Romanian Journal for Plant Protection, Vol. XVII, 2024
ISSN 2248 – 129X; ISSN-L 2248 – 129X

PRELIMINARY RESEARCH ON OPTIMAL GROWTH PARAMETERS UNDER CONTROLLED CONDITIONS FOR SOME SPECIES OF PREDATORY COCCINELLIDS

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Abstract. This study investigates the effects of temperature on the preimaginal development and aphid consumption rates of two predatory coccinellids species, *Coccinella septempunctata* and *Adalia bipunctata*, under controlled laboratory conditions. Populations were reared on natural diets consisting of aphids (*Acyrtosiphon pisum* and *Aphis fabae*) and an artificial diet developed for experimental purposes. Experimental conditions included three temperature regimes: 16°C, 20°C, and 25°C. The results demonstrated that a temperature of 25°C maximized aphid consumption and average lifespan of adults, with *C. septempunctata* exhibiting higher performance compared to *A. bipunctata*. Although natural diets outperformed the artificial alternative in terms of larval development, survival rates, and adult longevity, the artificial diet proved to be a viable option for *C. septempunctata* rearing. These findings provide a foundation for optimizing mass rearing protocols for predatory coccinellids intended to be used in augmentative biological control programs for aphids control on horticultural crops under protected spaces.

Key words: *aphidophagous Coccinellidae, predators, mass-rearing, diets*

INTRODUCTION

Coccinellidae beetles, commonly referred to “ladybugs” or “ladybird”, play a crucial role in the various ecosystem, especially in horticulture. The ladybird beetle *Coccinella septempunctata* Linnaeus, 1758 (COLEOPTERA: COCCINELLIDAE) is a significant predator of soft-bodied insects (Yi et al.,2022) an aspect of the natural phenomenon called biological control, that it is defined as the reduction of pest populations by natural enemies (Hala et al., 2019). The presence of *C. septempunctata* can also act as an indicator of ecosystem health. Both adults and larvae are predators, it was estimate that a single ladybug can consume up to 3,000 or more aphids in its lifetime, and also might feed on scale insects, mites, mealybugs, moth eggs etc. (Suleman & Riaz, 2023).

The two-spotted ladybird *Adalia bipunctata* (Linnaeus, 1758), native to Europe, Central Asia and North America, is also one of the most common coccinellids in orchards, known as a polyphagous predator with a wide range of prey (Omkar& Pervez, 2005). Besides other aphids’ species, *Aphis fabae* and *Acyrthosiphon pisum* are both recognized as supporting the growth and reproduction of *A. bipunctata* (Rana et al., 2002).

Among the alternative of chemical pest control practices, the most recommended is augmentative biological control, which involves using antagonists (predators or parasitoids) from the native fauna, mass reared and periodically released into crops. Many Coccinellidae species are aphid predators and effective for pest management in agricultural crop ecosystems.

For augmentative releases, large populations of high-quality individuals are required. From a practical perspective, the choice of species to be used and reared largely depends on the ease of mass rearing procedures. In many studies it is shown that *C. septempunctata* is a reputable predator and can be used for the biological control of wide range of aphids, found on a great diversity of plants and habitats (Hesler and Kieckhefer, 2008) while A. *bipunctata,* having also a large prey range, was mainly successful on arboreal species (Omkar and Pervez, 2005).

Mass production of *C. septempunctata* is usually carried out by feeding them with prey items such as the aphids: *Acyrthosiphon pisum, Aphis craccivora, A. fabae, A. gossypii, A. nerii, sambuci, Eucalipterus tiliae Metopolophium dirhodum, Myzus persicae, Uroleucon compositae* and so on; *Trichogramma chilonis* pupae, or *Ephestia kuehniella* eggs (Jin et al., 2010; Koch, 2003; Sun et al., 2001).

Also, various artificial diets have been developed for rearing several predators Coccinellidae (Ali et al., 2016; Bonte et al., 2010; Rojas et al., 2016; Tan et al., 2015; Yazdani & Zarabi, 2011; Sarwar & Saqib, 2010; De Clercq, 2004; Sun et al., 2017)). According to Cohen (2005), to support development, growth, and reproduction of insects, artificial diets basically contain protein, free amino acids, lipids, carbohydrates and vitamins.

