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## The feeding performance of *Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae) on cucurbit powdery mildew, *Erysiphe cichoracearum* (DC.) (Helotiales: Erysiphaceae)

*Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae)'nın Kabakgillerde külleme hastalığı *Erysiphe cichoracearum* (DC.) (Helotiales: Erysiphaceae) üzerindeki beslenme performansı

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### ABSTRACT

The feeding capacity of *Psyllobora vigintiduopunctata* on powdery mildew *Erysiphe cichoracearum* was investigated under laboratory (25±2°C, 70±5%RH, 12:12h photoperiod) and semi-field conditions. Under laboratory conditions, the total powdery mildew-infected area consumed approximately 19 cm<sup>2</sup> over the entire larval period. Over their lifetime, adult males consumed an average of 195.86±5.48 cm<sup>2</sup> of infected area, whereas females consumed 225.14±5.48 cm<sup>2</sup>. The semi-field experiments were performed to determine the efficacy of *P. vigintiduopunctata* against powdery mildew by releasing 10, 15, and 20 adults onto squash plants at the initial stage of infection. Distilled water was used as the control treatment. A commercial fungicide containing 200g/L azoxystrobin and 125g/L difenoconazole (Quadris® Maxx, 80ml/100L water) was applied to infected plants as the positive control treatment. The infected leaf area on squash plants was measured at five-day intervals, and the trials were terminated on day 20. In the control group, the plants were 100% infected with powdery mildew by day 20. The mean disease severity in plants treated with 10, 15, and 20 adult *P. vigintiduopunctata* was 16.99–17.07%, 12.88–13.42%, and 8.55–8.79%, respectively, on day 20. The average impact factors were calculated as 82.93–83.01%, 86.58–87.12%, and 91.21–91.45%, respectively. In squash plants that received the fungicide treatment, the severity of powdery mildew increased from day 15 onwards, reaching approximately 11% disease severity and 89% fungicide effectiveness by day 20. These results were in agreement with laboratory-based feeding capacity data and revealed that releasing 20 adult *P. vigintiduopunctata* per plant at the initial stage of infection can provide highly effective suppression of *E. cichoracearum*, representing a strong alternative to chemical control strategies.

## INTRODUCTION

Powdery mildew diseases are among the most common pathogens affecting cucurbit crops and represent a major group of diseases that spread rapidly among plants. In the chemical control of this disease in cucurbits, fungicides containing active ingredients such as boscalid, azoxystrobin, thiophanate-methyl, difenoconazole, as well as those belonging to the Strobilurin and Benzimidazole groups, are widely used (Newark et al. 2020). However, studies have reported that this pathogen has developed resistance to fungicides, particularly those in the Strobilurin and Benzimidazole groups, leading to an increase in the application rates of these compounds (Kooistra 1968, Lebeda and Křístková 1996, Elderfield et al. 2018, Newark et al. 2020). The implementation of alternative control methods such as disease-resistant plant cultivars, biofungicides, fertilisation, and biocontrol agents within integrated pest management strategies is of great importance (Yücesoy et al. 2020, Hanedar et al. 2023, Elagamey et al. 2023, Yalçın and Ülgentürk 2023). *Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae; Halyziini) is widely distributed across terrestrial climatic zones. It feeds on all structures of powdery mildew pathogens, including conidia, conidiospores, and mycelium. Previous studies have reported that it feeds on powdery mildew pathogens developing on various host plants, including *Erysiphe cichoracearum* (DC.), *Erysiphe convolvuli* (DC.), *Phyllactinia guttata* (Wallr.), *Sphaerotheca fuliginea* (Schltldl.), *Microsphaera alphitoides* (Griffon & Maubl.), *Uncinula necator* (Wallr.: Fr.) and *Oidium* sp. (Link) (Helotiales: Erysiphaceae) (Ratti 1996, Sutherland 2005, Sutherland and Parrella 2006, Sutherland 2009, Younes et al. 2015). The feeding capacity of *P. vigintiduopunctata* varies depending on both the host plant and the powdery mildew species; for example, larvae feeding on different powdery mildew species have been reported to exhibit the highest consumption on *E. convolvuli* and the lowest on *U. necator* (Younes et al. 2015). Yalçın et al. (2026) monitored two generations of *P. vigintiduopunctata* while feeding exclusively on cucurbit powdery mildew under laboratory conditions, and the predator completed its development within 26.7–28.4 days in both generations. The total lifespan of females ranged between 102–108 days, whereas that of males ranged between 106–107 days. The mean lifetime fecundity per female varied between  $317.48 \pm 142$  and  $376.55 \pm 146$  eggs. The intrinsic rate of increase ( $r$ ) of the predator on *E. cichoracearum* ranged between 0.0788–0.0829 females/day, while the mean generation time ( $T$ ) varied between 58.7–61.5 days. The gross reproductive rate (GRR), representing the total

reproductive output produced by a female per generation, was calculated as 219.5–228.5 individuals.

However, most of these studies have been conducted under observational or laboratory conditions, and the effectiveness of *P. vigintiduopunctata* against powdery mildew under field conditions has not yet been investigated.

The high survival and reproductive capacity of *P. vigintiduopunctata* when fed exclusively on cucurbit powdery mildew for two consecutive generations under laboratory conditions indicate that this species may represent a promising biological control agent against powdery mildew in cucurbit crops (Yalçın et al. 2026). Therefore, the present study aimed to evaluate the feeding capacity and efficacy of *P. vigintiduopunctata* on cucurbit powdery mildew under both laboratory and semi-field conditions in order to determine its potential for use in biological control programs.

