AGRICULTURAL UNIVERSITY PLOVDIV
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DEPARTMENT OF ENTOMOLOGY

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„Biology and control options of the southern green stink bug *Nezara viridula* (Linnaeus) and the brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)“

ABSTRACT
of the Dissertation

for the Award of the Educational and Scientific Degree
"Doctor of Philosophy"

Scientific field: 6.2. Plant Protection (Entomology)

Scientific supervisor:
Prof. Vili Harizanova, PhD

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The studies were conducted in the period 2018-2023 in the Insectary of the Department of Entomology, Agricultural University-Plovdiv and under field conditions in Pazardzhik and Plovdiv regions. The subject of the research is biology and the possibilities of control of two species of stink bugs from the Pentatomidae family, alien to Europe - the southern green stink bug (*Nezara viridula*) and the brown marmorated stink bug (*Halyomorpha halys*).

The dissertation has a volume of 126 pages and contains 17 tables and 92 photos and figures. The cited literature includes 252 sources, of which 1 in Cyrillic and 251 in Latin.

The defence of the dissertation will take place on .... of ………………. .. at ………a.m.…… of the Agricultural University-Plovdiv. The Specialized Scientific Jury was appointed by Order No. RD ……………

INTRODUCTION

Alien invasive species are one of the great challenges to global development and prosperity, especially for human health, healthy food, sustainable environments and sustainable economies. The southern green stink bug (*Nezara viridula*) and the brown marmorated stink bug (*Halyomorpha halys*) are just two of the many examples of alien insect species that accidentally entered the territory of Bulgaria, after which they established themselves as important pests of various crops. Both bugs are now ubiquitous and at the highest densities compared to all other indigenous members of the family Pentatomidae (Hemiptera; Suborder Heteroptera).

N. viridula is native to East Africa. For the conditions of Bulgaria, the stink bug has been observed since the beginning of the new millennium, but the first reports of its presence in the country are from 2007-2008. *H. halys* originates from East Asia. It was reported for the first time in Bulgaria in 2016. Finding suitable conditions for development and the presence of sufficient host plants, stink bugs reproduce quickly in new habitats. As a result, they occupied new territories and within a few years became economically important pests.

Their harmful activity is manifested in several aspects: direct harm from sucking juice, leading to a decrease in the quantity and quality of the produce; indirect harm from creating conditions for infection with phytopathogens, as an allergen for people, creating discomfort in households where they overwinter, etc.

Bearing in mind the importance of these two pests and the lack of information about their biology in Bulgaria and the possibilities of control, the present study was conducted.

1. GOAL AND OBJECTIVES

This dissertation aims to study the biology and control options of the southern green stink bug (*Nezara viridula* (Linnaeus)) and the brown marmorated stink bug (*Halyomorpha halys* (Stål)) for the conditions of Bulgaria.

To realize the set goal, the following more important tasks were identified:

1. To conduct observations and establish the most preferred species of cultivated and wild host plants in the Plovdiv and Pazardzhik regions.
2. To study various aspects of the life cycle: duration of development of individual stages, reproductive behaviour, number of generations per year, etc. under laboratory and field conditions.
3. To identify predatory and parasitoid species from natural populations associated with the southern green stink bug and the brown marmorated stink bug.
4. To study the regulatory possibilities of the established species of parasitoids.
5. To establish the biological efficacy of selected insecticides authorized for use in the European Union.

3. MATERIAL AND METHODS

The studies were conducted in the period 2018 - 2021 under laboratory and field conditions.

The laboratory experiments were carried out in the insectarium of Dept. of Entomology, Agricultural University-Plovdiv, at air temperature 25±2°C, relative humidity (RH) 50-60% and photoperiod 16L:8D without direct sunlight, at an intensity of artificial lighting (PAR) 150 µmol m^{-2} s^{-1}. The air temperature was chosen based on previous studies by other authors as the most favourable for the development of both species of stink bugs.

Observations under field conditions were carried out in the Plovdiv and Pazardzhik regions: the areas of the villages of Tsalapitsa, Isperihovo, Novo Selo, Trivoditsi, Joakim Gruevo, Kadievo, Kurtovo Konare, Zlatitrap, Yagodovo and Brestnik, the cities of Stamboliyski and Plovdiv.

Data from the trials were processed using the SPSS Statistics 26 for Windows and Microsoft Excel 365 programs.

3.1. DETERMINATION OF PREFERRED HOST PLANTS

The species composition of preferred host plants was studied under field conditions in the period 2018-2021. Annually, from April to November, a large number of cultivated and wild plant species were surveyed using standard entomological methods, such as scouting, visual observations, method of beating, and mowing with an entomological sac. A list of the examined plant species was compiled, in which the finding of eggs or nymphal instars of the respective bug species was noted and feeding was observed. The species of plants on which laid eggs or nymphs were found were determined as the most preferred hosts.

3.2. ESTABLISHMENT OF MORPHOLOGICAL FORMS OF *NEZARA VIRIDULA*

During the summer months of 2018, surveys were conducted in the Plovdiv region to identify different morphological forms of the southern green stink bug. The visual method, mowing with an entomological sac and the method of beating were used. Number and morphological form were noted. Based on the collected 1000 adult individuals of the stink bug, a ratio of colour forms was calculated.

3.3. STUDY OF THE BIOLOGICAL CHARACTERISTICS OF *N. VIRIDULA* AND *H. HALYS*

Under laboratory conditions

To create a laboratory population of *N. viridula* and *H. halys*, overwintering adult insects collected in the Plovdiv region in the spring of 2019 were used. The collected adult stink bugs were placed in Petri dishes to lay eggs. In each dish was placed filter paper and fresh raspberry shoots, the stems of which were wrapped in moistened cotton and tightly packed with Parafilm® to preserve moisture. Stink bugs were observed daily for copulation and the presence of laid eggs. From these eggs was created the laboratory population that was used in the various experiments**.**

Duration of development of individual stages

Egg stage

Each clutch of eggs laid under laboratory conditions was transferred, together with the material on which it was laid, to a separate Petri dish. A total of 419 eggs of the southern green stink bug and 385 eggs of the brown marmorated stink bug were observed daily for changes in morphology and hatching. The time (in days) from egg laying to hatching was taken as the duration of the egg stage (Figs. 1-2)**.**

Nymph stage

After establishing hatching of egg clusters individually distributed in Petri dishes, 1st instar nymphs were left in the same Petri dishes, and the date of hatching was noted. Observations on the duration of nymphal development from 1st instar (to first moult) were made on 286 nymphs of the southern green stink bug and 158 nymphs of the brown marmorated stink bug. After the transition to the 2nd instar, nymphs were reared individually in Petri dishes until adults emerged. Periodically, fresh raspberry shoots with fruits on them were placed in the Petri dishes. Nymphs were observed daily for changes in morphology and for the detection of a nymphal cuticle, which was taken as the beginning of the next nymphal instar. The studies were conducted with different numbers of nymphs for the two species of stink bugs: for the 2nd, 3rd, 4th and 5th nymphal instars of the southern green stink bug - 128, 122, 118 and 113 nymphs, respectively, and of the brown marmorated stink bug - 156, 131, 105 and 81 nymphs respectively.

The time from placement of a newly hatched nymph in a Petri dish until the emergence of an imago is taken as the duration of the nymphal stage (Fig. 1-2). The sex of the adults that emerged in the laboratory was determined by morphological features of the pygidium, and on this basis, the sex ratio was determined.

Figures 1 and 2. *Setups for establishing the duration of development of individual stages under laboratory conditions*

Adult life span

All newly emerged adults on one date were placed in separate Petri dishes with food provided. A total of 100 southern green stink bug adults and 80 brown marmorated stink bug adults were observed daily for the rest of their lives. The date of emergence and date of death were noted, and duration was calculated in days.

Under field conditions

The studies on the biological features of the southern green stink bug and the brown marmorated stink bug were conducted in 2020 in outdoor isolators in the yard of the insectarium of the Agricultural University-Plovdiv. The experiments were conducted on two-year-old hibiscus plants with a height of about 50 cm, planted in pots of Ø25 cm. The plant species was selected based on our observations of its preference by both species of bugs**.** The pots with the experimental plants were covered with organza (Figs. 3 and 4), which allowed easy observation and carrying out various manipulations. The population was created from adult bugs of both species collected from hibiscus on the campus of the Agricultural University - Plovdiv.

Duration of developmental stages

In late April and early May 2020, couples (female and male) of the collected stink bugs were placed in isolators for mating and egg-laying. After the detection of laid eggs, adults were removed from the isolator and placed in a new one. In this way, it was known that in each isolator there were eggs laid on a specific date. The duration of the egg stage was studied on 657 eggs of the southern green stink bug and 111 eggs of the brown marmorated stink bug. After the eggs hatch, the duration of development of the nymphal instars and adult emergence was monitored. After adults emerged, they were moved to a new isolator in couples (female and male).

Egg clusters laid by individual females were moved together with the leaf on which they were laid to a new isolator for observation. The number of eggs and the date of their laying were recorded. From this date, the monitoring of the duration of development of the individual stages of the next generation begins.

Nymphs that hatch from the respective egg clutch were observed daily until the first moult. As the nymphs pass into the 2nd instar, they are moved individually to a new isolator, where observation of their transformation into the next instar and adult continues. When an adult appears, the date of his emergence is recorded and in couples (male and female) they are placed in a new isolator.


Figures 3 and 4*. Isolators for studying the lifecycle parameters of the southers green stink bug and the brown marmorated stink bug at field conditions*

The life spans of 147 southern green stink bug adults and 10 brown marmorated stink bug adults obtained in previous experiments to establish the duration of development of the nymphal stage was tracked. Adults who emerged on one date were placed in a separate isolator, which was labelled and the date of emergence was recorded. Each isolator was checked daily for the rest of the adults' lives. The date of emergence and date of death were noted, and duration was calculated in days.

Studying the reproductive behaviour

Number of copulations and duration of copulation

Under laboratory conditions, 10 couples of both species of stink bugs were observed. Couples were formed from newly emerged adults and observed daily until the death of one of the two individuals. The beginning and end of copulation, as well as the number of copulations for the respective couple, are noted. The crimped filter paper was provided in the Petri dishes as a shelter as well as food (plant parts of the same species for all replicates in the series). Field trials were conducted in 2020 in the same manner as in the laboratory, but couples were placed in separate isolators.

Duration of pre-oviposition and oviposition period and egg productivity

Under laboratory conditions, couples (male and female) of newly emerged bugs of both species were placed in Petri dishes with provided food (top shoots of raspberry). Observations of their behaviour were made daily. The time from emergence to detection of the first eggs was taken as the duration of the pre-oviposition period. Plant parts were replaced with fresh ones until the death of the female individual, and the number of eggs laid by each female was recorded by date. After the death of the respective female, egg productivity was calculated as the sum of all eggs laid by one female and the average number of eggs in an egg cluster. Calculations were based on 11 females of the southern green stink bug and 7 females of the brown marmorated stink bug that lived more than 30 days.

Under field conditions, the experiment was conducted in 2020. In isolators, couples (female and male) formed by newly emerged bugs of both species from the experimental populations were observed. Observations and calculations of pre-oviposition and oviposition period duration and egg productivity were described under laboratory conditions.

3.3. SEARCHING FOR PREDATORY AND PARASITOID SPECIES FROM NATURAL POPULATIONS RELATED TO *N. VIRIDULA* AND *H. HALYS*

In 2019, 2020 and 2021, surveys were conducted in field plantations with different types of crops in the Plovdiv and Pazardzhik regions and in the cities of Stamboliyski and Plovdiv to detect predatory and parasitoid species from natural populations that have a trophic relationship with the two species of stink bugs.

Predatory insect species were identified by visual observations of feeding on any of the stink bug stages.

To detect parasitoids on the eggs, egg masses of both species of bugs were collected from different types of crops, where the atypical colouration of the eggs was observed and there was a possibility that they were parasitized. The collected egg masses, together with the material on which they were laid, were taken to the laboratory, where they were placed in Petri dishes and observed until the eggs hatched. Adult parasitoids that emerged from the parasitized eggs were collected for further identification.

Establishing rate of parasitism on eggs, nymphs and adults

In 2020-2021, from April to September, various field plantations in the region of the city of Plovdiv were surveyed monthly. The discovered egg masses were taken to the laboratory of the insectarium, where they were placed in Petri dishes and observed until the eggs hatched. The number of eggs in each egg cluster was recorded. After parasitoids were observed, the number of parasitized eggs and the number of parasitoids were recorded.

The identification of the species of egg parasitoids was carried out by Dr. Ovidiu Alin Popovici and Dr. Lucian Fusu from the Alexandru Ioan Cuza din Iași University, Romania (Universitatea Alexandru Ioan Cuza din Iași).

To establish the rate of parasitization by the parasitoid *Trichopoda pennipes* in 2019, various field plantations in the region of the city of Plovdiv were periodically surveyed. 5th instar nymphs and adults of the southern green stink bug were collected and transported in plastic containers (10x5x4 cm) to a laboratory in the insectarium where they were examined for the presence of eggs on the body.

Individuals with eggs laid on the body were housed in Petri dishes until the emergence of an adult parasitoid. Individuals from which no parasitoid has emerged were dissected after death (for possible development of a parasitoid that failed to complete its life cycle).

To detect this parasitoid on adults of the brown marmorated stink bug, all stink bug individuals collected for other experiments were carefully examined, but no parasitism was detected.

The species identity of the parasitoid was confirmed by two independent taxonomists: Dr. Hans-Peter Tschorsing of the Natural History Museum, Stuttgart, Germany, and Dr. Chris Raper, of the Natural History Museum, London, United Kingdom.

3.4. EVALUATION OF BIOLOGICAL EFFICACY OF PPPs

In 2019 and 2023, the biological efficacy of 7 insecticides (Table 1) with different active substances selected from the BFSA register of authorized plant protection products or with registrations in other EU countries was studied under laboratory conditions. The selected insecticides are biological, and 4 of them are plant-based: Neem Azal T/C, Biopren plus, Pyregard and Limocid, and 3 are based on microorganisms: Naturalis, Preferal and Sineis 480 SK. The chemical-based insecticide Mospilan 20 SP was chosen as the standard.

The preparations are registered against pests with piercing-sucking mouthparts on vegetables, fruit and other crops, such as whiteflies, thrips, cicadas, etc., but not against plant bugs. Of the preparations listed above, only Mospilan 20 SP is registered against the harmful grain bug.

The preparations were applied in 3 concentrations - ½ of the registered, registered and twice higher than the registered concentration, as follows: Neem Azal T/C - 0.1%, 0.2% and 0.4%; Bioprene plus - 0.075%, 0.15% and 0.3%; Preferential – 0.05%, 0.1% and 0.2%; Sineis 480 SK - 0.01%, 0.02% and 0.04%; Pyregard - 0.04%, 0.08% and 0.16%; Naturalis – 0.05%, 0.1% and 0.2%; Limocid - 0.2%, 0.4% and 0.8% and Mospilan 20 SP - 0.01%, 0.02% and 0.04% as a standard.

