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LIFE CYCLE PARAMETERS OF THE INVASIVE SOUTHERN GREEN STINK BUG (*Nezara viridula*) AT LABORATORY CONDITIONS

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Abstract

The life cycle parameters of Nezara viridula were studied in 2018 under laboratory conditions at the Department of Entomology, Agricultural University of Plovdiv. In the beginning of May field collected adults were placed in Petri dishes for mating and laying eggs (a couple of female and male per dish). A constant temperature of 25 ± 2ºC, RH 60-70% and a photoperiod of 16:8 hours L:D were maintained at the laboratory. The larvae, hatching from 15 egg groups, were reared individually in Petri dishes and monitored daily to determine the duration of each stage. The eggs were laid in groups of 27 to 83. The mean duration of the embryonic development was 6.04 ± 0.71 days. The five nymphal stages last as follows: 3.56 ± 1.56 days for the first instar, 9.75 ± 4.99 days for the 2nd instar, 7.16 ± 3.47 days for the 3rd instar, 10.36 ± 2.98 days for the 4th instar, and 13.46 ± 3.39 days for the 5th instar.

Key words*: color morphs, copulation, developmental stages, fecundity, Nezara viridula.*

INTRODUCTION

The geographical origin of the southern green stink bug *Nezara viridula* (Linnaeus) is a matter of debate. The most likely origin is the Mediterranean area and/or the African mainland (Hokkanen, 1986; Jones, 1988). According to DeWitt and Godfrey (1972) Bulgaria falls within the native range, nevertheless the species was mentioned for the first time in 1959 by Strawinski (1959) for the southern part of the country. It was mentioned later by Josifov (1963, 1999) and Josifov & Simov (2004) as part of the heteropteran entomofauna of the country, and only recently there were records in the northern part (Black Sea Coast in 2001, Sofia in 2011) (Simov et al., 2012).

The geographical distribution of the southern green stink bug extends across temperate and tropical areas (Todd, 1989; Waterhouse, 1998). It was reported as a common pest in most European countries like France, Germany, Greece (CABI/EPPO, 1998), Romania (Grozea et al., 2012; 2015), etc. *N. viridula* is classed by Todd (1989), as "... one of the most important pentatomid insect pests in the world ... It is cosmopolitan and highly polyphagous on many important food and fiber crops".

N. viridula causes economic damages to many horticultural and field crops such as tomatoe (*Lycopersicon esculentum*), corn (*Zea mays*), grape-vine (*Vitis vinifera*), pepper (*Capsicum annuum*), raspberry (*Rubus idaeus*) and apples (*Malus domestica*). Feeding causes malformations, discoloration and premature drop of fruits. Feeding on tomato fruits causes reduction of the yield and the fruits become unmarketable. Feeding punctures can also provide access for fungal and bacterial infections (Russin et al., 1988) which can be toxic to vertebrates feeding on maize kernels (Stringer et al., 1983; Payne and Wells, 1984). In USA *Nezara viridula* caused 34-53% fruit drop on pecan (Dutcher and Todd, 1983).

Regarding the pest biology, it is known that is mostly multivoltine with the extent of voltinism related to local differences in climate and the availability of host plants suitable for reproduction (Velasco et al., 1992; Velasco et al., 1995). *N. viridula* overwinter as an adult under bark of trees (Jones & Sullivan, 1981), inside buildings and facilitates. The adults start to emerge in spring, in the beginning of May. They leave their overwintering sites and migrate to different crops to feed and reproduce.

Though *N. viridula* has become a common pest on many agricultural crops in Bulgaria, especially on raspberries and field tomato, since the 90s of the last century (the region of Plovdiv, Harizanova, unpublished data)

(Figures 1 and 2.), it had not been studied so far. Simov et al. (2012) mentioned about some evidence that the species might be a pest in Bulgaria, recording damage (chlorosis) on tomatoes fruits in Gorno Spanchevo Vill., due to many piercings by larvae and adults of the southern green stink bug.

