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## The effect of biological periods and number of individuals on the damage amount of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)

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### Abstract

In this study, the relationships between the amount of damage and the phenology of the potato plant, and biological periods and the number of individuals of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) were investigated. For the relationship between the different biological stages of the insect and the amount of damage, individuals were allowed to be fed for 24 hours. The most fed in biological period on the potato plant was the third larval stage of *L. decemlineata* while the least period was recorded as adult individuals. For the relationship between the phenological period of the plant and the amount of damage, potato plants with a length of about 20 and 40 cm were used. Adult individuals of *L. decemlineata* averaged 0.46 g in leafy branch pieces of a 20 cm potato plant, and 0.36 g on average in 20 cm leafy branch pieces from the tip of a 40 cm potato plant. It was determined that adult individuals of *L. decemlineata* feed more on leaves of young potato plant. For the relationship between the number of individuals and the amount of damage, experiments were set up with adults in groups of 1, 2, 3, 4 and 5, respectively. While a single adult individual of *L. decemlineata* cause an average of 0.15 g feeding damage on the leafy branch parts of the potato plant, it was recorded as 0.13 g for two individuals, 0.24 g for three individuals, 0.21 g for four individuals, and 0.33 g for five individuals, respectively.

**Keywords:** *Leptinotarsa decemlineata*, Behavior, Potato, Damage amount

### Introduction

*Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) causes significant damage to many plants from the Solanaceae family in the world and in Turkey (Atak, 1973). Although the best host of this pest is potato, it can also cause damage on tomato, pepper and some weeds from this family (Atak, 1973; Has, 1992; Hare, 1990). Adults and larvae of potato beetle cause significant damage by feeding on all green parts of the potato plant. It has been noted that it causes significant damage to the plants, especially in the early development stages when the high population level is reached with the pest. In addition to the damage caused by direct feeding, this pest is also effective in the spread of potato brown rot, potato ring rot and spindle tuber viroid, which are important diseases of potatoes (Yuceer, 2011; Anonymous, 2017). If there is any struggle against this pest, the damage ratio can reach 100% (Has, 1992; Alyokhin et al., 2008; Cam et al., 2012).

In this study, the relationships between the phenological period of the potato plant, the biological period of *L. decemlineata*, the number of individuals and the amount of damage of the pest were separately investigated. For this purpose, it was presented that biological period of *L. decemlineata* caused more damage, phenological period the potato plant was fed more and how the increase in the number of individuals of *L. decemlineata* affected the change in the amount of damage.

### Material and Method

#### Plants and pest culture

Potato (*Solanum tuberosum* L.) tubers belonging to the Agria potato variety were planted in 1.5 l pots with a 1:1 soil: peat mixture. Daily care and irrigation were carried out when needed. During the growing of plant, no chemical control was made against disease, pest or fertilization. Adult individuals of *L. decemlineata* (Coleoptera: Chrysomelidae) were collected from potato growing experiment area in Isparta University of Applied

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Sciences, Faculty of Agriculture in May 2020. Collected adult individuals were brought to the laboratory in plastic culture containers and placed on 30x50x60 cm sized potato plants in a plexiglass cage covered with tulle on the sides and top. The eggs laid by the adults fed on these potato plants were taken from the cage together with the leaf on which they were laid, and placed in a 5x8x12 cm plastic culture container with blotting paper at the bottom. Larvae hatched from these eggs were infested on potato plants in another plexiglass cage. In order to increase the population of the pest and to ensure mass production, potato plants used as food were renewed when necessary.

#### **Establishment of Trials**

##### **The Relationship Between the Biological Period and the Amount of Damage**

Experiments were carried out according to the randomized plot design with 10 replications. All larval and adult individuals of *L. decemlineata* taken from cages where the insect is mass-produced were used in the applications. Insects of different biological periods taken from the mass-produced cages were transferred into a 5x8x12 cm plastic culture container with blotting paper at the bottom as one for each according to their biological periods. Then, weight measurements of leafy branch pieces taken from the potato plant were made with an electronic precision scales (Kern ABC 220-4 m, max.=220 mg, min.=10 mg, e=1 mg, d=0.1 mg). The cut parts of the plants were placed in 5 ml eppendorf tubes containing water and fixed in eppendorf with the assistance of cotton.

