FEEDING BEHAVIOR OF *LEPTINOTARSA DECEMLINEATA* (SAY) (COLEOPTERA: CHRYSOMELIDAE) (COLORADO POTATO BEETLE) AND THE AMOUNT OF DAMAGE ON POTATO PLANT

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Abstract. *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is the most destructive pest of potato. In this study, the feeding behavior of this pest and the relationship between the number of individuals and the amount of damage were investigated. Experiments were conducted in a controlled climate room at a temperature of 25 ± 1°C, relative humidity of 55 ± 5%, and photoperiod of 16:8 h using *L. decemlineata* individuals. According to the results obtained from the experiments on the feeding behavior of *L. decemlineata*, the longest total feeding time was observed in fourth-instar larvae. In the damage amount studies, the areas consumed by different biological stages of the potato beetle were calculated. According to the results obtained from these studies, the fourth-instar larvae were in the first place in terms of the area consumed. In addition, as a result of the studies on the damage amount, it was revealed that there was a correlation between the number of individuals of *L. decemlineata* and the amount of damage it caused.
Keywords: *Leptinotarsa decemlineata, feeding behavior, damage, potato plant*

Introduction

Potato (*Solanum tuberosum* L.) is an especially important crop worldwide. With 2020, the potato currently ranks fourth amongst the world's crops, following maize, wheat, and rice (Gikundi et al., 2023). Potato has a wide cultivation area in Türkiye. The provinces where production is intensively concentrated are Niğde, Kayseri, Afyonkarahisar, İzmir and Konya (TÜİK, 2024). Potato, which is widely used in human feeding, also has industrial raw material and export potential and, has made an important place in the national economy (Onaran et al., 2000; Camire et al., 2009). According to TÜİK 2024 data, the potato cultivation area in Türkiye reached 1.391.716 decares and the annual production amount was 6.500.000 tons.

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is the most widespread insect pest that causes major economic losses, especially on potatoes. *L. decemlineata* is also an insect pest of economic importance in the agricultural industry worldwide (Weber, 2003). The Colorado potato beetle is listed as an A2 quarantine pest (EPPO, 2024). Complete yield loss has been reported in potato crops when defoliation is greater than 75%. This underlines the damage that potato beetles can cause to potato fields (Hare, 1980). Damage is caused by larvae and adults (Kennedy, 2009). These individuals can cause complete defoliation of potato plant leaves and can lead to a large yield loss (Casagrande, 1987; Hare, 1980, 1990; Has, 1992). *L. decemlineata* was first observed in 1811 by Thomas Nuttall and formally described in 1824 by Thomas Say. This pest is found almost everywhere in the world (Pope and Madge, 1984; CABI, 2021; EFSA, 2023; EPPO, 2023). *L. decemlineata* is one of the most notorious insect pests of potatoes, and threatens potato farming in Turkey, as in other countries (Alyokhin et al., 2013).

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The adults and larvae of *L. decemlineata* cause damage to the leaves of potato plants in the form of holes of varying sizes, usually starting at the edges. In leaves, a skeleton consisting of veins and petioles is usually present. Consequently, leaf fall can occur. During the larval stage, a single potato beetle can consume 40 cm^2 of potato leaves (Ferro et al., 1985). When the leaves of the plant fall, adult individuals feed on the stems and exposed tubers (Alyokhin, 2009). The defoliation of potato plants by potato beetles can completely destroy potato crops and significantly reduce tuber production (Kennedy, 2009; Alyokhin et al., 2013).

In the present study, two important issues were discussed, which were planned to more clearly reveal the importance of *L. decemlineata* in potato cultivation. First, the aim of this study was to determine the feeding behavior of all larval stages of the potato beetle, males, and females in the pre-oviposition and oviposition periods, and to determine whether there were any differences between stages. The other was to determine the amount of damage in groups consisting of different numbers of individuals in each of the insect's biological periods. This study was carried out to contribute to the literature on these issues and to reveal information about the amount of damage that the pest can cause to potato plants.

