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EVOLUTION THE CURRENT SITUATION OF GREEN STINK BUGS POPULATIONS IN AGRICULTURAL AREAS OF TIMIS COUNTY AND EFFECTIVE CONTROL STRATEGIES

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Abstract

Green stink bugs (as polyphagous pest) are still active in agroecosystems in Romania producing negative effects in various types of agricultural and horticultural areas. In this paper, we focused on the population capacity of the pest in 8 localities belonging to Timis County, each representing a type of area, but also on the monthly dynamics during 5 months (June-October) from 2019-2022. We also focused on testing both chemical and non-polluting control products currently used by farmers and gardeners in the western part of the country. From the ones analysed, we found that in all the monitored localities the pest was present at varying levels from low to high. Regarding the monthly dynamics, the most specimens were observed in August and September, with a maximum recorded in 2020 followed by 2021. The most effective control strategy involves a chemical product applied individually, i.e Karate Zeon (lambda - cihalotrin) but also the Laser 240 SC (spinosad) product, 1-2 applications. Their combination is essential in keeping harmful populations under control.

***Key words**: control, agroecosystem, green stink bug, populations.*

INTRODUCTION

The origin of the green stink bug (*Nezara viridula* L.) (Hemiptera: Pentatomidae) is debatable, some mention that it comes from Asia area (Yukawa and Kiritani, 1965) and others from the African area (Hokkanen, 1986). One thing is certain, however, that it is expanding more and more in the temperate areas, not necessarily warm like the ones of origin. Later, the species spread everywhere, in Africa (Poutouli, 1995;), Asia (Kiritani and Hokyo, 1962; Kaul et al., 2007; Dehghani-Zahedani et al., 2022), Australia (Clarke, 1992), New Zeeland (Rea, 1999), South America (Panizzi, 2008), North America (Jones, 1988; Sosa-Gomez and Moscardi, 1995; Capinera, 2001) and Europe (Colazza et al., 1985; Rédei and Torma, 2003). In Romania, green stink bug has been present and identified as pest since 2010 (Grozea et al., 2012) and then it has spread to the east (Kurzeluk et al., 2015).

There is no complete list of plant species that it damages, but it certainly falls into the category of polyphagous with a wide spectrum of cultivated or wild plants (Solanaceae, Cruciferae, Malvaceae, Poaceae) (Panizzi and Slansky, 1991; Panizzi, 1997).

The great ability to spread in different places has made the pest species adapt to new and new plant species, both in the category of agricultural plants and horticultural plants.

Thus, *Nezara virdula* in all its damaging forms (nymphs and adults) was found on the aerial organs of vegetable (Uddin et al., 2013; Girish et al., 2014; Esquivel, 2016; Looney, 2019), agricultural (Costea and Grozea, 2022; Edde, 2022) or ornamental plants (Ciceoi et al., 2017).

In Africa, Asia and the U.S., the pest is considered of great economic importance, the production losses being high (Edde, 2022).

The control strategies adopted until now are diverse and applicable depending on the economic level or the geographical location. High costs with chemical treatments are associated with Africa (Taylor et al., 2018). But there are other approaches, such as biological ones (natural enemies, biopesticides) that are applied, but especially in the US, Europe and Australia (Knight and Gurr, Marcu and Grozea, 2018; 2007; Ademokoya et al., 2022).

Although it has been present in Romania for some time, there have been no comprehensive monitoring investigations (as far as we know) or in finding solutions to stop it except at the level of closed space as solar system (Marcu and Grozea, 2020). Continuous adaptation to new host plants could be a future problem for agro-horticultural crops.

In this context, we proposed that through this work we update the situation of the harmful species *Nezara viridula* and try to find combined solutions to keep it below the economic threshold of damage.