The prepared leafy twigs were placed in plastic culture containers containing different biological stages of the insect, as food. After the different biological stages of the insect were allowed to feed for 24 hours, the re-weights of the leafy branches given as food were measured with precision balances and recorded.

##### **The Relationship Between the Plant Phenology and the Amount of Damage**

Experiments were carried out according to the randomized plot design with 10 replications. Potato plants grown in 1.5 l pots with a 1:1 soil: peat mixture was carried out with plants reaching a length of approximately 20 cm and 40 cm. The weights of leafy branch pieces taken separately from plants with 20 cm and 40 cm lengths were made and the cut parts of the plants were placed in 5 ml eppendorf tubes containing water and fixed with the help of cotton so that they do not dry out. These tubes, in which plant parts were placed, were placed in a 5x8x12 cm plastic culture container with blotting paper at the bottom according to the different phenological periods of the plant parts taken from the plants. Then, the adult individuals taken from the insect mass production cages were transferred to the culture containers, one for each. After the pests were allowed to feed for 24 hours, the weights of the leafy branches given as food were recorded with precision scales again.

##### **The Relationship Between the Number of Individuals and the Amount of Damage**

Experiments were carried out in a randomized plot design with 5 replications. In the applications, the weight measurements of the leafy branch pieces taken from the potato plant were made. Then, the cut parts of the plants were placed in eppendorf tubes containing water and fixed with the help of cotton so that the leafy branch pieces taken from the potato plant did not dry out. These tubes were placed in a 5x8x12 cm plastic culture dish with blotting paper at the bottom. Adult individuals taken from cages where the insect was mass produced were placed in groups of 1, 2, 3, 4 and 5 in culture pots containing one leafy branch piece, respectively. After the pests were allowed to feed for 24 hours, the re-weights of the leafy twigs given as food were measured and recorded.

All experiments with plant and pest production were carried out in a climate room with 25°C temperature, 60±5% relative humidity and 16 hours of light and 8 hours of darkness.

#### **Data analysis**

After the analysis of variance (ANOVA) conducted on data which obtained from this trial, Tukey's HSD multiple comparison test was applied ( $P \leq 0.05$ ). Two-way t-test (Paired-samples t-test) was applied to the data to determine the relationship between plant phenology and amount of damage ( $P \leq 0.05$ ). In addition, correlation and regression analysis ( $P \leq 0.01$ ) between the number of individuals and the amount of feeding obtained in grams were performed. Statistical analyses were performed by IBM® SPSS® Statistics (Version 20.0, August 2011, SPSS Inc., Chicago, Illinois, USA) package program.

#### **Findings and Discussion**

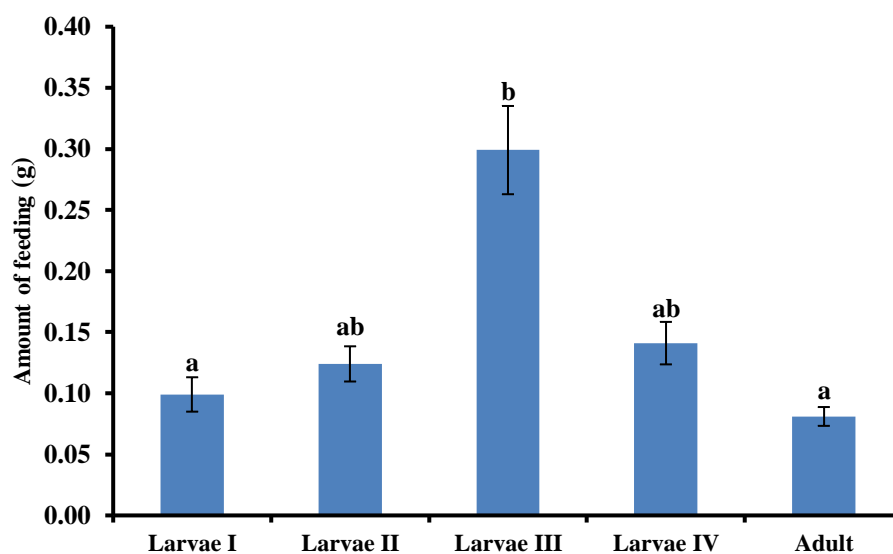
##### **The Relationship Between the Biological Period and the Amount of Damage**

The feeding amount of *L. decemlineata* on the potato plant was recorded as 0.10 g for the first larval stage, 0.12 g for the second larval stage, 0.30 g for the third larval stage, 0.14 g for the fourth larval stage and on average as 0.08 g. Third larval stage had the highest amount of feeding on the leafy branch parts of the potato plant and a statistically significant difference was found between the other periods. There was no significant difference between the first larval stage of *L. decemlineata* and the adult stages in terms of the amount of feeding on the potato plant.