Selecting the most appropriate diet, which should be inexpensive, continuously available, and should not affect the normal development and fitness of the predator, is one of the key challenges of insect mass-rearing programs (De Clercq 2004).

The aim of the study was to examine the effect of temperature on:

1. the adult longevity and prolificity parameters of two species of predatory coccinellids: *Coccinella septempunctata* and *Adalia bipunctata* as function of diet,
2. the average consumption rates of the aphids *Acythosiphon pisum* and *Aphis fabae* throughout the larval development of *C. septempunctata* and *A. bipunctata*,
3. the development and survival of the preimaginal stages of *C. septempunctata*, when the diet was the aphid *Acythosiphon pisum* or *Aphis fabae* compared to feeding on an artificial diet, under laboratory conditions.

MATERIALS AND METHODS

The research was conducted in the applied entomology laboratory at R.D.I.P.P. Bucharest, where populations of the species *C. septempunctata* and *A. bipunctata* were brought and reared on natural hosts represented by aphids (reared on peas and beans) and an artificial diet, at different temperatures: 16, 20, and 24 degrees Celsius.

The main experimental steps of the study were:

I. Obtaining the ladybug stock colonies

The adults of both ladybug species were collected in the spring of 2024 from an orchard in Agigea, Constanța County, and introduced for rearing in the applied entomology laboratory at R.D.I.P.P. Bucharest, in cages with aphids on peas ad libitum and oviposition substrate. The room was maintained under a photoperiod of 14:10 (L1) and a temperature range of 24 ± 2 degrees Celsius. Both ladybug species were reared using the same procedure. In short, the egg masses were collected every 1 to 2 days and placed in containers, while newly hatched larvae were transferred immediately after emergence. Second-instar larvae (L2) were transferred to

rearing containers containing aphids on peas as prey. Third-instar (L3) and fourth-instar (L4) larvae were reared individually, and pupae were transferred to new containers immediately after pupation. Newly emerged adults were transferred to cages with aphids in abundance and oviposition substrate.

The specimens from the second generation obtained in the laboratory were further used for the study.

Figure 1. Aspects of *Coccinella septempunctata* adults rearing on aphids in the isolator

II. *Obtaining the aphids stock colonies*

The colonies were established by collecting aphids from a private garden in May 2024. The aphids *Acythosiphon pisum* reared on peas and *Aphis fabae* reared on beans were obtained and kept under isolator conditions.

III. *Food provided to predator Coccinellidae*

i) Natural diet 1 – *Acythosiphon pisum* reared on peas.
ii) Natural diet 2 – *Aphis fabae* reared on beans.
iii) Artificial diet – A mixture composed of whey protein hydrolysate + casein + honey + others, according to a recipe currently under development for patenting purposes.

IV. Experimental setup

i) Adults were transferred in pairs into plastic containers (6 cm × 4 cm) with perforated lids for ventilation, lined with filter paper, and pre-filled with leaves containing aphids from each species or the artificial diet. Ten containers (each with one pair) were prepared for each food variant and one of the temperatures (16, 20, and 25 degrees Celsius). Observations were made regarding the adult longevity. The egg masses laid in each variant were separated, and observations were made regarding the duration of the period and the hatching percentage.

ii) Ten L2, L3, or L4 larvae were transferred to rearing containers lined with filter paper and contained 2 leaves of peas or beans infested with a known number of aphids as prey or 2 masses of the artificial diet. For each larval stage, 10 containers were prepared for each temperature (16, 20, and 25 degrees Celsius). Observations were made regarding the number of aphid larvae consumed, the duration of the larval stage, and survival rates.

Observations on egg laying, egg hatching, larval development duration, pupal stage duration and adult longevity were performed 3 times a day, at 4-hour intervals.

V. Data analysis

The experiment started with the *C. septempunctata* and *A. bipunctata* adults of the first generation obtained in controlled conditions. Average was calculated for adult longevity considering diets and temperature as factors, and consumption of aphids by larvae of the two predator species considering temperature as factors. The study continued for *C. septempunctata* population, observations was made and female fecundity, egg viability, development and survival of larval and pupal stages was calculated considering diets and temperature as factors. The data were analysed using two-way ANOVA, followed by determining the significant differences between means by Tuckey test using Minitab statistical software (version 22).

