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The Preliminary Study on the Biology of An Invasive Species, *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northwest Türkiye

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A B S T R A C T

The Brown marmorated stink bug (BMSB) [*Halyomorpha halys* (Hemiptera: Pentatomidae)] is a polyphagous pest causing significant damage to approximately 300 crops, such as nuts, vegetables, and ornamentals. In 2017, the BMSB was discovered for the first time in Türkiye. There is currently no published data available on the biology of the pest in Türkiye. This preliminary study was carried out in Sakarya province, where the BMSB has observed a low population since 2021. In this study, some biological parameters such as voltinism, mortality rates, egg hatching rates, and egg-adult developmental times of the 1st and 2nd generations of the insect populations were determined. The study was carried out under semi-field conditions and laboratory. Considering the BMSB's minimum temperature threshold, which has yet to be determined in Turkey, the accumulation of degree days was calculated using the references to four possible thresholds ($DD_{12,\ 12.5,\ 13,\ 13.5}$) and was determined to be between 536.91 - 608.69 DD. The average egg-adult developmental time was 47.85 days for the 1st generation at naturally fluctuating temperatures and 47.13 days for the 2nd generation under laboratory conditions at 25°C. The hatching rate of egg masses was determined to 90.63% in the 1st generation and 57.75% in the 2nd generation. Total mortality in the first generation was 22.97% and 90.24% in the second generation. It was revealed that the insect could produce two generations in Türkiye, but the number of egg-producing adults and eggs laid in the second generation was significantly lower than that in the first. This preliminary study was carried out with a limited number of samples due to the initial infesting in the region and, therefore more comprehensive research is needed to reveal the the biology of BMSB in the country.

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Introduction

The brown marmorated stink bug (BMSB), [*Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae)] is a significant invasive species that rapidly spreads around the world and has a severe impact on biodiversity and ecosystems (Simberloff et al., 2013). The invasive BMSB, which is native to North and South Korea, Japan, and China (Zhu et al., 2017), was detected for the first time in Pennsylvania (USA) in the mid-1990s (Hoebeke and Carter, 2003) and is now found in numerous US states and a few Canadian territories (Anonymous, 2018). It initially emerged in Switzerland in 2004 (Haye et al., 2014) and quickly spread to all European countries and several Asian countries (Kriticos et al., 2017). . The BMSB was first recorded in Türkiye in 2017 in Istanbul, faunistically (Cerci and Kocak, 2017) and then in Artvin (Kemalpasa) in neighboring Georgia, causing problems with large aggregations at overwintering sites (Guncan and Gumus, 2019).

BMSB, a polyphagous pest, has over 300 hosts and causes significant yield and quality losses by piercing and sucking plants, mainly agricultural products such as fruits, vegetables, and row crops (Nielsen and Hamilton, 2009a; ESA, 2011). Tissue damage, color change, necrosis, or chlorotic areas develop as a result of BMSB adults and nymphs feeding, and the injured fruits/products become unmarketable (Leskey and Nielsen, 2018). Due to its high flight capacity and wide range of possible hosts, the bug may move between its hosts during the season, thereby causing more damage (Wiman et al., 2015). BMSB has caused huge economic losses in agricultural production in different parts of the world. For instance, in the mid-Atlantic region in 2010, the invasive species caused $37 million in damage to apples alone, as well as losses of up to 100% in peaches in various states (Polk, 2011; Leskey et al., 2012), and significant damage to various vegetable crops (Kuhar et al., 2012), soybean (Nielsen et al., 2011;

Owens et al., 2013), and other crops (Basnet et al. 2014; Rice et al. 2014; Basnet et al. 2015). In Europe, it soon became the main pest of orchards after its detection (Maistrello et al., 2017), particularly in pear (Bariselli et al., 2016), peach (Pansa et al., 2013; Damos et al., 2020), kiwifruit (Francati et al., 2021), and cherry (Moore et al., 2019). BMSB has become an international concern because it has caused significant damage in USA, Italy, and Georgia, particularly in hazelnut production (Bosco et al., 2018; Murvanidze et al., 2018; Ozdemir and Tuncer, 2021). The mapping of the potential global range of the BMSB for Türkiye exposed that the coastline part of the Black Sea region offers the optimum climatic conditions for the insect, and hazelnut is the most intensive cultivated plant grown in these areas (Ozdemir and Tuncer, 2021). Many different crops, in addition to hazelnut, are potentially at risk in the near future given the pest's current distribution along this coastline covering a total of 10 province such as Artvin, Rize, Trabzon, Giresun, Samsun, Bartın, Düzce, Sakarya, Yalova, İstanbul (Ozdemir and Tuncer, 2021; Ak et al. 2023).