The feeding rates of the second larval stage and the fourth larval stage of *L. decemlineata* were found to be close to each other on the potato plant. The feeding amount of the third instar larvae of *L. decemlineata* on leafy stem parts of the potato plant is higher than the first larval stage and adult stages. Likewise, the feeding amount of the third larval stage is higher than the feeding amount of the second instar and fourth larval stage, and the difference between the feeding amounts is important. In this study, it was determined that the second and fourth larval stage of *L. decemlineata* fed more than the adult and first

instar larvae. While the third larval stage of *L. decemlineata* were the most fed biological period on

the potato plant, the least fed period was recorded as the adult individuals (Figure 1).



**Figure 1.** Feeding amounts of different biological stages of *Leptinotarsa decemlineata* fed on potato plant in 24 hours (The difference between the means ( $\pm$ standard error) represented by columns with the same letter is statistically insignificant (Tukey's HSD test  $P \leq 0.05$ ;  $F = 3.660$ ;  $sd = 4.45$ ;  $P = 0.012$ ).

Yaglıkçı and Karsavuran (2011) recorded the highest average live weight of *L. decemlineata* fed on potato plants in the fourth larval stage as 155.91 mg. This period was followed by the third instar larvae with 79.12 mg, the second larval stage with 27.25 mg and the first larval stage with 10.50 mg. The area consumed by *L. decemlineata* on potato leaves in different biological stages was recorded in the fourth larval stage with a maximum of 328.39 mm<sup>2</sup>. This period was followed by preoviposition with 317.54 mm<sup>2</sup>, oviposition with 281.75 mm<sup>2</sup>, young male with 280.41 mm<sup>2</sup> and third larval stage with 119.02 mm<sup>2</sup> (Ozsarı, 2018). Noronha et al., (2002) noted in their field study that the damage potential of *L. decemlineata* increased during the

third-fourth larval stage and the first adult emergence. In another study, the consumption amount of the second larval stage of *L. decemlineata* at 25°C was recorded as 1,313.32 mg. The consumption amounts of the third and fourth larval stage of the pest were determined as 1,097.72 and 711.22 mg. It was determined that the adults in the adult period are in the same statistical group with the consumption amount of 850.52 mg of the fourth larval stage (Cinar, 2020).

#### The Relationship Between the Plant Phenology and the Amount of Damage

The relationship between plant phenology and damage amount of *L. decemlineata* fed on potato plant is given in Table 1.

**Table 1.** Feeding amounts of *Leptinotarsa decemlineata* fed on potato plants in different phenological periods in 24 hours (g)\*

	Amount of feeding $\pm$ SE	t	sd	P
Plants 0-20 cm tall	0.46 $\pm$ 0.085	1.108	9	0.297
Plants 20-40 cm tall	0.36 $\pm$ 0.0230			

\*\*Two-way t test (Paired-samples t test) ( $P \leq 0.05$ ) was used.

Adult individuals of *L. decemlineata* fed on potato plant in different phenological periods average was recorded as 0.46 g on average in leafy branch pieces of a potato plant with a size of about 20 cm, and the amount of feeding in leafy branch pieces 20 cm from the tip of a 40 cm potato plant as 0.36 g. It was determined that the adult individuals of *L. decemlineata* were fed more on the leaves of the young potato plant and there was no statistically significant difference between the feeding amount