## MATERIALS AND METHODS

*Cultivation of host plants, establishment of Erysiphe cichoracearum culture, and establishment of Psyllobora vigintiduopunctata colony*

Cucurbit seeds were grown at regular sowing intervals, and when the plants reached the 8–10 leaf stage, the powdery mildew pathogen was inoculated onto the plants using a soft-tipped brush to ensure the continuous availability of both plant material and the pathogen. Individuals of *P. vigintiduopunctata* were reared in plexiglass cages measuring 70×50×60 cm. The infected plants were used both for mass rearing of the predator and for experimental applications (Yalçın et al. 2026).

*Determination of feeding capacity of Psyllobora vigintiduopunctata fed on Erysiphe cichoracearum*

The study was conducted using *P. vigintiduopunctata* adults cultured under laboratory conditions, and this generation was designated as F1. The same experiment was repeated with the new generation adults (F2) obtained from this generation. The powdery mildew-infected area consumed by *P. vigintiduopunctata* during the larval, prepupal, and adult stages was recorded daily, and individual consumption amounts were calculated using the ImageJ software developed by the National Institutes of Health (NIH) (<http://imagej.org>) (Figure 1). ImageJ is a computer-based image analysis program capable of detecting color differences and performing quantitative measurements. It is an open-source software that allows visualization, examination, measurement, and validation of scientific image data. During image acquisition, digital photographs of infected leaves were taken using a fixed measurement reference (e.g.,

a ruler), and the images were transferred to a computer for analysis. The powdery mildew-infected area on each leaf was measured, and after 24 hours of feeding by a larval or adult individual, the leaf was re-photographed. Based on these measurements, the total powdery mildew-infected area consumed by *P. vigintiduopunctata* was calculated in cm<sup>2</sup> for each 24-hour period and across all developmental stages. The number of individuals used at each developmental stage is presented in Table 1.

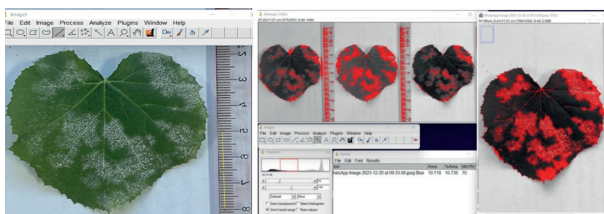


**Figure 1.** Measurement of the infected area with *Erysiphe cichoracearum* on squash leaves (Original: F.YALÇIN)

For each larval stage, total consumption was determined based on molting events. During the adult stage, individuals were monitored daily until death, allowing the total area consumed by a single adult over its lifespan to be determined. During the monitoring, fully powdery mildew-infected leaf sections (100% infected) were used. When these leaves became degraded or the mildew infection was no longer present, they were replaced with fresh infected leaves.

#### *Determination of the efficacy of Psyllobora vigintiduopunctata against Erysiphe cichoracearum under semi-field conditions*

Semi-field experiments were established at the experimental fields of the Directorate of Plant Protection Central Research Institute (Ankara, Türkiye) and were conducted in aerated mesh cages measuring 125×65×65 cm (Figure 2). The cultivation of squash seedlings and the inoculation of squash powdery mildew were carried out using the methods described above. In the experiment, each cage was considered one replicate. Four pots containing one powdery mildew-infected squash seedling each were placed inside each cage. The experiment was conducted with four replicates and the cages were positioned in the field at least 2 m apart from each other. Previous field observations in the experimental area indicated that *E. cichoracearum* caused the highest infection levels under Ankara conditions during May and September, which is consistent with the seasonal incidence patterns of cucurbit powdery mildew reported under similar continental climate conditions (Lebeda and Křístková 1996, Yücesoy et al. 2020). Therefore, the study was conducted twice, corresponding to the periods of high cucurbit powdery mildew incidence in Ankara, namely May (Replicate 1) and September (Replicate 2).

Applications to the control group and predator release were initiated when powdery mildew symptoms were present on 0–1% of the leaf area, corresponding to disease scale level '1' (MAF 2025a). The release doses were determined based on the feeding capacity of *P. vigintiduopunctata* under laboratory conditions and the average size of powdery mildew-infected squash leaves. Based on preliminary trials, and considering the phenological growth of plants within the cages and the rapid increase in powdery mildew severity, 10, 15, and 20 adult *P. vigintiduopunctata* individuals were released per plant. Accordingly, each cage (replicate) contained a total of 40, 60, and 80 adult (female:male = 1:1) *P. vigintiduopunctata* individuals, respectively.

Plants in the control group were sprayed only with distilled water, and no additional treatment was applied. As a positive control, a fungicide registered for powdery mildew control in cucurbits and for which no resistance development has yet been reported, containing 200g/L Azoxystrobin + 125g/L Difenoconazole (SC formulation, Syngenta Quadris® Maxx), was selected and applied on the same day as predator release to squash plants showing powdery mildew symptoms on 0–1% of the leaf area. The experiment was set up with four replicates, each replicate containing four plants. A total of 16 plants were used for each trial. The application was performed according to the standard pesticide trial method of the Ministry of Agriculture for powdery mildew in cucurbits [*Erysiphe cichoracearum* DC., *Podosphaera xanthii* (Castagne) U. Braun and Shishkoff] (MAF 2025a). Since the efficacy of this fungicide has been reported to persist for 12–14 days (Syngenta 2025, MAF 2025b), the fungicide-treated experimental group was divided into two subgroups. In the first subgroup, the fungicide was applied on the first day of the experiment, and the plants were monitored for 20 days. In the second subgroup, the fungicide was applied twice, at the beginning of the experiment and on day 14.



