

SUMMER DIAPAUSE AND WINTER QUIESCENCE OF *COCCINELLA SEPTEMPUNCTATA* (COL. COCCINELLIDAE) IN CENTRAL GREECE

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Coccinella septempunctata (Coleoptera: Coccinellidae) in Greece is a multivoltine species. In the lowlands, all instars are abundant in spring becoming scarce from July until the end of the warm period of the year; they are absent in winter. In June, most *C. septempunctata* adults migrate to mountainous aestivo-hibernation sites. Measurements of the duration of pre-oviposition period in females taken monthly from the summit of Mount Kitheron in central Greece (1993-1994) and transferred to laboratory conditions of high temperature (25°C), long day (16 hrs light/24 hrs), and presence of aphids, indicated that the *C. septempunctata* females were in diapause during July and August. The diapause gradually terminated from late August to late October and was followed by a period of quiescence extending from November to March of the following year.

During the summer diapause, arrest of ovarian development was indicated by immaturity of the ovaries in all sampled females and the complete absence of vitellogenic resorption signs. Also, adults were found with enlarged fat bodies, and the median duration of preoviposition period in females transferred to the above laboratory conditions was 90 days in early July and 82 days in early August. During the period of winter quiescence, arrest of ovarian development was characterized in most samples by immaturity of the ovaries in all females and, in some samples, by the presence of a few females with signs of vitellogenic resorption. In winter, adults were found containing fat body reserves of different levels, and the median preoviposition period of females transferred to optimal breeding conditions was 29 days in early November and 16 days in mid January.

KEY-WORDS: *Coccinella septempunctata*, Coleoptera, aestivo-hibernation, summer diapause, winter quiescence.

Diapause enables aphidophagous Coccinellidae (Coleoptera) to endure prolonged starvation during seasons when aphids are scarce. The biological importance of this has long been understood, and *Coccinella septempunctata* L. was recognized early as a species displaying an imaginal diapause (Dobrzanski, 1922).

“Aestivo-hibernation” occurs among aphidophagous Coccinellidae in hot, dry temperate and subtropical zones (Hagen, 1962). This term was used to describe the dormancy of the univoltine *C. septempunctata* in Turkey, where the beetles spend late summer, fall and winter on the tops of mountains (Bodenheimer, 1943). In Greece, the life cycle of *Exochomus quadripustulatus* L. (Coccinellidae), a scale- and aphid-feeding, non-migratory univoltine species, is characterized by an adult dormancy which consists of three distinct

parts: an obligatory summer diapause period (July, August); a period of suppression of diapause, with shifting of aggregation sites and perhaps some feeding (September, October, November); and a period of winter quiescence, from November-December to late February or early March (Katsoyannos, 1976).

C. septempunctata populations are heterogenous in their tendencies to univoltinism or multivoltinism (Hodek, 1986). In Greece they display a strong tendency to multivoltinism, completing up to 4 overlapping generations in rearings in outdoor cages (Katsoyannos *et al.*, in press). In the field in central Greece, reproductive activity of *C. septempunctata* occurs in spring in the lowlands. In June, massive migrations of adults are observed on the summits of nearby mountains. Aggregations are formed and are continually present on the mountains until the beetles begin emigrating, between March and mid April of the following year. From July until the end of the warm period of the year, *C. septempunctata* are scarce in the lowlands; during the winter months, they are absent altogether (Katsoyannos *et al.*, in press). The following study was conducted in order to obtain information on the development of *C. septempunctata* during April-June and to distinguish the types of dormancy it exhibits in Greece during July-August and December-February.

MATERIALS AND METHODS

Samples of 20-40 *C. septempunctata* adults were collected at regular intervals during 1991 and 1993 (once or twice per month) from two locations: the plain of Kopais (ca. 38° 20' - 38° 30' N.L.), central Greece, where they were found in the spring on maize, cotton, alfalfa and non-cultivated plants; and their aestivo-hibernation sites on the summit of Mount Kitheron (elevation 1 409 m), about 30 km to the southeast of Kopais, where they were found during all the other seasons of the year.

The physiological state of the beetles was checked by dissections in Ringer solution and laboratory examination. Data on the females' ovaries, as well as on the digestive tracts and fat bodies of all the adults were recorded.