Table 1. *List (in Alphabetical order) of the tested PPPs against N. viridula and H. halys*

Trade product/Producer	Active substance	Group according to MoA	Mode of Action (MoA)
Biopren Plus Althaller Italia S.r.l	S-methoprene – 6,74%, *Chrysanthemum cinerariaefolium*- 4,81%, PBO-10,17%	3 Piretroids	It causes hyper-arousal and blockage of the nervous system
Limocid Vivagro, France	Orange oil 60 g/l	UNE – plant extracts	
Mospilan 20 SP Summi Agro Romania. S.R.L.-Bulgarian Branch	acetamiprid-200 g/kg	4 Neonicotinoids	It causes hyper-arousal and blockage of the nervous system
Naturalis SBS Europe, S.r.l., Italy	*Beauveria bassiana*, strain ATCC 74040 - 0.185 g/kg	UNF – Fungal agents with unknown MoA	
Ним Азал Т/С Трифолио-М, Германия	azadirachtin A-1%+ azadirachtin B,V,G,D- 0,5%+ neem substance2,5%	UN – Compound with unknown MoA	-
Piregard Cerrus C.A.C., Italy	piretrini -40 g/l	3 Piretroids	It causes hyper-arousal and blockage of the nervous system
Preferal WG Biobest	Spores of *Izaria fumosorosea Apopka*	UNF - Fungal agents with unknown MoA	-

	Strain 97 (ATCC 20874) -20%		
Sineis 480 СК Corteva Agriscience Bulgaria ЕООД	Spinosad 480 g/l	5 nicotinic acetylcholine receptor (nachr) allosteric modulators - site i	Activates nAChRs causing overexcitation of the nervous system

The study was carried out as follows: clean and healthy apical tomato leaves of approximately the same size were used, the petioles of which were wrapped in moistened cotton and with Parafilm® to preserve moisture. Replicates were scored according to the biological efficacy test method developed by the Insecticide Resistance Committee (IRAQ – Method 022). Each tomato leaf was immersed for 5 seconds in the solution of the corresponding PPP with the selected concentration, after which it was dried on a mesh grill. The leaves for the controls are immersed in water. Before placing the treated leaves, filter paper was added to the experimental containers to absorb excess moisture. Using a fine brush, three 5th instar nymphs of the southern green stink bug and the brown marmorated stink bug were placed in each container (Fig. 5).

The biological efficacy of each of the listed products was determined by 3 replicates and a control for each concentration. The condition of the nymphs was recorded on days 3, 7 and 9 after treatment.

The biological efficacy was calculated according to the formula of Abbot (1925):

$$E = \frac{x-y}{x} . 100,$$ where:

E = biological efficacy, %;
x = per cent alive individuals in the control after the treatment;
y = per cent alive individuals in the variant after the treatment;
100 = percent.

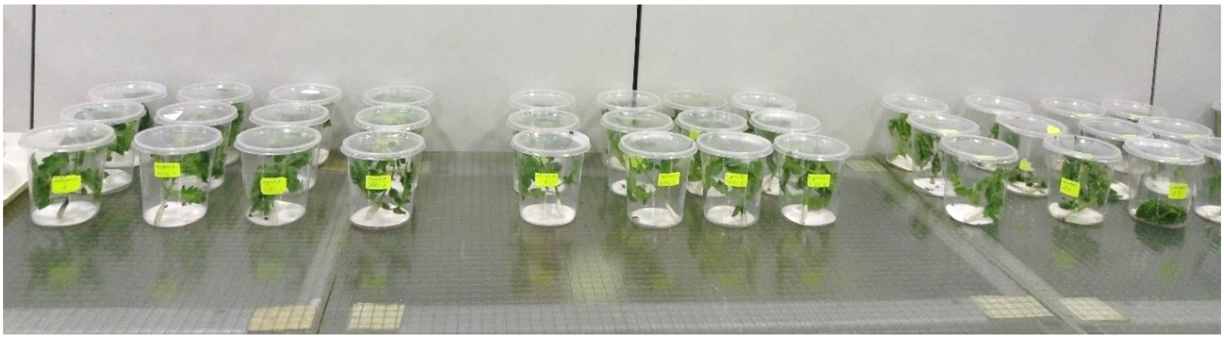


Figure 5. *Testing biological efficacy of PPPs on 5th instar nymphs of N. viridula and H. halys under laboratory conditions*

4. RESULTS AND DISCUSSION

4.1. PREFERENCE OF *N. VIRIDULA* TO HOST PLANTS

Of the 145 host plant species of *N. viridula* reported in the literature, surveys were conducted in the Plovdiv and Pazardzhik regions according to those listed in the Table 2 plant species. Although the species is polyphagous, not all host plants are equally suitable for its development and therefore equally preferred. As preferred hosts, we assume species on which females choose to lay eggs and species on which we find young nymphs (apparently hatched on the relevant plant). Of the investigated plant species, oviposition was not found on hot pepper, broccoli, hazelnut, pear, thuja, gladiola, hops and baconweed. No stages of the stink bug were detected, as well as damage to the plant organs, on tobacco, alfalfa, peach, plum, blackberry and linden.

Feeding and damage were most commonly observed on tomatoes, green beans, raspberries, corn, and hibiscus and eggs were most commonly laid on these plants.

In tomatoes, nymphs and adults damage mainly fruits, which confirms what was observed by other authors in the USA and Europe (Hoffmann et al., 1987; Grozea et al., 2012). Upon feeding, small pits are formed on the fruit surrounded by empty and air-filled cells that appear as white, sunken areas on green fruit and yellow on red fruit. Beneath the damaged areas, the tissues are white in color. Damaged fruits have deteriorated taste qualities and have no market value. In the conducted observations, no preference of bugs for green or red fruits was found, despite reports by some authors of a stronger attack of nymphs on green fruits (Grozea et al., 2012).

Table 2. Plant species on which observations were carried out for feeding and occurrence of stages of *N. viridula* in the region of Plovdiv and Pazardzhik

№	**Crops surveyed**	**Stages of *N.viridula* (+/-)**		**Damage on…**	**Location**
1	Tomato (*Lycopersicon esculentum* Mill, 1768)	+	E,N,A*	Fruits	Plovdiv, Tsalapitsa village, Isperihovo village, Novo selo village
2	Bell pepper (*Capsicum annuum* L., 1753)	+	E,N,A	Fruits	Plovdiv, Isperihovo village, Yagodovo village
3	Green beans (*Phaseolus vulgaris* Linnaeus, 1753)	+	E,N,A	Fruits	Stamboliyski, Trivodici village
4	Hot pepper (*Capsicum annuum L. var. Dzhulyunska shipka*)	+	N,A	Fruits	Yoakim Gruevo village
5	Broccoli (*Brassica oleracea L. var. italica* Plenck, 1794)	+	N,A	Fruits	Stamboliyski
6	Potatoes (*Solanum tuberosum* Linnaeus, 1753)	+	E,N,A	Leaves	Plovdiv, Stamboliyski
7	Tobacco (*Nicotiana tabacum* Linnaeus, 1753)	-			Novo selo village
8	Maize (*Zea mays* Linnaeus, 1753)	+	E,N,A	Fruits	Yagodovo village, Isperihovo village, Kadievo village, Zlatitrap village
9	Alfalfa (*Medicago sativa* L., 1753)	-			Tsalapitsa village, Kadievo village, Zlatitrap village, Trivodici village
10	Apple (*Malus domestica* Borkh., 1803)	+	E,N,A	Fruits	Plovdiv, Stamboliyski,

					Yagodovo village, Brestnik village
11	Peach (*Prunus persica* (L.) Batsch 1801)	-			Stamboliyski, Zlatitrap village, Brestnik village
12	Plum (*Prunus domestica* L., 1753)	-			Yoakim Gruevo village, Tsalapitsa village, Brestnik village
13	Hazelnut (*Corylus avellana* L., 1753)	+	N,A	Fruits	Yoakim Gruevo village
14	Wild plum (*Prunus cerasifera* Ehrh., 1789)	+	E,N	Fruits	Yoakim Gruevo village
15	Pear (*Pyrus communis* L., 1753)	+	N	Fruits	Tsalapitsa village, Yoakim Gruevo village
16	Vine (*Vitis vinifera* L., 1753)	+	E,N,A	Fruits	Brestovitsa village, Brestnik village,Tsalapitsa village, Yagodovo village
17	Raspberry (*Rubus idaeus* L., 1753)	+	E,N,A	Fruits	Plovdiv, Isperihovo village, Yoakim Gruevo village
18	Blackberry (*Rubus fruticosus* L., 1753)	-			Yoakim Gruevo village
19	Mulberry (*Morus alba* L., 1753)	+	E,N,A	Leaves, fruit	Stamboliyski
20	Hibiscus (*Hibiscus syriacus* L., 1753)	+	E,N,A	Buds	Plovdiv, Stamboliyski
21	Gladiolus (*Gladiolus dalenii* Van Geel, 1829)	+	N	Buds	Stamboliyski
22	Tuya (*Thuja orientalis* L., 1753)	+	N	Fruits	Plovdiv, Stamboliyski
23	Linden tree (*Tilia tomentosa* Moench., 1785)	-			Plovdiv
24	Hops (*Humulus lupulus* Linnaeus, 1753)	+	N,A	Leaves, inflorescens	Stamboliyski, Kurtovo Konare village
25	Bindweed (*Convolvulus arvensis* L., 1753)	+	E	Leaves	Plovdiv
26	Baconweed (*Chenopodium album* L., 1753)	+	N, A	Leaves	Plovdiv

*Legend: E-eggs; N-nymphs; A-adults

On green beans, nymphs and adults suck sap from the stems and pods, but damage is mainly observed on the pods, which is also described in the study by Kuhar et al., (2012). Damage to the young pods is manifested by the deformation of the seeds and a decrease in the yield. On raspberries, nymphs and adults prefer to suck the juice from the fruits in all stages of their development, also observed by other authors (Wiman et al., 2015).

On corn, feeding was observed only on the kernels of the cob (Figs. 6 and 7), despite reports of leaf and stem damage (Clower, 1958). Damaged cobs have discoloured and sunken kernels, resulting in quality deterioration and lower yield.


Figures 6 and 7. *Feeding on corn kernel and damage a week later.*

On hibiscus, bugs suck sap from the buds, which leads to drying, delayed development and premature dropping, especially at higher densities, which are observed in late summer. On apple fruits, sucking juice causes the appearance of a dark point at the puncture site, darkening the pericarp below the puncture site. If the puncture occurs at an earlier stage of the development of the fetus, it grows unevenly.

On pepper, the stink bug also feeds on the fruits, but only a single damage was found. The surveyed pepper areas in the village of Isperihovo, region Pazardzhik, were adjacent to tomatoes, which had significant damage from bugs. The visual observations confirm that stink bugs prefer tomatoes to pepper, which explains the lighter damage on pepper.

Nymphs and adults are found on mulberry and vine and fruit feeding is seen, but damage is minor. Visual observations in June show a high number of mulberry bugs. Copulating Couples and the presence of egg clusters were detected.

Observations predictably show that adult bugs move from one plant host to another depending on the availability of preferred plant parts. In late August to mid-September, nymphs and adults are found en masse on apples and vines, where they feed on the fruit.

The bug has been reported in the literature to damage nuts, including hazelnuts (Genduso, 1974). During the studies in the region of the city of Plovdiv, single damage to hazelnuts was found. The damage is expressed in the formation of spots or sinking of the nut, as well as their premature dropping.

Although N. viridula has been reported in the literature to cause severe damage to peaches and is one of the important enemies of this crop (Johnson et al., 1985), no attack was observed on this fruit species during our surveys in the study area, possibly due to the presence of more preferred hosts in the vicinity.

4.1.2. PREFERENCE TO HOST PLANTS OF *H. HALYS*

Of the 26 cultivated and wild plant species surveyed in the Plovdiv and Pazardzhik regions, only 16 were found to lay eggs and have young nymphs of *H. halys* (Table 3).

Table 3. Plant species on which observations were carried out for feeding and occurrence of stages of *H. halys* in the region of Plovdiv and Pazardzhik

№	Crop surveyed	Ocuurence of stages of *H. halys* (+/-)		Damage	Location
1	Tomato (*Lycopersicon esculentum* Mill, 1768)	+	*E,N,A	Fruits	Plovdiv, Tsalapitsa village, Isperihovo village, Novo selo village
2	Bell pepper (*Capsicum annuum* Linnaeus, 1753)	+	E	Fruits	Plovdiv, Isperihovo village, Yagodovo village
3	Green beans (*Phaseolus vulgaris* Linnaeus,1753)	-			Stamboliyski, Trivodici village
4	Hot pepper (*Capsicum annuum L. var. Dzhulyunska shipka*)	-			Yoakim Gruevo village
5	Broccoli (*Brassica oleracea L. var. italica* Plenck, 1794)	-			Stamboliyski
6	Potatoes (*Solanum tuberosum* Linnaeus, 1753)	-			Plovdiv, Stamboliyski
7	Tobacco (*Nicotiana tabacum* Linnaeus, 1753)	-			Novo selo village
8	Maize (*Zea mays* Linnaeus, 1753)	+	E,N,A	Fruits	Yagodovo village, Isperihovo village, Kadievo village, Zlatitrap village
9	Alfalfa (*Medicago sativa* Linnaeus, 1753)	-			Tsalapitsa village, Kadievo village, Zlatitrap village, Trivodici village
10	Apple (*Malus domestica* Borkh., 1803)	+	E,N,A	Fruits	Plovdiv, Brestnik village, Stamboliyski, Yagodovo village
11	Peach (*Prunus persica* (L.) Batsch, 1801)	-			Stamboliyski, Zlatitrap village, Brestnik village
12	Plum (*Prunus domestica* L., 1753)	+	E,N	Fruits	Yoakim Gruevo village, Tsalapitsa village, Brestnik village
13	Hazelnut (*Corylus avellana* L., 1753)	+	N	Fruits	Yoakim Gruevo village
14	Wild plum (*Prunus cerasifera* Ehrh., 1789)	-			Yoakim Gruevo village
15	Pear (*Pyrus communis* Linnaeus, 1753)	+	E,N	Fruits	Tsalapitsa village, Yoakim Gruevo village
16	Vine (*Vitis vinifera* L., 1753)	+	E,N,A	Fruits	Brestovitsa village, Brestnik village, Tsalapitsa village, Yagodovo village
17	Raspberry (*Rubus idaeus* L., 1753)	+	E,N,A	Fruits	Plovdiv, Isperihovo village, Yoakim Gruevo village

18	Blackberry (*Rubus fruticosus* L., 1753)	+	N	Fruits	Yoakim Gruevo village
19	Mulberry (*Morus alba* L., 1753)	+	E,N,A	Leaves, Fruits	Stamboliyski
20	Hibiscus *(Hibiscus syriacus* L., 1753)	+	E,N,A	Buds	Plovdiv, Stamboliyski
21	Gladiolus (*Gladiolus dalenii* Van Geel, 1829)	-			Stamboliyski
22	Tuya (*Thuja orientalis* L., 1753)	+	N,A	Fruits	Plovdiv, Stamboliyski
23	Linden tree (*Tilia tomentosa* Moench.,1785)	+	E,N	Leaves	Plovdiv
24	Hops (*Humulus lupulus* L.,1753)	-			Stamboliyski, Kurtovo Konare village
25	Bindweed (*Convolvulus arvensis* L., 1753)	-			Plovdiv
26	Baconweed (*Chenopodium album* L., 1753)	-			Plovdiv

*Legend: E-eggs; N-nymphs; A-adults

The species is newer to the country than the southern green stink bug, and so far its occurrence has been observed on a more limited range of host plants. As can be seen from the table. 3 stages and damage from feeding on beans, broccoli, hot pepper, alfalfa, tobacco, peach, Wild plum, gladiola, and hops, as well as on the weed species Bindweed and white Baconweed have not been established. Eggs have been found on tomato, pepper, corn, apple, plum, vine, mulberry, raspberry, hibiscus and linden.

