

REVIEW



on a dissertation work for obtaining the educational and scientific degree "doctor" in: field of higher education 6. Agricultural sciences and veterinary medicine, professional direction: 6.2. Plant protection, scientific specialty "Plant protection"

Author of the dissertation: **MARIYA VALERIEVA HRISTOZOVA**, full-time doctoral student at the Department of Entomology at the Agricultural University, Plovdiv

Dissertation topic: *Biology and control options of the green stink bug *Nezara viridula* (Linnaeus) and the brown marbled bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)*

Reviewer: Prof. Dr. **MARIYANA YORDANOVA IVANOVA**, University of Agribusiness and Rural Development (UARD) - Plovdiv; higher education field: 6. Agricultural sciences and veterinary medicine, professional direction: 6.2. Plant protection, the scientific specialty "Plant protection" (Entomology), designated as a member of the scientific jury by order of the Rector of the Agricultural University (No. RD-16-1262/06.12.2023).

1. Brief introduction of the candidate.

Mariya Valerieva Hristozova was born in the town of Stamboliyski on July 18, 1989. In the period 2011-2016, she graduated from the Bachelor's degree at the Agricultural University - Plovdiv, majoring in agronomy, after which in the period of 2016-2017 she obtained the Master's degree in Plant Protection at the same university. From 01.03.2018 to 01.03.2023 she was enrolled in PhD program (order No. RD-26-20/26.02.2018), full-time study at the Department of Entomology of the Agricultural University – Plovdiv for a period of 3 years under the supervision of Prof. Dr. Vili Harizanova.

Mariya Hristozova's work experience began in 2008 as a sales consultant at Petrol AD, where she worked until October 2016. In 2021, she started working as a chief expert at the Agricultural University - Plovdiv, and from 2022 - to the present she is an assistant at Department of Entomology.

She has carried out 2 specializations - in Novi Sad, Serbia in 2021, and in Podgorica, Montenegro in 2022.

She is fluent in English - written and spoken. She has good command of Microsoft Office™ (Word™, Excel™ and PowerPoint™); She has basic knowledge on graphic design (Adobe Illustrator™, PhotoShop™). Possesses good communication skills, loyalty and ethics in relations with others, an excellent teamworker.

The PhD student took part in the following scientific and scientific-practical conferences:

- 1st International Symposium on Climate Change and Sustainable Agriculture, 14-15 November – Plovdiv, Bulgaria.
- Agriculture for Life, Life for Agriculture, 4-6 June, 2020 – Bucharest, Romania.
- Plant Health in Sustainable Agriculture: Hot Spots and Solution Perspectives September 6 - 8, 2022 – Novi Sad, Serbia.
- Scientific - practical conference "The experience of yesterday, the actions of today, the confidence for tomorrow – November 7, 2023.

2. Relevance of the problem

The dissertation submitted for review is devoted to a study of the invasive species: southern green stink bug *Nezara viridula* (Linnaeus) and brown marbled bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Invasive species are characterized by their rapid spread into new regions where they were not previously present, and this is usually associated with negative impacts on ecosystems and native species with which they compete for food, and this can lead to significant crop losses. Their ability to easily spread and adapt to different climatic conditions makes them a global problem and requires coordinated efforts at the international level. Therefore, it is necessary to develop effective and sustainable control methods combining biological, chemical and integrated strategies to manage populations of these species. In this context, Mariya Valerieva Hristozova's PhD thesis is up-to-date and can be considered as a contribution to agricultural science.

3. Objective, tasks, hypotheses and research methods.

The **objective** of this dissertation is to study the biology and control possibilities of the southern green stink bug (*Nezara viridula* (Linnaeus)) and the brown marbled bug (*Halyomorpha halys* (Stål)) for the conditions of Bulgaria.

To realize the objective set, the following more important **tasks** have been identified:

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

The studies were conducted in the period 2018 - 2023 under laboratory and field conditions. The laboratory experiments were carried out in the insectarium of the Department of Entomology, Agricultural University - Plovdiv, at an air temperature of $25 \pm 2^{\circ}\text{C}$, relative

humidity (RH) 50-60% and a photoperiod of 16L:8D without direct sunlight, at artificial lighting intensity $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. The air temperature was chosen based on previous studies by other authors, as the most favorable for the development of both types of bugs.

The observations under field conditions were carried out in the Plovdiv and Pazardzhik regions in the areas of the villages of Tsalapitsa, Isparihovo, Novo Selo, Trivodici, Joakim Gruevo, Kadievo, Kurtovo Konare, Zlatitrap, Yagodovo and Brestnik, the cities of Stamboliyski and Plovdiv.

Standard methods for entomological science were used, which were correctly selected and correctly applied. In general, the methodology of the experiments is correctly established. Mathematical data processing was performed using SPSS Statistics 26 for Windows and Microsoft Excel 365 program.