The aim of the present study was to establish lifecycle parameters of the local populations of *N. viridula*.

MATERIALS AND METHODS

The study was conducted in 2018-2019 under controlled laboratory conditions at the premises of the Agricultural University - Plovdiv. A constant temperature of 25 ± 2ºC, RH 60-70% and a photoperiod of 16:8 hours L:D were maintained at the laboratory.

Insect rearing

The laboratory colony of *Nezara viridula* was reared using adults collected in the beginning of May from raspberry plants in a private garden in Stamboliyski, Plovdiv's region. The adults were placed in Petri dishes for mating and laying eggs (a couple of male and female per dish) (Figures 3 and 4). On the bottom of each Petri dish a filter paper for absorbing the moisture was placed. Pods of green bean (*Phaseolus vulgaris*) and pieces from apple (*Malus domestica*) were provided as food. Fan-like folded paper was placed inside the Petri dish where the adults could lay their eggs. The food in the Petri dish was replaced every two days with fresh one.

Developmental Time and Life Table Studies

Egg stage

After eggs were laid the egg group, together with part of the paper on which had been laid, they were transferred to new Petri dishes for hatching and determining the duration of the egg stage

Nymphal stage

Fifteen egg masses were obtained and placed in Petri dishes (one egg mass per dish). The larvae hatched from each egg group were reared individually in Petri dishes and monitored daily to determine the duration of each instar. On hatching first instars have a strong aggregation behavior (Simmons and Yeargan, 1988) and for this reason they were initially left on the egg shells until they molted to the second instar and began to disperse. Each nymph was moved individually to a Petri dish by using a thin brush. On the bottom of the dish a filter paper and a fresh pieces of apple (*Malus domestica*) as a food were placed, together with a folded pieces of paper where the nymphs could hide if disturbed.

Adult stage

At adult emergence, the sex of all individuals was determined by the morphology of their external genitalia. Newly emerged adults were placed in Petri dishes (a couple of male and female per dish) for mating, laying eggs, and to determine longevity and fecundity. The duration of each copulation was measured and recorded in hours. The number of eggs in each newly laid egg group were counted and number recorded for calculating the average fecundity of the females. The adult longevity was calculated by recording the time from emergence to the death. In each Petri dish fresh pieces of apple (*Malus domestica*) as food and folded pieces of paper were placed where the adults can hide for maiting and laying their eggs.

All nymphal instars and adult's development, behavior and survivorship were monitored and recorded at regular basis.

Data were analyzed using Microsoft Excel statistics 2010.

RESULTS AND DISCUSSIONS

Egg stage

The embryonic development lasts 6.04 ± 0.71 days (Table 1), similar to the results reported by Harris and Todd (1980). The eggs are light yellow in color, barrel shaped and with flat tops. They are laid in clusters and firmly glued to each other. Parallel to the embryonic development, changes are observed in the color - eggs become orange three days after laying and a small red triangle starts to appear on the top of each egg. The eggs turn into red color day before hatching (Figures 5 and 6). Young larvae hatch by opening the disk-shape cap. Hatching takes about five minutes for an individual egg, but the entire egg mass is hatching for 1 to 2 hours.

Nymphal stage

As described by Waterhouse (1998), the 1st instar nymphs did not feed and aggregated on the empty egg shells. Newly hatched larvae are light orange with red eyes. Larvae become red in color twelve hours after hatching and dark red twenty four hours after hatching.

Mean development time of the first instar is 3.56 ± 1.56 days (Table 1).

