoids, *Trissolcus cultratus*, *Trissolcus basalis*, *Anastatus bifasciatus*, *Ooencyrtus telenomicida*

INTRODUCTION

Invasive insect species are one of the great challenges to sustainability in agriculture, posing a threat to plant production. Direct damage from feeding on crops leads to significant economic losses and disrupts ecosystem functions. Additional indirect effects on human health, water, and the environment may occur after attempts to reduce the density of invasive species with intensive pesticide use. The brown marmorated stink bug *H. halys* is native to East Asia - Japan, Korea, China and Taiwan (Chu and Lu, 1982; Hoebeke and Carter, 2003). It was reported for the first time in Europe in 2004 (in Luxembourg), and in 2007 it was reported in Switzerland (Wermelinger et al., 2008). Finding suitable conditions for development, including the presence of sufficient host plants, *H. halys* reproduces rapidly in new habitats. As a result, the species occupied new territories and within a few years became an economically important pest. In 2012, Maistrello et al. (2014) reported it for the province of Modena, Italy. In 2013, Vetek et al. (2014) reported the occurrence of *H. halys* in Budapest. In the countries neighbouring Bulgaria, the species was found in Greece in 2012 (Milonas and Partsinevelos, 2014), in Serbia in 2015 (Šeat, 2015), and in the same year in Romania (Macavei et al., 2015). The first record from Bulgaria is from 2016, when Simov (2016) found 3 nymphs of *H. halys* in the center of Sofia.

H. halys is polyphagous and attacks more than 120 plant species (Haye et al., 2015; Bergmann et al., 2016). In the region of origin - Japan, Kobayashi (1967) reported various fruit and legume host species: peach (*Prunus persica*), cherry (*Prunus avium*), apple (*Malus domestica*), blue plum (*Prunus subg. Prunus*), fig (*Ficus carica*), persimmon (*Diospyros kaki*),

orange (*Citrus X sinensis*), vine (*Vitis vinifera*), mulberry (*Morus alba*), soybean (*Glycine max*) and burdock (*Arctium lappa*). Damage is caused by nymphs and adults sucking sap from fruits, pods, buds and stems of host plants (Kuhar et al., 2012). In apples, damage is expressed by the formation of brown crusted tissue under the skin of the fruit (Day et al., 2009). Damage is the greatest at the end of the season, before fruit harvest (Nielsen & Hamilton, 2009). As a result of sucking juice, dark green spots appear on the fruits of green varieties of apples, on red fruits - dark red spots (Brown, 2003).

A growing number of studies focus on the natural enemies of the pest and the possibilities of its biological control. In China, Yang et al. (2009) described *Trissolcus halyomorphae* as an excellent bioagent to control *H. halys*, which is the predominant species and among the 6 egg parasitoids found in the Beijing area by Lan-Fen (2010): *Trissolcus* halyomorphae, *Trissolcus* sp., *Telenomus* sp., *Acroclisoides* sp., *Anastatus* sp. and *Ooencyrtus* sp. The degree of parasitism by *Trissolcus halyomorphae* varies between 20 and 70%. In the USA and Europe, the main species of parasitoids on *H. halys* eggs belong to the *Scelionidae*, *Eupelmidae* and *Encyrtidae* families (Abram et al., 2017). In 2012-2013, in the USA (Maryland), the impact of local populations of *Anastatus reduvii, A. pearsalli, A. mirabilis, Trissolcus brochymenae, T. euschisti, Telenomus podisi* and *Ooencyrtus* sp., were assessed as potential bioagents to control *H. halys*. 61.17% of the total parasitism was due to *An. reduvii*, (Jones, 2013). In Maryland Cornelius et al. (2016) also found *Telenomus podisi, Anastatus reduvii, Trissolcus brochymenae, Gryon obesum, Trissolcus hullensis*, and *Gryon stewarti* and later (Cornelius et al., 2016a) added several more local parasitoid species: *Trissolcus euschisti, Trissolcus edesae, Ooencyrtus johnsoni* and one non-indigenous species - *Trissolcus japonicus*. In northern Delaware, the parasitism rate of *H. halys* eggs by *Trissolcus japonicus* exceeded 50%. (Hedstrom et al., 2017). Balusu et al. (2019) found *Trissolcus basalis* (*Hymenoptera*: *Scelionidae*) introduced in the United States parasitizing *Halyomorpha halys* (*Hemiptera*: *Pentatomidae*).

