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LABORATORY STUDY OF THE LIFECYCLE PARAMETERS OF THE BROWN MARMORATED STINK BUG (HALYOMORPHA HALYS STÅL.) – AN ALIEN PEST ESTABLISHED IN BULGARIA

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Abstract

The brown marmorated stink bug (*Halyomorpha halys*) is a pest found on agricultural crops, first reported in Bulgaria in 2016. In 2018 in the region of Plovdiv it was found feeding on a variety of agricultural and ornamental plants, such as corn, tomato, apple, vinegrapes, raspberry, mulberry, and hibiscus. The rapid spread and the threat for many potential host plants in Bulgaria were the reasons for the current study. The life cycle parameters of the Bulgarian population of H. halys were studied in 2019. The progeny of the field-collected adults from the region of Plovdiv were reared at laboratory conditions at constant temperature of 25 ± 2°C, RH 60 - 70% and a photoperiod of 16:8 hours L:D. Under these conditions the embryonic development was completed in 5.10 ± 1.02 days; the 1st nymphal instar in 5.32 ± 1.23 days; the 2nd - in 9.35 ± 2.88 days; the 3rd - in 7.18 ± 2.55 days; the 4th - in 7.40 ± 1.89 days, and for the 5th nymphal instar – in 10.87 ± 2.63 days. The adults lived an average of 17.53 ± 9.34 days with a maximum life span of 44 days.

Key words: alien pest, Halyomorpha halys, life span, fecundity, nymphal development

INTRODUCTION

The brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive polyphagous species native to China, Japan and Taiwan. Currently this invasive species is widespread in Europe and the USA thanks to the international trade between Asia and other parts of the world (McPherson, 2018). In Europe *H. halys* was first reported from Switzerland in 2007 (Wermelinger et al., 2008) and since then - in most of the European counties: Greece (Milonas, 2011), Italy (Maistrello et al., 2014), Hungary (Vetek et al., 2014), Romania (Macavei et al., 2015), Serbia (Šeat, 2015), Croatia (Šapina, 2018). In Bulgaria the brown marmorated stink bug was reported for the first time in 2016 (Simov, 2016).

Many regions of the world are environmentally suitable for this insect, and this pest has the potential to severely damage a wide range of crops (Zhu, et at. 2012). More than 100 plant species are mentioned as hosts of *H. halys* (Lee et al, 2013), including fruit trees, vegetable crops and ornamental plants. Soon after the first record in Bulgaria in 2016 the species was observed to feed on corn, tomato, apple, vinegrapes, raspberry, mulberry, and hibiscus in the region of Plovdiv (unpublished data).

The brown marmorated stink bug can feed on different parts of the plants, but prefers the generative organs for which it is particularly destructive (McPherson & McPherson, 2000). Its feeding, like of all the stink bugs (Pentatomidae), can cause many different types of damage. The saliva, which is secreted into the feeding site, contains a cocktail of digestive enzymes, including amylase, proteinases, and pectinase, which dissolve the cell walls and contents (Backus 1985; Peiffer & Felton, 2014). The salivary secretions dissolve the plant tissues and cause a subsurface corking damage on fruits, vegetables, and nuts (Miles, 1969;

Wiman et al., 2014); enzymatically disrupt the function of the plant tissues (Hori, 2000; Peiffer & Felton, 2014); or cause other deformities such as discoloration (Zobel et al. 2016). Feeding damages on apple fruit include black spots, corky tissue and deformation (Leskey et al. 2009; Nielsen and Hamilton 2009), on maize - discoloration on the kernels (Kuhar et al. 2012), on tomato - light-yellow, discolored spots and corky tissue (Zobel et al. 2016), on bell pepper - white or yellow areas and a spongy tissue (Zobel et al. 2016). Feeding during the early stages of fruit or nut development can cause shriveling or abortion (McPherson & McPherson, 2000). Besides the described direct injuries to host plants, as in all the representatives of the Pentatomidae family, when inserting their stylets into the plant tissues, a secondary infection from incidental or vectored yeasts can result in tissue decay or necrosis (Esquivel et al., 2010).