**Figure 2.** Cages used in the efficacy experiment (Original: F.YALÇIN)

The powdery mildew-infected area on squash leaves was measured every five days by adapting the methods

described by Lebeda et al. (2010). On each assessment date, a total of 10 leaves were randomly selected from each plant, photographed, and disease severity was quantified using the ImageJ software. The percentage efficacy was then calculated by applying Abbott's formula to the disease severity values obtained from the software (Abbott 1925).

In addition, considering that the mean developmental period of *P. vigintiduopunctata* under constant conditions is approximately 27 days (Yalçın et al. 2026), the treatment group in which 10 adults were released was monitored until fortieth day in order to evaluate environmental adaptation of the predator in the release area, assess its reproductive capacity, and monitor disease progression.

#### Statistical Analyses

Feeding capacity data were analysed using CONSUME-MSChart (Chi and Liu 1985, Chi 1988, Chi 2026). The software computes age-stage-specific consumption rates ( $c_{xj}$ ), age-specific consumption rates ( $c_x$ ), and the gross consumption rate ( $P$ ) within the two-sex life table framework. Differences in consumption between the F1 and F2 generations were tested using the paired bootstrap method (100,000 resampling replicates) (Chi 1988, Huang and Chi 2011). ImageJ software was used to measure the powdery mildew-infected leaf area and the area consumed by *P. vigintiduopunctata*. Percentage efficacy was calculated using Abbott's formula at each assessment date. Although the formula assumes a stable control group, applying it at discrete time points rather than cumulatively provides a reliable and widely used measure of treatment efficacy in plant pathology (McGrath 2001, Lebeda et al. 2010). Disease severity and efficacy data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) before analysis. Where necessary, arcsine square root transformation was applied. Means were compared using Duncan's Multiple Range Test ( $P < 0.05$ ). The relationship between the number of released insects and the proportion of powdery mildew-infected leaf area was assessed using regression analysis at each assessment date.

## RESULTS

#### Feeding capacity of *Psyllobora vigintiduopunctata* on *Erysiphe cichoracearum*

The areas fed upon by *P. vigintiduopunctata* larvae (Figure 3a,b) and adults (Figure 3c) showed a significant and measurable decline in visible signs of disease. An analysis of stage-specific consumption rates in both generations revealed that feeding capacity increased progressively throughout the larval stages, with daily consumption during the L4 stage ( $2.44 \text{ cm}^2$ ) reaching levels comparable to that

of adult males ( $2.49 \text{ cm}^2$ ). The total amount of powdery mildew-covered leaf area consumed during the L4 stage was recorded as  $9.6 \text{ cm}^2$ . Over the course of the entire larval period (L1 through prepupa), a single *P. vigintiduopunctata* individual consumed a total of approximately  $19 \text{ cm}^2$  of *E. cichoracearum*-infected leaf surface (Table 1). Adults began feeding immediately after emerging from the pupal stage, with peak feeding activity observed between days 30 and 35. Although feeding capacity showed slight fluctuations, a sharp decline was noted between days 100 and 110. Adult females consumed more powdery mildew ( $225 \text{ cm}^2$ ) compared to males ( $196 \text{ cm}^2$ ). Interestingly, in the second generation, while a decrease in feeding capacity was observed in females, an unexpected increase was recorded in males (Table 1, Figure 4) ( $P < 0.05$ ).

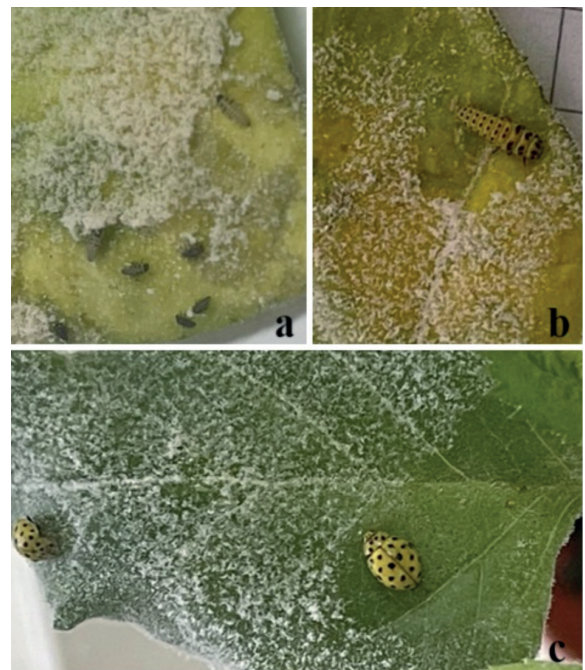


**Figure 3.** Larvae (a, b) and adults (c) of *Psyllobora vigintiduopunctata* feeding on *Erysiphe cichoracearum* (Original: F.YALÇIN)

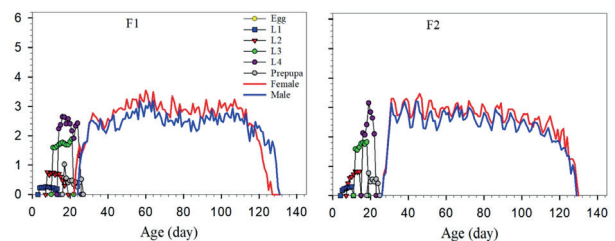


**Figure 4.** Age-stage-specific consumption rate ( $C_{xj}$ ) of *Psyllobora vigintiduopunctata* on *Erysiphe cichoracearum*