During the studies carried out in the region of the city of Plovdiv, damage was most often observed on the fruits of tomatoes, raspberries and mulberries and the flower buds of hibiscus.

In the surveyed areas with tomatoes, damage was found only on the fruit, which confirms observations in the USA (Kuhar et al., 2012). Damaged fruits have white to pale yellow spots and the tissue beneath the skin is hardened. It has also been observed that bugs attack tomatoes grown without stakes more than those grown with stakes.

On hibiscus, nymphs and adults damage the buds by sucking sap, which leads to their drying, delayed development and premature dropping. Damage is observed from the end of August to the end of September when the stink bugs are feeding heavily before moving to their wintering grounds.

In maize, damage is mainly observed on the kernels of the cobs (Figs. 8 and 9), which was also described by Kuhar et al. (2012). Young nymphs cluster in groups at the base of the leaves, but no damage is observed, while fourth and fifth instar nymphs suck sap from the top of the cobs and the kernels, causing discolouration and deformation of the kernels, and hence deterioration in quality. and yield reduction.

Figures 8 and 9. *Damage of H. halys on corn*

Bugs feed on mulberry by sucking juice from the leaves and fruits. During the visual observations carried out at the beginning of June, the presence of all stages of the stink bug on this host was established.

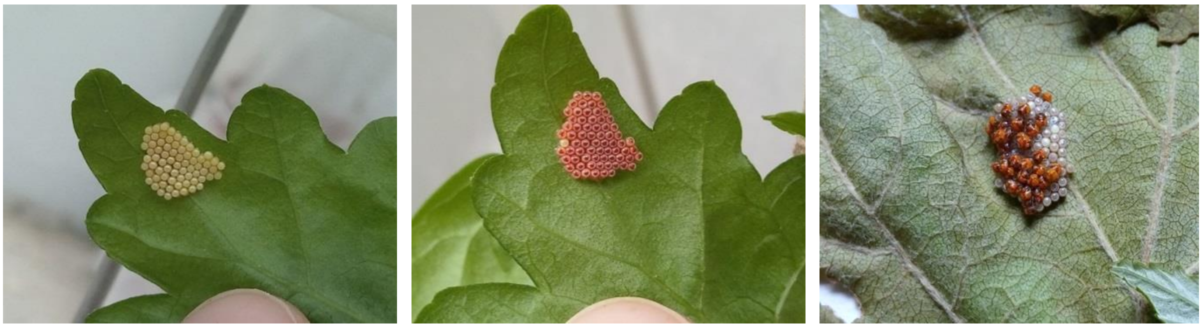
On apples, bugs attack the fruit, but the damage observed in the Plovdiv region is insignificant, despite reports of serious damage by the enemy on this crop in other countries (Brown, 2003).

4.2.MORPHOLOGY

4.2.1 Nezara viridula

The eggs are of the family's typical barrel shape and are laid in clusters tightly packed together in parallel rows, usually 9-12 eggs in a row. The shape of the egg mass is characteristic, which can be seen especially well under laboratory conditions. Freshly laid eggs are pale yellow in colour and average 1.3 mm in length and 0.9 mm in width.

During embryonic development, a change in colouring is observed - after the third day, they begin to acquire an orange colour, and the formation of red "solid triangles" is observed on the caps. On the fifth day, the eggs acquire an orange-reddish colour (Fig. 10-12).



Figures 10, 11 and 12. *Eggs of N. viridula after laying (left), before hatching (centre) and after hatching (right)*

The species has 5 nymphal instars. First-instar nymphs are orange-red, with red eyes and transparent legs and antennae (Fig. 13), and second-instar nymphs have a black head, breast, legs and antennae, with a yellow spot on each outer side of the thorax. The nymphs of the third instar are coloured like those of the second instar, but sometimes the black colour may be replaced by olive green (Fig. 14).

Their dimensions average 3.6 mm in length and 2.6 mm in width. Fourth-instar nymphs are larger, approximately 6.2 mm long and 4.7 mm wide, and the characteristic green colour begins to become visible (Fig. 15). In fifth-instar nymphs, the abdomen is yellowish-green, with red spots along its length (Fig. 16). When passing into the fifth age, variation in colouration is observed - "light" and "dark" form, with green and dark brown to the black colouration of the dorsal part, respectively. The rudiments of the wings are highly developed and the main part of the abdomen is covered by them. At the end of its development, the fifth instar nymph is about 10 mm long and about 7 mm wide.

Figure 13. *Nymph 1st instar of N. viridula*

Figure 14. *Nymph 3rd instar of N. viridula*

Figure 15. *Nymph 4th instar of N. viridula*

Figure 16. *Nymph 5th instar of N. viridula*

Of the 5th instar nymphs observed under laboratory conditions, light forms (with green colouration) predominate, the ratio being 60 light forms: 40 dark forms (Figs. 17 and 18).

Figures 17 and 18. *Nymphs 5th instar of N. viridula – light form (left) and dark form (right)*

Newly emerged adults are light green with a soft body covering that hardens after about 24 hours. Males have a body length of about 12.1 mm and females - 13.15 mm. The eyes are black, and the wings completely cover the abdomen. Males are smaller in size than females and differ in the last abdominal sternite (pygidium)(Figs. 19 and 20).

Figure 19. *Pygidium of female N. viridula*

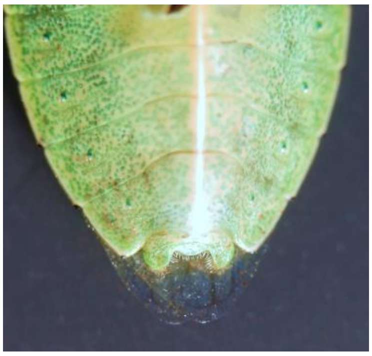
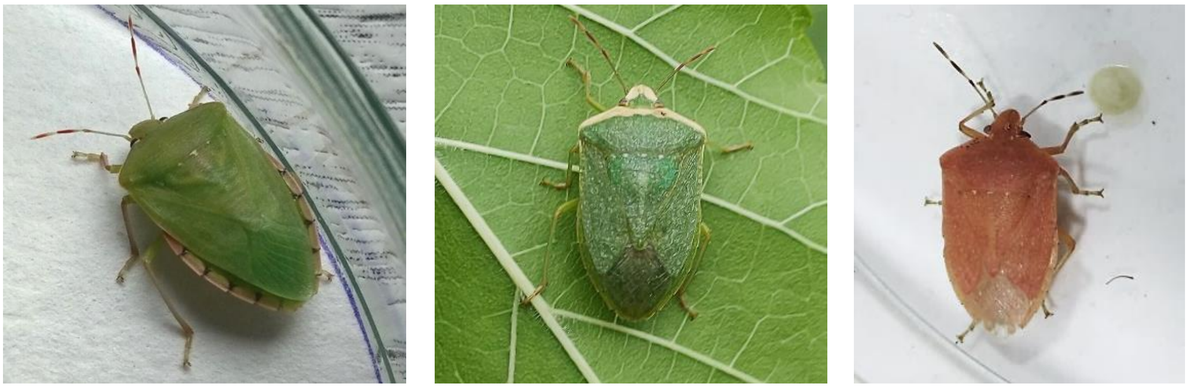


Figure 20. *Pygidium of male N. viridula*

Colour forms of adult *N. viridula*

Since the species is a relatively large insect, descriptions of its stages have been published since the beginning of the last century until today (Jones, 1918; Todd, 1989; Roychoudhury and Joshi, 1996; Squitier, 2011, etc.). Several colour morphological forms have been described in the adults, which bear the corresponding names: var. smaragdula F. (G-type, all green), var. torquata F. (O-type, mostly green body colour with a yellow stripe on the thorax and head), var. aurantiaca Costa (Y-type, all yellow coloured), blue form, duyuna form, typica form (Chen, 1980) and black form (Esquivel et al., 2015).

Of the 7 morphological forms of *N. viridula* reported in the literature, within our study for the region of the Plovdiv region we found only 3: var. smaragdula, var. torquta and var. aurantica (Figs. 21-23).



Figures 21, 22 and 23. *Adults of different colour forms: var. smaragdula (left), var. torquata (centre) and var. aurantica (right)*

Out of 1000 adult stink bugs collected, the predominant one is var. smaragdula (94.3%), while var. torquata (5.5%) and var. aurantica (0.2%) were found in very low numbers (Fig. 24).

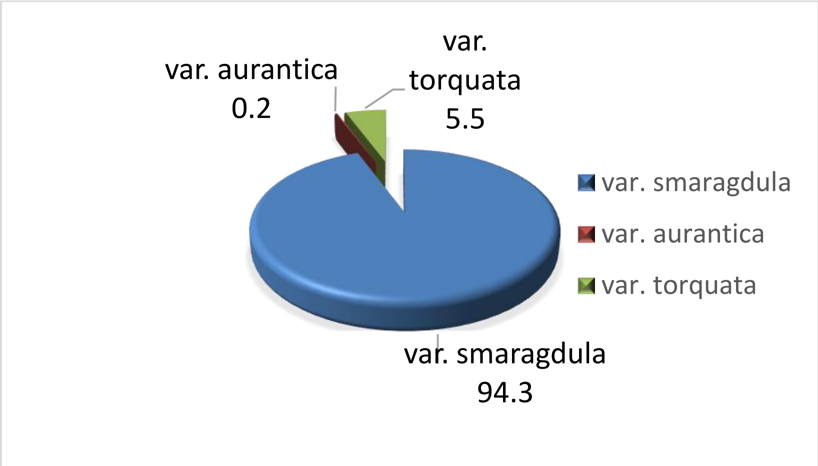
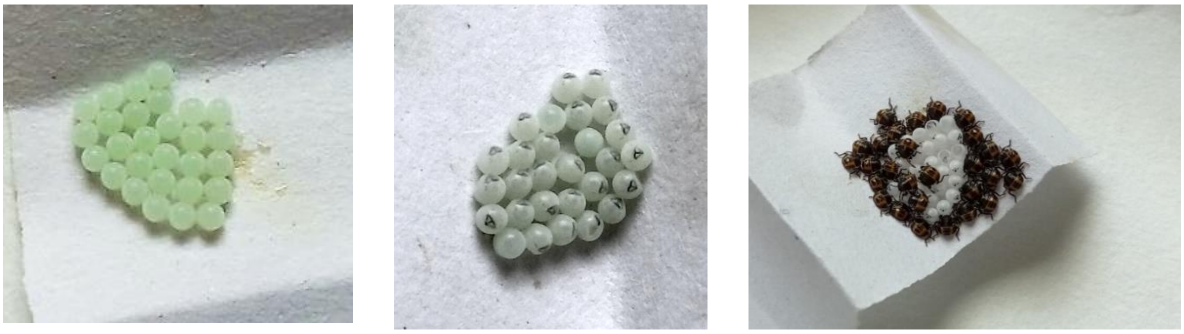


Figure 24. *Ratio of the different colour forms of N. viridula, collected in the region of Plovdiv*

4.2.2. *HALYOMORPHA HALYS*

The eggs are roughly spherical measure an average of 1.6 mm in height and an average of 1.3 mm in diameter, and are white or pale green. They are laid in piles, with one pile consisting of 20-30 eggs. During embryonic development, changes in colouration are observed as follows: after the third day, the eggs begin to fade, and two red dots appear on the caps and a black line below them. A day before hatching, the eggs acquire a white colour (Fig. 25-27).



Figures 25, 26 and 27. *Eggs of H. halys after laying (left), prior hatching (center) and излюпване (right)*

The brown marmorated stink bug has five nymph instars. 1st instar nymphs average 2.4 mm in length and elliptical in body shape. They are coloured light orange to red. The head, chest and legs are black, the abdomen is yellowish-red with 3 black horizontal stripes on the tergites, the eyes are dark red and the antennae are reddish-black. 2nd instar nymphs are approximately 3.7 mm in length. The body is egg-shaped and slightly flattened.

The head and dorsal part of the thorax are black, and the abdomen is whitish with reddish spots. The eyes are reddish-black, the antennae are also reddish-black, and the legs are blackish-brown (Figs. 28-29).

Figure 28. *Nymph 1st instar of H. halys*

Figure 29. *Nymph 2nd instar of H. halys*

Figure 30. *Nymph 3rd instar of H. halys*

The 3rd instar nymphs are about 5.5 mm long and the body is flattened, pear-shaped. The head is rectangular with a pair of horn-like projections in front of the eyes. The head and dorsal part of the thorax are brownish-black in colour. The abdomen is whitish with red spots connected between them. Coloration of eyes and antennae is as in 2nd instar nymphs. The legs are brownish-black in colour, except for the base of the femur and the middle of the tibia, which are white (Fig. 30).

The 4th instar nymphs measure approximately 8.5 mm, and the body is the same shape as the previous instar. Colouration is almost the same as in 3rd instar nymphs. The antennae are reddish-black, except for the upper edge of the third segment and the base of the fourth segment, which are yellowish-white. Thighs are brown with black spots except for the base which is yellowish white.

The tibiae and feet are black-brown except for the middle of the tibiae, which is whitish (Fig. 31). The 5th instar nymphs are about 12 mm long, and the body is pear-shaped and quite flattened. The head and dorsal part of the thorax are mostly brownish-black in colour with a metallic sheen, except for a few whitish spots on the head and thorax.

Figure 31. *Nymph 4th instar of H. halys*

Figures 32 and 33. *H. Nymph 5th instar of H. halys*

The abdomen is orange-white, covered with dense black dots with a slight metallic sheen and reddish spots. The eyes are red-black. The antennae are black, except for the apex of the third and the base of the fourth segment, which are whitish. The thigh is mottled black-brown and whitish at the base. The tibiae and feet are mostly brown, except for the middle of the tibiae, which are white, and the upper part of the feet, which are black (Figs. 32 and 33).