Monitoring the global invasion of BMSB indicates that the level of crop damage is directly related to the insect's phenology and voltinism. Abiotic factors have an impact on the number of generations produced by multivoltine insects like BMSB throughout the insect's growth season (Kistner et al. 2017). Furthermore, the fact that it is able to reproduce more than once per year, its high reproductive ability, the capacity of both adults and nymphs to cause damage, and its tolerance to climatic conditions are all factors contributing to the BMSB's damaging potential (Maistrello et al. 2017; Stoeckli et al. 2020). As an example, in Georgia, which borders Turkey and is an important hazelnut producer, it has caused significant damages by generating two generations per year because of having the favorable climatic conditions in the newly invaded areas (Murvanidze et al., 2018; Bosco et al., 2018).

Temperature and photoperiod are the primary determinants on insect phenology (Bale et al., 2002; Tobin et al., 2008). Temperature and photoperiod, which can vary depending on latitude and other geographical traits, are known to influence the development, survival, voltinism, population density, and size of BMSB, as well as its overwintering behavior (Nielsen and Hamilton, 2009b; Zhu et al., 2012; Haye et al., 2014; Costi et al., 2017). BMSB is univoltine or bivoltine in its native habitats in northern China, Korea, and Japan (Lee et al., 2013). It generates one generation every year in northern states such as Pennsylvania, New Jersey, and New York (Nielsen and Hamilton, 2009b; Nielsen et al., 2016), but two generations per year in southern states such as Virginia, North Carolina, and California (Bakken et al., 2015; Leskey et al., 2015; Ingels and Daane, 2018). The BMSB is univoltine in northern Switzerland (Haye et al., 2014), bivoltine in northern Italy (Costi et al., 2017), and in the subtropical zone of western Georgia bordering Turkey (Murvanidze et al., 2018).

Following the first detection of the BMSB in Sakarya province (Ozdemir and Tuncer, 2021), there has been an increase in complaints about it because of its overwintering behavior, which has caused nuisance problem for homes in recent years. BMSB poses a potential threat to corn and kiwi production, as well as hazelnut, the insect's most important host and an important agricultural product in the province. Therefore, it is critical to understand and reveal the biology and behavior of BMSB in order to effectively apply integrated pest management strategies. There is currently no data available on the biology of the pest in Türkiye. Some biological parameters of the first and second generations of BMSB, a new pest in Sakarya province, were studied in this preliminary study.

Material and Methods

Collection of Adults and Obtaining Egg Masses

Overwintering BMSB adults (102 individuals) were collected around the pheromone traps in the woodland region of Sakarya province's Arifiye County in early July, 2022 and placed in sleeve cages (50x70 cm). The branches on which the cages were placed have been chosen to have at least ten fruit clusters in the hazelnut orchard. The cages were visually inspected daily, and the egg masses laid on the leaf surface were collected and used in the experiment.

Determining Hatching Rate, Development Times, and Mortality in Outdoor Conditions

The hatching rate was assessed on 34 healthy egg masses (914 eggs) from sleeve cages. The selected egg masses (4 egg masses including 111 eggs) from these were used for determining nymph development. Egg masses were transferred into ventilated plastic boxes (10 × 10 × 5 cm), which were kept under sheltered outdoor conditions protected from rain and direct sunlight and perceiving photoperiod in Arifiye, Sakarya (40º69’88.98’’N, 30º34’76.44’’E), and their development was followed. The outdoor temperature data was obtained from the local meteorological station. The first instar nymphs were counted and mortality rates were calculated for each egg mass. Following the first molting, all second instar nymphs emerging from each egg mass were collected, transferred into ventilated plastic containers, and their development was monitored daily until the adult stage. As foods, hazelnut (*Corylus avellana* L.), apple (*Malus domestica* Borkh.), pear (*Pirus communis* L.), and bean (*Phaseolus vulgaris* L.) fruits and water were supplied to nymphs as described by Funayama et al. (2015). For each instar, developmental time and mortality were recorded by daily checking. For second generation, newly emerging adults from the first generation experiment (57 adults) were placed in boxes in pairs, and foods and water were provided as previously indicated in laboratory. A single pear leaf was placed to each of the boxes as oviposition substrate. Three egg masses (71 eggs) laid by first-generation females were reared under laboratory conditions (25 ± 1°C, 70 ± 5% RH, and 16:8 h light/dark). Egg hatching, development time and mortality were determined by daily checking.

