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Development, predation and reproduction by *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae) as predator of *Pulvinaria regalis* Canard (Homoptera: Coccidae) and its coincidence with the prey in the field

Entwicklung, Prädationsleistung sowie Reproduktion des Prädators *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae) bei Ernährung mit *Pulvinaria regalis* Canard (Homoptera: Coccidae) sowie Koinzidenz mit der Beute im Freiland

C. Sengonca*, C. Arnold
Department of Entomology and Plant Protection, Institute of Phytopathology, University of Bonn, Nussallee 9, D-53115 Bonn, Germany
* Corresponding author, email: C. Sengonca@uni-bonn.de

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Summary

The present study aimed to investigate the development, predation and reproduction of *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae) at alternating temperatures of 9/19 ± 1 °C and 12/24 ± 1 °C as well as constant 25 ± 1 °C, feeding exclusively on eggs and nymphs of *Pulvinaria regalis* Canard (Homoptera: Coccidae). Additionally, its coincidence with the prey in the city of Bonn was examined in 1995 and 1997, respectively.

In the laboratory, the mean developmental duration of *E. quadripustulatus* decreased significantly at increasing temperatures. It was 57.1 days at 9/19 °C and 44.7 days at 12/24 °C as well as 28.3 days at 25 °C. Duration of the 4th larval stage was always the longest. It took 17.3 days at 9/19 °C, 14.5 days at 12/24 °C and 9.0 days at 25 °C to complete this stage. Mean duration of the pupal stage was 16.2 days at 9/19 °C, 10.8 days at 12/24 °C and 7.5 days at 25 °C. The total number of *P. regalis* eggs consumed by *E. quadripustulatus* larvae decreased significantly with increasing temperatures. The predation was 44.4 mg (= 8698 eggs) at 9/19 °C and 43.1 mg (= 8451 eggs) at 12/24 °C as well as 40.2 mg (= 7882 eggs) at 25 °C. The mean weight of *E. quadripustulatus* larvae increased steadily during the larval development irrespective of temperature, but decreased slightly prior to the pupal moult. The mean weight of pupae was 0.0119 g at 9/19 °C and 0.0125 g at 12/24 °C as well as 0.0107 g at 25 °C. Hibernating *E. quadripustulatus* ♀♀ consumed 10.13 mg of biomass in the form of *P. regalis* nymphs at 9/19 °C and 9.69 mg at 12/24 °C, respectively. Predation of the ♂♂ was less with 2.78 mg biomass consumed at 9/19 °C and 2.95 mg at 12/24 °C. The food uptake by freshly hatched *E. quadripustulatus* ♀♀ was significantly higher than the uptake by ♂♂ but it was less than the uptake by the overwintering ♀♀. In total, freshly hatched ♀♀ consumed on average 9.94 mg at 9/19 °C and 8.66 mg at 12/24 °C, freshly hatched ♂♂ on average 5.65 mg at 9/19 °C and 5.61 mg at 12/24 °C. *E. quadripustulatus* ♀♀ laid a mean total number of 96.8 ± 53.7 eggs within a period of 36 days at 9/19 °C and 139.1 ± 75.0 eggs at 12/24 °C in a period of 30 days.

The coincidence of the different developmental phases of the predator and the prey could be observed in the field in 1995 as well as in 1997. *E. quadripustulatus* adapted its life cycle to the climatically induced postponement in the development of *P. regalis* observed in 1997.

Due to the very good predation of larvae and adult *E. quadripustulatus* on *P. regalis,* the sufficient reproduction of *E. quadripustulatus* while feeding exclusively on *P. regalis* and the coincidence with the prey observed on amenity trees in the city of Bonn, *E. quadripustulatus* has to be considered as a promising predator of *P. regalis.*

Key words: *Exochomus quadripustulatus; Pulvinaria regalis;* development; predation; reproduction; coincidence; predator; scale insect; biological control

Zusammenfassung

Ziel der vorliegende Arbeit war es, die Entwicklung, Prädation und Reproduktion von *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae) bei Wechseltemperaturen von 9/19 ± 1 °C und 12/24 ± 1 °C sowie bei konstanten 25 ± 1 °C und alleiniger Ernährung mit Eiern und Nymphen von *Pulvinaria regalis* Canard (Homoptera: Coccidae) zu untersuchen. Außerdem wurde die Koinzidenz mit der Beute an Park- und Alleebäumen im Stadtgebiet von Bonn in den Jahren 1995 und 1997 beobachtet.

Bei Laboruntersuchungen verkürzte sich die durchschnittliche Entwicklungsdauer von *E. quadripustulatus* signifikant mit steigender Temperatur und betrug 57,1 Tage bei 9/19 °C bzw. 44,7 Tage bei 12/24 °C und 28,3 Tage bei 25 °C. Das vierte Larvenstadium dauerte dabei mit 17,3 Tagen bei 9/19 °C bzw. mit 14,5 Tagen bei 12/24 °C und mit 9,0 Tagen bei 25 °C stets am längsten. Das Puppenstadium wurde bei 9/19 °C in 16,2 Tagen bzw. bei 12/24 °C in 10,8 Tagen und bei 25 °C in 7,5 Tagen durchlaufen. Insgesamt verzehrten die *E. quadripustulatus*-Larven bei niedrigen Temperaturen mehr *P. regalis*-Eier als bei hohen Temperaturen. Die Predation betrug 44,4 mg (= 8698 Eier) bei 9/19 °C bzw. 43,1 mg (= 8451 Eier) bei 12/24 °C und 40,2 mg (= 7882 Eier) bei 25 °C. Das Larvengewicht stieg bei allen Temperaturen gleichmäßig während der Larvalentwicklung an, um kurz vor der Verpuppung wieder leicht zurückzugehen. Das durchschnittliche Puppengewicht betrug 0,0119 g bei 9/19 °C bzw. 0,0125 g bei 12/24 °C und 0,0107 g bei 25 °C. Überwinternde Adulte ♀♀ nahmen bei 9/19 °C insgesamt 10,13 mg Biomasse in Form von *P. regalis*-Nymphen bzw. 9,69 mg Biomasse bei 12/24 °C auf. Die Prädation von ♂♂ war dagegen mit 2,78 mg bei 9/19 °C bzw. 2,95 mg bei 12/24 °C deutlich geringer. Frisch geschlüpfte *E. quadripustulatus*-Adulte ♀♀ verzehrten durchschnittlich mit 9,94 mg Biomasse bei 9/19 °C bzw. 8,66 mg Biomasse bei 12/24 °C signifikant mehr *P. regalis*-Nymphen als die ♂♂, aber deutlich weniger als die überwinternden ♀♀. Für die ♂♂ konnten Werte von 5,65 mg bei 9/19 °C bzw. 5,61 mg bei 12/24 °C ermittelt werden. Untersuchungen über die Reproduktion ergaben eine durchschnittliche Gesamtzahl von 96,8 ± 53,7 abgelegten Eiern bei 9/19 °C bzw. 139,1 ± 75,0 Eiern bei 12/24 °C. Die Eiablage dauerte bei niedrigeren Temperaturen 30 Tage, bei höheren Temperaturen 36 Tage.