**Table 1.** Daily and total feeding capacity (cm<sup>2</sup>) of *Psyllobora vigintiduopunctata* on *Erysiphe cichoracearum* across different developmental stages

| Stages       | Generation | n  | Daily consumption          | Total consumption          |
|--------------|------------|----|----------------------------|----------------------------|
|              |            |    | Mean±SE (cm <sup>2</sup> ) | Mean±SE (cm <sup>2</sup> ) |
| L1           | F1         | 75 | 0.247±0.04aA*              | 1.02±0.03aB                |
|              | F2         | 77 | 0.258±0.03aA               | 1.14±0.03aB                |
| L2           | F1         | 71 | 0.700±0.12aC               | 2.14±0.06aC                |
|              | F2         | 70 | 0.749±0.09aC               | 2.70±0.08aC                |
| L3           | F1         | 65 | 1.683±0.11aD               | 5.67±0.15aD                |
|              | F2         | 67 | 1.724±0.08aD               | 6.07±0.13aD                |
| L4           | F1         | 63 | 2.441±0.29aE               | 9.57±0.19aE                |
|              | F2         | 64 | 2.656±0.37aE               | 9.55±0.23aE                |
| Prepupa      | F1         | 61 | 0.422±0.01aB               | 0.52±0.04aA                |
|              | F2         | 61 | 0.521±0.01aB               | 0.63±0.04aA                |
| Adult Female | F1         | 30 | 2.797±0.21aF               | 225.14±6.97aF              |
|              | F2         | 30 | 2.788±0.10bF               | 207.43±5.48bF              |
| Adult Male   | F1         | 30 | 2.495±0.16aE               | 195.86±6.59aG              |
|              | F2         | 30 | 2.517±0.10bE               | 201.08±8.42bG              |

\* Values are means ± SE estimated by bootstrap resampling (100,000 replicates). Lowercase letters indicate differences between generations within the same stage; uppercase letters indicate differences among developmental stages (paired bootstrap test,  $P < 0.05$ ).

Evaluation of consumption rates indicated that feeding activity was lower during the first and second larval instars compared to the later developmental stages in both generations. Consumption rates increased markedly with the transition of the predator to the L3–L4 instars, and the highest and most stable consumption was observed during the adult stage, particularly in females. This pattern indicates that consumption capacity increases with advancing developmental stage. During the transition to the prepupal stage, feeding activity decreased as expected. While consumption rates remained relatively stable until the mid-adult stage, a pronounced decline was observed toward the end of the stage in both generations, indicating age-related physiological decline. The consumption patterns of the F1 and F2 generations were largely similar (Table 1). When consumption values were compared between generations at each stage, no statistically significant differences were detected in larval instars (L1–L4) or the prepupal stage ( $P > 0.05$ ). Adult females in the F2 generation consumed less than F1 (207.43 vs. 225.14 cm<sup>2</sup>), while F2 males consumed slightly more than F1 males (201.08 vs. 195.86 cm<sup>2</sup>) ( $P < 0.05$ ; Table 1). These differences between sexes across generations are noteworthy. Female consumption declined while male consumption increased in F2. This divergence likely reflects a shift in energy allocation between generations, possibly linked to differences in reproductive investment or adaptation to laboratory diet, as has been reported for other coccinellids reared continuously on a single food source (De Clercq et al. 2011).

#### *Effectiveness of Psyllobora vigintiduopunctata on Erysiphe cichoracearum*

When the development of powdery mildew on squash leaves was examined, it was determined that in the control group, where only distilled water was sprayed, the disease progressed rapidly throughout the experimental period, exceeded 90% on day 15, reached 100% in all plants on day 20, and the plants died (Table 2;  $P < 0.05$ ). In contrast, disease development was significantly suppressed in the groups where predators were released. On the fifth day of the experiment, disease symptoms on leaves were recorded as 7–8%, 6–6.5%, and 6% in the groups where 10, 15, and 20 adult *P. vigintiduopunctata* were released, respectively, and these values were found to be significantly lower than the control group. On days 10 and 15, as the release dose increased the rate of disease progression slowed down, and the lowest disease rates, ranging between 8–9%, were obtained in the group where 20 predators were released. This pattern remained consistent on day 20.

In the fungicide-treated groups, disease symptoms were found to be significantly lower than the control group on all sampling days. In the first-application treatment group, the disease rate ranged between 3.66–4.22% on day 5, gradually increased over time, and reached 10.86–11.00% on day 20 ( $P < 0.05$ ). In the second-application treatment group, disease symptoms remained at the lowest levels throughout the experimental period; the disease rate was between 3.26–4.08% on day 5 and decreased to very low levels of

1.86–3.12% on days 15 and 20. Especially in the later stages, a marked reduction in disease symptoms on leaves was observed in this group ( $P<0.05$ ).

When the treatments were compared in terms of disease symptoms, both predator releases and fungicide applications significantly suppressed powdery mildew compared to the control group. Among the groups with *P. vigintiduopunctata* releases, the highest success was achieved with the release of 20 predators. In fungicide applications, on day 20 the disease rate was at 11.00–10.86% in the first-application group, whereas in the group with the release of 20 predators this rate ranged between 8.55–8.79% ( $P<0.05$ ).

In the groups where predator was released, effectiveness increased depending on both time and release rate (Table 3). In the group where 10 predators were released, effectiveness was 38.93–42.45% on day 5, increased to 79.15–79.32% on day 10, and reached the highest level with 88.43–88.98% on day 15. On day 20, the values ranged between 82.93–83.01%. In the group where 15 predators were released, effectiveness was determined at 46.28–52.29% on day 5, increased to 82.57–82.82% on day 10, and reached 89.81–90.21% on day 15. On day 20, effectiveness remained stable at 86.58–87.12% ( $P<0.05$ ).