Calculating Degree-Days for BMSB

Daily temperature data was obtained from regional meteorological station, for Sakarya, Arifiye. The formula max.+min./2 was used to compute daily average temperatures. Accumulated degree-days (DD) were computed by subtracting development thresholds from daily average temperatures. DD calculations were performed by selecting four possible development thresholds ($DD_{12,\ 12.5,\ 13,\ 13.5}$) for BMSB, taking into consideration Haye et al. (2014) because the development threshold for BMSB is yet unclear in Türkiye. Because only a few individuals survived in the second generation, DD was not calculated.

Results and Discussion

Egg hatching and the mortality rates of developmental stages

The hatching rate of egg masses was determined as 90.63% in the 1st generation and 57.75% in the 2nd generation (Table 1). Nielsen et al. (2008) observed that egg masses had an average hatching rate of 81.60% for first generation. In a field study conducted in North Carolina by Ogburn et al. (2023), the egg hatching rate for the first generation was 90%, whereas about 26% for the second generation. As seen in our study, the hatching rate of 2nd generation eggs was lower than that of the first generation.

While overall mortality of nymphs in the first generation was 22.97%, it was 90.24% in the second generation (Table 1). Similarly, Rot et al. (2022) reported that the overall mortality rate in the first generation ranged between 25.07 and 78.57%, and that it ranged between 31 to 100% in the second generation. Haye et al. (2014) and Costi et al. (2017) reported that total mortality and mortality rates for nymphal stages demonstrated significant differences in the first and second generation, which is consistent with our findings (Tablo 1).

Development times of biological stages of Halyomorpha halys

In this study, the egg hatching time was determined to be 5.75 days for the first generation and 5.33 days for the second generation. Lee et al. (2013) showed that the hatching period for BMSB eggs is normally 5-6 days. The total development time from egg to adult was found to be 47.85 days in the 1st generation in outdoor conditions and 47.13 days at 25°C under laboratory conditions in this study (Table 2). Similarly, Rot et al. (2022) determined it to be 55.89 days for the 1st generation, and the longest development period was 81 days in the 2nd generation in outdoor conditions. However, in this study, 28 pairs of BMSB produced only 3 egg masses, and the mortality of second generation nymphs was very high (90%). Few numbers of eggs laid by the first-generation females can be explained by the decline in the proportion of females with fully developed ovaries as a result of the reduction in photoperiod (< 15-15.5 h) (Reznik et al. 2022). Rot et al. (2022) and Govidan and Hutchison (2020) pointed it out as the primary cause of the second generation's reduced fecundity and longer pre-oviposition period. On the other hand, Nielsen et al. (2008) noted that the genetic variations of individuals in the geographic populations, rearing conditions, or the collection dates of the individuals which were used for the study could be attributed to the differences in the pre-oviposition period and fecundity.

A few researches on the biology of BMSB have been carried out in Georgia and Greece, neighboring Turkey. The existence of two generations per year has been reported in Georgia (Murvanidze et al., 2018) and, in Greece (Andreadis, unpublished data). One of the main questions was whether BMSB could produce a second generation in Türkiye. Rearing of the first generation adults showed that adults can lay hatching eggs and nymphs can develop to the adult stage. This preliminary study reveals that BMSB can produce two generations per year in Türkiye. But, of the 28 pairs, only three pairs produced one egg mass per pair. On the other hand, it was observed that overwintering BMSB adults laid eggs until the end of August in this study, as well as in some other studies in Türkiye (unpublished).

Table 1. Egg hatching and mortality of 1st and 2nd generation of *Halyomorpha. halys* at different development stages

G	E	Egg hatch % (mean ± SE)	Number of nymphs (n)	Mean mortality rates of nymphs %					Total Mortality %
				First instar	Second instar	Third instar	Fourth instar	Fifth instar	
1st	914	90.63±3.17	74	6.76	7.25	3.13	6,45	1,72	22.97
2nd	71	57.75±19.44	41	82.93	14.29	16.67	20	0	90.24

G: Generations; E: No. of eggs

Table 2. Mean developmental times of immature stages of 1st generation of *Halyomorpha halys* in outdoor conditions and, 2nd generation of its under-laboratory conditions