Materials and methods

The main materials of the study consisted of the eggs of *L. decemlineata*, individuals in each larval stage, pupae, male individuals, female individuals in pre-oviposition and oviposition periods, and potato plants of the Agria variety. The experiments were carried out in climate rooms with a temperature of 25 ± 1°C, 55 ± 5% proportional humidity, and 16:8 L:D cycles (Boiteau and Alford, 1983).

Insect rearing

Adult individuals were collected from potato plants in Niğde Province, Türkiye by employees of the Potato Research Institute (Niğde). The collected adult individuals were cultured in a climate room, and a stock culture was established. The experiments were conducted on individuals from this culture. Glass cages with wooden frames of 43×75×65 cm and 18 cm diameter with nylon-coated ventilation holes on the front and sides were used for insect production. Potatoes with 18-10 cm tall and 8-10 leaves, placed in plastic pots. They were then placed in cages to feed and lay eggs. *L. decemlineata* individuals were collected from these plants and were allowed to feed. By consuming the leaves of potato plants used in stock culture, all individuals on these plants were collected with a sable brush or hand and transferred to new plants with 8-10 leaves which were placed into glass cages and jars.

Feeding behavior of individuals in different biological stages of L. decemlineata

Experiments were carried out separately and with one individual for all larval stages of the insect, male individuals, female individuals in the pre-oviposition period, and female individuals in the oviposition period. All larvae and adults used in the experiments were 24-h-old individuals.

The individual to whom the feeding behavior was determined, starved for 16 ± 2 h in a cage without food. This individual was then taken up on the leaves of 8-10 leaf potato plants and observed for 1 h. During the observation period, the plants and *L.*

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decemlineata individuals were not caged. The time from the moment an insect starts feeding to the end of feeding without interruption has been accepted as a meal (Mitchell and Low, 1994a, b; Simpson, 1995; Raubenheimer and Browne, 2000; Nagata and Nagasawa, 2006; Fedderwitz et al., 2015).

The observation period was initiated by placing the individual on the young leaf of the plant to be observed when the insect's starvation period was completed. With the start of the 1 h period, the start and end of each feeding and end of the 1 h observation period within that period were determined separately by the electronic timer in seconds. Thus, the feeding time, feeding intervals and feeding numbers were obtained. During the 1 h period, no such situation was encountered, as the larvae and the adults fell off the leaves and died.

Taking into account the values obtained during the 1-h observation period, total feeding time, total time spent without feeding, duration of one feeding and number of feedings were calculated.

Studies on the amount of damage

In the experiments related to determining the amount of damage of *L. decemlineata*, groups of individuals consisting of one, two, four and eight individuals from each larva and adult were considered. All larvae and adults used in the damage experiments were 24-h individuals.

Individuals from the same egg package of *L. decemlineata* produced in the climate room were included in the experiment. These individuals were kept in cages without food for 16 ± 2 h. After this period was completed, groups of individuals of the same age and period were left to feed separately on compound leaves of potato plants for 4 h. Considering the groups of individuals, to 1-4 compound leaves were given, more than the individuals could consume. The plants and *L. decemlineata* larvae were not placed in cages during feeding. The adults were kept in transparent plastic cylinder jars, considering that they could fly. At the end of the feeding period, individuals were removed from the leaves and transferred to a stock culture.

Photographs of each compound leaf of the potato plants used in the experiment were taken. To calculate the area of leaves on which the insects were fed, photographs of the leaves were taken again after the feeding was completed. Then, the images of these photographs were transferred to a computer and the leaf areas were determined using the program called ImageJ® (Abramoff et al., 2004). Differences between the leaf areas measured before and after feeding were recorded. In this way, the areas consumed by individuals belonging to different biological periods of the insect in a 4-h period were calculated.

Data analysis

All experiments were conducted using a single-factor randomized plot design. Feeding behavior experiments were performed in ten replicates, with each individual representing one repetition. To examine differences in feeding behavior among the biological stages of the insect, the collected data were subjected to analysis of variance (ANOVA). A 95% confidence interval with a significance level of < 0.05 was considered as a criterion for the Duncan's multiple range test., using the "agricolae" package in the R Statistical Programme (R version 4.2.2). $P < 0.05$ was established as the criterion for statistical analysis, utilizing the "agricolae" package in the R Statistical Software (R version 4.2.2) (de Mendiburu, 2019).