RESULTS AND DISCUSSIONS

Michaud (2005) considered female fecundity (egg number), fertility (determined by egg viability) and longevity as the appropriate criteria for determining relative prey suitability for adult coccinellids. The present laboratory study data revealed that the temperature influence the adult's longevity of both coccinellid species (Figure 2). In case of diet consisting of natural prey, the shortest lifespan was recorded for females reared at 25°C. For *Coccinella septempunctata*, the mean lifespan was 43 days when reared on *A. fabae*, 42 days when reared on *A. pisum*, and 20 days when reared on the artificial diet. The adults of *Adalia bipunctata*, reared at 25°C, had an average lifespan of 24 days when reared on *A. pisum*, 23 days when reared on *A. fabae*, and 12 days when reared on the artificial diet. The temperature of 16°C significantly reduced the adult's lifespan for both predators reared on the artificial diet. The result are in line with previous works that consider temperature interval 20- 25°C to be the most favorable for *C. septempunctata* development (Katsarou et al., 2005).

The average aphid consumption throughout the entire larval development was highest at 24°C (Figure 2). The average consumption of *C. septempunctata* larvae was 167.5, 130.2 and 65.4 aphids when fed on *A. pisum*, at 16, 20 and 25°C, respectively and 148, 111.4 and 53.9 aphids when fed on *A. fabae* at the same temperatures, respectively. The mean number of aphids consumed by a single larva of *A. bipunctata* was 93.2, 76.5 and 42.3 aphids when fed on *A. pisum*, at 16, 20 and 25°C, respectively and 77.5, 67.2 and 34.4 aphids when fed on *A. fabae* at the same temperatures, respectively (Figure 2). The data indicated that larvae of both predatory were significantly more voracious on *A. pisum* than on *A. fabae.*