Bei Freilanduntersuchungen an Park- und Alleebäumen in Bonn konnte in beiden Untersuchungsjahren die Koinzidenz der einzelnen Lebensphasen des Prädators mit denen der Beute *P. regalis* beobachtet werden, wobei *E. quadripustulatus* klimatisch bedingte Verschiebungen im Entwicklungszyklus von *P. regalis* im Jahre 1997 im Vergleich zu 1995 kompensierte und seine eigenen Entwicklungszyklus entsprechend anpasste.

Auf Grund der ermittelten Prädationsleistung von Larven und adulten *E. quadripustulatus*, der guten Reproduktion bei alleiniger Fütterung mit *P. regalis* und bedingt durch die Koinzidenz mit der Beute im Freiland ist *E. quadripustulatus* als erfolgversprechender Prädator von *P. regalis* anzusehen.

Stichwörter: *Exochomus quadripustulatus; Pulvinaria regalis;* Entwicklung; Predationsleistung; Reproduktion; Koinzidenz; Predator; Napfschildlaus; Biologische Bekämpfung

1 Introduction

The Horse Chestnut Scale insect *Pulvinaria regalis* Canard (Homoptera: Coccidae) was introduced into Europe in the late sixties of the last century (Harris 1970). It has been observed in the Rhineland area in Germany since 13 years (Sengonca and Faber 1995), attacking mainly trees and shrubs in urban areas. According to Sengonca and Faber (1995) and Faber and Sengonca (1996), horse chestnut, maple and lime are the tree species worst effected by this pest. Within the last few years, *P. regalis* has spread over vast areas in Germany due to its high fecundity and broad host range (Schmitz 1997; Sengonca and Arnold 1999). Recent observations have revealed that a further

spread of this noxious pest is most likely (Faber and Sengonca 1996; Sengonca and Arnold 1999; Arnold and Sengonca 2001).

The four-spotted ladybeetle *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae), an important polyphagous predator of *P. regalis*, is native to Germany and can be observed frequently from lower altitudes up to mountainous areas. It is also a common beneficial in Europe (Horion 1961) and Asia Minor (Uygun 1977). It can be found in a variety of habitats but grows optimally in pine forests (Klausnitzer 1967). Other habitats are silver firs (Hofmann 1938), sitka spruce (Franke-Grosmann 1950), juniper and tree of life (Buddeberg 1884), and english tree (Zacher 1919). In horticulture plantations, *E. quadripustulatus* was observed on tea (Bogdanowa 1956), citrus (Argyriou and Kourmadas 1980), olive grooves (Panis 1979) and apple orchards (Schmidt 1928; Lövei 1981). Recently, *E. quadripustulatus* was found in high numbers on shrubs and trees along roads in the affected area of *P. regalis* (Faber and Sengonca 1996; Arnold and Sengonca 1999).

E. quadripustulatus feeds on a variety of homopterous insects including different aphid and scale insect species (Iperti et al. 1977; Radwan and Lövei 1983). It is especially a voracious predator of newly introduced scale insects that multiplied enormously in its new environment. In Italy, it is found as a predator of *Nidularia pulvinata* (Planchon) on oak trees (Viggiani 1991) and *Eupulvinaria hydrangeae* Steinweden and *P. regalis* on horse chestnut, maple and lime trees in Belgium (Merlin 1993) and Germany (Sengonca and Faber 1995; Arnold and Sengonca 1999, 2001), respectively.

Since a conventional control of *P. regalis* is not appropriate in urban areas in Germany, it was aimed therefore, to develop a biological control strategy. Within this context, *E. quadripustulatus* was considered to be a very promising predator. However, very little information about its ecological and biological properties (Sengonca and Faber 1996) and efficiency against *P. regalis* was available in the literature.

The objectives of this work were, therefore, aimed to examine development, predation and reproduction by *E. quadripustulatus* with *P. regalis* eggs and nymphs as prey at different temperatures in the laboratory. Additionally, the coincidence of *E. quadripustulatus* with *P. regalis* under field conditions was examined.