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Experiments assessing the extent of damage were conducted with five replicates, with one replicate for each individual group. In these experiments, one-way analysis of variance (ANOVA), as well as correlation and regression analyses, was performed on the data measured in square millimeters to elucidate the differences in the extent of damage between the biological stages of the insect and among individual groups, as well as to explore the relationships between these variables. The R packages ggplot2 and Stat2 were used to generate graphical representations for regression and correlation analyses (de Mendiburu, 2019).

Results

Feeding behavior of Leptinotarsa decemlineata (Say) individuals

The total feeding time, total time spent without feeding, and duration of one feeding of *L. decemlineata* differed among individuals at each larval stage and among adults.

The total feeding time was 3,284.00 s on average and was the longest in individuals in the fourth larval stage. This period constituted 91.22% of the observation period and was statistically significant. When the total time spent without feeding was examined, this period was the longest for male and female individuals during the oviposition period. When the total time spent without feeding was examined, it was found to be the longest in male and female individuals during the oviposition period. The total time spent without feeding by male individuals was 2,515.90 s, which constituted 69.89% of the observation period. Female individuals in the oviposition period spent an average of 2,814.00 s without feeding during the 1-h observation period, which corresponded to 78.17% of the observation period. The values observed in male and female individuals during the oviposition period were statistically different from those in the other periods. When the duration of a feeding was examined, this period corresponded to 19.89; 17.55; 18.61; 16.73 and 17.86% of the observation period in second, third, and fourth-instar larvae, male individuals and female individuals in the pre-oviposition period, respectively. These periods were statistically included in the same group (*Fig. 1*).

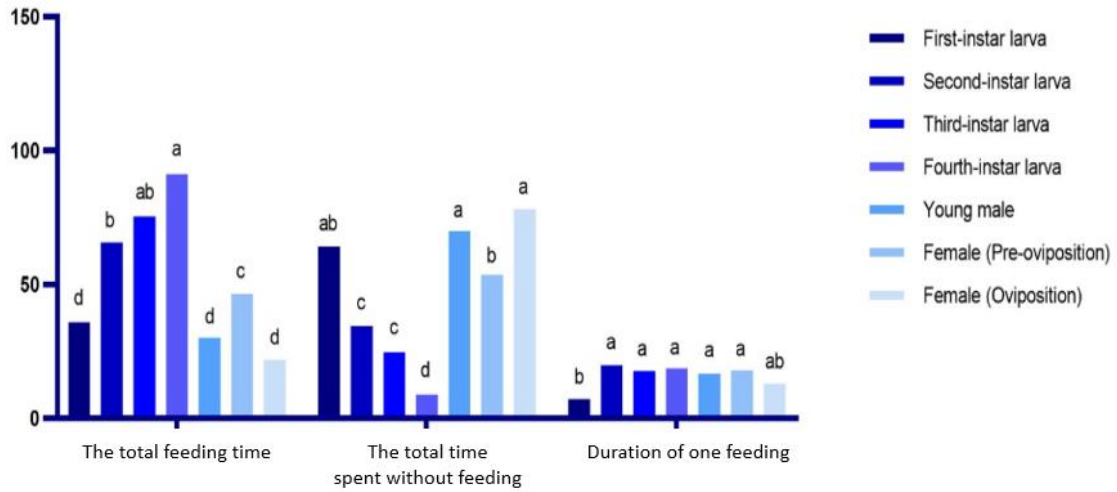

Figure 1. *The ratios (%) of the total feeding time, the total time spent without feeding and duration of one feeding of Leptinotarsa decemlineata (Say) in different biological stages, determined by keeping them on the leaves of the potato plant for 1 h. *Values followed by the same letter (s) are not significantly different at P ≤ 0.05, according to Duncan's multiple range test*

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The longest duration of one feeding was observed in individuals in the second larval stage, with an average of 716.06 s. This period lasts an average of 670.20 s for individuals in the fourth larval stage, and an average of 642.92 s for individuals in the pre-oviposition period. The average duration of one feeding in individuals in the third larval stage was 631.98 s. In young males, this period lasted an average of 602.28 s. When these values were considered, the differences were not statistically significant. The average duration of one feeding for females in the oviposition period was 462.35 s. This duration was the shortest in individuals in the first larval stage, with an average of 257.52 s. The difference between durations was statistically significant (*Fig. 1*).