The direct and indirect damage, caused by *H. halys* are negatively affecting the quantity and the quality of yield: lower berry weight and cracked berries in vine grapes rape (Mohekar, 2016), more than 50% deformed fruits reported from the orchards in Italy (Maistrello et al., 2017), losses in apple production estimated as 37 $ million in the USA (Rice et al., 2014).

Recognizing the economic importance of *H. halys*, Nielsen et al. (2008) study the parameters of its lifecycle and based on their findings, predict that over time its distribution in the United States will resemble that of other phytophagous stink bug species with similar host rage, such as *N. viridula, Acrosternum hilare, and Euschistus* sp., and recommends IPM programs to incorporate H. halys in the regions where it becomes established.

The very rapid dispersal of the pest in the region of Plovdiv, and the observed injuries in many crops, were the main reasons to initiate the current study on establishing the lifecycle parameters of the local population of *H. halys* at contstant temperature, RH and photoperiod, which could be later used for predicting the population dynamics and applying control measures.

MATERIALS AND METHODS

The study was conducted in 2019-2020 under the controlled conditions in a laboratory of the Department of Entomology at the Agricultural University - Plovdiv. A constant temperature of 25±2°C, RH 60-70% and a photoperiod of 16L:8D were maintained at the laboratory.

The laboratory population of *Halyomorpha halys* was reared using adults collected from *Hibiscus syriacus (Hibiscacea)* in the region of Plovdiv at the beginning of May 2019. The adults were placed in plastic containers measuring 10x5x4 cm (length, width, height) for mating and laying eggs (a couple of male and female per container). Fanlike folded paper was provided for the adults to lay their eggs. Slices of apple fruit *(Malus domestica)* were provided as food. Filter paper was placed on the bottom of each container for absorbing the excess moisture.

Egg stage

The eggs masses, produced by the females, together with the fanlike folded paper on which they have been laid, were transferred to Petri dishes, each egg mass being placed individually and checked upon on a daily basis for hatching (Fig. 1). The duration of the egg stage was estimated as an average of the days from egg laying to hatching.

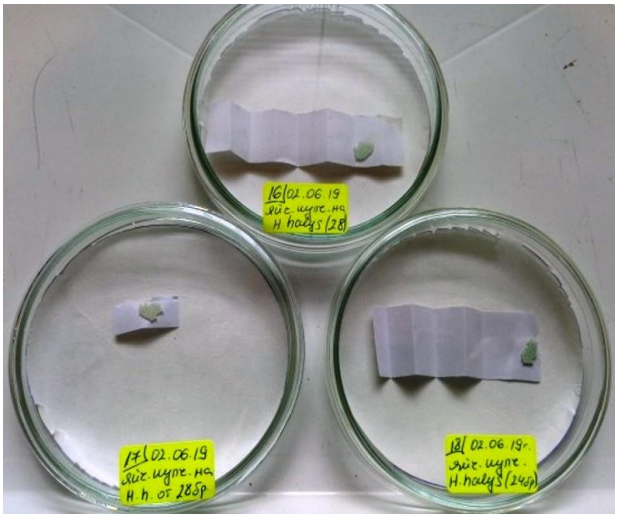

Figure 1. Petri dishes with egg masses for estimating the duration of the egg stage of Halyomorpha halys at laboratory conditions.

Nymphal stage

Twenty five egg groups laid by the females of the laboratory population were placed singly in plastic containers measuring 10x5x4 cm (length, width, height) with a lid. After hatching, the nymphs from each egg group were monitored daily to record the duration of each nymphal instar. First instar nymphs, which have a strong aggregation behavior and stay on the eggshells, were left in the same plastic containers until the first molting. After the molting each of the next-instar nymphs was placed individually in a plastic container until the emergence of an adult. Slices of fresh apple fruit were provided regularly as a source of food for the nymphs.

Adult stage

The newly emerged adults were transferred as couples to new containers for mating, laying eggs, and recording the life span and fecundity. The duration of each copulation was measured as the time from coupling to dispersal. The number of eggs in each newly laid egg group was counted and recorded for calculating the average fecundity.

For all nymphal instars and adults development, behavior and survivorship were monitored and recorded at regular basis. Data were analyzed using Excel for Microsoft 365.