2 Materials and methods

2.1 Developmental duration

Duration of *E. quadripustulatus* development was examined in climatic chambers at alternating temperatures of 9/19 ± 1 °C (10/14 h) and 12/24 ± 1 °C (8/16 h), a constant temperature of 25 °C ± 1 °C (24 h), a relative humidity of 65 ± 10 % and artificial light of 1800 lx intensity which was provided by neon tubes from Osram (universal-white). The alternating temperature regimes were chosen according to the temperatures prevailing in the egg-laying period of *P. regalis* in the Rhineland area in April and June compared to a constant temperature of 25 °C in the laboratory. Round plastic cages, 11 cm in diameter and 3 cm high, were used for the experiments. The lids of the cages were equipped with meshed holes to allow for fresh air exchange. Small pieces of sponge of 1 cm^2 size were used to provide water to the insects. The larvae were taken from of a stock culture, whose starting individuals had been collected in Bonn. Egg-masses and attached bodies of female scales were offered as food. Individual *E. quadripustulatus* larvae, at maximum 24 h of age, were transferred for the experiments to each box. Daily controls were conducted to guarantee the exact determination of each developmental stage. All experiments were replicated 15 times.

2.2 Predation and daily increase in weight of *Exochomus quadripustulatus* larvae and weight of pupae

In another set of experiment, daily predation and increase in weight of *E. quadripustulatus* larvae and weight of pupae was determined. The experimental set-up was similar to the one described above. To determine the daily food uptake by the *E. quadripustulatus* larvae, the weight of every single egg mass and the attached female was taken before and after they had been offered to the predator. Weights of the egg masses were recorded after each moulting to the next larval stage and after moulting to the

pupal stage, using a precision balance from SARTORIUS (BP210). Due to an inconsistent number of eggs per egg mass, it was necessary to determine the mean weight of a single *P. regalis* egg in a pilot test with 500 eggs. Afterwards the total number of consumed eggs was calculated. The larval weight was determined on a daily basis. The pupae were measured once, immediately after the last moulting. All experiments were replicated 15 times.

2.3 Predation by *Exochomus quadripustulatus* adults

Predation of *E. quadripustulatus* adults with *P. regalis* nymphs as the only food source was examined for both freshly hatched and for hibernating adults. The experiments were carried out under the same climatic conditions and set-up as mentioned above.

In one set of experiment, freshly hatched adults, less than 24 h of age, were kept under long day conditions at 16 : 8 h (L : D). In another set of experiment, hibernating adults were kept under a short day conditions of 8 : 16 h (L : D) for 30 days. This treatment was necessary to provide a period of reduced activity to *E. quadripustulatus* adults as in the winter months, which is necessary to stimulate the development of mature individuals (KATSOYANNOS 1976). After 30 days, the climatic conditions were changed to 16 : 8 h (L : D) as prevailing in the field in spring. For both the experiments, leaves of heavily infested maple and lime trees were collected in Bonn and brought into the laboratory. The leaves were kept in small vials filled with water and cotton wool to prevent rapid wilting and following escape of the *P. regalis* nymphs. Individual female or male *E. quadripustulatus* adults were transferred to singly placed leaves in each experimental box and provided with ad libitum prey. The number of scales consumed and killed was recorded every 24 h by the help of a magnifying glass. Since the size and body weight of the *P. regalis* nymphs increased continuously during the experiment, the mean body weight of a randomly collected sample of 20 scales was measured every 7 days. All experiments were replicated 10 times.

2.4 Reproduction

The reproductive capacity of *E. quadripustulatus* was investigated in climatic chambers under the two alternating temperature regimes and other conditions mentioned above. Singly mated females were placed in individual boxes. *P. regalis* nymphs placed on leaves were provided ad libitum. The number of eggs laid was counted each day. The experiments were replicated 10 times.

2.5 Coincidence of *Exochomus quadripustulatus* and *Pulvinaria regalis* in the field

The coincidence of the different developmental stages of *E. quadripustulatus* and *P. regalis* was examined on heavily infested trees in Bonn from March to November in 1995 and 1997, respectively. Stems, branches and leaves of lime *(Tilia cordata)* and maple trees *(Acer platanoides)* were examined thoroughly and the occurrence of the different developmental stages of *E. quadripustulatus* and *P. regalis* was recorded.

3 Results

3.1 Developmental duration

The mean post-embryonic developmental duration of *E. quadripustulatus* larvae feeding on *P. regalis* eggs and the mean duration of the pupal stage decreased significantly at higher temperatures (Table 1). The longest mean duration of development was 57.1 days at changing temperature of 9/19 °C. Complete development took 44.7 days at 12/24 °C and 28.3 days at 25 °C, which was significantly less than at 9/19 °C. The mean duration of the four larval stages and the pupal stage was significantly different among the three temperature regimes. Duration of the 4th larval stage was always the longest. It took 17.3 days at 9/19 °C, 14.5 days at 12/24 °C and 9.0 days at 25 °C to complete this stage. The first larval stage was longer with 8.2 days than the second with 7.1 days at 9/19 °C and also with 4.5 days at 25 °C longer than the second with 3.4 days. But it was shorter with 5.9 days than the second

with 6.3 days at 12/24 °C. Mean duration of the pupal stage was 16.2 days at 9/19 °C, 10.8 days at 12/24 °C and 7.5 days at 25 °C (Table 1).

3.2 Predation and increase in weight of *E. quadripustulatus* larvae and weight of pupae

The total amount of food consumed by *E. quadripustulatus* larvae during its post-embryonic development decreased significantly with the increasing temperature (Table 2). It was highest at 9/19 °C with 44.4 mg of consumed eggs. It was 43.1 mg at 12/24 °C and 40.2 mg at 25 °C. The mean weight of a single *P. regalis* egg, determined in a pilot test, was 0.00510 mg. Assuming that the *E. quadripustulatus* larvae fed only on eggs, one can calculate that at 9/19 °C it has consumed an average 8698 eggs during its development. While at 12/24 °C and 25 °C this number was 8451 and 7882 eggs, respectively.

No significant difference in the amount of food consumed by *E. quadripustulatus* larvae within a particular larval stage was observed between the two alternating temperatures of 9/19 and 12/24 °C. The only exception was observed for the first larval stage at 12/24 °C and 25 °C, where no significant difference was observed. The amount of food uptake was highest in the fourth larval instar, irrespective of the temperature. It was 22.95 mg at 9/19 °C temperature, 22.60 mg at 12/24 °C and 21.23 mg at 25 °C (Table 2).