**Table 2.** The percentage of *Erysiphe cichoracearum* disease symptoms on plants treated with *Psyllobora vigintiduopunctata* and fungicide

| Experimental Group            | n | Replicate | % Disease symptom (Mean±SE) (Min–Max) |                                    |                                  |                                     |
|-------------------------------|---|-----------|---------------------------------------|------------------------------------|----------------------------------|-------------------------------------|
|                               |   |           | Days                                  |                                    |                                  |                                     |
|                               |   |           | 5th                                   | 10th                               | 15th                             | 20th                                |
| Control                       | 4 | 1.        | 12.23±0.98 dA*<br>(11.83 – 12.93)     | 50.99±1.30 eB**<br>(45.83 – 57.43) | 96.10±0.31 fC<br>(94.90 – 97.18) | 100.00±0.00 fD<br>(100.00 – 100.00) |
|                               |   | 2.        | 13.33±0.33 dA<br>(12.95 – 13.83)      | 48.85±0.64 eB<br>(47.70 – 50.03)   | 92.88±0.42 fC<br>(92.00 – 93.40) | 100±0.00 fD<br>(100.00 – 100.00)    |
| First fungicide application   | 4 | 1.        | 3.66±0.18 aA<br>(3.25 – 4.05)         | 4.76±0.30 abB<br>(4.38 – 5.18)     | 7.41±0.36 bC<br>(6.10 – 8.30)    | 11.00±0.61 cD<br>(10.05 – 11.70)    |
|                               |   | 2.        | 4.22±0.28 aA<br>(3.90 – 4.53)         | 5.69±0.22 abB<br>(5.08 – 6.53)     | 7.39±0.3 bC<br>(6.83 – 7.83)     | 10.86±0.37 cD<br>(9.88 – 11.60)     |
| Second fungicide applications | 4 | 1.        | 3.26±0.09 aA<br>(3.15 – 3.35)         | 4.69±0.13 aB<br>(4.43 – 4.83)      | 2.97±0.06 aA<br>(2.83 – 3.08)    | 1.86±0.08 abA<br>(1.73 – 2.00)      |
|                               |   | 2.        | 4.08±0.22 aB<br>(3.98 – 4.20)         | 4.76±0.25 aB<br>(4.58 – 5.00)      | 3.12±0.2 aA<br>(2.78 – 3.53)     | 1.93±0.13 abA<br>(1.75 – 2.08)      |
| 10 predators                  | 4 | 1.        | 7.04±0.39 bB<br>(6.30 – 7.85)         | 10.63±0.23 bcC<br>(10.15 – 11.20)  | 10.59±0.20 cC<br>(9.95 – 10.95)  | 16.99±0.85 dD<br>(16.98 – 17.03)    |
|                               |   | 2.        | 8.14±0.31 bB<br>(7.90 – 8.43)         | 10.1±0.25 cC<br>(9.43 – 10.43)     | 10.75±0.32 cC<br>(10.18 – 11.33) | 17.07±0.22 dD<br>(16.80 – 17.30)    |
| 15 predators                  | 4 | 1.        | 6.57±0.3 bB<br>(5.60 – 7.25)          | 8.89±0.41 bC<br>(7.83 – 8.85)      | 9.41±0.39 bC<br>(8.78 – 10.38)   | 13.42±0.70 cD<br>(13.28 – 13.65)    |
|                               |   | 2.        | 6.36±0.21 bB<br>(5.75 – 7.45)         | 8.39±0.35 bC<br>(7.38 – 10.43)     | 9.46±0.41 bC<br>(8.28 – 10.95)   | 12.88±0.48 cD<br>(12.58 – 13.40)    |
| 20 predators                  | 4 | 1.        | 6.16±0.23 bB<br>(5.80 – 6.60)         | 8.06±0.39 bC<br>(7.23 – 8.88)      | 8.62±0.53 bC<br>(6.98 – 10.48)   | 8.55±0.29 bD<br>(8.40 – 9.00)       |
|                               |   | 2.        | 6.09±0.22 bB<br>(5.58 – 7.30)         | 8.04±0.35 bC<br>(6.90 – 9.40)      | 8.69±0.48 bC<br>(6.80 – 10.63)   | 8.79±0.28 bC<br>(7.98 – 9.13)       |

Values followed by different lowercase letters in the same column are statistically different from each other ( $P<0.05$ ).

\*\* Values followed by different uppercase letters in the same row are statistically different from each other ( $P<0.05$ ).

Low disease level: aA

High disease level: fD

n: number of replications; 40 readings were taken in each replication.

In the group where 20 predators were released, the highest biological control effectiveness was obtained on all days. In this group, effectiveness was 49.63–54.31% on day 5, increased to 83.54–84.20% on day 10, and reached 90.64–91.03% on day 15. On day 20, effectiveness remained stable at 91.21–91.45%. Overall, it was observed that the highest and most consistent effectiveness was obtained with the release of 20 predators (Table 3;  $P < 0.05$ ).

In the first-application fungicide group, effectiveness increased over time but showed a limited decrease at later stages. In this group, effectiveness was 68.34–70.07% on day 5, increased to 88.35–90.66% on day 10, and reached the highest level with 92.04–92.29% on day 15. On day 20, effectiveness decreased to 89.00–89.14%. In contrast, in the second-application fungicide group, effectiveness increased earlier and continued to increase throughout the experimental period. Effectiveness, which was 69.39–73.34% on day 5, increased to 90.26–90.80% on day 10, reached 96.64–96.91% on day 15, and reached the highest values with 98.07–98.14% on day 20 ( $P < 0.05$ ).

