

Parasitoids of the invasive *Nezara viridula* (Linnaeus) in Bulgaria

Паразитоиди по инвазивната *Nezara viridula* (Linnaeus) в България

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ABSTRACT

The southern green stink bug *Nezara viridula* (Linnaeus) is a polyphagous species that causes economic damages to many crops in Bulgaria. The aim of this study was to identify the parasitoid species that could suppress the populations of *Nezara viridula*. A lot of agricultural crops were inspected in the region of Plovdiv, Bulgaria, for the presence of egg parasitoids and imaginal parasitoids of the pest. Egg masses and adults of *Nezara viridula* were collected during the field surveys in 2019-2021 in Southern Bulgaria. The only parasitoid species emerging from adults of the green stink bug was *Trichopoda pennipes* (Tachinidae), which is reported for the first time in Bulgaria. The rate of parasitism at field conditions ranged from 2.83% to 35% in the different generations of the host. The egg parasitoids were from family Scelionidae – *Trissolcus basalis* (Wollaston), Eupelmidae – *Anastatus bifasciatus* (Geoffroy), and Encyrtidae – *Ooencyrtus telenomicida* (Vassiliev). It was found that at field conditions the rate of parasitism of the eggs in 2020 and 2021 was respectively 7.77% and 0.25% in May, 34.32% and 17.23% in June, 72.3% and 80.51% in July, and in August – reaching 100% and 89.79%.

Keywords: *Nezara viridula*, parasitoids, *Trichopoda pennipes*, *Trissolcus basalis*, *Anastatus bifasciatus*, *Ooencyrtus telenomicida*

АБСТРАКТ

Южната зелена дървеница *Nezara viridula* (Linnaeus) е многояден вид, който нанася икономически щети на много култури в България. Целта на това проучване е да се идентифицират видовете паразитоиди, които биха могли да потиснат популациите на *Nezara viridula*. Наблюдавани са много земеделски култури в района на Пловдив, България, за наличие на яйчни и имагинални паразитоиди по неприятеля. По време на теренните проучвания през 2019-2021 г. в Южна България са събрани яйца и възрастни на *Nezara viridula*. Единственият паразитоид, открит във възрастните на зелената миризлива дървеница, е *Trichopoda pennipes* (Tachinidae), който вид се съобщава за първи път в България. Степента на паразитиране при полеви условия варира от 2.83% до 35% при различните поколения на гостоприемника. Паразитоидите по яйцата са от семейство Scelionidae – *Trissolcus basalis* (Wollaston), Eupelmidae – *Anastatus bifasciatus* (Geoffroy) и Encyrtidae – *Ooencyrtus telenomicida* (Vassiliev). Установено е, че при полеви условия степента на паразитиране на яйцата през 2020 г. и 2021 г. е съответно 7.77% и 0.25% през май, 34.32% и 17.23% през юни, 72.3% и 80.51% през юли, и през август – достигайки 100% и 89.79%.

Ключови думи: *Nezara viridula*, паразитоиди, *Trichopoda pennipes*, *Trissolcus basalis*, *Anastatus bifasciatus*, *Ooencyrtus telenomicida*

INTRODUCTION

The southern green stink bug *Nezara viridula* (Linnaeus) is one of the most important pests from family Pentatomidae (Todd, 1989). Adults and nymphs cause significant damages on many agricultural crops worldwide. *N. viridula* may attack all parts of the plants, but adults and nymphs prefer to feed mostly on fruiting structures (Panizzi and Slansky, 1991). The feeding punctures form small, hard and brownish spots on the fruits. Feeding damages also can cause malformations (Johnson et al., 1985), premature drop of the fruits, affect the fruit qualities and decrease the market value of the fruits.

Different methods are used to control *N. viridula* including chemical control, Integrated Pest Management (IPM) programs and natural enemies. Several parasitoids from Hymenoptera and Diptera are known to provide biological control of *Nezara viridula*. Waterhouse (1998) described the majority of egg parasitoids including *Trissolcus basalis* (Hymenoptera) and imaginal and nymphal parasitoids of *Nezara viridula*, such as *Trichopoda pennipes* (Diptera). Ehler (2002) made an evaluation of some natural enemies of *N. viridula* in northern California during 1999 and 2000 and concluded that among the parasitoids *Trissolcus basalis* was the dominant species. The rate of parasitism ranged in 1999 from 0~40% and in 2000 – from 0~50%. In a study in Egypt (Draz et al., 2006) the rate of parasitism by *Trissolcus basalis* on *N. viridula* eggs reached 77.31%. *Ooencyrtus* spp. were also reported as egg parasitoids of *N. viridula* (Jones, 1988), but their role in reducing the population density of *N. viridula* was insignificant.