The weight gain of *E. quadripustulatus* larvae was inconsistent at different temperatures (Fig. 1). The mean body weight increased steadily during the larval development irrespective of temperature, but decreased slightly prior to the pupal moult. A period of high food uptake and a steady increase in body weight by the *E. quadripustulatus* larvae was observed until the 17th day at 25 °C and the 30th day at 12/24 °C, as well as the 37th day at 9/19 °C. The maximum mean larval weight recorded was 0.0115 g at 25 °C and 0.0159 g at 12/24 °C, as well as 0.0139 g at 9/19 °C. Weight of the pupae were taken immediately after the pupal moult. It was 0.0119 g at 9/19 °C and 0.0125 g at 12/24 °C, as well as 0.0107 g at 25 °C.

Table 1. Mean developmental duration of *Exochomus quadripustulatus* larvae with *Pulvinaria regalis* eggs as prey at three different temperatures

Temperature (°C)	Duration of larval development (days)					total (days)
	L_1 mean ± SD	L_2 mean ± SD	L_3 mean ± SD	L_4 mean ± SD	pupae mean ± SD	mean ± SD
9/19 (10/14h)	8.2 ± 2.4 a	7.1 ± 2.1 a	8.3 ± 2.0 a	17.3 ± 1.8 a	16.2 ± 1.7 a	57.1 ± 3.6 a
12/24 (8/16h)	5.9 ± 1.2 b	6.3 ± 1.3 b	7.2 ± 1.5 b	14.5 ± 0.7 b	10.8 ± 1.1 b	44.7 ± 3.2 b
25 (24h)	4.5 ± 1.0 c	3.4 ± 0.7 c	3.9 ± 0.9 c	9.0 ± 1.1 c	7.5 ± 1.9 c	28.3 ± 1.8 c

Means in columns followed by the same letter are not significantly different at the 5 % level (TUKEY's Multiple Range Test)

Table 2. Predation of *Exochomus quadripustulatus* larvae with *Pulvinaria regalis* eggs as prey at three different temperatures

Temperature (°C)	Consumed *P. regalis* eggs (mg)					Total number of consumed eggs mean ± SD
	L_1 mean ± SD	L_2 mean ± SD	L_3 mean ± SD	L_4 mean ± SD	Total mean ± SD	
9/19 (10/14h)	2.71 ± 1.3 a	6.54 ± 0.6 a	12.20 ± 0.9 a	22.95 ± 0.5 a	44.4 ± 5.3 a	8698 ± 1039 a
12/24 (8/16h)	2.33 ± 0.2 b	6.11 ± 0.1 a	12.06 ± 0.4 a	22.60 ± 0.4 a	43.1 ± 6.0 a	8451 ± 1176 a
25 (24h)	2.13 ± 0.2 b	5.26 ± 0.3 b	11.62 ± 1.1 b	21.23 ± 2.5 b	40.2 ± 10.5 b	7882 ± 859 b

Means in columns followed by the same letter are not significantly different at the 5 % level (TUKEY's Multiple Range Test)

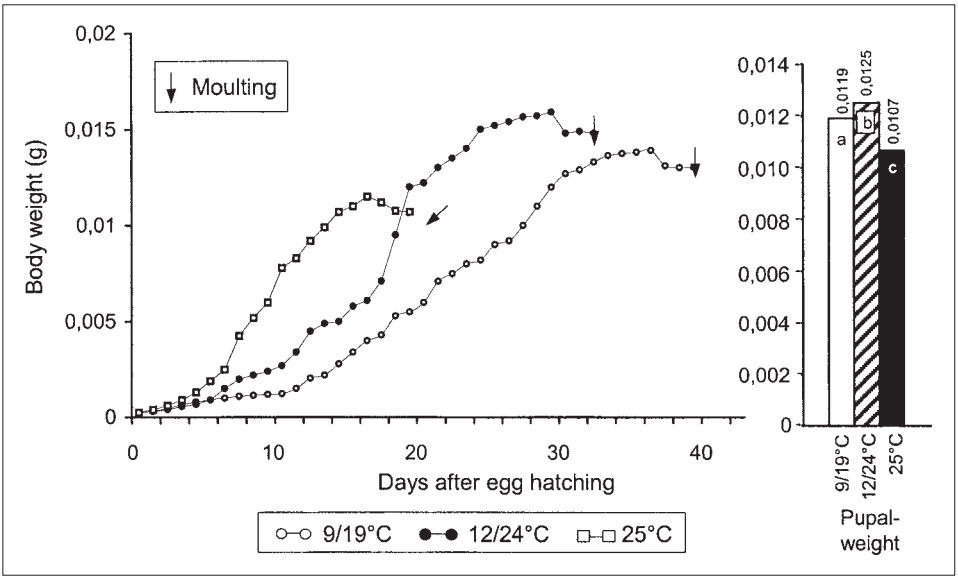

Fig. 1. Average daily increase in weight of *Exochomus quadripustulatus* larvae and weight of pupae with *Pulvinaria regalis* eggs as prey at three different temperatures. [Values followed by different letters are significantly different at 5 % level (T-Test)]

3.3 Predation of *E. quadripustulatus* adults

The hibernating *E. quadripustulatus* adults showed lower predation activities at the beginning of the experiments. On the first day after the hibernation period, the female consumed on average 0.065 mg of biomass at 9/19 °C and 0.072 mg at 12/24 °C (Fig. 2). While the males consumed significantly lower biomass than the females with 0.030 mg at 9/19 °C and 0.032 mg at 12/24 °C. No major changes in the food uptake occurred until day 32 at 9/19 °C and day 29 at 12/24 °C. Afterwards, the females started a period of distinct predation that lasted until day 63 at 9/19 °C and day 51 at 12/24 °C. Females consumed a maximum amount of biomass on day 52 at 9/19 °C with 0.306 mg and on day 40 at 12/24 °C with 0.432 mg. The consumption was less by males with 0.12 mg at 9/19 °C on day 37 and 0.14 mg at 12/24 °C on day 32. After this period, the adults consumed very little biomass until they died. The female consumed significantly higher amount of biomass at 9/19 °C with 10.13 mg than at 12/24 °C with 9.69 mg. The predation of the males differed just marginally between the two temperature regimes. They consumed 2.78 mg at 9/19 °C and 2.95 mg at 12/24 °C (Fig. 2).