3. Transparency and presentation of the obtained results.

The dissertation submitted for review has a total volume of 126 pages. The main text contains 17 tables and 92 images and figures. The cited literature includes 252 sources, of which 1 in Cyrillic and 251 in Latin.

The dissertation is well structured and contains all the generally accepted sections, namely: introduction, literature review, aim and objectives, material and methods, results and discussion, conclusions, literature.

The literature review is thematically well-ordered, consistent with the issues considered in the dissertation and demonstrates the PhD student's good knowledge on the research problem. It is divided into 2 main parts, in which information is given about the two considered types of observed species, touching on the following topics: origin and distribution, hosts, biological features, damage activity and picture of the damage, control methods. Contemporary literature sources have been used, which are in the time range from 1918 to 2021, with 63.5% being after 2000. In the course of the discussion, the cited literature is skilfully used in analyzing the results and formulating the conclusions.

5. Discussion of results and used literature.

As a result of the precisely performed experimental work, the obtained data are presented and discussed in the Results and Discussion section (which is the main one and written in 51 pages).

The preferences of the two species for host plants were observed, and the results were summarized in tables in which the examined crop, development stage of the respective species, damage to parts of the plants and the location of the examined crop were described. The species on which females choose to lay eggs and the species on which we find young nymphs (apparently hatched on the respective plant). 26 cultivated and wild plant species were observed in the Plovdiv and Pazardzhik region.

As a result of the observations, it was found that *N. viridula* shows the greatest

preference for feeding on the following plant species: tomatoes (*Lycopersicon esculentum*), raspberry (*Rubus idaeus*), green beans (*Phaseolus vulgaris*), pepper (*Capsicum annuum*), corn (*Zea mays*), hibiscus (*Hibiscus syriacus*) and apple (*Malus domestica*). Observations show that adult *N. viridula* move from one host plant to another depending on the availability of the preferred parts. For example, in late August to mid-September, nymphs and adults congregate en masse on apple and vine, where they feed on the fruit.

The brown marbled bug, *H. Halys*, in addition to the listed plant species, also prefers mulberry (*Morus alba*), vine (*Vitis vinifera*), plum (*Prunus domestica*), pear (*Pyrus communis*), hazel (*Corylus avellana*), linden (*Tilia tomentosa*) and thuja (*Thuja orientalis*).

The morphological features of both species were studied. The eggs of *Nezara viridula* have the barrelshaped shape typical of the family Pentatomidae, and those of *Halyomorpha halys* are roughly spherical in shape. Freshly laid eggs of the first species are light yellow in color, and white to pale green color in the second one. Both species show changes in coloration during embryonic development. Both species have 5 nymphal instars, which the PhD student observed and described in terms of size, coloration. From the 5th instar nymphs of *N. viridula* observed under laboratory conditions, it was found that the light forms (with green coloration) predominated in a ratio of 60: 40 with the dark form. Of the 7 morphological forms of *N. viridula* reported in the literature, 3 were found in the present study in the Plovdiv region: var. *smaragdula* (94.3%), var. *torquate* (5.5%) and var. *aurantica* (0.2%).

Extensive studies under laboratory and field conditions have been made to establish the biological characteristics of the two invasive species. Under laboratory conditions, embryonic development was found to take an average of 6.04 days in *N. viridula* and 5.10 days in *H. halys*. Under field conditions, this duration is respectively 7.57 days for *N. viridula* and 5.68 days for *H. halys*. It was found that *N. viridula* develops one generation under laboratory conditions and two generations - under field conditions (in isolators), *H. halys* develops only 1 generation per year.

The PhD student observed the reproductive behavior of the two species and found that *N. viridula* copulates 1 to 5 times in its lifetime, and *H. halys* – 1 to 3 times. Laboratory observations show that copulations start mostly at night, copulation itself lasts shorter under field conditions in both species.

Egg productivity was studied, which was higher in the southern green stink bug under both laboratory and field conditions.

The PhD student studied predator and parasitoid species and found 5 types of parasitoids and 1 predator on the southern green stink bug, and 5 types of egg parasitoids on the brown marbled bug. The degree of parasitism by the egg parasitoids of *Nezara viridula* under field conditions increased from spring to autumn - up to 100% in 2020 and up to 89.79% in 2021. The survival rate of egg parasitoids increases towards the end of summer and reaches 71.84% in 2020 and 47.28% in 2021. In *H. halys*, which is a newer species for Europe and Bulgaria compared to southern green stink bug, the degree of parasitization by the established egg parasitoid species was significantly lower and was observed only in the third year of the study - in 2021.

A single species parasitizing on adults has been identified - *Trichopoda pennipes* was also found in 2020 – 2021, which is new to Europe, and the parasitism rate of *N. viridula* adults of this species varies from 4.25% in early spring to 35% in autumn. In the case of the brown marmorated bedbug, no parasitization of adult bedbugs was detected within the scope of the present study.