A frequently reported parasitoid on the adult and the late-instar nymph of *N. viridula* is the tachinid parasitoid *Trichopoda pennipes* (Fabricius) (Diptera:Tachinidae) which is native to the Nearctic region (Jones 1988). According to Buschman and Whitcomb (1980), McPherson et al. (1982), Temerak and Whitcomb (1984) *T. pennipes* is one of the most successful natural enemies of *Nezara viridula*. The parasitism rate of *N. viridula* adults by *T. pennipes* affects female (44.9%) and male (36.4%) reducing their life span

(De Salles, 1992). In Hawaii, a 100% rate of parasitism of *N. viridula* has been observed (Davis 1964). Salerno et al. (2002) reported in Italy a rate of 24% parasitized *Nezara viridula* adults. In Switzerland, Petremand et al. (2015) described parasitism by *T. pennipes* on *N. viridula*. After *T. pennipes* was accidentally introduced into Italy (Colazza et al. 1996, Salerno et al., 2002), it was reported from several European countries, including Slovenia (Groot et al., 2007) and Greece (Kazilas et al., 2020). In 2019 in the region of Plovdiv *T. pennipes* was quite common, which makes us believe that it has been introduced years ago.

So far, there are no reports on parasitoids of *N. viridula* in Bulgaria. The aim of the present study was to find parasitoids, attacking different stages of *N. viridula*, which could contribute to the control of the pest's populations in Bulgaria.

MATERIALS AND METHODS

This study was conducted in 2019-2021 in the region of Plovdiv, Bulgaria. Different agricultural crops were surveyed for the presence of eggs and imaginal parasitoids of *Nezara viridula*. The parasitized adults and eggs of *N. viridula* were collected and reared in a laboratory at the Agricultural University - Plovdiv, at constant temperature of 25±2 °C, RH 60-70% and a photoperiod of 16:8 hours L:D.

Collecting and rearing of the parasitized adults of Nezara viridula

From the beginning of June to Mid-September different crops were surveyed in the region of Plovdiv using visual observations. Adults of *N. viridula* were collected from maize (*Zea mays*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*) and Rose of Sharon (*Hibiscus syriacus*) and inspected for the presence of eggs from Tachinid flies. The adults of *N. viridula* with laid eggs from Tachinidae were collected in plastic containers and transferred to the laboratory, where part of them were dissected and the rest were reared in Petri dishes (one adult per dish). Filter paper was placed on the bottom of the dish for absorbing the moisture, and pieces of apple fruit were provided as food for the stink

bug. After the completely developed parasitoid's larvae emerged from the bug between the posterior abdominal segments they were transferred to new Petri dish for pupating and emerging of the adult parasitoids.

Dissection of *Nezara viridula* adults

Proportions of the field collected adults of *N. viridula* were dissected with thin scalpel. Males and females were dissected by cutting and removing the ventral part of the integument and carefully examined for the presence of larvae.

Collecting and rearing of the egg parasitoids

Leaves from maize, potatoes, tomato and rose of Sharon were inspected for the presence of egg masses of *N. viridula*. The leaves or leaf sections with egg masses were collected and placed in Petri dishes (one egg mass per a dish). On the bottom of each dish filter paper was placed and the leaf petiole was wrapped in wet cotton and parafilm for maintaining the moisture. The egg masses were examined daily for the emerging of the egg parasitoids. The emerged adult parasitoids were collected and preserved in 90% alcohol until the species identification by Dr. Ovidiu Alin Popovici (Faculty of Biology, University of Alexandru Ioan Cuza UAIC, Romania).

RESULTS AND DISCUSSION

Imaginal parasitoids

In 2019 27 parasitized adults of *N. viridula* with laid eggs from Tachinidae on their bodies were collected from maize (*Zea mays*) in the village of Yagodovo (42.11059, 24.85028), Plovdiv's region, of which 21 males and 6 females. Five parasitized adults of *N. viridula* (3 males and 2 females) were collected from rose of Sharon in Plovdiv city (42.13415, 24.765441). A total of 325 parasitized adults of *N. viridula* (155 males and 170 females) were collected from rose of Sharon in the town of Stamboliyski (42.13331, 24.53767), Plovdiv's region. Nine parasitized 5th instar nymphs of *N. viridula* were collected from the same place. All collected adults were parasitized by the same parasitoid. The eggs of the parasitoid were

deposited on a different part of the body of the host – head, thorax, scutellum, abdomen, and very rarely on the legs. The eggs were laid mainly on the dorsal side of the thorax and some – on the ventral side. The eggs of the parasitoid are with whitish color and oval shape and are easily noticed against the green color of the bug's body. Few days later after placing the parasitized stink bugs in the Petri dishes the cream-colored third instar larva of the parasitoid emerges from the bug between the posterior abdominal segments.

The larvae of the parasitoid pupated in dark reddish-brown puparium formed from the last larval skin. The mean development time of the pupal stage of *Trichopoda pennipes* was 14.05 ± 1.59 days (Figure 1), with most of the adults emerging after 14-15 days.

A total of 129 adult flies emerged from 181 pupae (Figure 1) and all of them were from one species – *Trichopoda pennipes* (Fabricius). The species identity was confirmed by Dr. Hans-Peter Tschorsing from the Naturkundemuseum Stuttgart.