MATERIALS AND METHODS

The experimental design

In order to establish the population level of the pest *Nezara viridula* for the period 2019-2022, we made observations in 8 localities in Timis county (Figure 1). Their choice was made based on the availability and variety of cultures. Being known that the pest has a wide range of host plants, we focused on plants already established as host plants.

Figure 1. The positioning of the places where the observations were made

In order to facilitate the interpretation of population level results, each place was coded according to the data in Table 1. The study places, more precisely, localities were coded as follows: TI-1-GS, PI-2-F, PE-3-GA, GR-4-F, JI-5-GS, NI-6-F, DU-7-FO and IO-8-GA being associated with a certain category of crops or plants. Among these, we chose to focus on corn and sunflower (as agricultural field plants), tomato and pepper (as vegetables from garden) or lilac purple, magnolia and elderberry (as ornamental plants from green spaces or forest).

Depending on the category of the host plant, we delimited the surfaces to be analysed (Table 1). So, for the large field crops, $300m^2$, for those in the gardens, we chose smaller areas (available) of $100m^2$ and for the ornamental plants in green spaces (shrubs) we went for 5 plants/approximately $50m^2$.

Months of observations

For the quantitative and evolutionary establishment of green sting bugs from the above-mentioned localities, we chose to make monthly observations, between the 10th and 20th day of each month (June-October) covering the period of intense vegetation for plants/crops, the activity of nymphs and adults of the pest but also the movement to all the distant localities.

Testing of chemical products and bioinsecticides

In order to find the best control strategy, we tested in 2022 a chemical product and a bioinsecticide currently used by farmers and gardeners in the western part of the country.

Thus, in laboratory (Phytosanitary Diagnosis and Expertise Laboratory from ULST) we tested 1 chemical product (Karate Zeon, a.s lambda - cihalotrin) of 0.02%) and 1 bioinsecticide (Laser 240 SC, a.s spinosad in dose of 0.05%) in 3 repetitions (R1, R2, R3).

Since testing in the field would have been difficult to do because in an open field green stink bugs can migrate immediately after treatments, we resorted to testing in a closed space, using parts of corn plants 30 insects each collected from the field (GR-4-F) and the tomato garden (PE-3-GA).

Statistical interpretation of data

For the assessment of the population level of the pest, descriptive statistics were made regarding the mean, minimum, maximum value, standard deviation, quartiles, asymmetry coefficient and variance, and for the monthly evolution, comparative graphs.

In order to establish the effectiveness of the control products, we resorted to the Significance Test (Duncan) by which the active pests remaining in the terrariums were evaluated.

Table 1. Identification and characterization of study sites

Place (Code)	Identification of GPS coordinates	Category	Plants or crops	Areas or plants analyzed
Timisoara (TI-1)	45°46'55.2"N 21°13'48.1"E	Green space (GS)	Lilac purple	5 plants/50 m^2
Pischia (PI-2)	45°87'61.5"N 21°42'01.1"E	Field (F)	Corn	300 m^2
Pesac (PE-3)	45°99'45.8"N 20°83'02.4"E	Garden (GA)	Tomato	100 m^2
Grabat (GR-4))	45°52'26.9"N 20°45'13.7"E	Field (F)	Corn	300 m^2
Jimbolia (JI-5)	45°79'75.3"N 20°73'67.5"E	Green space (GS)	Magnolia	5 plants/50 m^2
Nitchidorf (NI-6))	45°58'62.1"N 21°51'49.7"E	Field (F)	Sunflower	300 m^2
Dumbravita (DU-7)	45°78'30.7" N, 21°24'19.8"E	Forest (FO)	Elderberry	5 plants/50 m^2
Iohamisfield (IO-8)	45°5685.5"N 52°75'53.7"E	Garden (GA)	Pepper	100 m^2

RESULTS AND DISCUSSIONS

Evaluation of the population level and monthly dynamics

In all the places analysed, the active stages were present, both larvae and nymphs. They were present on all the relevant aerial organs of plants, on leaves, inflorescences, corn cobs, stems and fruits (in the case of vegetables) (Figure 2).