RESULTS AND DISCUSSION

Egg stage

The embryonic development lasted 5.10 ± 1.02 days (Table 1), similar to the results reported by Nielsen, Hamilton and Matadha (2008). The eggs were light green or light blue in color, with round shape, laid in clusters (Fig. 2a). Parallel to the embryonic development, changes were observed in the color - eggs become whitish and two red spots started to appear on top of each egg. The eggs turned white in color 24 hours before hatching. Hatching took about five to ten minutes per individual.

Table 1. Mean developmental time (days ± STDEVA) of Halyomorpha halys (Stål) at constant temperature (25±2°C)

Duration (days)	Average	STDEVA	Min	Max	N
Egg stage	5.10	1.02	3	7	385
Nymphal stage					
1st instar	5.32	1.23	2	12	158
2nd instar	9.35	2.88	3	25	156
3rd instar	7.18	2.55	4	18	131
4th instar	7.40	1.89	3	17	105
5th instar	10.87	2.63	8	22	81

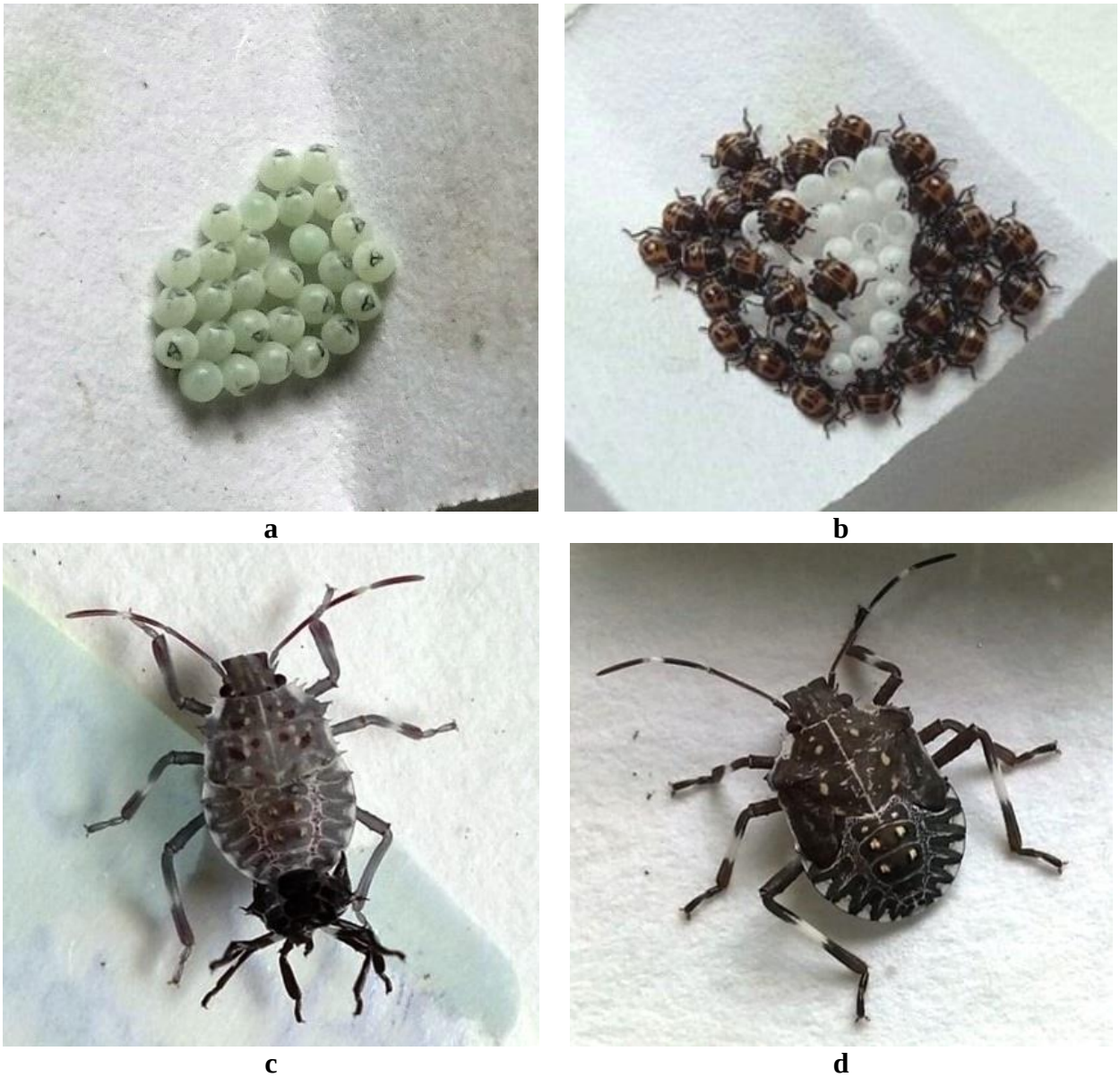

Fig. 2. Different developmental stages of *H. halys*: a-eggs; b-1st instar nymphs; c- 4th instar nymph just after molting; d- 5th instar nymph of *H. halys*

Nymphal stage

The first instar nymphs were averagely 2.4 mm in length with black head and thorax, and yellowish red abdomen. The 1st instar nymphs remained on the egg chorion until the molting without feeding (Fig. 2b). The mean development time of the first instar was 5.32 ± 1.23 days (Table 1), similar to the results reported by Nielsen et al. (2008).

The color of the body in the second instar was getting darker with visible rough spiny projections along the lateral edge of the thorax. The 2nd instar nymphs started to feed and aggregate. The mean development time until the next molt was 9.35 ± 2.88 days (Table 1).

The third instar nymphs were bigger in size compared to the second instar nymphs. The nymphs were darker in color. Third instar nymphs started to disperse, feed and live individually. Nymphs preferred to feed mostly with fruiting structures and when disturbed they

immediately would hide under the leaf surface. Wing pads began to appear after each successive molt (Fig. 2c). The mean development time of the third instar was 7.18 ± 2.55 days (Table 1).

The forth instar nymphs were moving very fast on the plant surface. The mean development time of the fourth instar was 7.40 ± 1.89 days (Table 1).