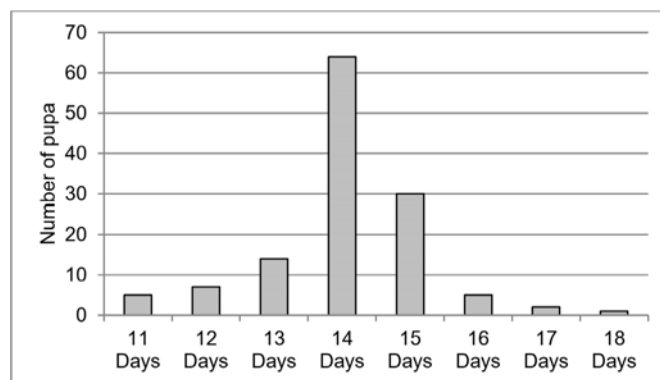


Figure 1. Development time (days) for the pupal stage of *Trichopoda pennipes* at constant temperature of 25 ± 2 °C

Dissection of parasitized *Nezara viridula* adults

A total of 240 adults of *N. viridula* with laid eggs on their bodies were dissected (123 males and 117 females) and examined for the presence of imaginal parasitoids inside. A total of 38 larvae were found in 38 adults of *N. viridula* (one larva per adult). Of the parasitized adults, 22 were males and 16 – females. In the male adults, 13 larvae were found in the abdomen and 9 in the thorax.

In the female adults, 9 larvae were located in the abdomen and 7 in the thorax. Many of the parasitized adults of *N. viridula* had more than one laid egg on their bodies. Although several larvae often enter the host's

body, only one is able to complete its development (Shahjahan, 1968) most probably because of cannibalism. All the emerging adults were from one species – *Trichopoda pennipes* (Figure 2-6).



Figure 2 and 3. Eggs of *T. pennipes* laid on a nymph (left) and an adult (right) of *N. viridula*



Figure 4. Larva of *T. pennipes* leaving the host through posterior abdominal segments



Figure 5. Larvae and pupa of *T. pennipes*



Figure 6. Adult *T. pennipes* emerged from the pupa



Figure 7. Parasitized eggs of *N. viridula*

Rate of parasitism at field conditions

In 2020, the rate of parasitism in the field populations of overwintering *N. viridula* was low and varied from 4.25% in March to 11.65% in April. In September, in adults from the second generation a higher rate of parasitism was observed, reaching 35%. In 2021 the rate of parasitism was lower in general – 2.83% in the overwintering population, 12.38% in the first generation in July and 10.17% in the second generation at the end of August.

Egg parasitoids

In August 2019 a total of 1880 eggs of *N. viridula* were collected: 14 egg masses with a total of 988 eggs from maize (*Zea mays*) in Yagodovo, Plovdiv's region, 3 egg masses with 188 eggs from rose of Sharon in Plovdiv city and 9 egg masses from rose of Sharon in Stamboliyski, Plovdiv region with 624 eggs. A total of 482 of them were parasitized (25.6%). Under laboratory conditions 42 adult parasitoids emerged, which were identified as *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), and *Trissolcus basal* (Wollaston), (Hymenoptera: Scelionidae) (Figure 7-9). The two species are mentioned for the first time in Bulgaria as parasitoids of *Nezara viridula*. *Anastatus bifasciatus* is an egg parasitoid reported from more than thirty hosts in the orders Hemiptera and Lepidoptera (Stahl et.al. 2018).



Figure 8 and 9. Adults of the egg parasitoids *Trissolcus basal* (left) and *Anastatus bifasciatus* (right)

Trissolcus bassalis is an egg parasitoid and one of the main biological control agents of *Nezara viridula*. It has been used in many biological control programs worldwide (Jones, 1988; Colazza and Bin, 1995). From egg masses collected from potato and tomato plants in 2020 in the region of Plovdiv another parasitoid was identified - *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) (Figure 10).



Figure 10. Adult of the egg parasitoid *Ooencyrtus telenomicida*

Rate of parasitism at field conditions

From field collected eggs of *N. viridula* (a total of 5520 eggs in 2020 and 9301 eggs in 2021) it was found that the degree of parasitism was lowest in May and was gradually increasing to August. In 2020 in May 7.77% of the eggs were parasitized, in June – 34.32%, in July – 72.30%, and in August – 100%. Similarly, in 2021 the rate of parasitism was increasing from 0.25% in May, 17.23% – in June, 80.51% – in July and reaching 89.79% in August. The most prevailing among the three egg parasitoid species was *Trissolcus bassalis*.

CONCLUSIONS

The Southern green stink bug *Nezara viridula* (Linnaeus) is wide spread in Bulgaria and is a major pest of crops and ornamentals. In the region of Plovdiv 4 species of parasitoids were found and reported for the first time in Bulgaria on *N. viridula*: the imaginal/nymphal parasitoid *Trichopoda pennipes* (Fabricius) (Diptera: Tachinidae) and the egg parasitoids *Trissolcus bassalis* (Wollaston) (Hymenoptera: Scelionidae), *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae).

The rate of parasitism by *T. pennipes* at field conditions ranged from 2.83% to 35% in the different generations of the host. The rate of parasitism of the eggs in 2020 and 2021 was respectively 7.77% and 0.25% in May, 34.32% and 17.23% in June, 72.30% and 80.51% in July, and in August – reaching 100% and 89.79%.

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