

## Effect of Atmospheric Carbon Dioxide Applications on Adult Emergence Time of *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae): A Laboratory Simulation Study

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**ABSTRACT:** This study was conducted to determine the effects of two different doses (450 ppm, 600 ppm and 670 ppm) of intermittent carbon dioxide (CO<sub>2</sub>) application on the time required for individuals of *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) to reach the adult stage, under controlled conditions, targeting the 1st and 3rd nymphal instars. As a result of the study, it was evaluated that CO<sub>2</sub> applications made at both doses had a statistical effect on nymphal development times compared to the control application (450 ppm). It was determined that in the control dose, the time from the 1st nymphal stage to the adult varied between 44-55 days, while in 600 ppm dose this period was 60 days, and in 670 ppm this period was approximately 67 days. It was determined that in the control dose, the time from the 3rd nymphal stage to the adult varied between 34-39 days, while in 600 ppm it was 44 days, and in 670 ppm it was 51 days. According to the obtained results, it was understood that the time to reach the adult increased depending on the dose compared to the control application. In studies, revealing the differences in different nymphal stages of the pest and different durations of exposure to carbon dioxide is important in terms of determining both the effects of gas exchange on the behavioral parameters of the pest and the effects of atmospheric gas exchange in the control of the pest.

**KEYWORDS:** *Halyomorpha halys*, Nymph development periods, Carbon dioxide application, Adult emergence time

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## INTRODUCTION

Atmospheric gases have significant effects on plant species, pests and natural enemy species, including behavioral parameters. Carbon dioxide gas is also among the atmospheric gases that have significant effects on insect populations and communities. Pest diversity and their adaptation to the environment, biology, and physiology are affected by global warming. It is still not known exactly how and to what extent this effect will occur. It is also a matter of curiosity how carbon dioxide gas, which is thought to have a significant effect on global warming, will affect the adulthood of pests, and this situation should be determined by different biological studies. In particular, in parallel with these gas changes, the effect of this gas on temperature changes and changes in atmospheric gas levels will cause pests to form different biotopes. It is also expected that these biotopes will be affected by physiological changes in the plant and increase their damage status (Özgen, et al., 2024).

*Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) is a species that is spreading rapidly especially in Black Sea Region and causes significant damage to cultivated plants (Çerçi and Koçak, 2017; Göktürk and Tozlu, 2019; Göktürk et al., 2018). In addition to the name 'Brown skunk', this species is also called 'Brown stink bug' in Türkiye. The pest goes through 5 nymphal stages. The most characteristic feature of the nymphs is the presence of spines on their pronotums in later stages (Hoebeke and Carter, 2003). Although *Halyomorpha halys* can produce 1-5 generations per year depending on temperature and photoperiod (Niva and Takeda, 2003, Lee, 2015), in the Eastern Black Sea Region, it mostly produces 1 generation per year in Artvin province and sometimes 2 generations in coastal areas. The number of generations varies depending on the year, the plant the species feeds on, especially the temperature and humidity conditions, and the habitat in which it lives.

This study was carried out in the Bio-

engineering Department of Firat University Faculty of Engineering in order to determine the effects of three different doses of intermittent atmospheric CO<sub>2</sub> applications on the adult development times of *Halyomorpha halys* nymphs produced under laboratory conditions.

## MATERIALS AND METHODS

### Production of *Halyomorpha halys* individuals

Individuals were produced in a climate chamber where 28±1 °C temperature, 60±10% humidity, and 16/8 photoperiod conditions were provided (Figure 1). The main culture was created with adult *Halyomorpha halys* individuals collected from hazelnut fields in Black Sea Region, where the pest has a high population. The pest was collected from the region provinces (Artvin, Samsun, Trabzon) on 12-13 January 2024 as approximately 60 male and 60 female individuals that had not entered diapause. The pest was kept in room conditions for 2 days, healthy individuals were separated and kept in culture cages as 5 males and 5 females individuals, and a total of 10 individuals were kept together in 12 different culture containers. 10 adults of *Halyomorpha halys* were placed in a container measuring 25 cm in diameter × 9 cm in height (with four 2 mm diameter ventilation holes opened along the edges of the container to provide air circulation). Other individuals were also placed in a square plastic container measuring 30 cm in length × 23 cm in width × 10 cm in height. Paper towels were placed in the containers to provide hiding places and additional surface area for adults to lay eggs easily, and filter paper was placed in the holes opened on the sides of the containers. The open end of the filter paper was placed in pure water and the closed end was placed in the container to meet the water needs of the individuals. In order for the adults brought from nature to adapt to the environmental conditions, the culture containers were checked regularly 3 times a day, and it was checked whether the humidity conditions in the container remained stable and

whether the adults drank water regularly through the filter paper and water pores. During this period, the dead individuals in the containers were collected daily and care was taken to keep the male and female ratio in the container as 1/1. The egg packages left daily were collected by hand. In order not to damage the eggs by the adults, the eggs were collected regularly on a daily basis. Approximately 1 month later, on February 8, 2024, eggs were collected from the adult individuals

brought from nature. The eggs were placed in smaller containers and their hatching was monitored, and the nymphs that hatched from the eggs were fed with carrots, soybeans, and kiwi until they became adults. After the individuals became adults, the adults obtained from the laboratory offspring were separated into 5 males and 5 females again, and trial and application studies were started with the eggs obtained from these individuals (Figure 2).



**Figure 1.** Climate chambers where *Halyomorpha halys* (Stål, 1855) individuals were produced

## RESULTS AND FINDINGS

### Experimental application results

In the study, the effects of one control and two different CO<sub>2</sub> doses on the development of 1st and 3rd instar nymphs of *Halyomorpha halys* were determined and air samples in the cabin were taken within a 24-hour period to determine the changes in gas ratios.