When the number of feedings was taken into consideration, this number was the highest in the first-instar larvae, with an average of 5.00, followed by the fourth-instar larvae with an average of 4.90 feedings. No statistically significant difference was observed in the number of feedings during either periods. The average number of feedings was seen as an average of 4.30 in the third-instar larvae and an average of 3.30 in the second-instar larvae. The average number of feedings in the pre-oviposition period was recorded as 2.60 and there was a statistical difference between them. This number was determined as 1.80 in the young male period. The lowest number of feedings was observed in females during the oviposition period, with an average of 1.70. The difference between these two values was not statistically significant (*Fig. 2*).

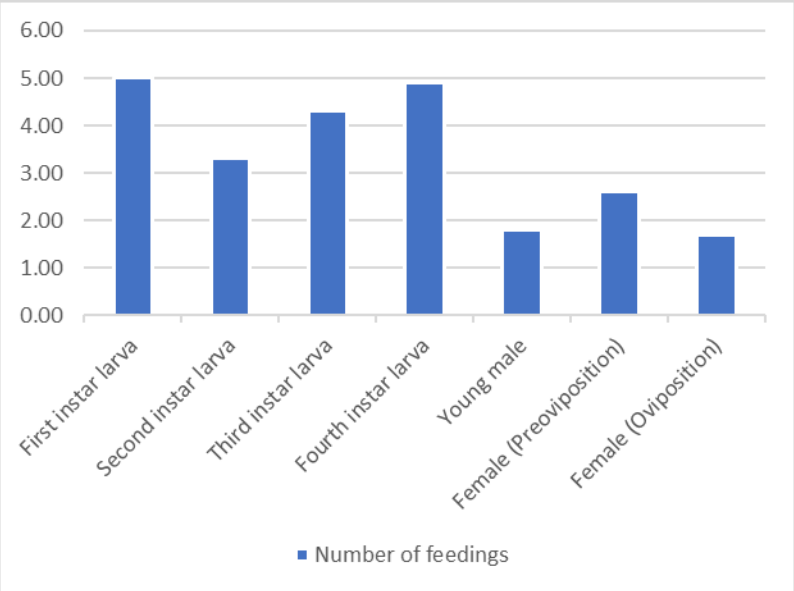

Figure 2. *Number of feedings of Leptinotarsa decemlineata (Say) in different biological stages, determined by keeping them on the leaves of the potato plant for 1 h*

The amount of damage of Leptinotarsa decemlineata individuals in different biological stages

The areas consumed on potato leaves by groups of one, two, four, and eight individuals of *L. decemlineata* (Say) individuals in all larval stages and male, pre-oviposition and oviposition female individuals were calculated.

The areas consumed by *L. decemlineata* larvae, which were kept on the detached leaves of potato plants for 4 h, were examined by considering the individual groups.

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Accordingly, the areas consumed by a single individual were calculated as 7.47 mm^2, 20.94 mm^2 and 119.02 mm^2 on average in the 1st, 2nd and 3rd instar larvae, respectively. The areas consumed by a single individual were found to be the highest in the fourth-instar larvae and were calculated as 328.39 mm^2 on average. In adult individuals, the areas consumed were found to be 280.41 mm^2 and 281.75 mm^2 for young males and females in the oviposition period, respectively. In adult individuals, the areas consumed by females in the pre-oviposition period was the highest, with an average of 317.54 mm^2. When the areas consumed by a single individual were considered, the averages of fourth-instar larvae, young males, pre-oviposition, and oviposition females were in the same statistical group. The average area consumed by a single individual of the third-instar larvae were found to be statistically different from the other stages. When the areas consumed by a single individual in the first and second-instar larvae were examined, there was no statistical difference between these two stages; it was observed that they were statistically different from the other biological periods.