Adults vary in size and colouration. The body is shield-shaped and is 12 to 17 mm long and 7-10 mm wide. It is mostly grey-brown, but it can also be grey-ochre, tile red or chestnut, with dark points. In the upper part, the head is broadly rounded, the eyes are large, and the proboscis reaches the second abdominal segment. Hemieletris are mottled in brown, densely punctate, with a slightly reddish tint. The apical membrane is yellowish transparent, with dark brown veins. The legs are brown and have whitish stripes. The abdomen along the periphery is coloured in alternating white and dark stripes.

Males are smaller than females. Sex is determined by the shape of the pygidium (Figs. 34 and 35).

Figure 34. *Pygidium of female H. halys*

Figure 35. *Pygidium of male H. halys*

In Europe, *H. halys* can be confused with another species of the same family - *Rhaphigaster nebulosa* (Figs. 36 and 37). Key characters for distinguishing the two species include the white stripes on the antennae and legs, the shape of the prothorax, and the alternating dark and light stripes along the periphery of the abdominal segments (in *H. halys*, the dark stripes are separated by a thin light longitudinal line).

Figures 36 and 37. *Adult of H. halys (left) and of Rhaphigaster nebulosa (right)*

4.3. INVESTIGATION OF BIOLOGICAL PARAMETERS OF *N. VIRIDULA* AND *H. HALYS*

4.3.1. DURATION OF DEVELOPMENT OF THE DIFFERENT LIFECYCLE STAGES

Egg stage

As is known, the duration of development of each stage depends mainly on temperature (Waterhouse, 1998). The embryonic development of *N. viridula* under laboratory conditions with a constant temperature of 25±2°C, 60-70% RH and a photoperiod of 16L: 8D lasts an average of 6.04±0.71 days (Table 4). The results obtained are similar to those of Chanthy et al. (2015), who found that at a temperature of 25°C and a humidity of 40%, embryonic development lasted an average of 7 days.

The established average duration of the egg stage of *H. halys* under laboratory conditions is 5.10±1.02 days (Table 4), which is within the limits of what was also established by other authors, such as Kawada and Kitamura (1983) (average 4- 5 days) and Haye et al. (2014) (mean 5.80 days).

Table 4. *Duration of egg stage (days) of N. viridula and H. halys at laboratory conditions (25±2°C, 60-70% RH, 16 L:8Dand at field conditions in 2019-2020*

Species	Mean±Std. Deviation	Std. Error	Minimum	Maximum	Number
At laboratory conditions					
N. viridula	6,04±0,71	0,03	5	8	419
H. halys	5,10±1,02	0,05	3	7	385
At field conditions					
N. viridula	7,57±2,17	0,08	4	14	657
H. halys	5,68±1,24	0,12	4	8	111

Under field conditions in isolators, the duration of the egg stage of *N. viridula* and *H. halys* was 7.57±2.17 days and 5.68±1.24 days, respectively (Table 4).

Nymphal stage

Under laboratory conditions, the duration of the 1st nymphal instar of *N. viridula* averaged 3.56±1.55 days (Table 5), which is similar to that found by Harris and Todd (1980) - 3.8 days. 1st instar nymphs do not feed, which is characteristic of most stink bug species (Jones, 1918). After the first moulting, the nymphs begin to feed on plants. The duration of development of the 2nd nymphal instar was 9.75±4.98 days on average and on average 7.16±3.47 days in the 3rd instar (Table 5).

In our experiments, the nymphs of the 4th instar completed their development in an average of 10.36±2.98 days (Table 5). The 2nd and 3rd instar nymphs continue to cluster, and after the 4th instar, they begin to disperse, a behaviour described by some authors (Knight and Gurr, 2007). The duration of development of the 5th nymphal instar was 13.47±3.39 days, which is similar to that found in experiments by Harris and Todd (1980) - 11.9 days and by Chanthy et al. (2015) - 11.1 days.

In *H. halys*, the development of 1st instar nymphs takes an average of 5.32±1.23 days (Table 5). The results of the study are very similar to those obtained by Medal et al. (2013) and Haye et al. (2014). To pass through the 2nd instar, nymphs required an average of 9.35±2.89 days, similar to that found by Nielsen et al. (2008) - 9.62 days. After passing into the third instar, the nymphs in this species no longer feed in groups, but migrate in search of food independently. This stage of development takes an average of 7.18±2.56 days, as observed by Nielsen et al. (2008) is almost the same -7.08 days.

It is noteworthy that for the development of the 4th instar, the nymphs of this species need less time compared to those of the southern green stink bug - 7.41±1.89 days, but this is similar to what was found by Nielsen et al. (2008) - 7.38 days. The last 5th instar was also of shorter duration than that found for the southern green stink bug - an average of 10.88±2.64

days (Table 5), which almost coincides with the study of Nielsen et al. (2008) - an average of 10.44 days.

Under field conditions, the development of 1st instar nymphs of *N. viridula* lasted an average of 4.17±1.47 days, while that of the brown marmorated stink bug was 5.17±0.38 days (Table 6). In both species, the typical behaviour of newly hatched nymphs was observed in the isolators to crowd onto the empty egg chorions and remain on them until they passed into the second nymphal instar.

It is interesting that in both species the development of the 2nd instar occurs faster under field conditions than under laboratory conditions - on average 6.66±3.42 days for *N. viridula* and 6.95±1.68 days for the Brown marble stink bug, which can be explained by the higher air temperatures during this period.

Table 5. *Duration of nymphal stage of N. viridula and H. halys at laboratory conditions (25±2°C,u 60-70% RH, 16 L:8D)*

Nymphal instar	**1st**	**2nd**	**3rd**	**4th**	**5th**
N. viridula					
Mean ± Std. Deviation	**3,56±1,55**	**9,75±4,98**	**7,16±3,47**	**10,36±2,98**	**13,47±3,39**
Std. Error	0,09	0,44	0,31	0,27	0,32
Minimum	2	3	3	5	5
Maximum	8	29	20	19	27
Number	286	128	122	118	113
H. halys					
Mean ± Std. Deviation	**5,32±1,23**	**9,35±2,89**	**7,18±2,56**	**7,41±1,89**	**10,88 ±2,64**
Std. Error	0,09	0,23	0,22	0,18	0,29
Minimum	2	3	4	3	8
Maximum	12	25	18	17	22
Number	158	156	131	105	81

After the 2nd instar, a difference was observed in the duration of the remaining nymphal instars between the two species of stink bugs (Table 6). For the southern green stink bug, development is faster than under laboratory conditions, averaging 5.94±2.37 days, but for the brown marmorated stink bug it takes 19.03±3.74 days.

The same features are also observed in the development of the 4th nymph instar - again for the southern green stink bug under field conditions it takes place a little faster - on average 6.99±5.20 days compared to laboratory conditions, and for the brown marmorated stink bug it is much longer – 17.18±2.58 days.

The duration of the 5th instar under field conditions for the southern green stink bug was slightly longer than that under laboratory conditions - 12.38±3.77 days, while for the brown marmorated stink bug it was 30.7±5.14 days. The large difference in the duration of development of the nymphal stage under laboratory and field conditions is due to variable air temperatures especially those below 15°C or above 35°C, at which development stops (Haye et al., 2014).

Table 6. *Duration of the nymphal stage of N. viridula and H. halys at field conditions in the region of Plovdiv in 2020*

Nymphal instar	**1st**	**2nd**	**3rd**	**4th**	**5th**
		N. viridula			
Mean ± Std. Deviation	**4,17±1,47**	**6,66±3,42**	**5,94±2,37**	**6,99±5,20**	**12,38±3,77**
Std. Error	0,06	0,19	0,13	0,31	0,23
Minimum	3	3	3	3	5
Maximum	8	23	14	31	31
Number	624	339	338	281	273
		H. halys			
Mean ± Std. Deviation	**5,17±0,38**	**6,95±1,68**	**19,03±3,74**	**17,18±2,58**	**30,7±5,14**
Std. Error	0,04	0,21	0,59	0,63	1,63
Minimum	5	5	11	11	20
Maximum	6	10	25	23	36
Number	111	64	39	17	10

During field trials in isolators, the minimum air temperature was below 15°C from June 1 to 11, 2020, June 23, June 27 and 28, July 10 and 11, and July 14 and 15 July (Appendix 1). The maximum air temperature exceeded 35°C on 30 June, 29, 30 and 31 July.

Duration of the development of one generation

In our experiments in laboratory conditions at a temperature of 25±2°C, *N. viridula* develops one generation in an average of 41.92 days (Table 7). Under similar but not the same conditions, Harris and Todd (1980) found that one generation developed in 36.7 days (25-28°C), and Kariya (1961) had an average of 34.2 days. According to Roychoudhury & Joshi (1996), *N. viridula* requires an average of 49.59 days to complete its life cycle.

We found that under the laboratory conditions in our experiments, for the development of one generation (from laying the egg to an adult), the brown marmorated stink bug needs an average of 43.23 days (Table 7). A similar duration was found in the study by Medal et al. (2013) - from 33 to 55 days or an average of 43 days, as well as in a study by Haye et al. (2014) - 42.31±0.53 days.

Under field conditions, as temperatures rise in the spring, adult stink bugs leave their hibernation sites and begin feeding. Overwintering females of the southern green stink bug lay their eggs in the first half of May, and the first generation develops by mid-July, with an average life cycle duration of 45.69±14.91 days (Fig. 38).

The increase in temperatures (average temperature 24.8°C) and the small amount of precipitation (average 17.8 mm) in August (Appendix 1) favour the faster development of the individual stages of the second generation. The average duration of development of the second generation was shorter, which is expected at the higher temperatures in this period and was 35.43±3.23 days (Fig. 38).

The average duration of development from egg to adult of the brown marmorated stink bug is 79.7±6.63 days (Fig. 38). The relatively low temperatures (average temperature 17.8°C) and significant rainfall (average 80.7 mm) in May, as well as the slow gradual rise in temperatures (average temperature 21.4°C) and prolonged rainfall (average 55, 4 mm) in June are a prerequisite for delaying the development of individual stages of the enemy, as a result of which the duration of development of one generation is significant.

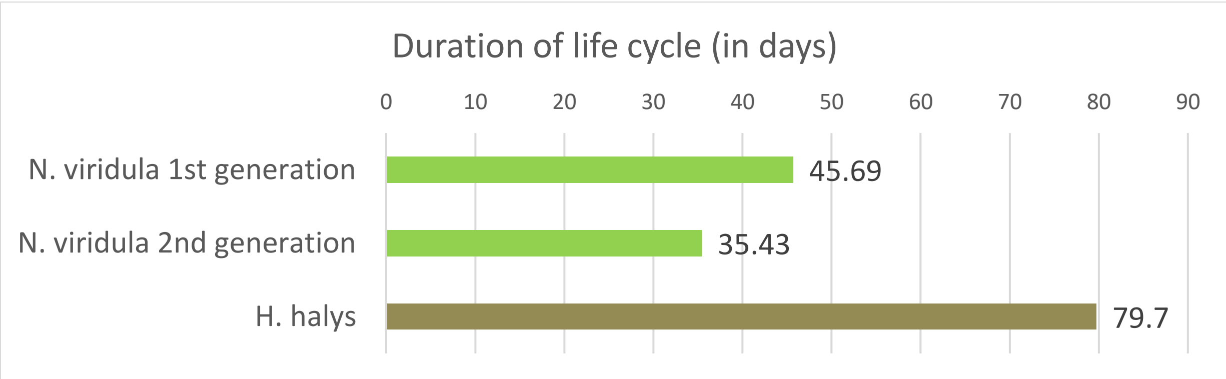
Regarding the number of generations that the southern green stink bug develops outdoors in a year, there is variation among different studies. In the conditions of the state of Louisiana, USA, the species develops 4 generations per year (Jones, 1918), and according to Drake (1920), in the southern parts of the state of Florida, it is possible to develop 5 generations. In the southern part of Japan, *N. viridula* develops 3 generations and rarely a fourth (Kiritani and Hokyo, 1962), in Egypt, it develops 3-4 generations per year (Ali et al., 1979), in Australia (Queensland) - 2 generations per year (Velasco et al., 1995).

Table 7. *Duration of the development (days) of one generation of N. viridula and H. halys at laboratory conditions (25±2°C, 60-70% RH, 16 L:8D)*

	Number	Minimum	Maximum	Mean ± Std. deviation	Std. Error
N. viridula	25	35	67	41,92±6,87	0,37
H. halys	70	34	50	43,23±3,56	0,43

Under the climatic conditions in the region of the city of Plovdiv, *N. viridula* develops 2 generations per year.

Figure 38. *Duration of the life cycle of the different generations of N. viridula and H. halys at field conditions in isolators in the region of Plovdiv in 2020*



	N	Min	Max	Mean ± Std. Deviation	Std. Error
N. viridula **1st generation**	16	28	66	45,69±14,91	3,73
2nd generation	256	29	43	35,43±3,23	0,20
H. halys	10	64	87	79,7±6,63	2,09

Продължителност на живот на възрастните и съотношение на половете

In our laboratory studies, females of *N. viridula* lived longer than males, as also observed by Chanthy et al. (2015). The duration of life in both sexes varies widely - in females from 10 to 88 days, and in males from 11 to 75 days.

The established average life span for females was 47.35±25.58 days and for males 34.81±20.97 days (Table 8). The results obtained are different from those reported by Chanthy et al. (2015), according to which females live an average of 76.1 days and males 55.6 days.

The duration of life in both sexes of *H. halys* varies widely - in females from 3 to 44 days, and in males from 2 to 36 days. The established mean life expectancy for females was 18.34±9.40 days and for males was 16.66±9.33 days, (Table 8) despite the reports of Medal et al. (2013) that the lifespan of females ranged from 9 to 16 weeks (average 84 days) and that of males from 8 to 18 weeks (average 119 days).

Table 8. *Longevity of adults of N. viridula and H. halys at laboratory conditions (25±2°C, 60-70% RH, 16 L:8D)*

Number	Sex	Minimum	Maximum	Mean ± Std. Deviation	Std. Error
N. viridula					
64	Females	10	88	47,35±25,58	5,02
36	Males	11	75	34,81±20,97	5,24
H. halys					
40	Females	3	44	18,34±9,40	1,53
40	Males	2	36	16,66±9,33	1,58

The maximum lifespan of adults in nature is different from that in laboratory conditions, which is also confirmed by our field experiments at varying minimum and maximum air temperatures. Under field conditions in isolators, the lifespan of first-generation adults of the southern green stink bug in both sexes varies widely, with a maximum of 149 days for females and 151 days for males.

The average lifespan for females is 93.74±39.24 days and for males 89.51±36.19 days (Table 9). Adults of the second generation remain to overwinter and have not been studied for life span.