Figure 2. Green stink bugs in different forms on various plants: 1. adult on the lilac plant; 2. nymph in advanced stage on corn; 3. nymph in early stage on tomato

The population level was varied, and from what we found, a high level was recorded in populated cities and less so in smaller villages or open countryside (Figure 3). Which also explains the results of other researchers who integrate green stink bugs into the category of cosmopolitan pests (Aldrich et al., 1987).
The size of the populations over the entire period is varied from 8 to 464 specimens (Figure 3).
We also found that the type of plants or space is important, in gardens and green spaces, forests the pest was more present than in the field.
The most adults and nymphs were found in 2020 (550 specimens), followed by 2021 (256 specimens), then 2019 (239 specimens) and 2022 (152 specimens) (Figure 3). A sudden increase is observed in the period 2019-2020, then a gradual decrease (2021-2022).
From the descriptive analysis of the raw data (Table 2) it was extracted that the most pests were present in places with green spaces, namely x = 116.5 /TI-1-GS followed by forest with an average value of x = 49.7/DU-7-FO and then of gardens with x = 24.2/PE-3-GA. Regarding the open fields with corn and sunflower, the values were lower (x = 2.0/PI-2-F and x = 0.80/NI-6-F0.800). Intermediate values from all categories of spaces were placed at the average value of 48.2/ IO-8-GA, 20.1/GR-4-F) but also x = 38.1/(JI-5-GS).
The monthly evolution shows a gradual increase starting with June and reaching the peak in August (287 specimens) then a sudden increase until September (Figure 4). The fewest green stink bugs were recorded in June (4 specimens) but also in September (7 specimens).
Practically, it is a natural evolution that follows the course of the development of the stages of hemimetabolous insects from hibernating adults, nymphs and then the new adults to which the availability of food and climatic conditions have contributed. August is known as the hottest month of the year and green sting bugs are thermophilic species.
However, it was not possible to quantify the possibility that some of individuals temporarily migrated to other plants, especially in large spaces, such as those of corn and sunflower, therefore the values can be considered estimates. There is still no clear and efficient method for quantifying of green stink bugs in general.

Table 2. Descriptive statistics for number of green stink bugs observed in the study sites in Timis County

Place (code)	Descriptive elements[1] No. stink bugs/2019-2022							
	Mean	Min.	Max.	Low.Q.	Up. Q	R	Var.	SD
Timisoara (TI-1-GS)	116.500	0.00	366.000	0.00	180.000	366.000	299.10	176.5423
Pischia (PI-2-F)	2.0000	0.00	26.000	0.00	12.500	26.000	19.00	11.160
Pesac (PE-3-GA)	24.2000	0.00	142.000	0.00	71.000	142.000	4056.30	69.0811
Grabat (GR-4-F)	20.1000	0.00	102.000	0.00	55.000	103.000	2999.20	57.1600
Jimbolia (JI-5-GS)	38.100	0.00	166.000	0.00	20.000	166.000	3911.10	79.125
Nitchidorf (NI-6-F)	0.800	0.00	1.9000	0.00	0.9000	1.9000	8.07	10.005
Dumbravita (DU-7-FO)	49.700	0.00	222.000	0.00	45.000	222.000	890.40	94.1570
Iohamisfield (IO-8-GA)	48.200	0.00	102.000	0.00	59.000	102.000	44.20	12.160

[1]mean, minimum, maximum, quartiles (lower and upper), range, variance standard deviation