The freshly hatched adult started a distinct food uptake directly after the end of their post-embryonic development (Fig. 3). The females consumed significantly more *P. regalis* nymphs than the males at both temperature regimes. During the first day, the *E. quadripustulatus* female consumed 0.088 mg of biomass at 9/19 °C and 0.096 mg at 12/24 °C. While the males consumed 0.079 mg at 9/19 °C and 0.088 mg at 12/24 °C. The food uptake by the adults increased steadily within the next days. The maximum amount of biomass uptake by the females was recorded on day 9 at 9/19 °C with 0.611 mg and on day 10 at 12/24 °C with 0.637 mg. The maximum uptake for males was recorded on day 7 at 9/19 °C with 0.32 mg and on day 11 at 12/24 °C with 0.365 mg. After this period, the predation by both the adults decreased till the end of the experiment. Thus, the females consumed 0.065 mg at 9/19 °C and 0.070 mg at 12/24 °C on the 30th day of experiment. On this date, the males consumed 0.030 mg at 9/19 °C and 0.035 mg at 12/24 °C. The total amount of consumed biomass was significantly higher at 9/19 °C than at 12/24 °C irrespective of the gender. In total, females consumed on average 9.94 mg at 9/19 °C and 8.66 mg at 12/24 °C. However, no significant differences were recorded for the total consumed biomass by males. It was 5.65 mg at 9/19 °C and 5.61 mg at 12/24 °C (Fig. 3).

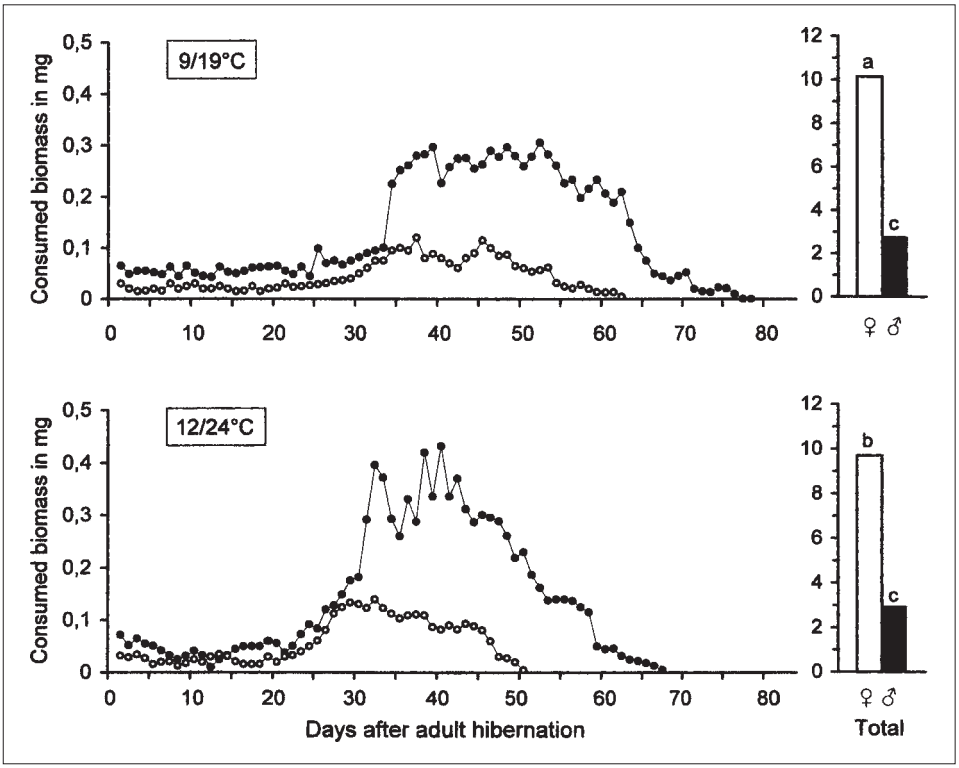

Fig. 2. Average daily predation of hibernating *Exochomus quadripustulatus* adults with *Pulvinaria regalis* nymphs as prey at two different temperatures. [Values followed by different letters are significantly different at 5 % level (T-Test)]

3.4 Reproduction

The *E. quadripustulatus* females layed their eggs in a chronological irregular pattern, with changing numbers of eggs deposited within one single day, ranging from 1 to 11 eggs at 9/19 °C and from 1 to 16 eggs at 12/24 °C. Significant differences in the total number of eggs laid at the two temperatures could be observed, also. The females laid a mean total number of 96.8 ± 53.7 eggs within a period of 36 days at 9/19 °C, and 139.1 ± 75.0 eggs at 12/24 °C in a period of 30 days (Fig. 4). The eggs were mostly laid in small groups but sometimes individually.

At 9/19 °C a sequence of days with and days without egg-laying was observed during the whole examination period. The number of eggs laid varied between a maximum of 11 eggs at day five and one egg at day 36 (Fig. 4). At this temperature 54.8 % of the total number of eggs were laid within the first 14 days of the experiment, the last egg on day 36. At 12/24 °C the egg-laying period was shorter than at 9/19 °C and ended on day 30. Here 82.0 % of the total eggs were deposited within the first 14 days. In the following 16 days until the cease of the egg-laying incidents of egg-laying occurred seldom and was reduced greatly as compared to the first 14 days (Fig. 4).