Under field conditions in isolation, the lifespan of adults of the brown marmorated stink bug was found to be 86 to 145 days (mean 116.75±29.60 days) for females and 105 to 151 days, mean 135±26 days for male individuals (Table 9). Because under our conditions this species developed only one generation per year, and in nature, adults remain overwintering, our observed life spans in isolators do not provide insight into that of overwintering individuals.

Table 9. *Longevity of adults of N. viridula and H. halys at field conditions in isolators in the region of Plovdiv*

Number	Sex	Minimum	Maximum	Mean ± Std. Deviation	Std. Error
N. viridula					
74	Females	7	149	**93,74±39,24**	3,29
73	Males	22	151	**89,51±36,19**	3,45
H. halys					
5	Females	86	145	**116,75±29,60**	14,80
5	Males	105	151	**135±26**	15,01

The sex ratio of the emerged adults of the southern green stink bug under laboratory and field conditions is in favour of females: of 100 adults who emerged in the laboratory, females are 64% (64♀:36♂), and of 272 emerged under field conditions, females predominate individuals with 56% – 151♀: 121♂ (Fig. 39).

For the brown marmorated stink bug, the sex ratio based on 80 adult bugs that emerged in our laboratory experiments was equal to female to male (40♀:40♂).

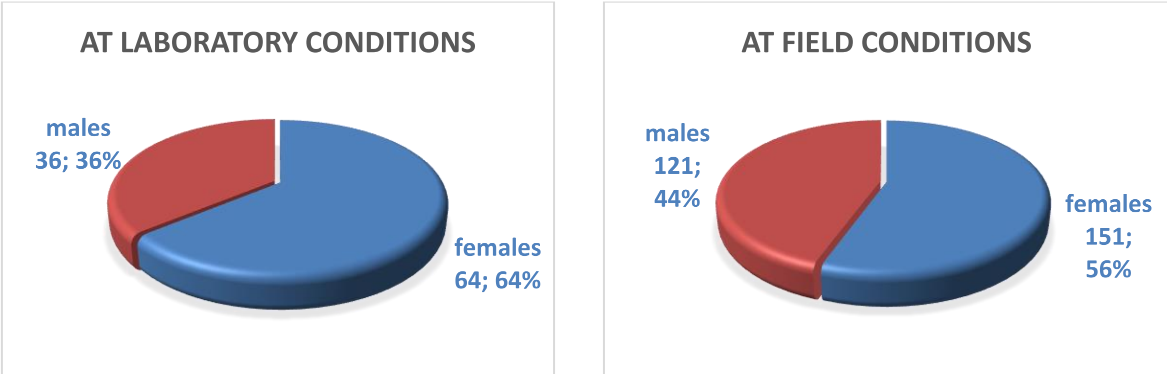
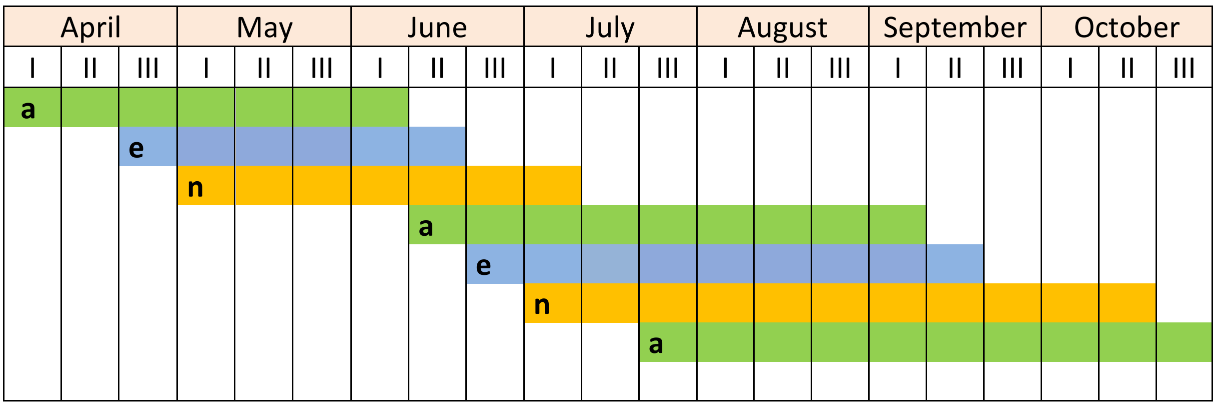


Figure 39. Ration between males and females of *N. viridula*

Observations on the phenological development under field conditions from 2018 to 2021 are summarized in Fig. 40. Adults of the overwintering generation of the southern green stink bug begin laying eggs in late April and continue until the end of the second ten days of June. Nymphs of the first generation are found from the beginning of May until the end of the first ten days of July. Adults of the first generation appear from the beginning of the second ten days of June and meet until the second ten days of September. Females of this generation start laying their eggs at the end of June, with the last eggs being found by the end of the second ten days of September.

Nymphs of the second generation can also be found until the third ten days of October. The adults of the second generation emerge in the third ten days of July, feed for a while and retire in search of wintering sites.

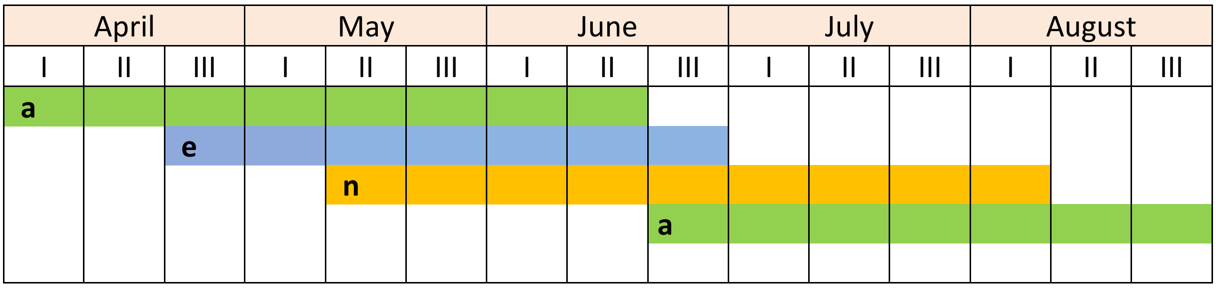
Figure 40. *Phenological development of N. viridula in the region of Plovdiv in 2018-2021*



a-adult; e-egg; n- nymph

Nymphs of the second generation can be found until the third ten days of October. During the observations made on the phenological development of the brown marmorated stink bug under field conditions, it was established that for one calendar year, under the climatic conditions of 2019 - 2021 in the region of the city of Plovdiv, the enemy managed to develop only 1 full generation (Fig. 41). Adults of the second generation appear in the third ten days of July, feed for some time and withdraw in search of places for wintering. Overwintering adults that spend the winter months in reproductive diapause live very long. After reactivation in the spring, they feed for a while and start laying eggs in the third ten days of May. Eggs are discovered by the end of July, and nymphs begin to hatch in the second ten days of June. Nymphs of the last 5th instar were found until the end of the first ten days of September.

Фиг. 41. *Phenological development of H.halys in the region of Plovdiv in 2019 -2021*



a-adult; e-egg; n- nymph

The first adults of the new generation appear in the third ten days of July. They feed for some time and at the end of September, they head to the wintering grounds.

4.3.2.REPRODUCTIVE BEHAVIOUR

Our observations show that males and females of *N. viridula* begin to copulate on average 14.73±5.39 days after emergence under laboratory conditions and on average 16.17±1.47 days in the field (Table 10).

Table 10. *Time from emergence to the first copulation in N. viridula and in H. halys at laboratory and field conditions (in days)*

Species	Mean±Std. Deviation	Std. Error	Minimum	Maximum	Number
At laboratory conditions					
N. viridula	14,73±5,39	1,62	7	23	11
H. halys	17±6,98	2,64	8	31	7
At field conditions					
N. viridula	16,17±1,47	0,60	14	18	6
H. halys	20,4±5,55	2,48	15	27	5

According to Mitchell and Mau (1969), females begin to copulate 5 days after emergence and males after 6 days. In another study, Chanthy et al. (2015) found that at 25°C newly emerged adults began to copulate after 25.8 days, and at 33°C after 11.5 days. In our experiments, the brown marmorated stink bug began to copulate 17±6.98 days after emergence under laboratory conditions and later under field conditions – 20.4±5.55, respectively (Table 10).

Observations on the behaviour of adults show that before copulation, male and female individuals "court" for about 10-15 minutes. There are conflicting opinions about the role of a pheromone secreted by males of the southern green stink bug, which according to Mitchell and Mau (1971) strongly attracts females, but according to Harris and Todd (1980), this substance plays the role of an aggregation pheromone rather than of sex pheromone. Without commenting on its role, Brennan et al. (1977) stated that males secrete the pheromone most strongly by the seventh day after emergence.

In the southern green stink bug, copulation under laboratory conditions lasted an average of 32.67±15.27 hours (Table 11), which is similar to that found by Harris and Todd (1980). In contrast to *N. viridula*, copulations are much shorter in the brown marmorated stink bug - an average of 4.71±1.98 hours, and a female copulates 1 to 3 times in her lifetime (Fig.42-43).

Figure 42. *Mating adults of N. viridula*

Figure 43. *An egg-laying female of N. viridula*

Table 11. *Duration of copulation in N. viridula and H. halys at laboratory and field conditions (in hours)*

Species	Mean±Std. Deviation	Std. Error	Minimum	Maximum	Number
At laboratory conditions					
N. viridula	32,67±15,27	3,59	8	68	18
H. halys	4,71±1,98	0,75	2	8	7
At field conditions					
N. viridula	26,67±8,05	2,68	22	48	9
H. halys	1,99±1,04	3,38	0,17	4,66	7

In the laboratory observations made, it was found that copulations mostly begin at night. Similar behaviour was also observed by Kawada Kitamura (1983).

Under field conditions, copulation in both bug species is shorter - in the case of the southern green stink bug, it varies from 22 hours to 48 hours, and its average duration is 26.67±8.05 hours (Table 11). Copulations take place on all aerial parts of plants.

The duration of copulations in the brown marmorated stink bug varies widely - from 10 minutes to 4 hours and 40 minutes, and the average duration is 1 hour and 59 minutes (Table 11). All observed copulations took place in shaded areas on all aerial parts of the plants.

Under laboratory conditions, Kawada and Kitamura (1983) found that *H. halys* adults copulated several times a day (5.28 times on average), with copulations being longest at night (2-3 hours). According to Medal et al. (2013), copulations were of short duration (between 8.43 to 11.00 minutes or an average of 10.15 minutes).

Duration of pre-oviposition and oviposition period and fecundity

The average length of the pre-oviposition period of *N. viridula* under laboratory conditions is 32.54±5.92 days (Table 12), which is similar to that found by Jones (1918) - about 4 weeks. Nielsen et al. (2008) found that at a temperature of 25°C the pre-oviposition period was 13.35±0.72 days. Hay et al. (2014) also investigated the length of the pre-oviposition period and found that at 20°C and 16 hours of light, females started laying on average after 25.17 days, and at 25°C after 12.17; days.

The egg-laying period of N. viridula observed in our experiments lasted an average of 19.82±5.19 days, whereas according to Mitchell and Mau (1969), it was 7 to 8 days.

One female of *N. viridula* lays 1 to 3 egg clusters. The eggs are laid close together, sticking together with a secretion that the females secrete during laying. One female lays an average of 57.27±19.64 eggs in one clutch, and the average fecundity is 89.88±16.61 eggs (Table 12). These results largely confirm those published by Roychoudhury and Joshi (1996), according to which a female can lay from 72 to 101 eggs, and from Waterhouse (1998) from 80 to 120 eggs.

Table 12. *Pre-oviposition period and fecundity of N. viridula and H. halys at laboratory conditions (25±2°C, 60-70% RH, 16 L:8D)*

	Pre-oviposition period (days)	**Fecundity (number)**	**Number of eggs in a cluster**
N. viridula			
Mean ± Std. Deviation	**32,54±5,92**	**89,88±16,61**	**57,27±19,64**
Minimum	23	29	27
Maximum	43	201	83
Std. Error	1,79	16,62	5,92
Number	11	9	11
H. halys			
Mean ± Std. Deviation	**17±6,98**	**45,08±16,42**	**23,96±6,76**
Minimum	8	17	3
Maximum	31	80	29
Std. Error	2,64	4,74	1,21
Number	7	12	31

The pre-oviposition period of the brown marmorated stink bug under laboratory conditions lasts an average of 17±6.98 days. The observations of other authors are similar - 13.35±0.72 days according to Nielsen et al. (2008) and 12.17 days, at 25°C and 16 h photoperiod according to Haye et al. (2014).

In the laboratory, females lay their eggs mostly in the evening on folded sheets of filter paper. Eggs are laid in groups, sticking firmly to the substrate. During the observations, it was found that one female lays from 1 to 3 egg clusters, and the average fecundity is 45.08±16.42

eggs (Table 12). These results differ from the observations of other authors, according to which a female lays an average of 8 egg clutches and the average fecundity is 212.25 eggs (Nielsen et al., 2008). An average of 23.96±6.76 eggs was counted per egg cluster, which is similar to that reported by Sargent (2011), who reported that an egg cluster contained 20 to 30 eggs.

Table 13. *Pre-oviposition period and fecundity of N. viridula and H. halys at field conditions in isolators in the region of Plovdiv in 2020*

	Pre-oviposition period (days)	Fecundity (number)	Number of eggs in a cluster
N. viridula			
Mean ± Std. Deviation	**25±1,41**	**78,21±19,46**	**47,60±27,93**
Minimum	24	18	12
Maximum	26	279	106
Std. Error	1, 97	19,46	5,82
Number	9	14	23
H. halys			
Mean ± Std. Deviation	**20,4±5,50**	**30,4±14,52**	**21,67±7,00**
Minimum	15	14	13
Maximum	27	54	28
Std. Error	2,48	6,49	2,86
Number	5	5	6

Under field conditions, the pre-oviposition period of the southern green stink bug lasts an average of 25±1.41 days, and of the brown marmorated stink bug - an average of 20.4±5.50 days (Table 13). In both species, females prefer to lay their eggs on the underside of leaves. Females of the southern green stink bug lay 1 to 5 clutches of eggs, and those of the brown marmorated stink bug lay 1-2 clutches of eggs. In one egg cluster of *N. viridula,* there are on average 47.60±27.93 and of *H. halys* - 21.67±7.00 eggs. The average fecundity of *N. viridula* was 78.21±19.46, and of *H. halys* - 30.4±14.52 eggs (Table 13).