Trials were initiated with nymphs that hatched on the same day as the eggs

hatched. The trials began on April 1, 2024, and the newly hatched *Halyomorpha halys* nymphs were given the same amount of carrots, soybeans and kiwi (Figure 2).

For the experiments, the individuals that reached the nymphal stages were placed in the culture boxes with 5 replications for each gas dose and the experiments were started and the individuals were placed in the gas unit for CO<sub>2</sub> applications (Figure 3).



**Figure 2.** Biological stages of *Halyomorpha halys* used in the experiments



**Figure 3.** Carbon dioxide applications to nymphs

The development of first and third instar nymphs of *Halyomorpha halys* was examined in the study, along with the impacts of one control and two distinct CO<sub>2</sub> doses. Air samples were collected in the cabin over the course of a day to ascertain variations in gas ratios. Two different doses of carbon dioxide were applied intermittently in the cabin. These doses were control (450 ppm), 600 ppm, and 670 ppm CO<sub>2</sub> gas concentrations. The gas was given once and the nymphs were kept in the cabin for 24 hours and then the 1st and 3rd instar nymphs were taken to the air-conditioning cabin. The temperatures of the cabin where the nymphs were kept and the air-conditioning cabin were monitored in 24-hour time periods and kept at the same temperature. In the study, the first air sample was taken at the 8th minute, and the other samples were taken at the 4th, 18th, 21st, and 24th hours. For each application,

25 individuals were left as controls. No gas treatment was applied to the control environment. In the measurements, the CO<sub>2</sub> concentration in the control room was measured as approximately 450 ppm. After the application, the nymphs were monitored daily in the air conditioning cabinet and followed according to the time they took to become adults.

### Statistical analysis results

In this article, 'Shapiro Wilk Goodness of Fit Test and Kolmogorov Smirnov Test' were used to test whether the data obtained were normally distributed. Descriptive statistics such as frequency and percentage (%) values were given for categorical data in the study, and mean and standard deviation were given for continuous data. Since normality assumptions were met, 'Independent Samples T Test' was preferred for comparing groups. In the

analyses, the statistical significance level Differences between the periods' was accepted as  $p < 0.05$ . Statistical transition times to adulthood after dose analyses were performed using the SPSS application and the control groups are (Statistical Package for Social Sciences; shown in Table 1. SPSS Inc., Chicago, IL) 21 package program.

**Table 1.** Statistical comparison of the differences between the transition times of the periods to adulthood according to the doses and the control treatments

Groups	N	Mean	Standard deviation	t	p-value
Control (450 ppm 1st period)	25	54.76	3.58	-7.040	<b>0.000</b>
600 ppm 1st period	22	60.73	2.12		
Control (450 ppm 3rd period)	25	39.08	3.55	0.446	0.658
600 ppm 3rd period	24	43.71	2.14		
Control (450 ppm 1st period)	25	54.76	3.58	-13.964	<b>0.000</b>
670 ppm 1st period	19	66.42	1.86		
Control (450 ppm 3rd period)	25	34.08	3.55	-9.380	<b>0.000</b>
670 ppm 3rd period	21	51.52	1.63		

When Table 1 is examined, the adult transition times of 600 ppm 1st stage and 3rd are significantly higher than the pests are significantly higher than the adult transition times of the pests in the control group ( $p < 0.05$ ). There is no statistically significant difference between the adult transition times of 600 ppm 3st stage pests and the adult transition times of the pests in the control group ( $p > 0.05$ ).

The adult transition times of 670 ppm 1st and 3rd are significantly higher than the adult transition times of the pests in the control group ( $p < 0.05$ ). The statistical differences in the adult transition times of the periods between doses and the adult transition times of different nymphal stages are shown in Table 2.

**Table 2.** Statistical analysis of differences between doses and their periods

Groups	N	Mean	Standard deviation	t	p-value
600 ppm 1st period	22	60.73	2.12	27.091	<b>0.000</b>
600 ppm 3rd period	24	43.71	2.14		
670 ppm 1st period	19	66.42	1.86	26.944	<b>0.000</b>
670 ppm 3rd period	21	51.52	1.63		
600 ppm 1st period	22	60.73	2.12	-9.061	<b>0.000</b>
670 ppm 1st period	19	66.42	1.86		
600 ppm 3rd period	24	43.71	2.14	-13.636	<b>0.000</b>
670 ppm 3rd period	21	51.52	1.63		

When Table 2 is examined, the transition times of 600 ppm 1st stage pests to adulthood are significantly higher than 670 ppm 3rd stage pests to adulthood are

significantly higher than the transition times of 600 ppm 3rd stage pests ( $p < 0.05$ ).

When the general results of this study are considered, the transition times of pre-adult periods with CO<sub>2</sub> application to adulthood are shorter than the control applications in the same period. It is known that this species reaches adulthood in 43 days without reporting food in laboratory conditions in previous studies (Fawad, et al., 2022).

In some studies, conducted under laboratory conditions, it has been reported that the transition period from egg to adult of the species varies between 44 and 52 days (Saito, et al., 1964; Kobayashi, 1967; Watanabe, et al., 1978; Yanagi and Hagihara, 1980; Oda, et al., 1981; Fujiie, 1985; Chu, et al., 1997; Qiu, 2007). These data are parallel to the data of

control applications. The time it took for individuals not treated with carbon dioxide to reach adulthood also varied between 44 and 55 days. It has been determined that these periods were extended with intermittent CO<sub>2</sub> applications applied in the 1st and 3rd periods. These results are important in terms of determining the differences in different nymphal periods of the pest and different periods of exposure to carbon dioxide in future studies, both in terms of the effects of gas exchange on the behavioral parameters of the pest and the effects of atmospheric gas exchange on the control of the pest.

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