Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Orijinal araştırma (Original article)

Interactions between *Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae) and Cucurbit powdery mildew

Psyllobora vigintiduopunctata (L.) (Coleoptera: Coccinellidae) ile Kabakgil külleme hastalığı arasındaki ilişkiler

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ARTICLE INFO

Article history:

DOI: 10.16955/bitkorb.1758992

Received : 05-08-2025

Accepted : 18-09-2025

Keywords:

The 22-spotted ladybird, two-sex life table, mycophagous, *Erysiphe cichoracearum*, biological control

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ABSTRACT

In cucurbit production, fungicides used to control powdery mildew, which significantly reduces product yield and quality, generate environmental pollution and residues. Sustainable approaches are therefore needed to control this disease. To evaluate the potential of *Psyllobora vigintiduopunctata* (Linnaeus) (Coleoptera: Coccinellidae) as a biological control agent against *Erysiphe cichoracearum* (DC.) (Erysiphales: Erysiphaceae), biological parameters and population dynamics were determined under controlled conditions (25±2°C, 70±5% humidity, 12:12 photoperiod) for two generations. A two-sex, age-stage life table approach was used to analyse the data. The results indicated that mean lifetime fecundity ranged from 317.48±142 to 376.55±146 eggs per female. *P. vigintiduopunctata* completed its development in 26.7–28.4 days in both generations. The total longevity of females was 102 to 108 days, while males had a lifespan of 106 to 107 days. The intrinsic rate of increase (*r*) of the predator on *E. cichoracearum* was found to be between 0.0788 and 0.0829 females/day, while the mean generation time (*T*) varied between 58.7 and 61.5 days. The gross reproductive rate (*GRR*), which represents the number of offspring produced per female per generation, ranged from 219.5 to 228.5 individuals. These laboratory results indicate a promising potential for *P. vigintiduopunctata* as a biological control agent against *E. cichoracearum* in cucurbits.

INTRODUCTION

One of the most serious fungal diseases affecting cucurbit plants is powdery mildew, which causes substantial yield losses in both greenhouses and field crops. Given the detrimental impacts of pesticides on the environment, biodiversity, and human health, there is an increasing focus on minimising their use and embracing alternative pest management methods (Hanedar et al. 2023, Yalçın and Ülgentürk 2023). The efficacy of commonly used fungicides

for controlling these diseases is often inadequate, and they pose additional challenges such as chemical residues and the development of resistance (McGrath and Shishkoff 1999, Altın et al. 2018, Kavyashree 2019). Biocontrol is the main alternative to chemical control of powdery mildew. The mycophagous ladybeetles in the tribe Halyziini are potentially attractive agents for the biological control of powdery mildew, but the trophic ecology of these beetles is poorly understood (Cruz et al. 1990, Almeida and Milleo 1998, Takeuchi et al. 2000, Ahmad et al. 2003, Krishnakumar et al. 2004, Sutherland 2005, Cividanes et al. 2007, Giorgi et al. 2009, Sutherland and Parrella 2009, Kumar and Mittal 2010, Illahi et al. 2011, Younes et al. 2015, Dharshini and Jagadish 2018, Garai and Sarkar 2020). *Psyllobora vigintiduopunctata* (Coleoptera: Coccinellidae: Halyziini) for example, is widely distributed and is better known as an aphid predator (Hodek 1973, Smaili et al. 2009, Kaplan et al. 2019, Alaserhat and Bozbek 2021). It has also been recorded feeding on *Erysiphe cichoracearum* (DC.), *Erysiphe convolvuli* (DC), *Phyllactinia guttata* (Wallr.), *Sphaerotheca fuliginea* (Schltdl.), *Microsphaera alphitoides* (Griff. and Maubl.) and *Uncinula necator* (Wallr) and *Oidium* sp. (Erysiphales: Erysiphaceae) (Ratti 1996, Sutherland 2005, Sutherland and Parrella 2006, Ülgentürk et al. 2011, Younes et al. 2015) in Türkiye and around the world. Karataraki et al. (2015) documented the feeding activity of *P. vigintiduopunctata* on *E. cichoracearum* in cucumber greenhouses in Greece. Younes et al. (2015) emphasise that *P. vigintiduopunctata* is one of the main predators of powdery mildew in Syria's coastal regions, with both adult insects and larvae present from April to November.