4.4. PREDATORY AND PARASITOID SPECIES FROM NATURAL POPULATIONS

4.4.1. PREDATORY AND PARASITOID SPECIES FEEDING ON *N. VIRIDULA*

During the study period, a total of 5 species of parasitoid on the southern green stink bug and 1 predator were identified. Although a large number of polyphagous predators such as ladybirds, goldeyes, ants, and predatory bugs, some polyphagous species such as crickets that can feed on eggs and nymphs of the southern green stink bug are reported in the literature, in our 3-year observations under field conditions, feeding on eggs of the southern green stink bug was found only by adults of the earwig (*Forficula auricularia* (Linnaeus, 1758)).

The established parasitoids belong to four families: four species of egg parasitoids from the families Scelionidae, Eupelmidae and Encyrtidae and one parasitoid of the adults from the family Tachinidae (Table 14).

Only one of the parasitoids has been reported in Bulgaria on eggs of the southern green stink bug - *Anastatus bifasciatus* (Mirchev, 2014). The species *Ooencyrtus telenomicida* has been reported as a parasitoid on the eggs of the processionary moth T*haumetopoea solitaria*

(Lepidoptera: Notodontidae) (Boyadzhiev et al., 2017). This, as well as the other species of parasitoids (Table 14) we report for the first time in Bulgaria for the southern green stink bug as a host.

The first parasitized eggs of *N. viridula* were observed in raspberry plantations in the village of Joakim Gruevo, from where all imaginal adult parasitoids were identified as *Trissolcus basalis* (Wollaston) (Figs. 44 and 45). This species was reported by Jones (1988) as the most common and most effective egg parasitoid of *N. viridula*.

On hibiscus and mulberry in 2020 in the city of Stamboliyski, the presence of parasitized egg clusters was found. When reared under laboratory conditions, two species of egg parasitoids were isolated from these eggs, which were subsequently identified as *Anastatus bifasciatus* (Geoffroy) (Figs. 46 and 47) and *Trissolcus basalis* (Wollaston).

Table 14. *Predatory and parasitoid species on N. viridula from natural populations in the region of Plovdiv and Paradzhik in 2019-2021*

Order	Family	Species
Hymenoptera	Scelionidae	*Trissolcus basalis* (Wollastone, 1858)
	Eupelmidae	*Anastatus bifasciatus* (Geoffroy, 1785)
	Encyrtidae	*Ooencyrtus telenomicida* (Vassiliev, 1904)
		Ooencyrtus sp. (Ashmead, 1900)
Diptera	Tachinidae	*Trichopoda pennipes* (Fabricius, 1781)
Dermaptera	Forficulidae	*Forficula auricularia* (Linnaeus, 1758)

Parasitized eggs were collected on tomatoes and potatoes in the city of Plovdiv, from which three species of egg parasitoids were subsequently isolated - *Trissolcus basalis*, *Ooencyrtus telenomicida* (Fig. 48 and 49) and *Ooencyrtus* sp. *Anastatus bifasciatus* and *Ooencyrtus telenomicida* are polyphagous and parasitize the eggs of many species of Hemiptera (Randoni et al., 2017).

Regulating capacity of the established parasitoid species

The degree of egg parasitism by established egg parasitoids in 2020 gradually increased towards the end of the season - from 7.77% in May, 34.32% and 72.30% in June and July to 100% in August (Fig. 50). In 2021, the same trend is observed - a low degree of parasitism in May - only 0.25%, and the highest degree of parasitism was recorded in July 80.51% and August 89.79% (Fig. 51).

Figures 44 and 45. *Adults of the egg parasitoid Trissolcus basalis*

Figures 46 and 47. *Adult of the egg parasitoid Anastatus bifasciatus*

Figures 48 and 49. *Adults of the egg parasitoid Ooencyrtus telenomicida (female – left, and male- right)*

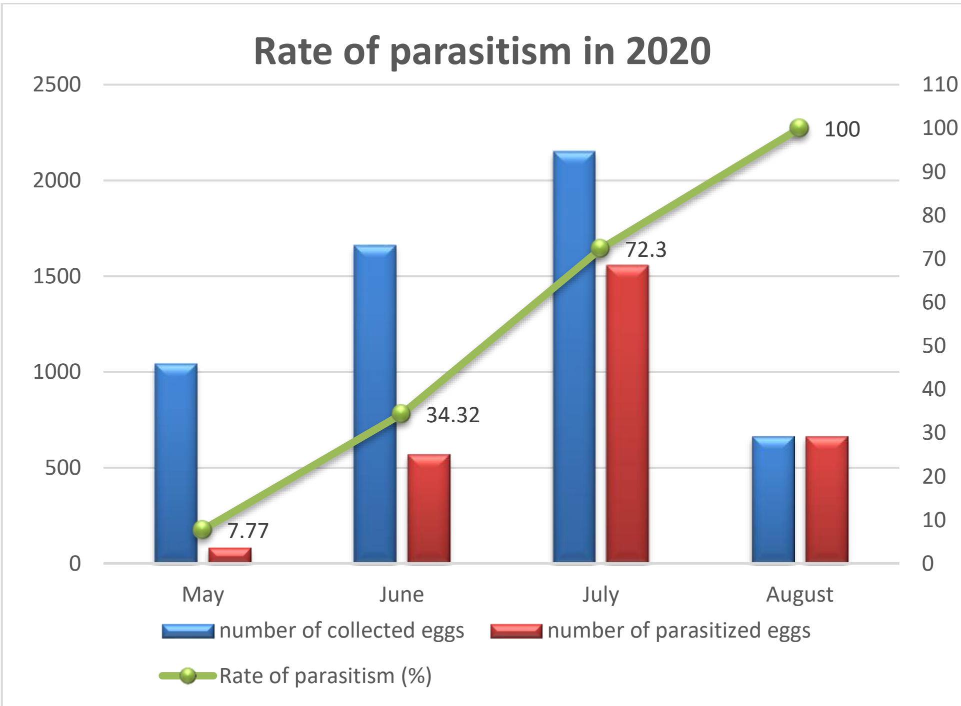


Figure 50. *Rate of parasitism on the eggs of N. viridula in 2020*

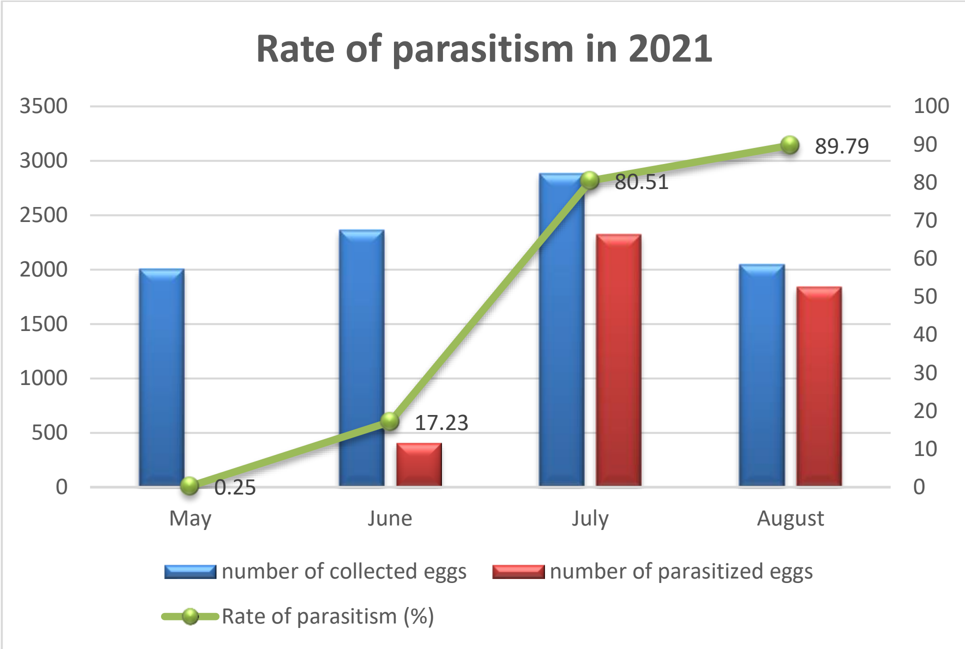


Figure 51. *Rate of parasitism on the eggs of N. viridula in 2021*

In addition to the degree of parasitization of the eggs, the degree of visualization of the egg parasitoids from the parasitized eggs was also determined (Table 15). This is an indicator that provides information on the survival of the egg, larval and pupal stages of the respective egg parasitoid. In July and August 2020, a higher degree of egg parasitoid visualization was observed - 67.80% and 71.84%, respectively, while in 2021 it did not exceed 47.28%.

Table 15. *Rate of emergence of the parasitoids from the eggs of N. viridula in 2020 - 2021*

Month	Parasitized eggs (number)		Emerged parasitoids (number)		Rate of emergence (%)	
	2020	2021	2020	2021	2020	2021
May	81	5	1	1	1,23	20
June	570	407	292	139	51,23	34,15
July	1556	2322	1055	975	67,80	41,99
August	664	1838	477	869	71,84	47,28

It was found that under field conditions, *Trissolcus basalis* was the predominant species, followed by *Ooencyrtus telenomicida* and *Ooencyrtus* sp. (Fig. 52), while in urban environments in Stamboliyski and Plovdiv, the prevailing species is *Anastatus bifasciatus*, followed by *T. basalis* (Fig. 53).

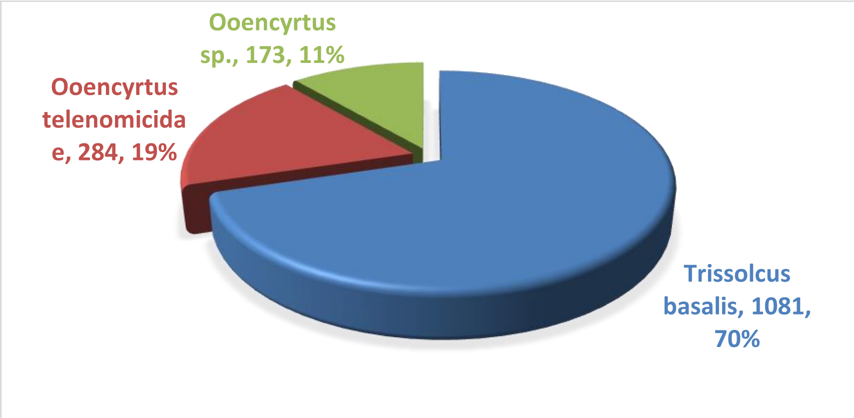


Figure 52. *Ration of the parasitoid species emerged from eggs of N. viridula (number and %) in tomato and potato crops in Plovdiv in 2020*

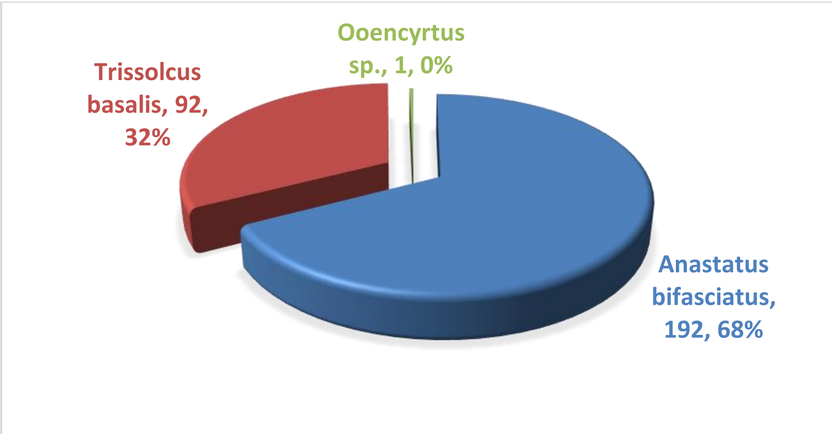


Figure 53. *Ration of the parasitoid species emerged from eggs of N. viridula (number and %) in biocenoses of hibiscus and mulberry (number and %) in the region of Plovdiv in 2020*

Investigation of the parasitoid *Trichopoda pennipes* (Fabricius)

According to Colazza et al. (1996), who first reported the species in Europe, it is new to the continent. In our observations, it was detected for the first time on stink bugs collected from corn near the village of Yagodovo, Plovdiv region in 2019, from where 27 parasitized adult specimens of N. viridula were collected, of which 21 were male and 6 were female. 5 parasitized adult specimens of N. viridula (3 males and 2 females) were collected from hibiscus bushes in the city of Plovdiv.

A total of 325 parasitized adult specimens of *N. viridula* (155 males and 170 females) were collected from hibiscus bushes in the town of Stamboliyski. Nine parasitized 5th instar nymphs of *N. viridula* were collected from the same location. All collected adults were parasitized by one species of parasitoid. The eggs of the parasitoid are found on different parts of the body of the southern green stink bug - head, chest, breast shield, abdomen and very rarely on the legs.

Eggs are laid mainly on the dorsal side of the thoracic segments and some on the ventral side (Figs. 54 and 55). The eggs of the parasitoid are whitish in colour and oval in shape and are easily spotted against the green colour of the stink bug's body.


Figures 54 and 55. *Eggs of the parasitoid Trichopoda pennipes laid on an adul and 5th instar nymph of N. viridula*

The newly hatched larva of the parasitoid penetrates the body cavity of the stink bug by gnawing an opening in the body covering at the place where the egg was laid (Fig. 56). A few days later, after placing the parasitized bugs in the Petri dishes, the parasitoid larva exits the host's body through an opening at the end of the bug's abdomen (Fig. 57).

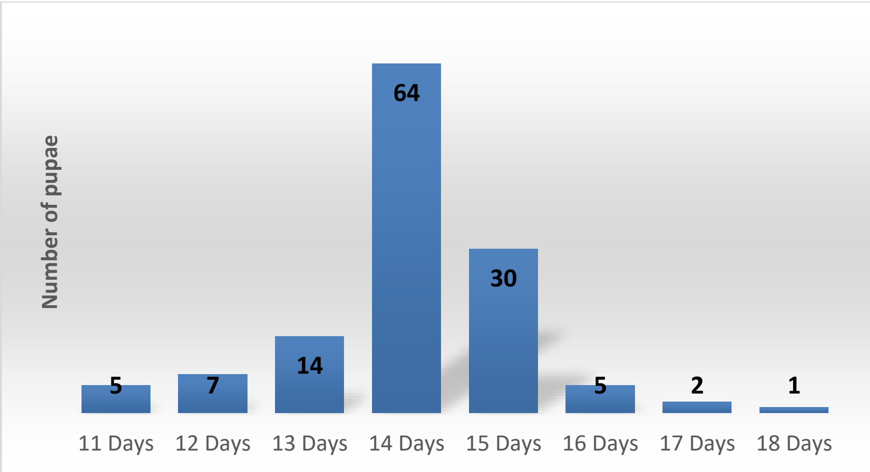

Figures 56 and 57. *Eggs and freshly hatched larva of the parasitoid Trichopoda pennipes on the scutellum of N. viridula (left) and larva of T. pennipes leaving the body of N. viridula (right)*

Within a few hours, the larvae of the parasitoid metamorphose into a dark reddish-brown puparium formed by the last integument of the larva (Figs. 58-59).