The fifth instar nymphs had a black or gray coloration with noticeable spines along the humeral margins, as well as white bands on the antennae and legs (Fig. 2d). The mean development time of the fifth instar nymphs was 10.87 ± 2.63 days (Table 1). They were feeding only on fruits. The 5th instar nymphs molted in 20 to 30 minutes to become adults. The newly emerging adults were white in color with red eyes. The typical brown coloration became visible two to three hours after the last molting. An average of 45.22 days was needed for H. halys to complete its development from hatching to adult.

The ratio F:M of the resulting adults was 38:35.

The mean duration of the copulation was 4.71 ± 1.98 hours, which was similar to the observed by Nielsen et al. (2008) and McPherson (2018). For mating (Fig. 3) the adults were choosing shaded places (under the folded paper inside the plastic containers). If disturbed, adults immediately stopped mating and dispersed. Copulation was observed mainly at night. The females deposited their eggs on the underside of the folded paper, firmly glued to each other.

The females laid 1 to 3 egg masses. The average number of eggs per egg mass was 23.97 and the average fecundity was 45.08 eggs per female (Table 2), which was much lower than the one, reported by Nielsen et al. (2008) - 243.78 ± 27.48 eggs.

The longevity of the males and the females was 16.65 to 18.34 days, respectively.

Fig. 3. Mating adults of *H. halys*

Table 2. Fecundity and adult longevity of Halyomorpha halys (Stål) at constant temperature (25±2°C)

	mean	STDEVA	min	max	Sample size
Female longevity (days)	18.34	9.40	3	44	38
Male longevity (days)	16.65	9.33	2	36	35
Fecundity (number)	45.08	16.42	17	80	12
Eggs per egg group (number)	23.97	6.76	3	29	31
Development from hatching to adult (days)	45.22	3.56	34	50	68
Duration of copulation (hours)	4.71	1.97	2	8	10

CONCLUSION

The brown marmorated stink bug Halyomorpha halys is a new pest on the agricultural crops in Bulgaria. At constant temperature of 25±2ºC, RH 60-70% and a photoperiod of 16:8 hours L:D the egg stage of the population in the region of Plovdiv was completed in 5.10 days, the nymphal stage – in 40,12 days, and one generation (from egg to adult) – in 45.22 days. This information could be used for predicting the population dynamics and applying control measures.

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