The average area consumed by the two individuals in the first-instar larvae after feeding was 20.26 mm^2. This was followed by the average areas consumed by the second and third-instar larvae as 63.18 mm^2 and 241.23 mm^2, respectively. For young males, females in the oviposition period and females in the pre-oviposition period, the average areas consumed were calculated as 609.18 mm^2; 627.36 mm^2 and 725.04 mm^2, respectively. When the areas consumed by the two individuals were examined, it was observed that the maximum areas consumed were formed by the fourth-instar larvae, with an average of 835.82 mm^2. When the areas consumed by the two individuals were examined, the highest areas consumed were observed in the fourth-instar larvae, with an average value of 835.82 mm^2. When the areas consumed by two individuals from the individual groups were examined, no statistical difference was found between the averages of the areas consumed only by the first and second-instar larvae. The differences between the average values of all the other biological stages were statistically significant.

The areas consumed by the four individuals at different biological stages after 4 h of feeding were calculated. The least consumed area was recorded as 32.79 mm^2 on average in the first-instar larvae. This was followed by the second and third-instar larvae and young males and females in the oviposition period, respectively. The areas consumed by these individuals were calculated as 148.83 mm^2, 585.97 mm^2, 1108.94 mm^2 and 1157.74 mm^2 on average, respectively. The areas consumed by the four individuals from the individual groups were mostly observed in the fourth-instar larvae and in the female individuals in the pre-oviposition period. While the average area consumed by the fourth-instar larvae was 1313.37 mm^2, this value was found to be 1310.38 mm^2 on average in female individuals in the pre-oviposition period. The averages of the fourth-instar larvae and pre-oviposition female individuals were in the same statistical group. The difference between the average values of the areas consumed by the third-instar larvae, young males, and oviposition females was statistically significant. No statistical difference was found between the areas consumed by the first and second-instar larvae, and the mean values of these periods were found to be statistically different from those of other biological stages.

Considering the areas consumed by the eight individuals from the individual groups, the minimum value was 60.20 mm^2 on average for the first-instar larvae. This was followed by the areas consumed by the second and third-instar larvae with an average of 197.50 mm^2 and 737.80 mm^2, respectively. The areas consumed in other biological

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stages were greater. The average areas consumed for females in the oviposition period, young males, fourth-instar larvae and females in the pre-oviposition period were found to be 1894.37 mm^2, 2076.29 mm^2, 2141.57 mm^2, and 2225.64 mm^2, respectively. The difference between the average areas consumed by the fourth-instar larvae, young males, pre-oviposition females, and oviposition females was found to be statistically insignificant. The average areas consumed by the eight third-instar larvae were found to be statistically different from the other stages. When the areas consumed by eight individuals of the first and second-instar larvae were taken into consideration, no statistical difference was observed between these two stages; it was noted that they were statistically different from the other biological stages (*Fig. 3*).