Figures 58 and 59. *Larvae and pupae of Trichopoda pennipes*

The average duration of the pupal stage of *Trichopoda pennipes* is 14.05 ± 1.59 days, with most adults emerging after 14-15 days. As seen in fig. 60, only 1 and only 2 pupae had developmental durations of 18 and 17 days, respectively. A total of 129 adult flies emerged from 181 pupae and they were all of one species - *Trichopoda pennipes* (Fabricius) (Figs. 61 and 62).

Figure 60. *Duration of the pupal stage (in days) of Trichopoda pennipes under laboratory conditions at 25±2ºC*



Mean	14,05
Standard Error	0,10
Standard Deviation	1,16
Minimum	11,00
Maximum	18,00

Figures 61 and 62. *Adults of T. pennipes in the laboratory and at field conditions on a tomato leaf*

Regulating potential of *Trichopoda pennipes*

In 2020, based on the adults of the southern green stink bug collected from different biocenoses, it was found that the degree of parasitism observed in overwintering stink bugs was low and varied from 4.25% to 11.65% (Table 16). In September, the adults of the second generation had a higher degree of parasitism, which reached 35%.

In 2021, a lower degree of parasitism was observed, reaching only 2.83% in the overwintering bugs (Table 17), 12.38% in the adults of the 1st generation, and 12.38% in the adults of the 2nd generation - 10.17%.

Table 16. *Rate of parasitism of the adults of N. viridula in the region of Plovdiv in 2020*

Month	Number collected	Number parasitized adults	Males	Females	Rate of parasitism (%)
Март	94	4	1	3	4,25
Април	103	12	5	7	11,65
Септември	120	42	9	33	35
Общо	317	58	15	43	-

Table 17. *Rate of parasitism of the adults of N. viridula in the region of Plovdiv in 2021*

Month	Number collected	Number parasitized adults	Males	Females	Rate of parasitism (%)
Май	106	3	2	1	2,83
Юли	113	14	13	1	12,38
август	118	12	5	7	10,17
Общо	337	29	20	9	-

It cannot be stated with certainty that the parasitoid prefers to lay eggs on the body of females or males, since in 2020 a predominance of parasitized females was observed, the ratio being 43♀:15♂, but in 2021 .– exactly the opposite – 9♀:20♂. However, it can be safely concluded that the parasitoid *T. pennipes* parasitizes mainly the adult stink bugs and much less often the nymphs, and then only the nymphs of the last 5th instar.

Regardless of the number of eggs laid on the body of a host, only one larva of the parasitoid completes its development. In all probability, cannibalism occurs between the hatched larvae within the body of the host, and only the strongest or first hatched succeeds in completing development, but this would be the subject of a different study.

4.4.2. PREDATORY AND PARASITOID SPECIES ON *H. HALYS*

It should be noted that during the first two years of the study, not a single parasitized egg of the brown marmorated stink bug was found. In 2021, however, we found a total of 5 species of egg parasitoids on the brown marmorated stink bug from the families Scelionidae, Eupelmidae and Encyrtidae (Table 18).

Table 18. *Predatory and parasitoid species, found on H. halys in the region of Plovdiv and Pazardzhik in 2021*

Order	Family	Species
Hymenoptera	Scelionidae	*Trissolcus basalis* (Wollastone, 1858)
		Trissolcus cultratus (Mayr, 1879)
	Eupelmidae	*Anastatus bifasciatus* (Geoffroy, 1785)
	Encyrtidae	*Ooencyrtus telenomicidae* (Vassiliev, 1904)
		Ooencyrtus sp. (Ashmead, 1900)
Dermaptera	Forficulidae	*Forficula auricularia* (Linnaeus, 1758)

None of the established species has been reported in Bulgaria on the host Brown marmorated stink bug. An undetermined species of the genus Ooencyrtus was reported by Boyadzhiev et al. (2017) on eggs of the processionary moth *Thaumetopoea solitaria* (Lepidoptera: Notodontidae).

The first parasitized eggs were found on mulberry trees in the city of Stamboliyski (Fig. 63). Under laboratory conditions, parasitoids were imaged from the collected parasitized eggs, which were identified as *Anastatus bifasciatus* (Geoffroy). According to Haye et al. (2015), *Anastatus bifasciatus* is a valuable potential candidate for biological control because, at the time of the study, it was the only European species that successfully developed in the eggs of the brown marmorated stink bug.

Four types of egg parasitoids - *Trissolcus cultratus* (Mayr)(Fig.64), *Trissolcus basalis* (Wollaston), *Ooencyrtus telenomicida* and *Ooencyrtus* sp. were isolated from parasitized eggs collected from different areas in Plovdiv and Pazardzhik regions.

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 in eggs of *N. viridula*.

Ooencyrtus telenomicidae and *Anastatus bifasciatus* are polyphagous and parasitize the eggs of many species of Hemiptera (Rondoni et al., 2017). In our observations, the parasitoid *Anastatus bifasciatus* predominates on eggs of the brown marmorated stink bug laid on plants in urbanized environments, while *Ooencyrtus telenomicidae* is mostly isolated from eggs laid on crops.

During our observations, a study by Rot et al was published. (2021), who reported the same preference of *Anastatus bifasciatus* for bug pollinations inhabiting plants in an urbanized environment.

Figure 63. *Parasitized eggs of H. halys*

Figure 64. *Adult of the egg parasitoid Trissolcus cultratus*

Egg parasitism rates in 2021 were relatively low, ranging from 10.57% reported in June to 21.17% in July (Fig. 65).

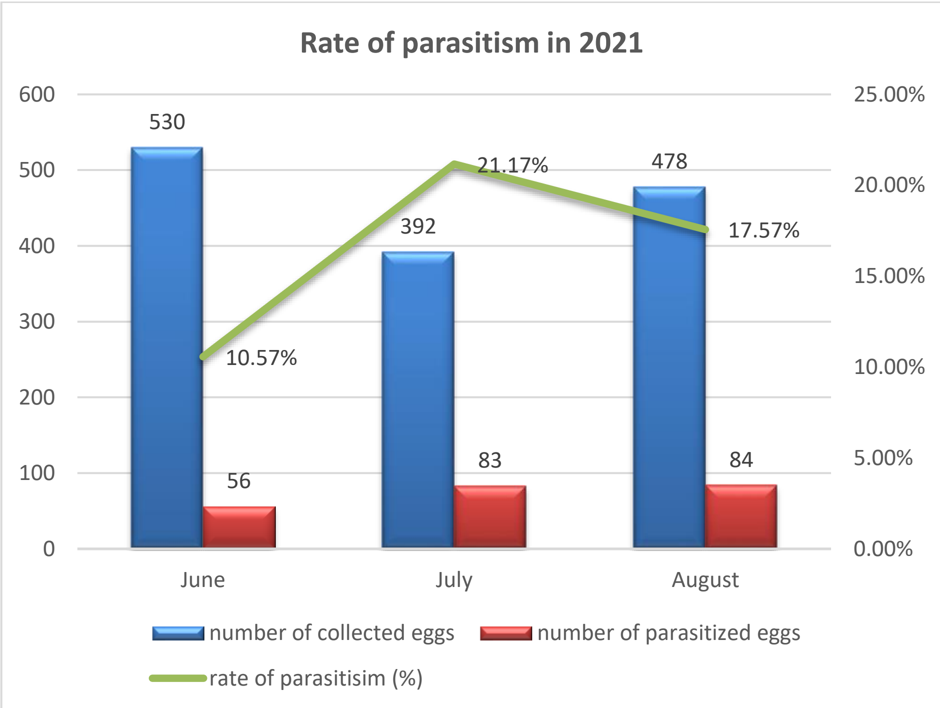


Figure 65. *Rate of parasitism of H. halys in the region of Plovdiv in 2021.*

The predominant parasitoid species is *Ooencyrtus telenomicidae*, followed by *Trissolcus cultratus, Trissolcus basalis* and *Anastatus bifasciatus*.

4.5.BIOLOGICAL EFFICACY OF PLANT PROTECTION PRODUCTS (PPPs)

4.5.1. BIOLOGICAL EFFICACY OF PPPs AGAINTS *N. VIRIDULA*

The tested biological preparations on a plant basis - Biopren plus, Neem Azal, Pyregard and Limocid - show different biological efficacy against the nymphs of the 5th instar of the southern green stink bug. It is noteworthy that on the 3rd day after treatment with Neem Azal in the two-fold increased concentration of 0.4%, mortality was 0%. We believe it is due to the observed and described by Abudulai et al. (2003) repellent action. Apparently, by the third day, the repellent effect is manifested and the nymphs avoid feeding, which explains the lack of mortality.

With Pierregard, nymph mortality ranged from 44% to 77.8% on the seventh day. An increase in mortality was observed with Neem Azal, again at concentrations of 0.1% and 0.2% it was higher and ranged from 44.4% to 66.7%, compared to the high concentration of 0.4%, where it reaches 11.11%. they avoid feeding, which explains the lack of mortality. On the 9th day after treatment, 100% mortality of the nymphs was reported for the products Neem Azal and Biopren plus, while for Pyregard, 71.43% mortality was reported at a concentration of 0.04%, and at 0.08% and 0.16% she is 100%. The weakest effect against stink bug nymphs is demonstrated by Limocid. On the 9th day after its application, the mortality of nymphs at concentrations of 0.2% and 0.4% varied between 46.67% and 53.33%, and at 0.8% it reached 86.67%.

On day 3, 55.6% to 66.7% nymph mortality was observed with the standard Mospilan, but on days 7 and 9, 100% nymph mortality was observed at all three concentrations (Fig. 66) .

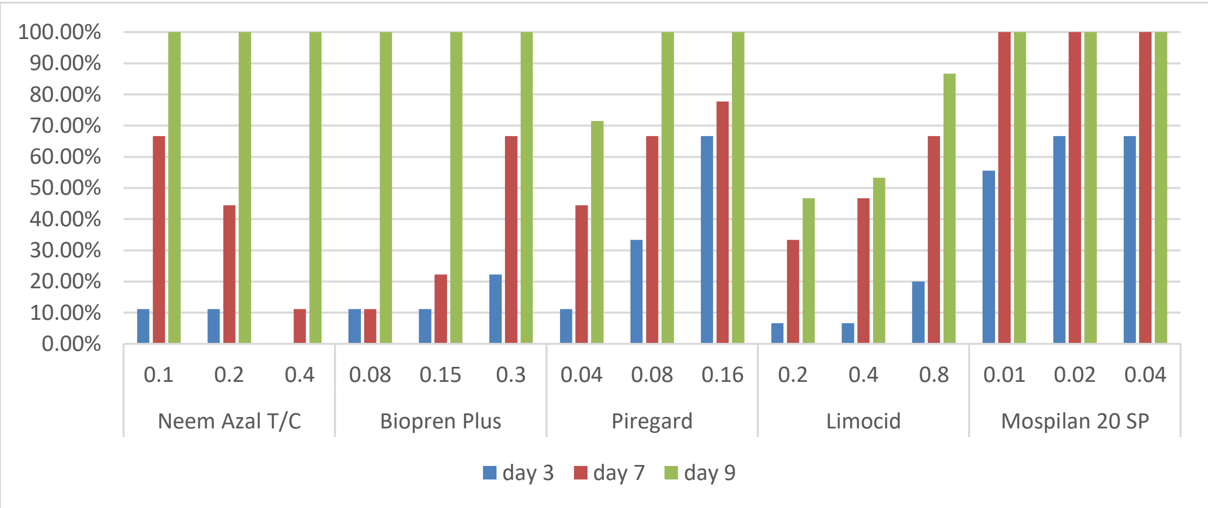


Figure 66. *Biological efficacy of plant-based insecticides against 5th instar nymphs of the southern green stink bug under laboratory conditions*

Of tested biological preparations based on microorganisms, on the 7th day after treatment, Preferal showed the best effect. At concentrations of 0.05% and 0.1%, mortality was 88.9%, and at 0.2%, mortality was 100%. For Naturalis, mortality at individual concentrations varied between 26.67% (at 0.05%) and 86.67% (at 0.2%). In Sineis, nymph mortality ranged from 44% to 77.8% on the seventh day. On the 9th day after treatment, 100% mortality of the nymphs was reported for the products Preferal and Sineis. The weakest effect against stink bug nymphs was observed with Naturalis. On the 9th day after its application, it caused 66.67% and 86.67% mortality at concentrations of 0.05% and 0.1%, while at a concentration of 0.2% the mortality of nymphs reached 100% (Fig. 67). On the 9th day after treatment, all tested insecticides reached 100% efficacy at doubled concentrations, but at the officially registered, they all had less efficacy compared to that of the reference Mospilan.

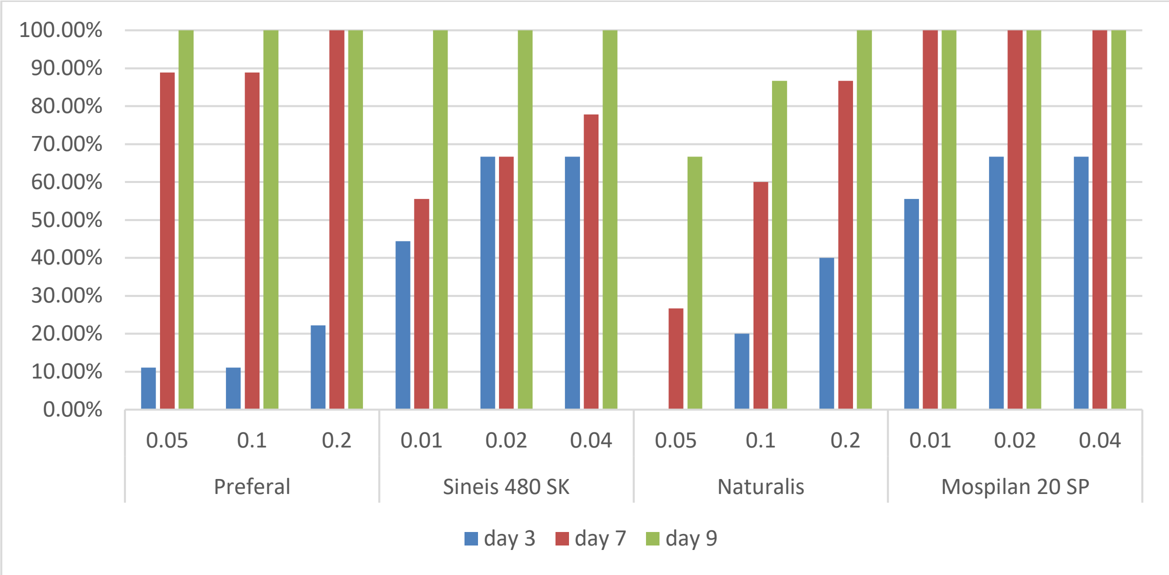


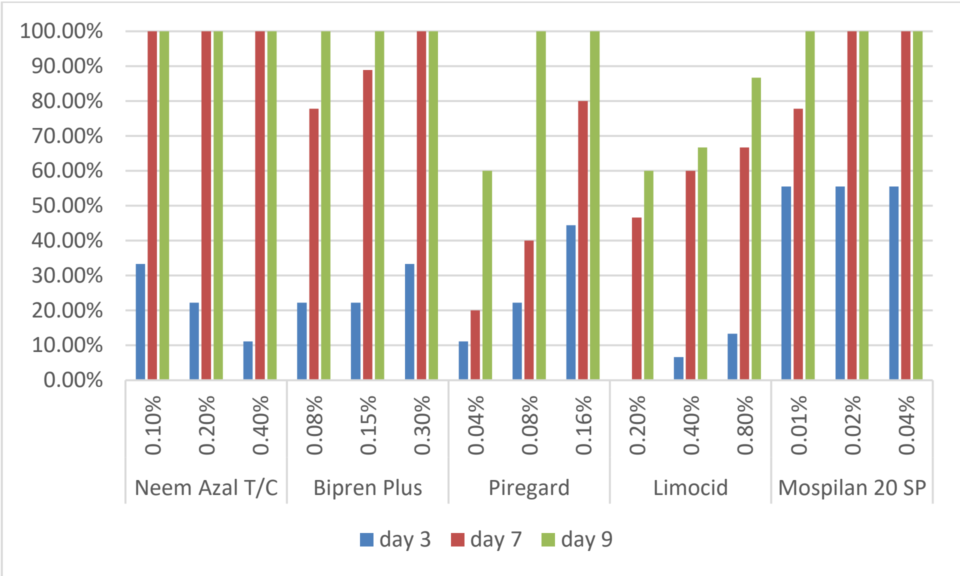
Figure 67. *Biological efficacy of microorganism-based insecticides against 5th instar nymphs of the southern green stink bug under laboratory conditions*

EVALUATION OF BIOLOGICAL EFFICACY OF PPPs AGAINST *H. HALYS*

The plant protection products selected for testing do not have official registration in the Republic of Bulgaria for use in the control of the brown marmorated stink bug.