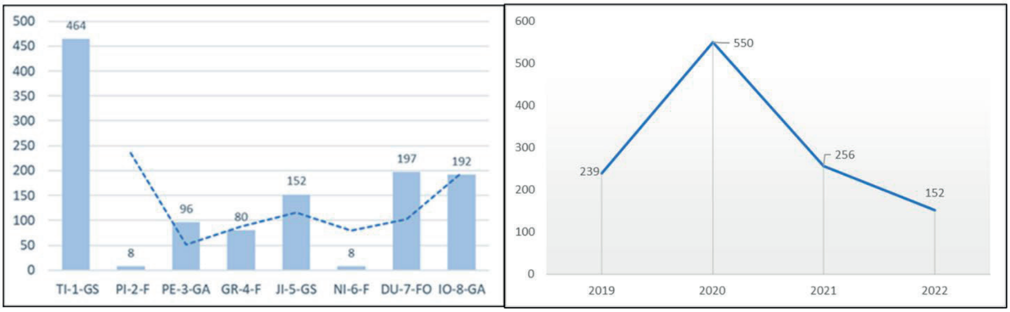

Figure 3. Total number of green stink bugs recorded in the period 2019-2022
in the study sites and annually where GS- Green space, F-Field, GA- Garden, FO-Forest

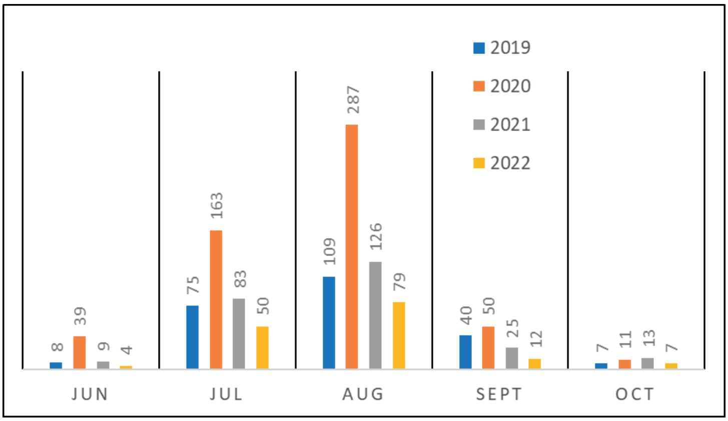

Figure 4. The monthly evolution (June-October) of the pest in the period 2019-2022

Assessing the effectiveness of treatments

From the analysis of the descriptive statistical data between the variants representing untreated, treated with insecticides and with bioinsecticides at different intervals (Table 3), the values were varied. Thus, in the Control variant, the most active stink bugs were recorded both at 3, 7 and 10 days (x = 25,000), while in the variants with insecticide Karate Zeon, lambda - cihalotrin the values were much lower, more chosen 7 and 10 days after their application (x = 6.50 and x = 1.50/Karate Zeon). In the versions with bioinsecticide (Laser 240 SC) it was found that the number of green stink bugs remained at medium and high values. On the 3rd day after the application of the bioinsecticide there were on average 23.00 individuals, then after 7 days there were 15.70 and on the 10th day, 12.35 individuals remained.

The statistical analysis of the results by expressing the significance test ($p<0.05$, $p>0.05$) from Table 4, shows that between all the Control variants (a, b, c) and the variants with the insecticide Karate Zeon on 7th day and the 10th day after the treatment, there is significant differences, i.e. p=0.039 (a-e), p=0.048 (b-e), p=0.047 (c-e) and p=0.034 (a-f), p=0.035 (b-f) and p=0.0345 (c-f). And between the Control variants and those in which the Laser 240 SC bioinsecticide was applied at 10 days, there were significant differences (p=0.042 (a-i), p=0.041(b-i) and p=0.039(c-i).