When biological and chemical control methods were compared in terms of effectiveness, both applications were found to be effective in controlling powdery mildew disease. On day 20, effectiveness was determined as 89.00–89.14% in the first fungicide application group, whereas effectiveness was 91.21–91.45% in the group where 20 *P. vigintiduopunctata* adults were released. Accordingly, it was

observed that the effectiveness of releasing 20 predators on day 20 was higher than that of a first fungicide application ( $P < 0.05$ ).

Regression analysis between predator number and powdery mildew infected area showed that, at all measurement times, the proportion of powdery mildew infected area decreased as the number of released predators increased, indicating an inversely proportional relationship between the number of released predators and the powdery mildew infected area (Figure 5).

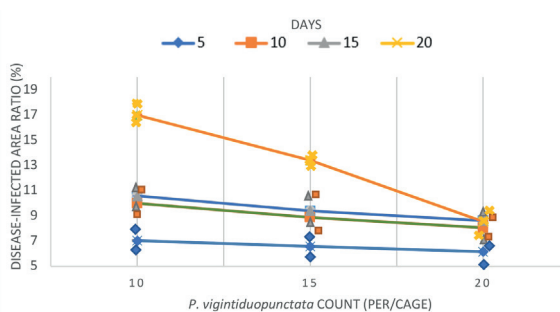


**Figure 5.** Regression graph showing the effect of *Psyllobora vigintiduopunctata* release dose on *Erysiphe cichoracearum*

In the study, the experimental group in which 10 predators were released was maintained until day 40, and the phenological status of the plants, the *P. vigintiduopunctata* population within the cages, and the progression of powdery mildew were monitored. It was determined that the plants

**Table 3.** Percentage reduction of *Erysiphe cichoracearum* following treatment with *Psyllobora vigintiduopunctata* and fungicide

| Experimental Group            | n | Replicate | % EFFECT        |                  |               |                |
|-------------------------------|---|-----------|-----------------|------------------|---------------|----------------|
|                               |   |           | Days            |                  |               |                |
|                               |   |           | 5th             | 10th             | 15th          | 20th           |
| First fungicide application   | 4 | 1.        | 70.07±1.86 abD* | 90.66±1.01 abB** | 92.29±0.56 bA | 89.00±0.37 bC  |
|                               |   | 2.        | 68.34±1.52 dD   | 88.35±0.76 bcC   | 92.04±0.42 bA | 89.14±0.33 bB  |
| Second fungicide applications | 4 | 1.        | 73.34±0.48 aC   | 90.80±0.20 aB    | 96.91±0.19 aA | 98.14±0.12aA   |
|                               |   | 2.        | 69.39±0.69 abD  | 90.26±0.29 aB    | 96.64±0.21 aA | 98.07±0.11 aA  |
| 10 predators                  | 4 | 1.        | 42.45±3.42 dD   | 79.15±1.11 dD    | 88.98±0.91 cB | 83.01±0.76 dC  |
|                               |   | 2.        | 38.93±4.08 dD   | 79.32±1.08 dD    | 88.43±0.84 dB | 82.93±0.68 dC  |
| 15 predators                  | 4 | 1.        | 46.28±2.22 cdD  | 82.57±0.44 cdC   | 90.21±0.71 bB | 86.58±0.69 cdC |
|                               |   | 2.        | 52.29±2.87 cC   | 82.82±0.36 cdC   | 89.81±0.66 bB | 87.12±0.55 cdB |
| 20 predators                  | 4 | 1.        | 49.63±1.23 cD   | 84.20±0.47 bcB   | 91.03±0.52 bA | 91.45±0.23 bA  |
|                               |   | 2.        | 54.31±2.66 cC   | 83.54±0.83 bcC   | 90.64±0.43 bA | 91.21±0.19 bA  |

\*Values with different lowercase letters in the same column are statistically different from each other ( $P < 0.05$ ).

\*\*Values with different uppercase letters in the same row are statistically different from each other ( $P < 0.05$ ).

High effect: aA

Low effect: dD

n: number of replications; 40 readings were taken in each replication.

inside the cages continued their phenological development and reached flowering and fruit set stages (Figure 6). In the cages where 10 predators per plant (40 in total) were initially released, it was determined that the beneficial population reached an average of  $199 \pm 13$  individuals (eggs+larvae+adults) at the end of day 40 (Table 4).

consumption on squash, similar to values for other hosts infected with *E. cichoracearum*. On *Picris echioides* (L.) infected with *E. cichoracearum*, total larval consumption was  $21.97 \pm 3.15 \text{ cm}^2$ ; on *Helianthus annuus* (L.) it was  $19.83 \pm 2.21 \text{ cm}^2$  (Younes et al. 2015). At the larval stage of *P. vigintiduopunctata*, its feeding capacity on *E. cichoracearum*

**Table 4.** Number of *Psyllobora vigintiduopunctata* in cages at the end of the experiment (units)

| Life Stage       | 1st Cage | 2nd Cage | 3rd Cage | 4th Cage | Mean (min-max) |
|------------------|----------|----------|----------|----------|----------------|
| Egg              | 54       | 48       | 35       | 28       | 41.25 (28-54)  |
| L1               | 15       | 27       | 15       | 20       | 19.25 (15-27)  |
| L2               | 21       | 14       | 17       | 17       | 17.25 (14-21)  |
| L3               | 18       | 23       | 25       | 24       | 22.50 (18-25)  |
| L4               | 22       | 12       | 16       | 13       | 15.75 (12-22)  |
| Pupa             | 18       | 15       | 21       | 17       | 17.75 (15-21)  |
| Adult ♀          | 30       | 33       | 32       | 34       | 32.25 (30-34)  |
| Adult ♂          | 33       | 37       | 30       | 32       | 33.00 (30-37)  |
| Total Population | 211      | 209      | 191      | 185      | 199 (191-211)  |



**Figure 6.** Image of control group plants on day 20 (a) and image of plants released with 10 *Psyllobora vigintiduopunctata* on day 40 (b) (Original: F.YALÇIN)

In the measurements, in the experimental group where 10 predators were released, the disease symptom level was determined as  $16.59 \pm 0.44\%$  (mean $\pm$ SE) on day 40. Considering the day 20 data ( $16.99 \pm 0.85\%$ ), despite the rapid increase tendency of the disease level, it was observed that the predators were able to suppress the powdery mildew pathogen under cage conditions and had the potential to maintain the disease level at similar levels.