In 2023, 22% of Türkiye's total agricultural output was made up of cucurbit crops (TÜİK 2024). The production of squash and watermelon in glasshouses or fields has been economically and historically important to agriculture in Ankara for a long time. The surveys in Ankara Province in 2022 revealed that *P. vigintiduopunctata* is commonly found in groups on plants infected with powdery mildew, particularly on species belonging to the Cucurbitaceae family. Field-collected adults and larvae were confirmed to feed on powdery mildew. Therefore, the relationship between cucurbit powdery mildew and *P. vigintiduopunctata* was prioritised. The aim of this study was to evaluate the fecundity, development time and longevity of *P. vigintiduopunctata* when fed exclusively on powdery mildew in laboratory conditions, as well as its population dynamics. Data were analysed using a two-sex, age-stage life table approach. The primary objective was to elucidate the biological interactions of this mycophagous species with *E. cichoracearum*, as well as to assess its potential for use in biological control programmes.

MATERIALS AND METHODS

Identification of powdery mildew and mycophagous coccinellid species

Adult coccinellids were collected from pumpkin leaves infested with powdery mildew in Ankara (Kahramankazan). The mildew samples and coccinellids were prepared for morphological identification. It was determined that *E. cichoracearum* was the only species of powdery mildew present on Cucurbitaceae species in the Ankara region (Boesewinkel 1980, Braun 1995, Braun et al. 2002). Based on Uygun's (1981) diagnostic criteria, the coccinellid adults were identified as *P. vigintiduopunctata*.

Cultivation of host plants and establishment of Erysiphe cichoracearum culture

The seeds of the commercially preferred sakız squash (*Cucurbita pepo* (L)) were cultivated by the host plant at 25 ± 2°C temperature, 70 ± 5% relative humidity, and a 12:12 h light-dark photoperiod in climate-controlled chambers (Younes et al. 2015). The plants were grown in 14×12.5 cm pots, and once the seedlings developed their first true leaves, they were transferred to a powdery mildew production chamber the specified conditions. The plants were inoculated with the powdery mildew pathogen, *E. cichoracearum*, using a soft-tipped brush to ensure successful infection. This procedure was repeated continuously to generate the necessary powdery mildew-infected squash plants for the study.

Establishment of Psyllobora vigintiduopunctata colony

Adults of *P. vigintiduopunctata* collected from the field were reared in ventilated plexiglass cages (70×50×60 cm) under controlled environmental conditions (25±2°C, 70±5% RH, 12:12 h photoperiod) as described by Younes et al. (2015). Powdery mildew-infected cucurbit plants grown in the same environmental conditions were placed inside the cages as a food source, along with moistened paper towels to maintain humidity and provide water. As mildew coverage on the plants decreased, fresh infected plants were introduced to ensure a continuous food supply and colony maintenance.

Determination of the biological parameters of Psyllobora vigintiduopunctata fed on Erysiphe cichoracearum

A total of 25 mating pairs of *P. vigintiduopunctata* adults were selected from the culture and placed into plastic containers (5.5×7×15 cm) for monitoring. To feed these pairs, powdery mildew-infected leaves were provided daily, and to meet their water and humidity needs, soaked drying papers (5×5 cm) were placed inside the containers. Any damaged or depleted leaves were replaced with fresh ones. The pairs in these containers were monitored daily, and biological

observations began as soon as the first egg was laid. This generation was designated as the first (F1). The study began with the monitoring of 505 eggs. Each egg was tagged and placed in a glass petri dish (9 cm) that was numbered, with a small piece of wet cotton inside to provide humidity. Once egg hatching began, powdery mildew-infected leaf segments were added to the petri dish for feeding the first larval instars, and the insects were monitored until they reached the adult stage. These adult insects, obtained from the egg stages were then transferred as pairs into plastic containers (5.5×7×15 cm). The eggs laid by these pairs on the same day were grouped to obtain life table data. To specifically investigate the effect of feeding solely on powdery mildew, the same study was repeated with individuals from the second generation (F2). A total of 510 eggs from the second generation were followed. The duration of all developmental stages, survival, and mortality rates were obtained through daily observations.