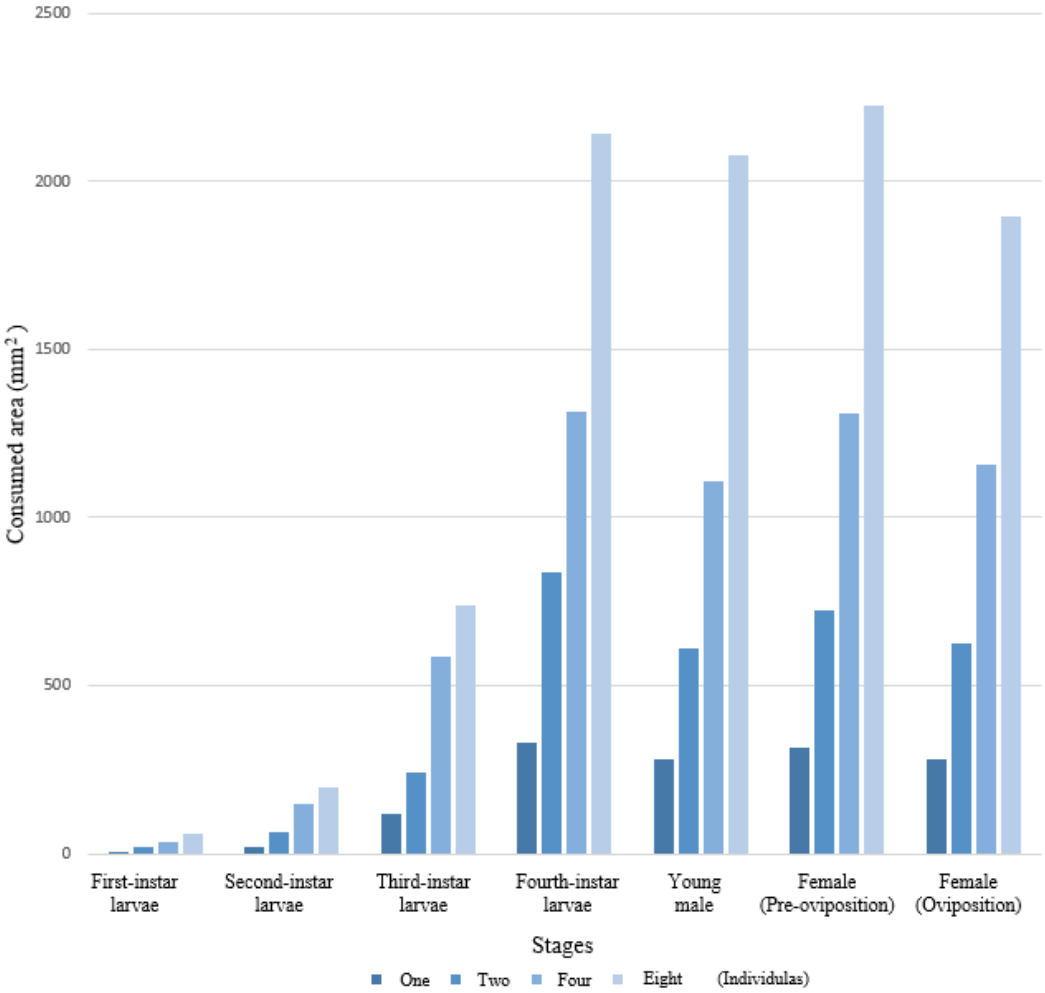

Figure 3. *The area consumed by Leptinotarsa decemlineata (Say) individuals in all larval stages after being kept on the detached leaves of the potato plant for 4 h (mm^2)*

Relationship between the number of individuals and the amount of damage in different biological periods of Leptinotarsa decemlineata (Say)

The areas consumed on potato plant leaves by individuals at all larval stages of *L. decemlineata*, male individuals, and female individuals in the pre-oviposition and oviposition periods were examined separately using regression and correlation analyses (*Fig. 4*).

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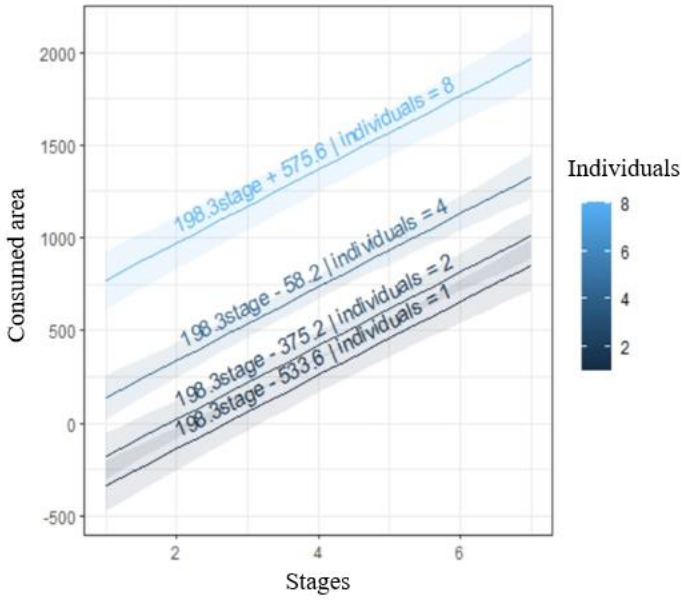

Figure 4. *Regression graph of the relationship between the number of individuals of Leptinotarsa decemlineata (Say) on the leaves of potato plants and the amount of damage*

According to the value in the significance column of the variance analysis table of the data obtained from studies on the amount of damage of individuals in all biological stages of the potato beetle examined, the relationship between the number of individuals and the amount of damage variables was statistically significant at $P < 0.01$ level.