3.5 Coincidence of *Exochomus quadripustulatus* and *Pulvinaria regalis* in the field

The *E. quadripustulatus* adults, which hatched from the previous year hibernating stage, were observed at the beginning of March on lime and maple trees. They were mostly located on the southern side of the trees, in small cracks and scars of the bark, hiding alone or in small groups. In 1995, the egg-laying

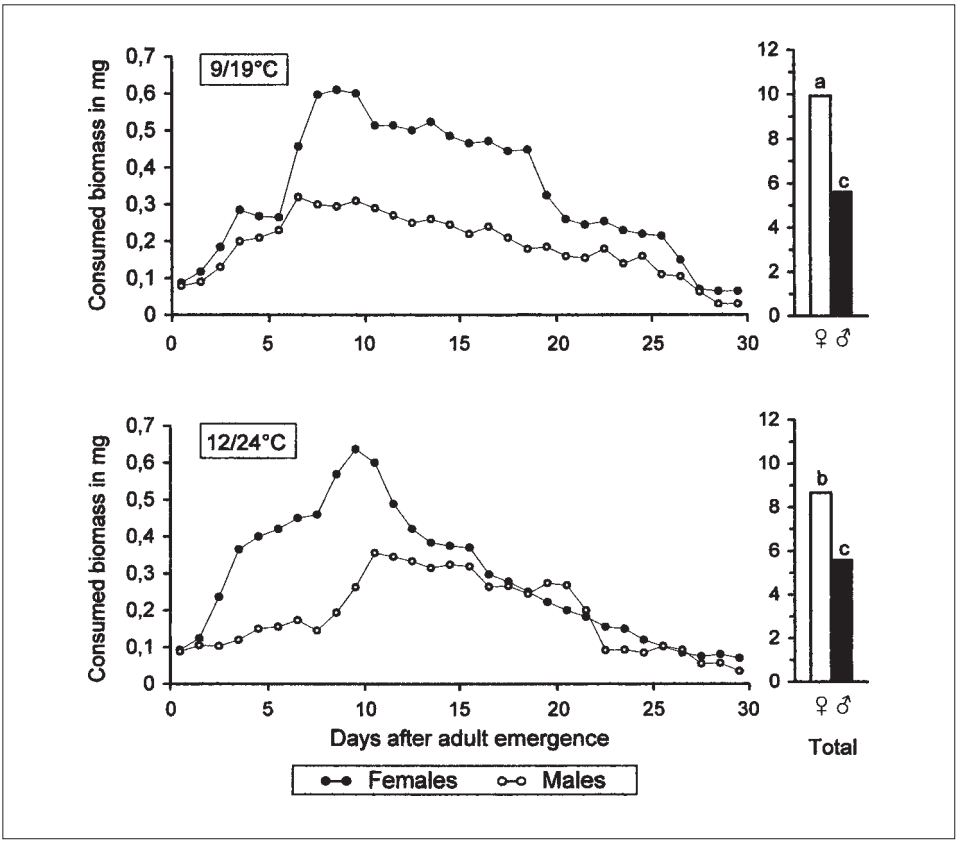

Fig. 3. Average daily predation of freshly hatched *Exochomus quadripustulatus* adults with *Pulvinaria regalis* nymphs as prey at two different temperatures. [Values followed by different letters are significantly different at 5 % level (T-Test)]

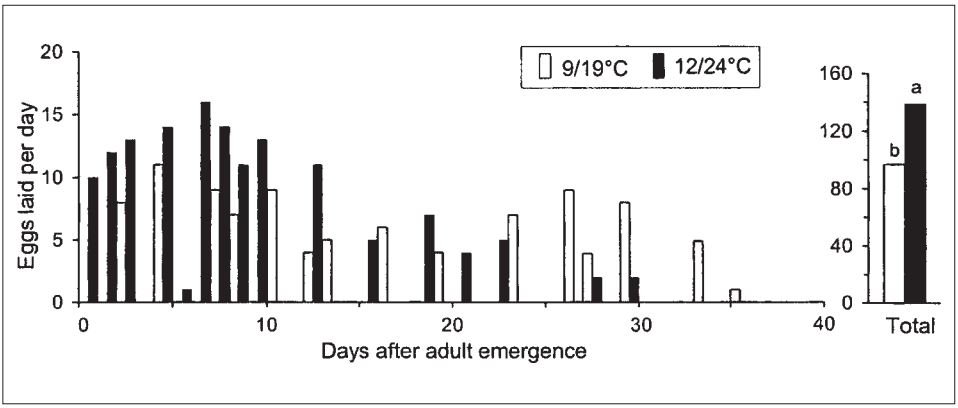

Fig. 4. Average daily reproduction of *Exochomus quadripustulatus* with *Pulvinaria regalis* nymphs as prey at two different temperatures. [Values followed by different letters are significantly different at 5 % level (T-Test)]

period of *E. quadripustulatus* took place from the 4th week of April until the 3rd week of June. The eggs were deposited either in small crevices adjacent to the ovisacs of *P. regalis* or directly beneath the body of a female scale insect, which had already started with the production of the ovisac at that point of time (Fig. 5). The first *E. quadripustulatus* larvae were observed from the beginning of the 2nd week of May, the last at the end of the third week in July. They fed exclusively on *P. regalis* eggs and stayed within the filamentous wax-like structures beneath the bodies of the dead *P. regalis* females. Normally, the *E. quadripustulatus* larvae consumed all eggs of an ovisac before they switched to another one. In some cases, more than one larvae fed on a single *P. regalis* ovisac. *E. quadripustulatus* pupae were observed from the beginning of 4th week in June until the end of July on the trees, mainly in close vicinity of the *P. regalis* colonies on the barks of stems and branches. Adult beetles were observed during the whole examination period. They were found mainly on the leaves before summer but after mid of June on all parts of the infested trees. They fed on *P. regalis* settlers and nymphs and other insects also. The first freshly hatched *E. quadripustulatus* appeared in the middle of July, recognizable by their incomplete coloration.