Parasitoids indigenous to Europe are also the subject of intensive research. In Switzerland, Haye et al. (2015) found that *Trissolcuss semistriatus* and *T. chloropus* parasitized on brown marmorated stink bug eggs, but failed to develop offspring, while at low numbers *T. cultratus* and *T. scutellaris* succeeded in developing and parasitizing 1-2% of the eggs. *Anastatus bifasciatus* developed successfully and the rate of parasitism was 29-34%. In a laboratory study, Federico et al. (2017) found that the parasitization rate of *O. telenomicida* was 35.56%, while that of *A. bifasciatus* was 20.36%. *Telenomus chloropus* was also tested as a potential bioagent of *H. halys*, but a low parasitism rate of 5.91% was reported. *O. pityocampae* succeeded in parasitizing 20.88% of the eggs, but parasitoids failed to develop from the parasitized eggs. Tunca et al. (2020) found that *Ooencyrtus kuvanae* can parasitize *H. halys* eggs and complete its development on this host. The duration of development of *Ooencyrtus kuvanae* in the eggs of *H. halys* is 18-19 days.

The current study aimed to establish the egg parasitoids of *Halyomorpha halys* in the region of Plovdiv and to assess the rate of naturally occurring egg parasitism.

MATERIALS AND METHODS

Surveys to detect parasitism on the eggs of *H. halys* were carried out in the region of Plovdiv in 2019, 2020 and 2021. The egg clusters of the brown marmorated stink bug were collected from the end of May to the beginning of September from different plants, and transported in plastic containers with the leaves on which they had been found to the Insectary of the Agricultural University – Plovdiv (at a constant temperature of 25±2 ºC,

RH 60-70% and a photoperiod of 16:8 hours L:D). On the day of the collection, the eggs with the plant part on which they had been laid were placed in Petri dishes and the number of eggs in each egg cluster was recorded. Paper was placed on the bottom of each dish filter and the leaf petiole was wrapped in wet cotton and parafilm for maintaining the moisture. Observations for egg hatching or emergence of adult parasitoids were made on a daily basis. The rate of parasitism was calculated based on the total number of eggs collected. The emerged parasitoids were stored in 96% ethanol until the identification done by Dr. Ovidiu Alin Popovici and Dr. Lucian Fusu from the University Alexandru Ioan Cuza, Iași, Romania.

RESULTS AND DISCUSSION

Although the surveys began in 2019, not a single parasitized egg of the brown marmorated stink bug was found in the first two years. In 2021, however, we found a total of 5 species of egg parasitoids on the brown marmorated stink bug from the Scelionidae, Eupelmidae and Encyrtidae families (Table 1).

Table 1. Species composition of the egg parasitoids of *Halyomorpha halys* in the region of Plovdiv

Species	Family	Order
Trissolcus basalis (Wollastone, 1858)	*Scelionidae*	*Hymenoptera*
Trissolcus cultratus (Mayr, 1879)	*Scelionidae*	*Hymenoptera*
Anastatus bifasciatus (Geoffroy, 1785)	*Eupelmidae*	*Hymenoptera*
Ooencyrtus telenomicidae (Vassiliev, 1904)	*Encyrtidae*	*Hymenoptera*
Ooencyrtus sp. (Ashmead, 1900)	*Encyrtidae*	*Hymenoptera*

None of the established species have been reported on the eggs of the brown marmorated stink bug as a host in Bulgaria. A species of the genus *Ooencyrtus* was reported by Boyadzhiev et al. (2017) parasitizing the eggs of the processionary moth *Thaumetopoea solitaria* (*Lepidoptera*: *Notodontidae*), but as it was not identified to species level, we are not able to confirm whether it was the same species as the unidentified one in our study.

The first parasitized eggs were found on mulberry trees in the city of Stamboliyski in 2021 (Fig. 1). Under laboratory conditions, parasitoids emerged from the collected eggs, and were identified as *Anastatus bifasciatus* (Geoffroy) (Fig. 2) According to Haye et al. (2015) *Anastatus bifasciatus* is a valuable potential candidate for biological control as it is the only European species that successfully develops in the eggs of the brown marmorated stink bug.

Four species of egg parasitoids - *Trissolcus cultratus* (Mayr) (Fig. 89), *Trissolcus basalis* (Wollaston), *Ooencyrtus telenomicida* and *Ooencyrtus* sp. were isolated from parasitized eggs collected in tomato and potato plantations in the region of the city of Plovdiv.