The adult longevity, egg production, oviposition, and sex ratio were determined for 30 randomly selected pairs from each generation. The sex ratio of *P. vigintiduopunctata* was calculated based on the first 100 individuals of each generation that reached adulthood and copulated. In addition, after the death of these individuals, their reproductive organs were dissected to confirm their sex (Uygun 1981).

Statistical analyses

Statistical analyses of biological parameters were conducted using the TWOSEX-MSChart (Chi 2025a) programs. Life history traits—including survival rate (l_x), fecundity (m_x), intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T), as well as predation rate (Cxj) were analysed following the methods of Chi (1988), Chi and Liu (1985), Goodman (1982), Huang and Chi (2011), and Tuan et al. (2013). Data from both generations were obtained and compared accordingly. The population growth of *P. vigintidupunctata* on *E. cichoracearum* was projected using the computer program TIMING-MSChart (Chi 2025b). All of the figures were drawn with SigmaPlot program (Version 14.0).

RESULTS

Biological parameters of Psyllobora vigintiduopunctata fed on Erysiphe cichoracearum

Among 100 randomly selected individuals, the F1 generation consisted of 53 females and 47 males, whereas the F2 generation included 46 females and 54 males. These data suggest a sex ratio approximating 1:1. Females of *P. vigintiduopunctata* are capable of multiple matings throughout their lifespan. Oviposition typically occurs on the undersides of leaves, generally in clusters of seven, though solitary eggs have also been observed.

The eggs hatched after an average of 5.6–6.0 days and the difference in hatching times between the two generations was statistically significant ($P<0.05$). The durations of the larva (14–15 days), prepupa (1.23–2.21 days), and pupa (5.73–5.83 days) stages, as well as the total developmental period (26.7–28.4 days), were similar in both generations ($P>0.05$). However, the APOP (12.24–10.17 days) and TPOP (40.40–37.76 days) were significantly shorter in the second generation ($P<0.05$). In the study, the oviposition period (69.40 and 64.62 days, respectively), total longevity of females (102.57 and 108.07 days), and males (106.45 and 107.80 days), and overall longevity (76.60 and 77.13 days) were observed to be statistically similar across generations (Table 1).

The age-stage-specific survival rate (s_{xj}), representing the probability of a newly laid *Psyllobora vigintiduopunctata* egg surviving to age x and stage j, is illustrated in detail in Figure 1. Examining the l_x curve, which reflects survival rates across all developmental stages for both generations, reveals that the trend is similar in both cohorts, remaining almost constant for the first 100 days after birth (Figure 2). After the 100th day, the number of deaths began to increase. The fecundity (m_x) curve displays that egg production gradually increases from the first day of oviposition, reaching peak values on day 65 in the F1 generation and on day 63 in the F2 generation.

In both generations, egg production declined with increasing age. The maternity curve (l_xm_x), which reflects effective reproduction by combining survival and fecundity rates, remained lower than the fecundity curve due to cumulative losses in the population over time, and displayed a continuous decline after day 100.

In both generations, the highest mortality rate was observed during the egg stage and molting from the first larval stage (L1) to the second larval stage (L2), among all biological stages. The lowest mortality rate occurred during development from pupa to adult (Figure 1).