In 1995, the eggs and ovisacs of *P. regalis* were observed from the 4th week of April until the 4th week of June, mainly on stems and branches of the host plants. Crawlers and settlers could be found in the period from the 2nd week of May till the 1st week of August and nymphs from the beginning of last week in July, located on the leaves. Male *P. regalis* appeared in the middle of March and were found till the 3rd week of April. The first female *P. regalis* was observed at the end of March and the last at the end of May. The females died after the end of the egg-laying period and the simultaneous production of the ovisacs.

Great differences were found in the incidence of *E. quadripustulatus* and *P. regalis* between 1997 and 1995 (Fig. 5). In 1997, the egg-laying period of *E. quadripustulatus* took place from the 3rd week of May until the 2nd week of July. The *E. quadripustulatus* larvae were observed from the end of May until the end of July. The first pupae were recorded from the 1st week of July and the last at the end of the first week in August. Freshly hatched adults appeared for the first time at the beginning of the third week in July.

In 1997, the *P. regalis* females started egg-laying in the third week of May, almost three weeks later than in 1995. The last egg-laying female was observed at the end of July. This delay resulted in a subsequent delay in the following developmental stages of *P. regalis* in 1997. Therefore, the first crawlers and settlers were found not until the end of May. The last settlers were recorded in the 3rd week of August. Nymphs were observed first in the fourth week of July (Fig. 5).

4 Discussion

There was a lack of information before this work regarding the developmental duration, the predation of *E. quadripustulatus* larvae and adults and the reproduction of adults with *P. regalis* as prey. The present results showed that the duration of development of *E. quadripustulatus* larvae is significantly influenced by temperature with reduced duration at higher temperature. Uygun (1978) reported that larval development was exclusively possible with temperatures ranging between 17 and 30 °C, at least for parts of the development. The importance of the diet in larval development was underlined by Katsoyannos (1976) and Radwan and Lövei (1983). According to their results, one can conclude that the duration of development of *E. quadripustulatus* larvae feeding solely on *P. regalis* at 25 °C is comparable to larvae feeding on the scale insects *Planococcus citri*, *Saissetia oleae* and *Aspidiotus nerii*. Furthermore, the *E. quadripustulatus* larvae consumed a significantly higher amount of *P. regalis* eggs at lower than at higher temperatures. The feeding period at lower temperatures was also longer due to the extended developmental period. And ultimately this resulted in higher body weight of the *E. quadripustulatus* larvae. The total number of consumed *P. regalis* eggs was relatively high compared to other scale insect species at 25 °C. Uygun (1978) observed that *E. quadripustulatus* consumed a total number of 3812 *P. citri* eggs during the whole post-embryonic development. However, the results obtained in the present experiments indicate a total number of 7882 consumed *P. regalis* eggs.

In the first few days, the overwintering adults showed lower predation. This behaviour changed dramatically after the end of the hibernation period. In field, this period of intensive food uptake coincided with copulation and egg-laying and was induced by a time of relatively little activity.