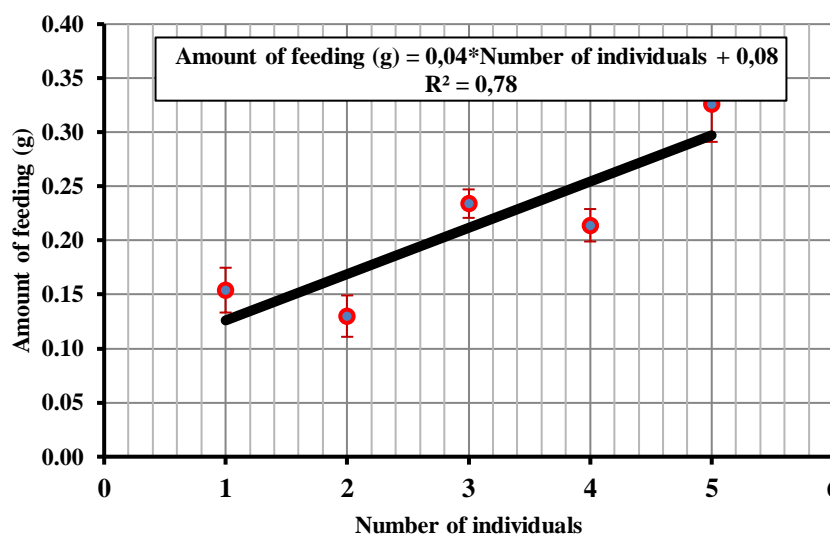
on the leaves of the partially older potato plant. In a study, it was determined that young leaves of *L. decemlineata* preferred more than old leaves, respectively, 55.00% for the first larval stage, 64.22% for the second larval stage, 81.67% for the third larval stage, 54.17% for the fourth larval stage and 65.00% for individuals (Telli, 2012). However, it was determined that feeding on young leaves was high with weight gains between the larvae transitions of the potato beetle (Cibula et al., 1967). In females

of *L. decemlineata* fed with old potato leaves, symptoms such as immobility and reproductive arrest were recorded (de Wilde et al., 1969).

#### The Relationship Between the Number of Individuals and the Amount of Damage

While a single adult individual of *L. decemlineata* causes an average of 0.15 g feeding damage on leafy branch parts of the potato plant, the average feeding damage was recorded as 0.13 g for two individuals, 0.24 g for three individuals, 0.21 g for four individuals, and 0.33 g for five individuals. According to the results of variance analysis on amount of damage to the adult individuals of *L. decemlineata* fed on the potato plant. The relationship between the number of individuals and the amount of damage variables is significant at the  $P < 0.01$  level. The functional expression of this relationship is  $F(1, 18) = 312.556$ ;  $P < 0.01$ . As a result of the correlation analysis, it was found that the correlation coefficient between the number of individuals and the amount of damage was  $R_{(\text{Number of individuals, Amount of damage})} = 0.883$ . According to this

correlation coefficient, the relationship between the number of individuals and the amount of damage is accepted as a strong and positive relationship. Accordingly, it can be said that the increase in the number of individuals fed on the potato plant may cause a significant increase in the amount of damage. According to the coefficient of determination obtained as a result of the correlation analysis, approximately 78% of the damage amount varies depending on the number of individuals. The regression equation obtained as a result of the regression analysis;  $\text{Damage amount} = 0.08 + 0.04 * \text{Number of individuals}$ . As a result of the study, it has been determined that there is a strong relationship in terms of the number of individuals and the amount of damage and this relationship is positive. In addition, it has been observed that the amount of damage will increase as the number of individuals increases up to a certain level. It was seen that there is a correlation between the number of individuals and the amount of damage caused by *L. decemlineata* (Figure 2).



**Figure 2.** Linear regression of feeding amounts of *Leptinotarsa decemlineata* in different numbers of individuals fed on a potato plant in 24 hours and the correlation between the number of individuals and the amount of feeding ( $P \leq 0.01$ ).

#### Results

In the study, it was founded that the feeding amounts of different biological periods of *L. decemlineata* on the potato plant differed. In this experiment, it was seen that while the third larval stage of *L. decemlineata* were fed the most, there was no significant difference between the feeding amounts of the other periods. It was observed that there was no statistical difference in terms of feeding amounts on leafy branch pieces taken from approximately 20 cm and 40 cm potato plants of *L. decemlineata* in the experiment using different plant phenologies. In the experiment conducted on the relationship between the number of individuals and the amount of damage of *L. decemlineata*, it was determined that there was a relationship between the number of individuals and the amount of feeding

( $R^2_{(\text{Number of individuals, amount of damage})} = 0.78$ ). It was observed that there was a linear increase in the amount of feeding as the number of individuals increased.

#### Compliance with Ethical Standards

##### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

##### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

**Ethical approval**

Not applicable.

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**Data availability**

Not applicable.

**Consent for publication**

Not applicable.

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