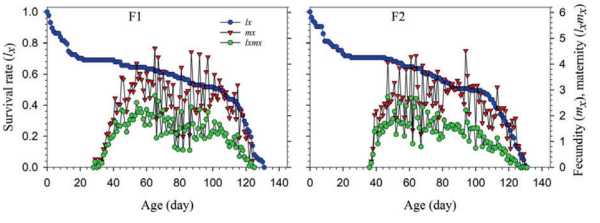


Figure 1. Age-specific survival rate (l_x), fecundity (m_x), and net maternity (l_xm_x) of *Psyllobora vigintiduopunctata* the first (F1) and second (F2) generations fed on *Erysiphe cichoracearum*

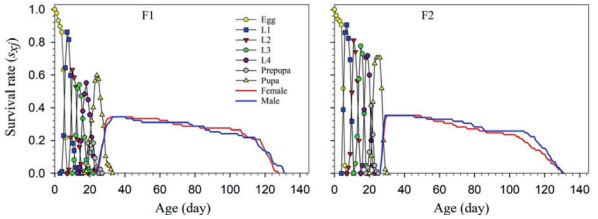


Figure 2. Age-stage-specific survival rate (s_{xj}) of the first (F1) and second (F2) generations of *Psyllobora vigintiduopunctata* fed on *Erysiphe cichoracearum*

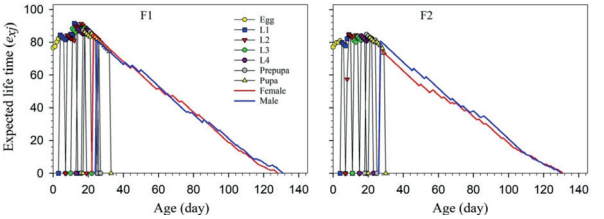


Figure 3. Expected life time (e_{xj}) of *Psyllobora vigintiduopunctata* of the first (F1) and second (F2) generations fed on *Erysiphe cichoracearum*

The life expectancy of an individual that successfully develops from a newly laid egg to adulthood was found to be approximately 130 days. This trend was consistent across both generations (P<0.05) (Figure 3).

The age-stage-specific life table also includes the reproductive value (v_{xj}), which represents the expected future contribution of a *P. vigintiduopunctata* individual at age *x* and stage *j* to the population. For a newly laid egg, this value corresponds to the finite rate of increase (λ), ranging between 1.080 and 1.086. At the end of the fourth larval stage (days 30–33), the reproductive value was approximately day 40, and it peaked at around day 73 during the adult female stage, between days 55 and 60. Although a similar trend was observed in the second generation, the curve remained more stable between days 40 and 90 (Figure 4).

Table 1. Development, longevity, and oviposition durations (days) of the first (F1) and second (F2) generations of *Psyllobora vigintiduopunctata* fed on *Erysiphe cichoracearum*

Stages	Generations	n	Mean±SE (Day)	p
Egg	F1	75	5.624±0.066b	0.001*
	F2	77	5.974±0.087a	
Larva 1	F1	71	4.212±0.102b	0.001
	F2	70	4.557±0.087a	
Larva 2	F1	65	3.046±0.107b	0.000
	F2	67	3.686±0.064a	
Larva 3	F1	63	3.413±0.080a	0.150
	F2	64	3.562±0.066a	
Larva 4	F1	61	3.934±0.098a	0.016
	F2	61	3.622±0.084b	
Prepupa	F1	61	1.230±0.054	0.833
	F2	61	1.213±0.053	
Pupa	F1	60	5.834±0.067	0.274
	F2	60	5.733±0.062	
Preadult	F1	60	27.767±0.276	0.716
	F2	60	28.050±0.104	
APOP	F1	30	10.167±0.597	0.009
	F2	30	12.236±0.506	
TPOP	F1	30	37.758±0.773	0.004
	F2	30	40.403±0.464	
Oviposition	F1	30	69.396±7.570	0.655
	F2	30	64.618±7.705	
Female Longevity	F1	30	102.569±4.598	0.376
	F2	30	108.070±4.198	
Male Longevity	F1	30	106.450± 4.722	0.833
	F2	30	107.796±4.370	
Total	F1	87	105.183±3.128	0.951
	F2	85	107.250±3.160	

*The difference between means indicated by the same letter in each row is statistically not significant (P>0.05).