## DISCUSSION

In a study, the total area consumed across four larval stages of *P. vigintiduopunctata* was approximately  $20.01 \pm 0.45 \text{ cm}^2$

on squash is intermediate compared to its feeding capacity on other powdery mildew species on different host plants.

The feeding capacity of *P. vigintiduopunctata* was changed depending on the powdery mildew species it feeds on and their host plant species. The consumption capacity of this predator would be ranked from highest to lowest as follows:  $29.90 \pm 3.97 \text{ cm}^2$  of *E. convolvuli* on *Convolvulus arvensis* (L.),  $28.08 \pm 5.94 \text{ cm}^2$  of *P. guttata* on *Morus alba* (L.),  $25.06 \pm 5.81 \text{ cm}^2$  of *S. fuliginea* on *Hibiscus esculentus* (L.),  $18.36 \pm 2.17 \text{ cm}^2$  of *M. albitoides* on *Quercus alleppica* (L.), and  $16.43 \pm 2.98 \text{ cm}^2$  of *U. necator* on *Vitis vinifera* (L.) (Younes et al. 2015).

Feeding capacities of mycophagous coccinellids vary not only by powdery mildew and host plant species but also among predator species. For example, *P. vigintimaculata* consumed  $6.3 \pm 3.3 \text{ cm}^2$  of *E. cichoracearum*-infected *Zinnia elegans* (Sessé and Moc.) during development (Sutherland and Parrella 2006), much lower than the  $19 \text{ cm}^2$  recorded in our study. On *H. esculentus*, *Psyllobora bisoctonotata* (Mulsant) reduced conidial density by 92%, consuming  $12.3 \text{ cm}^2$  of powdery mildew during development at  $25^\circ\text{C}$  (Soylu and Yiğit 2002). Five adults of *Illeis cincta* (Fabricius) reduced mildew severity from 92.8% to 32.4% on *Phyllactinia corylea* (Sacc.) -infected mulberry leaves within 10 days

(Krishnakumar and Maheswari 2004), *Halysia hauseri* (Mader) feeding on *Podosphaera leucotricha* (Ellis ve Everh) (Erysiphales: Erysiphaceae)-infected apple consumed 99.72 cm<sup>2</sup> over its lifespan, with L4 and adult stages having equal daily feeding capacities (Liu 1951). Ashraf et al. (2010) reported in their study on *Coccinella septempunctata* (L.) that females fed on more aphids than males and that this was associated with egg production. In the present study, the higher feeding rate of female *P. vigintiduopunctata* compared to males supports the idea that females require more energy for egg production. In the present study, females of *P. vigintiduopunctata* (F1) consumed an average 225.14 cm<sup>2</sup> of *E. cichoracearum* infected squash leaf area over their lifetime, while males consumed 195.86 cm<sup>2</sup>. These values are considerably higher than those recorded *P. vigintimaculata* (6.3±3.3 cm<sup>2</sup>) on *Zinnia elegans* infected with *E. cichoracearum* (Sutherland and Parrella 2006), and lower than the 367.07 cm<sup>2</sup> consumed by *P. vigintiduopunctata* females feeding on *S. fuliginea* on *H. esculentus* (Younes et al. 2015). The higher consumption of females compared to males is consistent with the greater energy demands associated with egg production, as previously reported for predatory coccinellids (Ashraf et al. 2010).

This study also evaluated the impact of releasing doses of predators on powdery mildew disease. In the control group, the disease increased rapidly and continuously due to the absence of treatment. This is because powdery mildew is a parasitic pathogen that spreads quickly when it encounters no obstacles on a plant (Pérez-García et al. 2009).

In predator release applications, it was determined that as the number of *P. vigintiduopunctata* adults increased, disease development was significantly limited. In the group where 10 adults of *P. vigintiduopunctata* were released, the pathogen was clearly suppressed compared to the control group, and disease development progressed quite slowly. In the group where 15 adults of *P. vigintiduopunctata* were released, although the disease level was lower, powdery mildew severity remained in the range of 12.88–13.42% at the end of the experiment. In contrast, in the group where 20 predator adults were released, the development of powdery mildew on leaves remained stable at 8.55–8.79%, and the disease was effectively controlled. This group showed the closest effect to two fungicide applications. In all replications, the stability of behavioral and feeding efficiency in *P. vigintiduopunctata* release treatments throughout the experiment supports the applicability of the method and confirms that the predator maintains its feeding consistency observed under laboratory conditions also under semi natural conditions.