According to the correlation coefficients (R) between the number of individuals and the amount of damage as a result of the correlation analysis, there is a strong and positive relationship between the number of individuals and the amount of damage. Accordingly, an increase in the number of individuals feeding on potato leaves will also cause a significant increase in the amount of damage. When the coefficients of determination obtained from the correlation analysis were examined, a rate of over 80% was observed for all periods of the insect. Accordingly, it can be said that the amount of damage varies depending on the number of individuals for all biological periods of the insect. The regression equations obtained between the amount of damage and number of individuals as a result of the regression analysis are also given in the table (*Table 1*).

Table 1. *Table of regression equations illustrating the relationship between the number of individuals and the extent of damage*

Stages	Correlation coefficient (R)	Coefficient of determination (%)	Regression equation (Y = a + Bx)
First-instar larvae	0.903	81	-12.5 + 17.07 × a*
Second-instar larvae	0.919	84	-46.22 + 61.53 × a*
Third-instar larvae	0.909	83	-129.27 + 220.11 × a*
Fourth-instar larvae	0.939	88	-324.52 + 591.72 × a*
Young male	0.954	90	-453.14 + 588.74 × a*
Female (Pre-oviposition)	0.971	94	-432.76 + 630.97 × a*
Female (Oviposition)	0.954	91	-351.76 + 536.82 × a*

*a: Number of individuals

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Discussion and conclusion

Leptinotarsa decemlineata is one of the most devastating insect pests responsible for the declining potato production in Türkiye. In the present study, studies on the feeding behavior and damage amount of *L. decemlineata* were conducted. As a result of studies on feeding behavior, the total feeding time of *L. decemlineata* was observed to be the longest among individuals in the fourth larval stage. These individuals were followed by third-instar larvae, second-instar larvae, and females in the pre-oviposition period, respectively. The differences between the periods were statistically significant. It was noted that the total feeding time was the shortest in females during the oviposition period. However, the differences between the total feeding time values of female individuals in the oviposition period, individuals in the first larval stage, and young male individuals were found to be statistically insignificant. Nagata and Nagasawa (2006) reported in their study on the feeding behavior of *Bombyx mori* (L., 1758) (Lepidoptera: Bombycidae) that the longest time spent feeding was observed in fifth-instar larvae. They noted that the shortest feeding time was observed in the first and second-instar larvae. In our study, we observed that the fourth-instar larvae of *L. decemlineata* were fed for the longest time. It can be said that the obtained result is similar to the result of the study conducted by Nagata and Nagasawa (2006). Raubenheimer and Browne (2000) reported that fourth- instar larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) spend more time feeding than fifth instar larvae. They noted that this change and behavioral mechanisms, such as meal duration and frequency, can be explained by increased nutritional requirements with development. However, they emphasized that further studies would be helpful in explaining the reasons for the observed developmental changes.

In studies of *Hylobius abietis* (L.) (Coleoptera: Curculionidae), Fedderwitz et al. (2015) found the average duration of a feeding session was 843 s in adult females and 362 s in adult males. In our study, the average meal duration in adult individuals was found to be 602.28 s in males, 642.92 s in pre-oviposition females and 462.35 s in oviposition females. It was observed that male *H. abietis*, which has a biting and chewing mouth structure, had shorter meal times than *L. decemlineata*. In female *H. abietis*, it was observed that meal times were longer. Reynolds et al. (1986) investigated the feeding behavior of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) larvae and reported a strong relationship between the length of the feeding period and the length of the previous non-feeding period.

Mitchell and Low (1994b) investigated the feeding behavior of 18 adult *L. decemlineata* females during the pre-oviposition period. They observed 18 adult females for a total of 49.871 s (13.85 h) over two days and recorded a total of 346 meals. In our study, when the number of meals of *L. decemlineata* was examined after 1 h of observation, it was found that the average number of meals in females in the pre-oviposition period was 2.60 and in females in the oviposition period was 1.70. When these results are examined, it is seen that this study is consistent with the study conducted by Mitchell and Low (1994b).