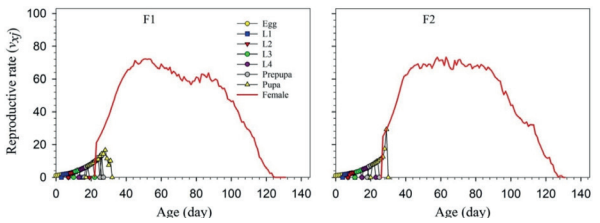


Figure 4. Age-stage-specific reproductive value (v_{xj}) of the first (F1) and second (F2) generations *Psyllobora vigintiduopunctata* fed on *Erysiphe cichoracearum*

In the study, the intrinsic rate of increase ($r^{(d-1)}$)—indicating the number of female offspring produced per female per day, considering both survival and fecundity—was calculated to be 0.0829–0.0788 females/day in both generation, respectively. The gross reproductive rate (*GRR*), representing the total number of offspring produced by a female over her lifetime, was determined to be 228.5–219.5 individuals. The daily finite rate of increase ($\lambda(d-1)$) was found to be 1.080–1.086. The net reproductive rate (R_0), which reflects the average number of female offspring that a female can produce during her lifetime, was measured as 120.3–129.7 offspring/female. The mean generation time was 58.7–61.5 days in both generations, and the difference between them was statistically significant (P<0.05) (Table 2). Although a slight decrease was observed in the most important parameters, such as the intrinsic rate of increase, the finite rate of increase and the net reproductive rate, as well as in other parameters such as fecundity and gross reproductive values, in the second generation compared to the first, these differences were not statistically significant.

The average number of eggs laid per female *Psyllobora vigintiduopunctata* was found to be 376.4 eggs/female in the first generation and 341.1 eggs/female in the second generation. Although the number of eggs was lower in the second generation, the difference between the two generations was not statistically significant. The reduced egg count in the second generation was attributed to early female mortality observed in 30 monitored females.

Using the TIMING-MSChart program, population growth was estimated over a 120-day period for a culture initiated with 10 eggs, based on the intrinsic reproductive capacity of *P. vigintiduopunctata* fed on *E. cichoracearum*. The numerical changes in all developmental stages throughout this period are presented in Figure 5, while the maximum number of individuals expected to be reached at each stage is shown in Table 3. Given the intrinsic rate of increase ($r^{(d-1)}$) was 0.0829 in the first generation and 0.0788 in the second generation. The population simulation projected that the number of adults would reach 4,214 individuals in the first generation and 2,236 individuals in the second generation by day 120. The population is estimated to consist of around 1,621 females and 1,605 males, based on the average of both generations. It is emphasized that these theoretically calculated values should be validated through separate empirical studies. Furthermore, since environmental factors in nature were not considered in the simulation, it is suggested that the results be tested under both laboratory and semi-field conditions.

Table 2. Population parameters of first (F1) and second (F2) generations of *Psyllobora vigintiduopunctata*

Population parameters	Generations	n	Mean±SE	p
The intrinsic rate of increase, r (d^{-1})	**F1**	**87**	0.0829±0,0035a	0.273
	F2	**85**	0.0788±0,0031a	
The finite rate of increase, λ (d^{-1})	**F1**	**87**	1.086±0.0038a	0.273
	F2	**85**	1.080±0.0032a	
Net reproductive rate, R_0 (egg)	**F1**	**87**	129.770±21.253a	0.748
	F2	**85**	120.398±20.095a	
Fecundity (egg)	**F1**	**30**	376.377±26.590a	0.352
	F2	**30**	341.100±27.255a	
Preadult survival rate (%)	**F1**	**87**	0.690±0.005a	0.555
	F2	**85**	0.705±0.005a	
Survival rate (%)	**F1**	**87**	0.982±0.0038a	0.555
	F2	**85**	0.985±0.0034a	
Mean generation time, T (day)	**F1**	**87**	58.724±1.022a	0.046
	F2	**85**	61.552±0.974a	
Gross reproductive rate, *GRR* (egg)	**F1**	**87**	228.556±31.950a	0.848
	F2	**85**	219.548±33.141a	

*The difference between means indicated by the same letter in each row is statistically not significant (P>0.05).