SD	MT	E	Egg	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Total nymph period	Total development
Egg-To-Adult of First Generation (Days±SE)										
6 July	24.4	28	7±0.00	5±0.00	6.29±0.34	7.38±0.31	8.48±0.38	14.80±0.69	41.95	48.95
12 July	24.7	28	6±0.00	5±0.00	7.88±0.42	7.80±0.22	9.57±0.58	13.43±0.70	43.68	49.68
15 July	24.9	27	5±0.00	4±0.00	6.74±0.25	7.05±0.33	9.16±0.53	14.44±1.00	41.39	46.39
15 July	24.9	28	5±0.00	3±0.00	7.25±1.57	8.63±1.64	8.88±1.04	13.63±1.15	41.39	46.39
Mean			5.75±0.48	4.25±0.48	7.04±0.34	7.72±0.34	9.02±0.23	14.08±0.33	42.1±0.60	47.85±0.86
Egg-To-Adult of Second Generation (Days±SE)										
23 Aug.	25	20	5±0.00	5.29±0.13	9.67±0.67	8.33±0,33	9±0.00	10±0.00	42.29	47.29
2 Sept.	25	19	5±0.00	5±0.00	9.67±1.67	9±3.00	14.50±0.50	5.50±3.50	43.67	48.67
8 Sept.	25	32	6±0.00	4.44±0.18	8±0.00	8±0.00	8±0.00	11±0.00	39.44	45.44
Mean			5.33±0.33	4.91±0.25	9.11±0.56	8.44±0.29	10.50±2.02	8.83±1.69	41.8±1.16	47.13±0.94

SD: Starting Date; MT: Mean Temp. (°C); E: No.of eggs;

Table 3. Total degree days ($DD_{12, 12.5, 13, 13.5}$) required for first generation of *Halyomorpha halys* development in outdoor conditions, Sakarya province

Theoretical thresholds (°C)	Egg-To-Adult Development (DD)	Calculated DD in some other countries
12.0	608.69±7.68	537.63 DD (Nielsen et al. 2008)
12.5	584.76±7.31	588.24 DD (Haye et al. 2014)
13.0	560.84±6.94	590 DD (Musolin et al. 2019)
13.5	536.91±6.59	530 DD (Rot et al. 2022)

In all the studies mentioned, it was observed that the first-generation adults produced fewer egg masses compared to overwintering adults, similar to some other countries. According to some researches (Nielsen and Hamilton, 2009a; Nielsen et al. 2016; Costi et al. 2017), the adult stages of two generations may overlap in field. It is obvious that our limited data is insufficient to decide the number of progeny implicitly and, further detailed studies are needed in Türkiye. But, at least, this data showed that the first generation adults could generate the partial second generation. Climate appropriateness and photoperiod are critical in this context (Nielsen et al., 2008; Lee et al., 2013). For this reason, the insect developed one generation/year in Zurich, Switzerland (Haye et al., 2014), but it developed two generations/year in Italy's Southern Alps (Costi et al. 2017) and similarly in Sochi Region of Russia (Reznik et al. 2022).

Predicting Degree-Days for BMSB

Considering the BMSB's the minimum temperature threshold, which has yet to be determined in Turkey, the accumulation of degree days were calculated using the references to four possible thresholds ($DD_{12, 12.5, 13, 13.5}$) as the shown in Table 3. Rot et al. (2022) reported that development from egg to adult requires 530 DD for the first generation and 545 DD for the second generation in Slovenia, based on the minimum temperature threshold of 12.2 °C determined by Haye et al. (2014). The egg-to-adult development in the US population was determined to be 538 DD using 14.14 °C as the threshold (Nielsen et al. 2008) and 530 to 590 DD in the Caucasian population for the developmental threshold of 13.3 °C (Musolin et al. 2019). In our study, DD values for first generation of BMSB, based on theoretical development thresholds, changed between 536.91 and 608.69 DD for the lowest (12 °C) and highest (13.5 °C) developmental thresholds respectively. Calculated DD values were consistent, in general, with other determined DD values in the countries (Table 3). Due to the limited number of individuals reaching adult stage (n=4), DD estimations for the second generation were not calculated in this study.

Conclusion

BMSB poses a significant risk to many cultivated plants, particularly hazelnuts, along the Black Sea coastline, where it spreaded in Türkiye. BMSB showed a high egg hatching rate in the first generation and a low rate in the second. Also, nymph mortality was much higher in the second generation. Development time for the first generation was about 50 days, and DD values changed between 537 and609 for first generation under semi-field conditions. This study indicated that the BMSB could generate a partial second generation in Sakarya province, Türkiye; however the number of fertile adults and eggs laid in the second generation was much lower than that in the first generation. These results consisted of preliminary data with a limited number of individuals, and therefore it is necessary to study the biology and seasonal phenology of BMSB in more detail with a larger number of individuals in Türkiye.

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