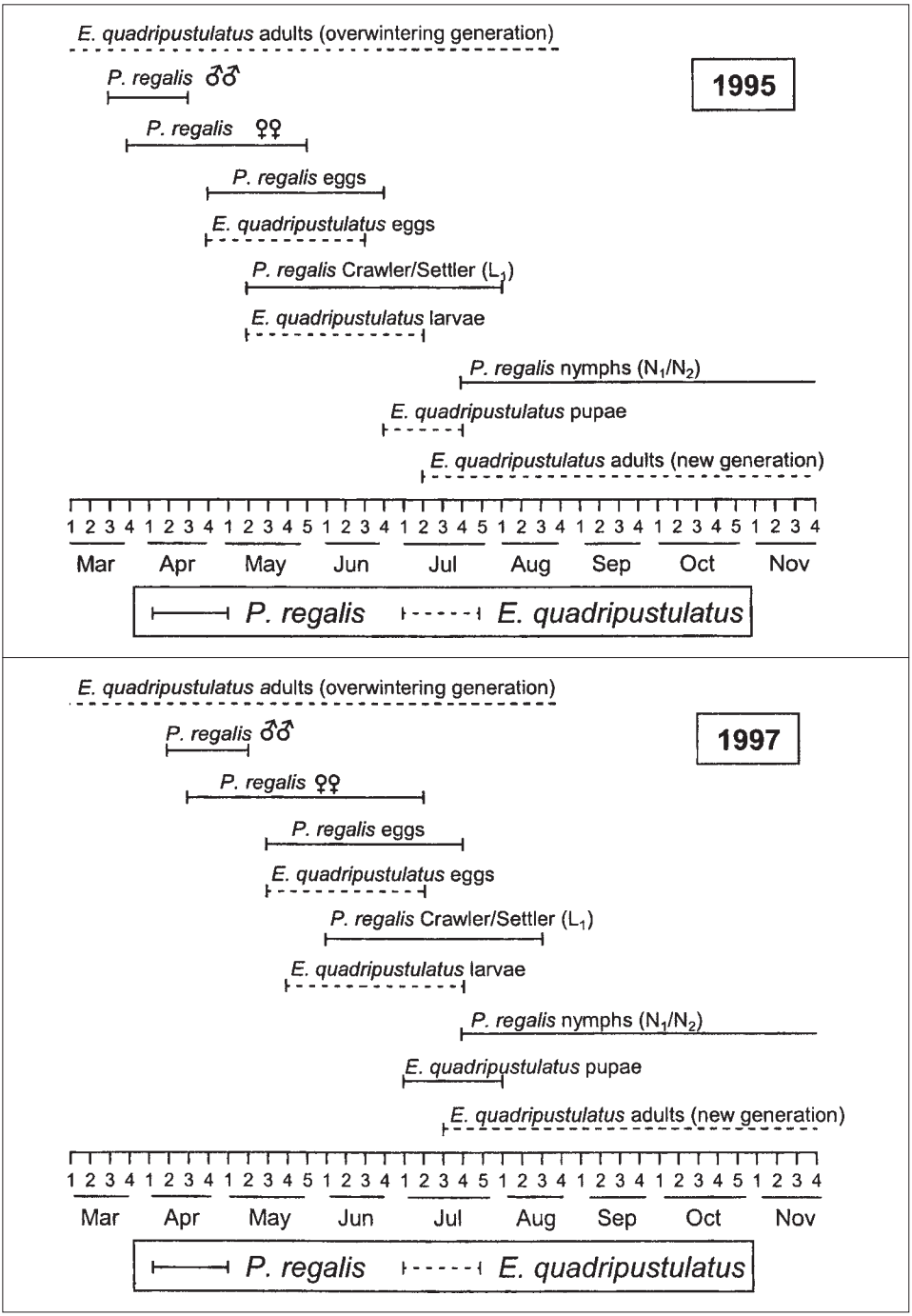

Fig. 5. Coincidence of developmental stages of *Exochomus quadripustulatus* and *Pulvinaria regalis* in the city of Bonn in 1995 and 1997.

Katsoyannos (1976) regarded this dormant form of the beetles as the quiescent form. The predation increased at later age with more number of adult scale insects were killed by *E. quadripustulatus* but not consumed in total. This lead to a very promising success in the control of *P. regalis* adults in springtime either before or after the egg-laying of the beetles.

The freshly hatched *E. quadripustulatus* adults started with a distinct food uptake. It is important to mention that at this time the individual *P. regalis* nymphs are small and light and the number of consumed *P. regalis* nymphs is very high. The potential control of *P. regalis* by *E. quadripustulatus* during this period is therefore very promising. Katsoyannos (1976) reported that the *E. quadripustulatus* adults initially build up a reserve depot of fat tissue before the final maturation of their sexual organs takes place. In the field, there is subsequently a period of low activity that belongs to the dormant form of the diapause (Fytizas and Katsoyannos 1982). In the northern and middle parts of Europe, this period passes directly into the hibernation. The final maturation of the sexual organs, the copulation and egg-laying takes place not before the end of this period (Katsoyannos 1976).

E. quadripustulatus females laid 96.8 ± 53.7 eggs at 9/19 °C and 139.1 ± 75.0 eggs at 12/24 °C. The presently recorded reproductive capacity corresponds with the earlier works reported in the literature. Katsoyannos (1976) found that *E. quadripustulatus* female laid an average of 130 eggs when fed exclusively on *Planococcus citri* at 25 °C. With *Aphis fabae* as prey, the total number of deposited eggs increased to an average of 185 eggs, with a maximum number of 479 eggs. Radwan and Lövei (1983) reported significantly lower fecundity for *E. quadripustulatus* with *Dysaphis plantaginea* and *Acyrtosiphon pisum* as preys with an average of 91.3 and 172.8 eggs, respectively. Merlin (1993) observed that *E. quadripustulatus* laid 114 ± 90.6 eggs while feeding on *Eupulvinaria hydrangeae.*

In field, the deposition of the *E. quadripustulatus* eggs took place in coincidence with the egg-laying period of *P. regalis.* This was obvious in 1997, when very cool temperatures in spring, compared to the longstanding average temperatures (Anonymus 1997), led to a late migration of *P. regalis* females to the stems and consequently late deposition of eggs. Despite the fact that *E. quadripustulatus* adults could be found on the trees throughout the spring, they started to deposit their eggs not before the *P. regalis* females had started to produce their ovisacs. Therefore, the preferred diet of *E. quadripustulatus* larvae is obviously scale insect eggs, though other potential prey insects can be found on the trees in high numbers. A similar observation was made by Zacher (1919) who found *E. quadripustulatus* larvae feeding exclusively on eggs of *Parthenolecanium corni.* Merlin (1993) examined *E. quadripustulatus* in Belgium and found eggs, larvae and pupae for longer periods of time than in Bonn. The reason for the earlier beginning of the egg-laying might be the milder climate in that region. And the prolongation of the egg-laying period could also be due to the availability of the preferred prey, the Hydrangea scale insect *Eupulvinaria hydrangeae,* in Belgium.

The location where the *E. quadripustulatus* adults deposited their eggs also plays a very important role from management point of view because of the limited mobility of the larvae. Schmutterer (1952) reported *E. quadripustulatus* adults feeding on a wide variety of different scale insect species in different places, but observed larvae only in bigger colonies of scales. The eggs are only deposited in such locations, where the freshly hatched larvae can find enough food for their entire development. In Bonn, the freshly hatched adults were found feeding solely on crawlers/settlers and the first nymphs but not on eggs due to time constraints. However, adult beetles were found in the urban environment during the whole summer. A migration into other biotopes was obviously not necessary for the preservation of the species, since *E. quadripustulatus* feeds on instars of scale insects and other homopterous insects. According to Radwan and Lövei (1983), *E. quadripustulatus* has a broad spectrum of potential prey insects, including different aphid species.

The present results clearly indicate that the indigenous ladybeetle *E. quadripustulatus* can be very efficient in the control of the horse chestnut scale insect due to its constant appearance in the urban environment, its preference for exotic prey organisms, its predation and reproductive capacity and its coincidence of development with *P. regalis.* Lower temperatures in spring result in a high predation of the *E. quadripustulatus* larvae on the *P. regalis* eggs. However, since the *E. quadripustulatus* larvae need bigger scale colonies for their development, the optimal predation can be achieved only at high scale population densities. Therefore, it would be of great help to install and devise places for overwintering of *E. quadripustulatus* population close to infested trees or to release artificially mass-reared *E. quadripustulatus* in spring.

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