Table 3. Statistical interpretation of values through descriptive elements expressing number of live green stink bugs left after treatments

Variable	Descriptive elements[1] Number of live green stink bugs left after treatments (control, insecticide and bio-insecticide)							
	Mean	Min.	Max.	Low.Q.	Up. Q	R	Var.	SD
Control (3rd day)	25.000	10.000	30.000	10.000	30.000	30.000	100.00	9.469
Control (7th day)	25.000	10.000	30.000	10.000	30.000	30.000	100.00	9.469
Ins (3rd day)[2]	20.350	7.000	24.000	7.500	24.000	6.000	87.326	8.824
Ins (7th day)[2]	6.550	2.000	14.000	2.500	12.500	12.000	42.351	5.569
Ins (10th day)[2]	1.500	1.000	3.000	1.500	3.500	6.000	13.000	3.155
BioIns (3rd day)[2]	23.000	8.000	27.000	9.500	27.500	22.000	93.153	8.588
BioIns (7th day)[2]	15.700	7.000	22.000	7.500	20.500	17.000	42.693	7.234
BioIns (10th day)[2]	12.350	5.000	17.000	5.500	17.000	7.000	14.336	5.119

[1]mean, minimum, maximum, quartiles (lower and upper), range, variance standard deviation
[2]Ins- Karate Zeon, BioIns- bioinsecticide Laser 240 SC

Table 4. Approximate probabilities by Duncan's Test for evaluating the effectiveness of treatments according to the number of live green bugs at different day intervals

Variable 1 (Var.2)	The Duncan test for evaluating the effectiveness of treatments by the number of green stink bugs alive. at different day intervals Approximate Probabilities for Post Hoc Test								
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Co(3rd d)(a)		1.00	1.00	0.55	0.039	0.034	0.89	0.13	0.042
Co(7th d)(b)	1.00		1.00	0.58	0.048	0.035	0.88	0.140	0.041
Co(10th d(c)	1.00	1.00		0.62	0.047	0.0345	0.785	0.11	0.039
Ins1(3rd d)(d)	0.55	0.58	0.62		0.35	0.56	0.099	0.25	0.41
Ins1(7th d)(e)	0.039	0.048	0.047	0.35		0.76	0.048	0.046	0.035
Ins1(10th d)(f)	0.034	0.035	0.0345	0.56	0.76		0.01	0.022	0.25
BioIns(3rd d)(g)	0.89	0.88	0.785	0.099	0.048	0.01		0.44	0.21
BioIns(7th d)(h)	0.13	0.130	0.11	0.25	0.046	0.022	0.44		0.09
BioIns(10th d)(i)	0.042	0.041	0.039	0.41	0.035	0.25	0.21	0.09	

(a)-Control variant after 3 days; (b)-Control variant after 7 days; (c)-Control variant after 10 days; (d)-variant with Karate Zeon insecticide after 3 days; (e)-variant with Karate Zeon insecticide after 7 days; (f)-variant with Karate Zeon insecticide after 10 days; (g)-variant with Bio-insecticide Karate Zeon after 3 days; (h)-variant with Bio-insecticide Karate Zeon after 7 days; (i)-variant with Bio-insecticide Karate Zeon after 10 days

Between the variants with bioinsecticide at 3 and 7 days and those with Karate Zeon insecticide at 7 and 10 days there were significant differences, where $p<0.05$, namely p=0.048 (g-e), p=0.01 (g-f) and p=0.046 (h-e), p=0.022 (h-f). And between the bioinsecticide applied at 10 days and Karate Zeon insecticide applied at 7 days, with p=0.035 (i-e) there are differences.

CONCLUSIONS

From the results in this paper, it appears that green stink bugs are still present in agricultural and horticultural crops as well as forests, and the population level is one to be taken into account, even if the trend is of a slight decrease.

It is obvious that they prefer places such as cities or gardens near people's houses, and which we suspect are protected from the wind and retain warm air.

Regarding the control, from our tests we found that insecticide Karate Zeon (lambda-cihalotrin) are effective after 7-10 days after application. Likewise, the bioinsecticide Laser 240 SC (spinosad) can be considered effective to a lesser extent.

With all this in mind, we consider that application with tested insecticide (Karate Zeon) alternating with the tested bioinsecticide could be the ideal combination for keeping the pest under control and avoiding over pollution of the environment.

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