As a result of the present study, it was found that in addition to *N. viridula*, *Trissolcus basalis* also parasitizes the eggs of *H. halys*. Around the same time, Balusu et al. (2019), who first reported *Trissolcus basalis* as an egg parasitoid of the brown marmorated stink bug, also found it from the eggs of *N. viridula*.

Ooencyrtus telenomicidae and *Anastatus bifasciatus* are polyphagous and parasitize the eggs of many species of the order Hemiptera (Rondoni et al., 2017). In our observations, the parasitoid *Anastatus bifasciatus* predominates on the eggs of the brown marmorated stink bug laid on plants in urban environments (*Morus alba* and *Hibiscus syriacus*), while *Ooencyrtus telenomicidae* is mostly isolated from eggs laid on agricultural crops (*Lycopersicon esculentum*). During our observations, a study by Rot et al. (2021) was published The researchers reported the same preference of *Anastatus bifasciatus* towards

urban and suburban regions. The egg parasitism rates in 2021 were relatively low, ranging from 10.57% reported in June to 21.17% in July (Fig. 8). The predominant parasitoid species is *Ooencyrtus telenomicidae*, followed by *Trissolcus cultratus*, *Trissolcus basalis* and *Anastatus bifasciatus*.

a

b

Figure 1. Parasitized eggs of *H. halys* (a) and exit holes of an egg parasitoid (b).

Figure 2. Adult of the egg parasitoid *Anastatus bifasciatus* (Geoffroy, 1785).

Figure 3. Adults of the egg parasitoid *Trissolcus basalis* (Wollastone, 1858).

a

b

Figure 4. Adult of *Ooencyrtus telenomicida* (Vassiliev, 1904): female (a) and male (b).

Figure 5. Adult of *Trissolcus cultratus* (Mayr, 1879).

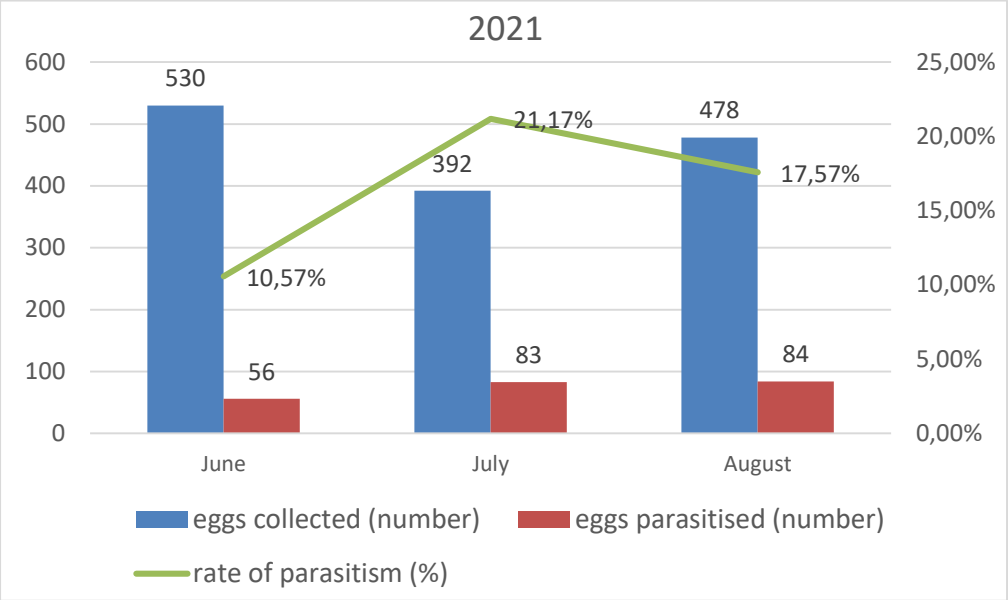

Figure 6. The rate of naturally occurring parasitism on the eggs of *H. halys* in Plovdiv's region in 2021.

CONCLUSION

In a survey for naturally occurring parasitism on the eggs of the Brown marmorated stink bug carried out in Plovdiv's region, in 2021 a total of 5 species of egg parasitoids were identified: *Trissolcus cultratus* and *Trissolcus basalis* from the *Scelionidae* family, *Anastatus bifasciatus* from *Eupelmidae* family, *Ooencyrtus telenomicida* and *Ooencyrtus* sp. from *Encytidae* family. The rate of parasitism at field conditions in 2021 varied from 10.57 % in June to 21.17% in July. As no parasitized eggs were detected in 2019 and 2020, we believe that, as a result of the adaptation to this new host, parasitoid species parasitization rates from natural populations will gradually increase in the coming years.

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