When fungicide applications were evaluated, it was understood that a single application significantly slowed disease severity, but due to the duration of the fungicide effect, repetition at day 14 was necessary, which is consistent with both experimental findings and usage instructions. It is also stated in the literature that fungicide effectiveness may decrease over time depending on environmental conditions and application intervals, and repeated applications are required for optimal control (McGrath 2001, Horst 2013). Second fungicide applications provided high phytosanitary control by minimizing disease level. However, the necessity of repeating applications at two-week intervals for powdery mildew control, the risk of resistance development, residue problems, economic cost, water use limitations, and potential risks for human and environmental health should be considered (Sarhan et al. 2020). Difenoconazole (Score 25% EC\*), which is included in the fungicide mixture, has been reported to have the highest efficacy in the control of the powdery mildew pathogen *P. xanthii* in cucumber; however, it also causes the highest phytotoxicity in cucumber plants, leading to morphological changes in leaves and pollen sterility (Elagamey et al. 2023). In addition, while fungicide application was repeated in the 20-day experiment, no additional release was performed in the predator release group. The initial lower effectiveness of *P. vigintiduopunctata* in reducing powdery mildew increased steadily over time. Depending on the number of predator released, it suppressed the disease and maintained its effectiveness, as our results on the effects of releasing 10 adult predators on cucurbit powdery mildew over 40 days show. The mycophagous species *I. cincta* was released onto mulberry plants infected with *P. corylea* powdery mildew, while fungicide was applied to a separate plant group. When these two treatments were compared, no statistically significant difference was observed between the two methods after 20 days in disease control. However, it was determined that the pathogen increased over time in fungicide-treated plants, whereas it tended to decrease in insect-released plants (Krishnakumar and Maheswari 2004).

## CONCLUSION

It is clear that chemical applications suppress plant diseases in a shorter time compared to biological control agents. However, problems such as negative effects on the ecosystem and resistance development eliminate this advantage. With an integrated perspective, biological control applications combined with other control methods, when applied at the right time and with well-adjusted release doses, can increase control effectiveness. In this study, when *P. vigintiduopunctata* adults were released at increasing doses at the lowest disease

level, the disease suppression effect increased, and especially in applications where 20 individuals were released, results very close to chemical control were obtained. In addition, the continuous consumption pressure of *P. vigintiduopunctata* on the pathogen offers a more persistent effect potential compared to chemicals. As a result, the application of biological control with *P. vigintiduopunctata* against powdery mildew pathogens in squash provides important advantages such as eliminating residue and resistance problems, supporting the conservation of natural enemies, and creating a sustainable long-term control mechanism. These findings reveal that although chemical control has a rapid effect advantage, releasing natural enemies at sufficient density can provide an environmentally friendly and long-term alternative for controlling powdery mildew disease in cucurbits. Furthermore, research is required into how *P. vigintiduopunctata* release should be harmonised with other plant protection methods and into the potential side effects of pesticide applications in fields where other harmful pests and disease agents may be present.

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## ÖZET

*Psyllobora vigintiduopunctata*'nın *Erysiphe cichoracearum* üzerindeki beslenme kapasitesi, laboratuvar ( $25\pm 2^\circ\text{C}$ ,  $70\pm 5$  bağıl nem, 12:12 saat fotoperiyot) ve yarı-tarla koşullarında araştırılmıştır. Laboratuvar koşullarında, larva dönemi boyunca tüketilen toplam külleme ile enfekteli alanın yaklaşık  $19\text{ cm}^2$  olduğu belirlenmiştir. Erginlerin yaşam süreleri boyunca tüketim miktarı incelendiğinde, erkek bireylerin ortalama  $195.86\pm 5.48\text{ cm}^2$ , dişilerin ise  $225.14\pm 5.48\text{ cm}^2$  enfekteli alan tükettiği saptanmıştır. Yarı-tarla denemeleri, küllemeyle karşı *P. vigintiduopunctata*'nın etkinliğini belirlemek amacıyla enfeksiyonun başlangıç döneminde kabak bitkilerine bitki başına 10, 15 ve 20 ergin birey salınarak yürütülmüştür. Kontrol uygulaması olarak yalnızca saf su kullanılmıştır. Pozitif kontrol olarak, 200g/L azoksistrobin ve 125g/L difenokonazol içeren ticari bir fungusit (Quadris® Maxx, 80ml/100L su) enfekteli bitkilere uygulanmıştır. Kabak bitkilerindeki enfekteli yaprak alanı beş günlük aralıklarla ölçülmüş ve denemeler 20. günde

sonlandırılmıştır. Kontrol grubunda bitkilerin 20. günde %100 oranında külleme ile enfekte olduğu belirlenmiştir. Bitki başına 10, 15 ve 20 ergin *P. vigintiduopunctata* salınan uygulamalarda ortalama hastalık şiddeti 20. günde sırasıyla %16.99–17.07, %12.88–13.42 ve %8.55–8.79 olarak belirlenmiştir. Ortalama etki ise sırasıyla %82.93–83.01, %86.58–87.12 ve %91.21–91.45 olarak hesaplanmıştır. Fungisit uygulanan kabak bitkilerinde ise külleme hastalığı şiddeti 15. günden itibaren artmış, 20. günde yaklaşık %11 hastalık şiddeti ve %89 etkinlik düzeyine ulaşmıştır. Elde edilen bulgular, laboratuvar koşullarında belirlenen beslenme kapasitesi verileri ile uyumlu olup, enfeksiyonun başlangıç döneminde bitki başına 20 ergin *P. vigintiduopunctata* salımının *E. cichoracearum*'un baskılanmasında oldukça etkili olduğunu ve kimyasal mücadele stratejilerine güçlü bir alternatif oluşturabileceğini ortaya koymuştur.

Anahtar Kelimeler: 22 benekli uğur böceği, salım, beslenme kapasitesi, mikofag, *Erysiphe cichoracearum*, *Cucurbita pepo*.

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