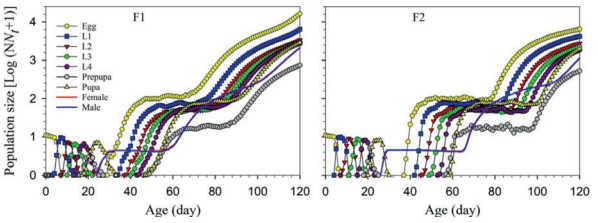


Figure 5. Age-stage-specific theoretical population size of the first (F1) and second (F2) generations *Psyllobora vigintiduopunctata* fed on *Erysiphe cichoracearum* by age and developmental stage

M. nigra, it laid 109.44±77.61 eggs/female over two months, with a maximum of 250 eggs per female (Younes et al. 2015). In contrast, our study displayed higher fecundity (341–376 eggs/female), despite a slight reduction in the second generation. These results indicate that *P. vigintiduopunctata* can survive and reproduce successfully when fed only on squash mildew, showing high adaptability to this diet.

The results obtained from this study during two generations of *P. vigintiduopunctata* indicate that feeding on squash powdery mildew is quite sufficient in terms of life

Table 3. Population growth simulation of *Psyllobora vigintiduopunctata* (individuals)

	Egg	L1	L2	L3	L4	Prepupa	Pupa	Female	Male	Total Adult	Total population size
F1	16.354	6.408	3.408	2.932	2.757	738	2.867	2.142	2.072	4.214	39.678
F2	6.490	4.211	2.747	2.274	1.854	526	1.994	1.099	1.138	2.236	22.333

DISCUSSION

In terms of biological findings, *P. vigintiduopunctata* fed *E. cichoracearum* on squash completed development in about 28 days, while the development time was shorter (21.97±1.89 days) when fed on *Morus nigra* infected with *Phyllactinia guttata* under the same temperature, humidity and photoperiod conditions used in our study (Younes et al. 2015). Conversely, Satti (2015) found that the mycophagous coccinellid *Psyllobora bisoctonotata* (Mulsant) required 25.28±1.57 days to develop from egg to adult on *Xanthium brasilicum* (Vell.) (Asterales: Asteraceae) infected with powdery mildew (Erysiphaceae).

The developmental time of mycophagous coccinellids feeding on the same species of powdery mildew varies depending on the temperature and coccinellid species. To illustrate, *Psyllobora confluens* (Fabricius) feeding on *E. cichoracearum* on okra, *Abelmoschus esculentus* (L.) completed development in 20.5±0.45 days at a low temperature of 23.6°C and in 16.6±0.53 days at a high temperature of 27.4°C (Cividanes et al. 2007). The lifespan of *P. vigintiduopunctata* is significantly affected by feeding on different mildew species on various hosts. Younes et al. (2015) found that females feeding on *P. guttata*-infected M. nigra had shorter lifespans than those feeding on E. cichoracearum-infected squash (males: 74±45.24 days; females: 48.22±18.71 days). However, in our study, the average lifespan was longer, with males surviving for 107 days and females for 105 days.

Diet differences also significantly affect egg production. When *P. vigintiduopunctata* was fed on *P. guttata*-infected

expectancy, survival and fecundity rates, finite rate of increase, net reproductive rate, and gross reproductive values of the coccinellid. In the present study, 80-85% of the larvae of *P. vigintiduopunctata* fed exclusively on squash mildew reached adulthood and displayed high reproductive capacity. In the first generation, there was a slight decrease in the intrinsic rate of increase, finite rate of increase, net reproductive rate of the life tables and fecundity, and gross reproductive values of *P. vigintiduopunctata*. On the other hand, in the second generation, these differences were not statistically significant. In contrast with first generation.

Halyziini species are obligate mycophagous insects that have been recorded feeding on various powdery mildew species (Sutherland and Parrella 2009). However, it is not yet known whether *P. vigintiduopunctata* would continue to feed on *E. cichoracearum* in the presence of other powdery mildew species or whether it would turn to alternative food sources such as pollen or honeydew if its main food source were unavailable. In a preliminary experiment, however, *P. vigintiduopunctata* feed on offered pollen. It is important for this coccinellid to feed on pollen or honeydew secretions in order to survive and remain in the garden or greenhouse where it is released in the absence of its main food.