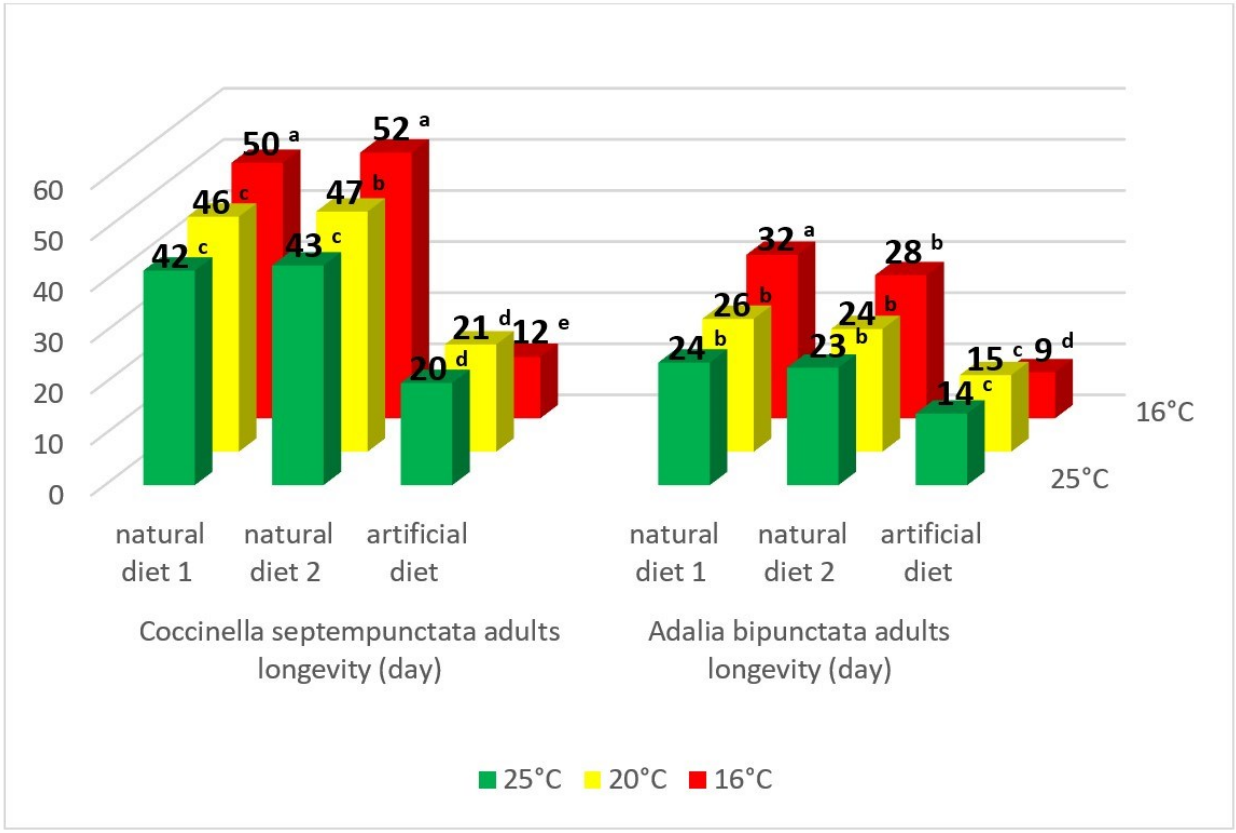

Figure 2. Longevity of females from the two ladybug species reared on the three types of diets as a function of temperature

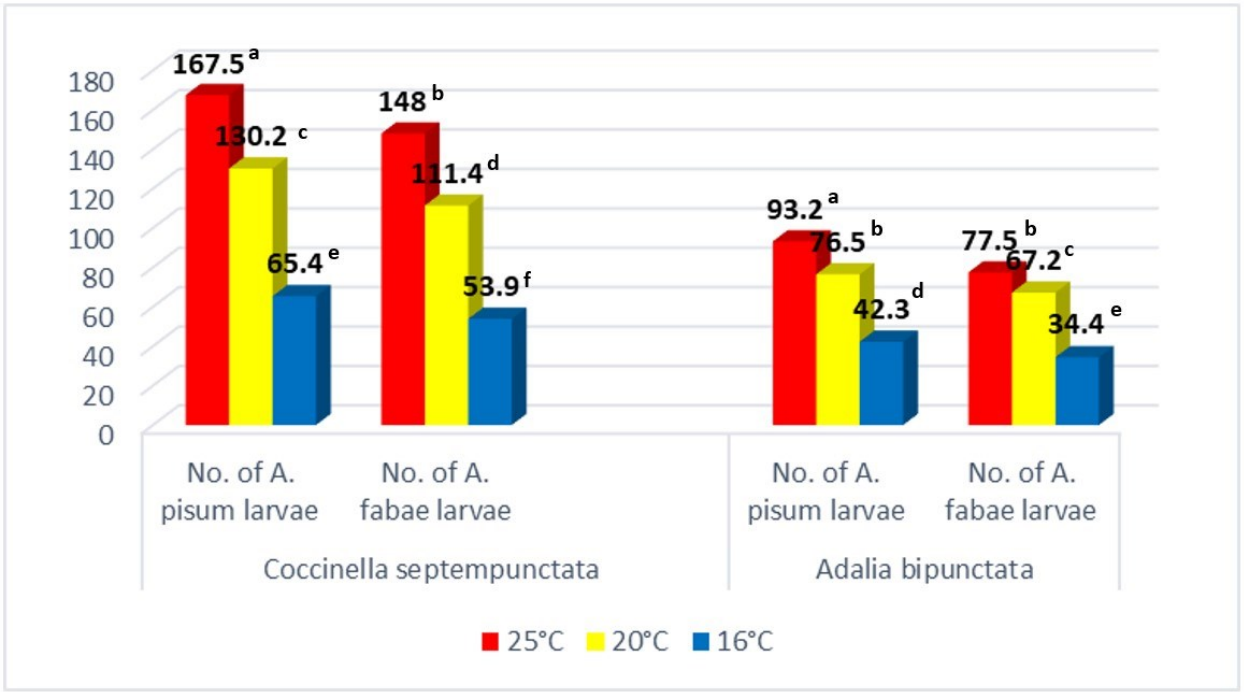

Figure 3. Average aphid larval consumption throughout the larval development of *Coccinella septempunctata* and *Adalia bipunctata* species as a function of temperature