Table 1. Mean developmental time (days ± STDEVA) for *Nezara viridula* (Linnaeus) at constant temperature of 25 ± 2ºC

Duration (days)	Average	STDEVA	Min.	Max.	n
egg stage	6.04	0.71	5	8	419
nymphal stage					
1st instar	3.56	1.56	2	8	286
2nd instar	9.75	4.99	3	29	128
3rd instar	7.16	3.47	3	15	122
4th instar	10.36	2.98	5	19	118
5th instar	13.46	3.39	7	25	113
from egg to adult	41.92	6.87	35	67	52

The mean development time for the second instar was 9.75 ± 4.99 days (Table 1). Third and fourth in stars are different from second in color. The overall green color starts to appear. Third instar nymphs start to disperse and feed mainly on fruits. When disturbed they immediately start to disperse and hide under the leaf surface. The mean development time was 7.16 ± 3.47 days (Table 1).

Fourth instar nymphs live alone. The mean development time of the fourth instar as 10.35 ± 2.98 days. Fifth instar nymphs differ in color among the individuals - part become black in color and the others - green. The percentage of the green against black forms was 60%. The mean development time of the fifth instar was 13.46 ± 3.39 days. A 5th instar nymph molted about 20 to 30 minutes to become an adult.

N. viridula required averagely 41.92 days to develop from first instar to adult. The ratio males: females of the resulting adults was 36: 64.

Males and females start to mate 12.3 ± 4.85 days after becoming adults. Adults mated only in shaded places. This happened under the folded paper inside the Petri dishes. The egg-laying period of a female lasts throughout her lifespan. The mean duration of mating was 32.66 ± 15.26 hours. Females deposited their eggs under the folded paper mainly at night. The eggs were firmly glued to each other. The females laid 1 to 3 egg masses.

Figures 1 and 2. Nymphs and adults of *N. viridula* on raspberry and tomato fruits in Plovdiv's region

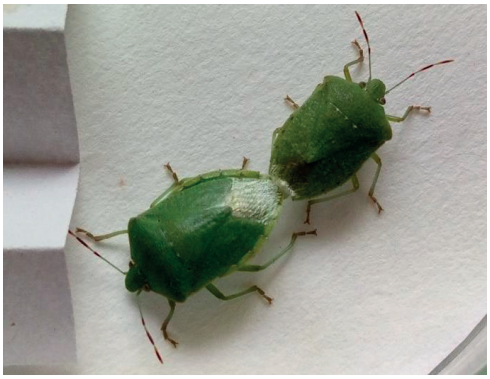

Figure 3. Mating in Petri dishes

Figure 4. Eggs laying at laboratory conditions

Figure 5. Freshly laid eggs

Figure 6. Day before hatching

Table 2. Adult longevity, pre-oviposition period and fecundity (days ± standard error) for *Nezara viridula* (Linnaeus) at constant temperature of 25 ± 2ºC

	Female adult longevity (days)	Male adult longevity (days)	Preoviposition period (days)	Fecundity (number)	Eggs per egg group (number)
Mean	47.35	34.81	19.82	89.88	57.27
Standard error	5.02	5.24	1.57	16.61	5.92
Min.	10	11	14	29	27
Max.	88	75	30	201	83
Sample size	26	16	11	11	13

The females lived longer than the males (an average of 47.35 days against 34.81 days) (Table 2). The pre-oviposition period was 19.82 days, the average fecundity – 89.88 eggs per female and the average number of egg per egg group - 57.27.

As described by McPherson (2018) the species has several 'color types' (i.e., genetic morphs) of adults but two main morphs exist (i.e., numerically dominant in most regions): var. *smaragdula* F. (G-type, completely green coloration) and var. *torquata* F. (O-type, predominantly green body with anterior yellowish coloration). During the entire study in Plovdiv,s region, the most prevailing was the G-type, *N. viridula* var. *smaragdula* F., comprising 95.4% of all the collected adults.

CONCLUSIONS

Nezara viridula is a pest of many horticultural and field crops in Bulgaria. The populations in the southern part of Bulgaria are predominantly var. *smaragdula* F. (G-type, completely green coloration), and rarely var. *torquata* F.

At constant temperature of 25 ± 2ºC, RH 60-70% and a photoperiod of 16:8 hours L:D one generation may develop for 41,92 days. Female live longer than males and lay an average of 89.88 eggs.

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