In studies of the amount of damage caused by *L. decemlineata*, the ImageJ® program was used to determine the consumed areas. Different researchers (Alchanatis et al., 2000; O'neal et al., 2002; Ji et al., 2017) have also used different programs for leaf area measurements. From the damage amount studies, it was observed that the highest consumption in all biological periods was a result of the feeding groups of eight individuals. However, it was noted that the area values consumed by the larvae in the

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third period as a result of the feeding groups of four and eight individuals were in the same statistical group. In all other biological periods except the third-instar larvae, the values of the areas consumed as a result of the feeding of eight individuals were found to be statistically different from the values of the areas consumed by other individual groups. Pernal et al. (1996) studied the amount of damage caused by feeding larvae of *Epitrix cucumeris* (Harris) (Coleoptera: Chrysomelidae) on the leaves of potato plants. In their study, as in this study, they examined groups of individuals consisting of one, two, four, and eight. As a result of their studies, these researchers also stated that increasing the number of insects linearly increased the average number of holes per leaf, that is, the amount of damage.

In terms of biological periods, the largest leaf area was consumed by fourth-instar larvae, followed by the areas consumed by female individuals in the pre-oviposition and oviposition periods. The values of the areas consumed by the fourth-instar larvae, pre-oviposition, and oviposition female individuals, as a result of feeding in groups of eight individuals, were included in the same statistical group. The same situation was observed for the feeding behavior of one individual. The average areas consumed by adult individuals as a result of feeding for a period of 4 h was found to be 280.42 mm^2 for young males, 317.54 mm^2 for females in the pre-oviposition period and 281.75 mm^2 for females in the oviposition period. Kennedy (2009) stated that the average area consumed by adult *L. decemlineata* individulas per day varies between 130 and 1200 mm^2. When the areas consumed by adult individuals in this study are compared with the results of Kennedy (2009), it can be seen that there is harmony between them. Hare (1980) investigated the effects of *L. decemlineata* leaf consumption on the yield of potato plants. Hare (1980) noted that the plant was most affected by the consumption of leaves by potato beetles when adult emergence began. They also stated that this effect continued when female individuals in the oviposition period were observed. According to the data found in this study, we should state that the areas consumed by fourth-instar larvae are as much as those consumed by adults. Noronha et al. (2002) investigated the damage potential of *L. decemlineata* in potato-producing areas in 1999 and 2000. In their study, they stated that the damage increased from the moment the third and fourth-instar larvae began to appear, and continued to increase when adults emerged from the pupa. In our study, the areas consumed by the fourth-instar larvae were the highest, followed by the areas consumed by the adults. From this point of view, similar results were observed with the studies conducted by Hare (1980) and Noronha et al. (2002).

By investigating the feeding behavior of this important pest, information has been obtained regarding the damage it causes. The length of the feeding period provided preliminary information regarding the amount of food consumed. The longer the feeding period, the more the insect feeds and the more damage it causes. This study contributes to the literature. It is also thought that this study can be a model for studies conducted not only on the potato beetle but also on other similar biting and chewing species, especially insects in the Chrysomelidae family. Although feeding behavior has only been determined for some insects, collecting data from more species will facilitate conclusions regarding differences in feeding behavior based on phylogeny, ecology, and physiology. Basic information about feeding behavior can help in the design and interpretation of studies on phytophagous insect-plant interactions. This could also facilitate the evaluation and development of methods to protect plants against phytophagous insects.

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The amount of damage is related to the amount of leaf area consumed by the insect and varies according to the biological stages of the insect and the number of individuals. Therefore, an attempt was made to reveal the relationship between the amount of damage and the number of individuals at different biological stages. According to the values obtained as a result of this study, the total feeding period was the longest for the fourth-instar larvae. As a result of the damage amount studies, it was noted that the areas consumed by the fourth-instar larvae were the highest in all individual groups. In the light of these results, it is clear that long-term feeding of fourth-instar larvae will cause serious consumption in the leaf area.

Regression graphs were created to reveal the relationship between the number of individuals and amount of damage. With the help of these graphs, the amount of leaf area *L. decemlineata* individuals at a certain population density can be estimated at that density. As a result, comments can be made in terms of controlling this pest. These studies are expected to shed light on the threshold of economic damage. Therefore, studies on this pest are necessary to determine the time of control and prevent unnecessary spraying. It is expected that this study, which was conducted in line with these objectives, will provide data for future studies on this pest.

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