The highest mean fecundity of *C. septempunctata* fed with aphids was 262 eggs /female when it was reared on *A. pisum*, at a temperature of 25°C, while the lowest one was 200.8 eggs/ female when reared on *A. fabae* at 16°C. In case of artificial diet the mean fecundity of *C. septempunctata* was between 55 eggs /female at a temperature of 25°C, while the lowest one was 18 eggs/ female when reared at 16°C (Table 1). As for the hatchability percentages of the eggs, those recorded after rearing on the two natural preys (*A. fabae* and *A. pisum*) (80 and 78%, respectively) were significantly higher than those recorded on the artificial diets (35,9%). Sarwar and Saqib (2010) developed an artificial diet containing yolk, sucrose, honey, casein, and protein hydrolyzate for adults and larvae of *C. septempunctata* proposed to serve as a substitute food. They reported that *C. septempunctata* females fed with aphids (*Rhopalosiphum padi/SchizAphis graminum*) laid in average 290.2 eggs/female while in case of artificial diet produced 56.5 eggs/female.

Table 1. The influence of temperature on *C. septempunctata* fertility parameters as function of diets

Parameter	Natural diet 1			Natural diet 2			Artificial diet		
	25ºC	20 ºC	16 ºC	25 ºC	20 ºC	16 ºC	25 ºC	20 ºC	16 ºC
Average number of eggs/female	262 [a]	250 [b]	221 [d]	234 [c]	210,1 [d]	200,8 [e]	55 [f]	33 [g]	18 [g]
Hatchability rate	78 [a]	75 [b]	68 [d]	80 [a]	74 [c]	65 [d]	35,9 [e]	30,2 [f]	22 [g]

Note: different letter indicates significant differences between treatments ($p < 0.05$). $p \leq 0.05$ (Tukey's test).

Temperature also influenced the duration of larval development, ranging from 8.5 days for *C. septempunctata* reared at 25°C on natural food (the aphid *A. pisum*), to 36.5 days at 16°C when reared on the artificial diet (Table 2). It was significantly shorter at 25°C for both predators and increases as the temperature decreased. At 25°C, the highest survival rate was recorded for all three diets offered. Similar results were obtained for the pupal stage duration, with the shortest being around 7 days when feeding on both aphid species at 25°C.

Table 2. Development parameters obtained for the rearing of *C. septempunctata* on the three diets, depending on the temperature

Parameter	Natural diet 1			Natural diet 2			Artificial diet		
	25ºC	20 ºC	16 ºC	25 ºC	20 ºC	16 ºC	25 ºC	20 ºC	16 ºC
Larval development duration(days)	8,5 [f]	11,6 [d]	18,9 [c]	9,6 [e]	12,2 [d]	19,6 [c]	21,2 [c]	26,3 [b]	36,5 [a]
% Larval survival	90 [a]	85 [b]	82 [c]	89 [a]	83 [c]	80 [d]	65 [e]	60 [f]	54 [g]
Pupal stage duration (Days)	7,5 [g]	7,8 [f]	12,5 [d]	7,2 [g]	8,4 [e]	12,9 [d]	15,6 [c]	16,5 [b]	19,3 [a]

Note: different letter indicates significant differences between treatments ($p < 0.05$). $p \leq 0.05$ (Tukey's test).

The data are in line with those of Katsarou et al. (2005) that studied the *C. septempunctata* L. development under four temperatures (14, 17, 20 and 23 °C) and found that the larval or pupal stages development of *C. septempunctata* were significantly reduced with temperature increase, and total larval and pupal mortality was highest at 14 °C.

CONCLUSIONS

Coccinella septempunctata and *Adalia bipunctata* are two predator species with high potential as biological control agents of aphids in horticulture.

The knowledges achieved during this study provide a foundation for optimizing mass rearing protocols for predatory coccinellids intended to be used in augmentative biological control programs for aphids control on horticultural crops under protected spaces.

Although we found that natural diets represented by *Aphis fabae* and *Acyrthosiphon pisum* exhibited superior performance compared to the artificial diet, preliminary results showed that the latter can be a promising alternative for predatory ladybugs continuously rearing system.

Further research is needed to study the influence of the proposed diets on a larger number of successive generations of predators raised in the laboratory.

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