The tested plant-based PPPs Neem Azal, Biopren plus, Limocid and Pyregard showed different biological efficacy against the 5th instar nymphs of the brown marmorated stink bug. On day 3, nymph mortality in Neem Azal, Biopren plus and Pyregard treatments ranged from 22% to 44%. In Neem Azal, higher nymph mortality was observed at the low concentrations (0.1% and 0.2%), similar to that observed in the southern green stink bug experiment, at 22% and 33%, respectively. At a concentration of 0.4%, mortality was 11%. As with the nymphs of the southern green stink bug, the preparation also has a repellent effect here. The weakest biological efficacy against nymphs was observed with Limocid. At low concentrations, no dead nymphs were recorded, and at high concentrations, mortality reached only 13.33%.

On the 7th day, Neem Azal and Biopren Plus showed higher efficacy compared to Pyregard. In the Neem Azal treatment, 100% nymph mortality was reported at all three concentrations (0.1%, 0.2 and 0.4%), and with Biopren Plus it varied from 77% at the low concentration (0.08%) up to 100% at the high concentration (0.3%). With Pyregard, the mortality varies between 20.00% and 80.00% depending on the concentration (Fig. 68).



Фигура 68. *Biological efficacy of plant-based insecticides against 5th instar nymphs of the brown marmorated stink bug under laboratory conditions*

On day 9, 100% nymph mortality was reported in the variants treated with Biopren plus, while Pyregard had 60% mortality at the low concentration (0.04) and 100% mortality at the other two concentrations. With Limocid, the highest mortality of nymphs was observed at the high concentration of 0.8%, but it did not exceed 86.67% (Fig. 68).

Of the tested biological preparations based on microorganisms, on the 3rd day after treatment, Sineis showed the highest efficacy, with mortality ranging from 33 to 67%. The lowest biological efficacy against nymphs was observed with naturalis. At low concentrations, no dead nymphs were recorded, and at high concentrations, mortality reached only 26.67%.

On day 7, with Sineis the mortality varied between 20.00% and 80.00% depending on the concentration. On day 9, 100% nymph mortality was reported in the Sineis-treated variants (Fig. 69). Naturalis showed 46.47% nymph mortality at the low concentration (0.05%), and at the other two concentrations it reached 93.33% (0.1%) and 100% (0.2%).

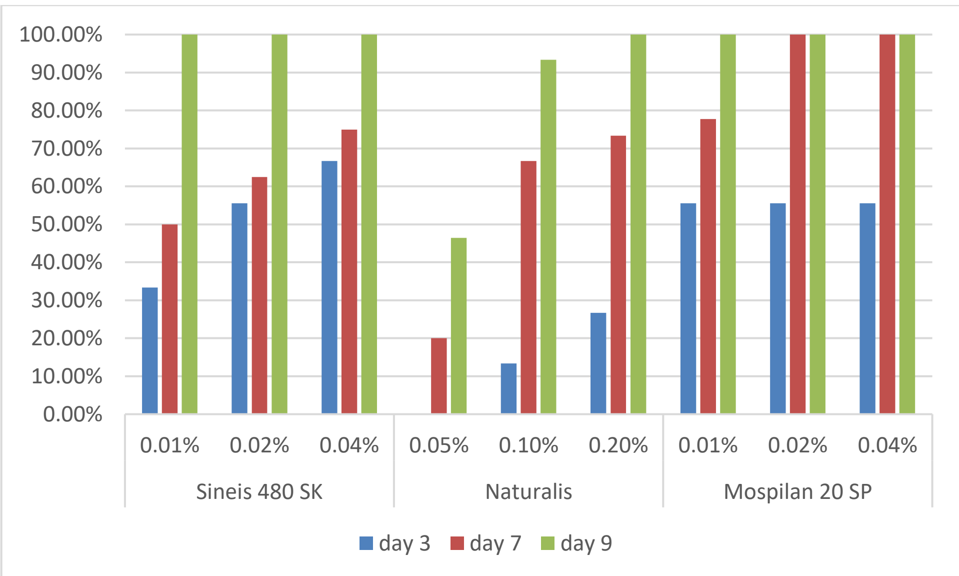


Figure 69. *Biological efficacy of microorganism-based PPPs on 5th instar nymphs of the brown marmorated stink bug under laboratory conditions*

With the standard Mospilan, which has a systemic effect and provides a long aftereffect, 56% mortality of the nymphs was recorded on the third day at all three concentrations. After day 7, mortality reached 78% at the 0.01% concentration and 100% at the other two concentrations.

Compared to the standard, the tested microorganism-based products had lower efficacy at the recorded concentrations on the 3rd and 7th day after treatment, but on the 9th day, Sineis was equivalent to Mospilan at all tested concentrations. Naturalis reaches 100% efficacy only at a doubled dose on the 9th day after treatment.

CONCLUSIONS

As a result of the conducted observations and studies, the following more important conclusions can be drawn:

1. For the conditions of Bulgaria, the two alien polyphagous species of bugs *Nezara viridula* and *Halyomorpha halys* show the greatest preference for feeding on the following cultivated and wild plant species:

N. viridula - tomato *(Lycopersicon esculentum),* raspberry *(Rubus idaeus),* green bean *(Phaseolus vulgaris),* pepper *(Capsicum annuum),* maize *(Zea mays),* hibiscus *(Hibiscus syriacus)* and apple *(Malus domestica)*

H. halys - besides those listed, mulberry *(Morus alba),* vine *(Vitis vinifera),* plum *(Prunus domestica),* pear *(Pyrus communis),* hazel *(Corylus avellana),* linden *(Tilia tomentosa)* and thuja *(Thuja orientalis).*

2. In the natural populations of *Nezara viridula* in the Plovdiv region, 3 morphological forms have been established: var. smaragdula, var. torquata and var. aurantica, of which the former predominates.

3. Under laboratory conditions (temperature 25 ± 2°C, RH 50 - 60% and photoperiod 16 L:18D), embryonic development takes an average of 6.04 days for *N. viridula* and 5.10 days for *H. halys*. Under field conditions, the duration of embryonic development was 7.57 days in *N. viridula* and 5.68 days in *H. halys*, respectively.

4. The duration of development of individual nymphal instars of *N. viridula* under laboratory conditions was the shortest for the 1st instar (3.56 days) and the longest for the 5th instar (13.47 days). The same was found for *H. halys* – 5.32 days and 10.88 days, respectively. Under field conditions, the 1st instar of *N. viridula* lasted 4.17 days and the 5th instar lasted 12.38 days. The first nymphal instar of *H. halys* has almost the same duration - 5.17 days, but the 5th - much longer - 30.7 days.

5. Under laboratory conditions, *N. viridula* develops one generation (from egg to adult) in 41.92 days, and *H. halys* - in 43.23 days.

6. Under field conditions in isolators in the Plovdiv region, *N. viridula* develops 2 generations per year, with the duration of the life cycle of the first generation being 45.69 days, and the second generation being 35.43 days. *H. halys* manages to develop only 1 generation per year and its development from egg to adult requires an average of 79.7 days.

7. The reproductive behavior of both bugs was studied and it was found that *N. viridula* adults copulate 1 to 5 times and *H. halys* adults 1 to 3 times during their lifetime. The duration of the pre-oviposition period under laboratory conditions is 32.54 days for *N. viridula* and 17 days for *H. halys*, and under field conditions - 25 and 20.4 days, respectively.

8. The southern green stink bug laid an average of 89.88 eggs under laboratory conditions and 78.21 eggs under field conditions, and the brown marmorated stink bug laid an average of 45.08 eggs under laboratory conditions and 30.4 eggs under field conditions.

9. Parasitoid species were isolated from the two species of stink bugs, which are reported for the first time in Bulgaria on these hosts:

- A total of 5 species of parasitoids were found on the host *Nezara viridula*, four of which are egg parasitoids: *Trissolcus basalis, Anastatus bifasciatus, Ooencyrtus telenomicida* and *Ooencyrtus* sp., and one is a parasitoid on the imago and nymph of the 5th instar - *Trichopoda pennipes*.
- 5 species of egg parasitoids were found on the host *Halyomorpha halys: Trissolcus cultratus, Trissolcus basalis, Anastatus bifasciatus, Ooencyrtus telenomicida* and *Ooencyrtus* sp.

10. *Nezara viridula* egg parasitoid parasitoid rates under field conditions increased from spring to autumn - up to 100% in 2020 and up to 89.79% in 2021. Egg parasitoid survival rates also increased towards the end of summer and reached 71.84% in 2020 and 47.28% in 2021.

11. On the eggs of *H. halys*, which is a recently established species for Europe and Bulgaria compared to the southern green stink bug, the degree of parasitism by the species of egg parasitoids is significantly lower - in 2021 it varied from 10.57% to 21.17%. As no parasitized eggs were detected in 2019 and 2020, we believe that, as a result of adaptation to this new host, parasitoid species parasitization rates from natural populations will gradually increase in the coming years.

12. The rate of parasitism of *N. viridula* adults by the parasitoid *Trichopoda pennipes*, new to Europe, in 2020 -2021; varies from 4.25% in early spring to 35% in autumn. No parasitization of adult *H. halys* bugs was detected in the present study.

13. Under laboratory conditions, the biological efficacy of 7 biological plant protection products was tested, of which plant-based: Neem Azal, Biopren plus, Piregrad and Limocid, and based on microorganisms: Preferal, Sineis 480 SK and Naturalis, with a chemical-based standard – Mospilan 20 SP. Of the biological insecticides, the highest biological efficacy against the nymphs of *N. viridula* was recorded with the fungal preparation Preferal, and against the nymphs of *H. halys* Neem Azal and Biopren Plus. On the 9th day after treatment, in both species, all tested products showed 100% efficacy in the registered concentrations, except for Naturalis and Limocid, and in a doubled concentration - all except Limocid.

SCIENTIFIC AND SCIENTIFIC-APPLIED CONTRIBUTIONS

I. Scientific contributions of an original nature

1. 1. For the first time in Bulgaria, different morphological forms of the southern green stink bug are reported, which are found in Pazardzhik and Plovdiv regions, as well as their percentage ratio.

2. For the first time in Bulgaria, the phenological development of *N. viridula* and *H. halys,* the duration of development of their individual stages and the number of generations per year under field conditions for the Plovdiv region have been studied.
3. For the first time in Bulgaria, the following species of parasitoids from local populations that parasitize stages of *N. viridula* are reported: *Trissolcus basalis, Ooencyrtus telenomicida, Ooencyrtus sp.,* and *Trichopoda pennipes*.

4. For the first time in Bulgaria, the following species of parasitoids from local populations that parasitize stages of *H. halys* are reported: *Trissolcus cultratus, Trissolcus basalis, Anastatus bifasciatus, Ooencyrtus telenomicida* and *Ooencyrtus* sp.
5. The degree of parasitization of the eggs by the parasitoids established for the southern green stink bug and the brown marmorated stink bug in natural conditions in different biocenoses in the Plovdiv and Pazardzhik regions was studied.
6. The degree of parasitism of adults and nymphs of the 5th instar of the southern green stink bug in different biocenoses in Plovdiv and Pazardzhik regions was studied.
7. The biological efficacy of plant protection products based on plant extracts and microorganisms, for which there are no previous studies in our country, was tested for both pest species.

II. Scientific contributions of a confirmatory nature

1. The established species of host plants in Pazardzhik and Plovdiv regions largely confirm what was observed by other authors in European countries.
2. The studies of the life cycle parameters of both species under laboratory conditions at temperature 25 ± 2°C, RH 50 - 60% and photoperiod 16 L:18D rather confirm the findings of other authors.
3. It has been confirmed that the egg parasitoid *Anastatus bifasciatus* from natural populations in Bulgaria successfully develops on the eggs of the southern green stink bug.

APPLIED CONTRIBUTIONS

1. The obtained results on the biological efficacy of the tested plant protection products can find practical application in the development of programs for IPM or in the organic farming.
2. The data on the phenological development of the two species and more specifically on the beginning of the hatching of the nymphs can be used when choosing the moment for treatment with PPPs - for the southern green skink bug it is most suitable after the first ten days of May, and for the brown marmorated stink bug – after mid-June.

Publications related to the dissertation:

Hristozova, M. (2020). Life Cycle Parameters of the Invasive Southern Green Stink Bug (*Nezara Viridula*) at Laboratory Conditions. Scientific Papers. Series A. Agronomy, 63(2).

Participation in scientific conferences:

1. 1-st International Symposium on Climate Change and Sustainable Agriculture 14-15 November 2019 – Plovdiv, Bulgaria
2. Agriculture for Life, Life for Agriculture June 4-6, 2020 – Bucharest, Romania

3. Plant Health in Sustainable Agriculture: Hot Spots and Solution Perspectives 6 - 8 September 2022 - Novi Sad, Serbia