The biological efficacy of 7 insecticides with different active substances, selected from the BFSA register for authorized plant protection products or with registrations in other EU countries, was tested under laboratory conditions, of which plant-based ones are: Neem Azal, Biopren plus, Piregrad and Limocid, and based on microorganisms are: Preferal, Sineis 480 SK and Naturalis. As a standard, Mospilan 20 SP was used, which is chemically based and registered in our country against the sunn pest (corn bug).

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* Nim Azal and Biopren Plus.

For *N. viridula*, of the tested biological products 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%.

In the case of the brown marbled bug, 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%.

On the 9th day after treatment, in both species, all tested products showed 100% efficacy in the registered concentrations, with the exception of Naturalis and Limocid, and in a doubled concentration - all except Limocid.

With Mospilan (used as a standard), 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 (0.02% and 0.04%).

As a result of the precisely performed experimental work under laboratory and field conditions and the analyzes and summaries made, 13 conclusions were formulated.

6. Contributions of the dissertation work.

As a result of the good theoretical preparation, the precisely derived laboratory and field experiments, as well as the objective analysis of the data from them, the PhD student has presented scientific and scientific-applied contributions that reflect her personal achievements.

Scientific contributions

- ✓ For the first time in the country, 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.
- ✓ For the first time in the country, 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 were studied.

- ✓ For the first time in our country, the following types of parasitoids from local populations that parasitize stages of *N. viridula* are reported: *Trissolcus basalis*, *Ooencyrtus telenomicida* and *Ooencyrtus* sp., *Trichopoda pennipes*.
- ✓ For the first time in our country, the following types of parasitoids from local populations that parasitize stages of *H. halys* are reported: *Trissolcus cultratus*, *Trissolcus basalis*, *Anastatus bifasciatus*, *Ooencyrtus telenomicida* and *Ooencyrtus* sp.
- ✓ The degree of parasitization of the eggs by the parasitoids found in the southern green stink bug and the brown marbled bed bug in natural conditions in different biocenoses in the Plovdiv and Pazardzhik regions was studied.
- ✓ The degree of parasitism of the adults and nymphs of the 5th instar of the southern green stink bug in different biocenoses in the Plovdiv and Pazardzhik regions was studied.
- ✓ 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 species.

Scientific and applied contributions

- ✓ The obtained results on the biological efficacy of the tested plant protection products can find practical application in the development of schemes for integrated plant protection (IPP) or for biological production.
- ✓ The data on the phenological development of the two species, and more specifically on the beginning of the nymph hatching, can be used when choosing the moment for treatment with plant protection products - for the southern green stink bug, it is most suitable after the first ten days of May, and for the brown marbled bug – after mid-June.

I accept the contributions as actually proven in the course of the research and in the publication related to the dissertation work. I believe that they are the personal work of the PhD student.

7. Critical Notes and Questions.

Research on invasive insect species is a real challenge for the now-a-days scientists. In this context, I admire the choice of topic that the PhD student and her supervisor have chosen.

I recommend that Mariya Hristozova's future scientific research to continue in this direction. In this regard, I would like to ask the following questions:

- 1) *Does the PhD student have observations on other invasive insect species in the area or in the country?*
- 2) *Can the PhD student justify why Biopren plus and Piregard are referred to the group of pyrethroids (Table 1)?*

8. Published articles and citations.

According to the minimum scientometrics requirements specified in the Regulations for the Application of the Law on the Development of the Academic Staff, PhD student Mariya Hristozova has indicated 1 publication:

- ✓ Hristozova, M. (2020). Life Cycle Parameters of the Invasive Southern Green Stink Bug (*Nezara viridula*) at Laboratory Conditions. Scientific Papers. Series A. Agronomy, Vol. LXIII, No. 2, 113 – 117, 2020. ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785.

The publication is related to the topic of the dissertation and it summarizes and publishes the results of the conducted research. It is published in a publication indexed in the Web of Science Core Collection.

The presented abstract (48 pages) has been prepared according to generally accepted requirements and objectively reflects the structure and content of the dissertation work.

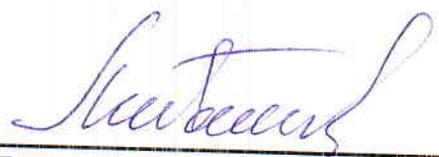
CONCLUSIONS

Based on the various research methods learned and applied by the PhD student, the correctly performed experiments, the generalizations and conclusions made, I believe that the presented dissertation work on the topic: „Biology and control options for the green stink bug *Nezara viridula* (Linnaeus) and the brown marbled bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)“ meets the requirements of the Law for development of academic staff in Republic of Bulgaria and the Regulations of the Agricultural University for its application, which gives me reason to evaluate it **POSITIVELY**.

I take the liberty of proposing to the honorable Scientific Jury to also vote positively and award Mariya Valerieva Hristozova the educational and scientific degree "doctor" in the scientific specialty 6.2. Plant protection.

Date: 09.02.2024
Plovdiv

REVIEWER:


(Prof. Mariyana Ivanova, PhD)