CONCLUSION

This study demonstrates that *Psyllobora vigintiduopunctata*, which has completed its development on *Erysiphe cichoracearum* and has maintained stable reproduction for two generations, has displayed significant adaptation to

this host. These results indicate that *P. vigintiduopunctata* has potential for use in integrated pest management against squash powdery mildew. However, it is essential to validate these findings under semi-field and natural conditions and to examine how this predator could be incorporated into strategies involving chemical and biological fungicides employed in powder mildew control.

ACKNOWLEDGEMENTS

This study was supported by the Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies. We would like to thank Prof. Dr. Fikret DEMİRCİ (Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara, Türkiye) and Dr. Durmuş ERDURMUŞ (Directorate Of Plant Protection Central Research Institute, Ankara, Türkiye) for the identification of the powdery mildew pathogen, and Dr. Derya ŞENAL (Department of Plant Protection, Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University, Bilecik, Türkiye) for the identification of the coccinellid.

ÖZET

Kabakgiller üretiminde, ürün verimini ve kalitesini önemli ölçüde azaltan külleme hastalığını kontrol etmek için kullanılan fungisitler çevre kirliliğine ve kalıntı oluşumuna neden olmaktadır. Bu nedenle bu hastalığın kontrolünde sürdürülebilir yaklaşımlara ihtiyaç vardır. *Psyllobora vigintiduopunctata* (Linnaeus) (Coleoptera: Coccinellidae)'nın, *Erysiphe cichoracearum* (DC.) (Erysiphales: Erysiphaceae)'a karşı biyolojik mücadele etmeni olarak potansiyelini değerlendirmek amacıyla, iki nesil boyunca kontrollü koşullarda (25±2°C sıcaklık, %70±5 nem, 12:12 fotoperiyot) biyolojik parametreleri ve popülasyon dinamikleri belirlenmiştir. Verilerin analizinde iki eşeyli, yaş ve döneme özgü yaşam çizelgesi yaklaşımı kullanılmıştır. Sonuçlar, ortalama yaşam boyu yumurta veriminin dişi başına 317,48±142 ile 376,55±146 yumurta arasında değiştiğini göstermiştir. *P. vigintiduopunctata* her iki nesilde de gelişimini 26,7–28,4 gün içinde tamamlamıştır. Dişilerin toplam yaşam süresi 102–108 gün, erkeklerin ise 106–107 gün olarak belirlenmiştir. Predatörün *E. cichoracearum* üzerinde kalıtsal üreme yeteneği (*r*) 0,0788–0,0829 dişi/gün arasında bulunurken, ortalama nesil süresi (*T*) 58,7–61,5 gün arasında değişmiştir. Dişi başına nesil başına üretilen toplam yavru sayısını ifade eden brüt üreme gücü (*GRR*) ise 219,5–228,5 birey arasında belirlenmiştir. Laboratuvar sonuçları, *P. vigintiduopunctata*'nın kabakgillerde *E. cichoracearum*'a karşı biyolojik mücadele etmeni olarak umut verici bir potansiyele sahip olduğunu göstermektedir.

Anahtar kelimeler: 22 noktalı uğur böceği, iki eşeyli yaşam tablosu, mikofag, *Erysiphe cichoracearum*, biyolojik mücadele

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Cite this article: Yalçın, F., Ülgentürk, S., & Özgökçe, M. S. (2026). Interactions between *Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae) and Cucurbit powdery mildew. Plant Protection Bulletin, 66-1. DOI: 10.16955/bitkorb.1758992

Atıf için: Yalçın, F., Ülgentürk, S., & Özgökçe, M. S. (2026). *Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae) ile Kabakgil külleme hastalığı arasındaki ilişkiler. Bitki Koruma Bülteni, 66-1. DOI: 10.16955/bitkorb.1758992