Female reproductive activity is indicated by the presence of either vitellinized oocytes in the ovarioles or signs of vitellogenic resorption in the ovaries. In males, since the activity of the follicular tissue in the testes begins at the pupal stage and does not cease in diapausing individuals (Hodek & Landa, 1971), sexual state was not considered in this study as a reliable indicator of the presence or absence of diapause.

The mid-guts of adults recently fed on aphids are distended and packed with aphid parts. In contrast, the mid-guts of beetles in a state of dormancy are reduced to tubes containing a brown fluid (Mc Mullen, 1967) or are empty (Hodek & Cerkasov, 1961). Greatly developed reserves of fat body are another conspicuous feature of diapausing coccinellids (Hodek, 1973).

In 1993-1994, batches of 40 *C. septempunctata* adults were periodically transferred (once or twice per month) from their dormancy sites on Mount Kitheron to the Kifissia laboratory, under 25° ± 1°C constant temperature, 60 ± 10% R.H. and 16 hrs light per 24 hrs conditions. They were reared in male-female pairs in cylindrical plastic vials (5.5 cm in diameter; 6.5 cm in length) and fed on an abundance of either *Aphis fabae* Scopoli (Homoptera: Aphididae) reared on *Vicia faba* L. (Leguminosae) seedlings or *Dysaphis crataegi* (Kaltenbach) (Homoptera: Aphididae) reared on fruits of *Cucurbita maxima* Duch. (Cucurbitaceae). The duration of preoviposition period of each female was measured and recorded.

RESULTS

ACTIVITY OF ADULTS AT THE LOWLAND PLAIN OF KOPAIS

In fig. 1, dissection results of *C. septempunctata* adults collected from Kopais are presented. During April and May, in both years of the study, the great majority of *C. septempunctata* females were reproductively mature and active; by June and July, however, the great majority of females were sexually immature (fig. 1a). Alimentary activity during the same period was indicated by the presence of food or fluid in the mid-guts of many of the adults dissected (fig. 1B). The reserves of fat body of different levels showed greater difference between the years 1991 and 1993 than between spring and summer in each of these two years (fig. 1C).

AESTIVO-HIBERNATION OF ADULTS AT THE SUMMIT OF MOUNT KITHERON

The summer diapause

Fig. 2 presents dissection results of *C. septempunctata* adults collected from the summit of Mount Kitheron throughout the year 1990-1991 and during the summer-months of 1992 and 1993. As shown in fig. 2A, 100% of the females sampled at their aestivo-hibernation sites in July and August 1991, 1992 and 1993 had immature ovaries, with ovarioles consisting of undifferentiated germaria only. No signs of the resorption of vitellogenic oocytes were ever found during July and August. The reduction of alimentary activity during these months is shown (fig. 2B) by the empty or fluid-filled midguts of all the adults. Almost, all adults dissected during this period had abundant reserves in the fat body. The samples from February through April 1991 indicate a lower amount of reserves in the later part of dormancy (fig. 2C).

The preoviposition periods of the females in batches of adults collected from the summit of Mount Kitheron and transferred to the laboratory are presented in fig. 3. A long preoviposition period during the summer-months (fig. 3A, B, C, D), progressively decreases in autumn (fig. 3F, G, H, I). This is indicated also by the median, minimal and maximal values for females in this experiment, presented in table 1.

The winter quiescence

As shown in fig. 2A, the great majority of females sampled at their hibernation quarters between December 6, 1990 and February 27, 1991 had immature ovaries, with ovarioles consisting of undifferentiated germaria only. In the December 1990 sample, ovarian activity was noticed in 3.3% of the dissected females, in the form of resorption of vitellogenic oocytes. At the end of the winter period, ovarian activity of this type was noticed again, in 8.3% of females collected on March 20 and March 27, 1991. Resorption of vitellogenic oocytes in 8.3% of the females collected, noticed as early as September 19, 1991, following their summer diapause.

During the winter dormancy period, the mid-guts (fig. 2B) of all dissected adults were either empty or fluid-filled. Also, in the adults collected between December 6, 1990 and February 27, 1991 (fig. 2C), a decrease in the proportion of individuals with abundant reserves of fat body was observed.

As fig. 3J, K and table 1 show, the duration of preoviposition period of females collected from the summit of Mount Kitheron and transferred to the laboratory in December and January was about four times shorter than in July and early August.

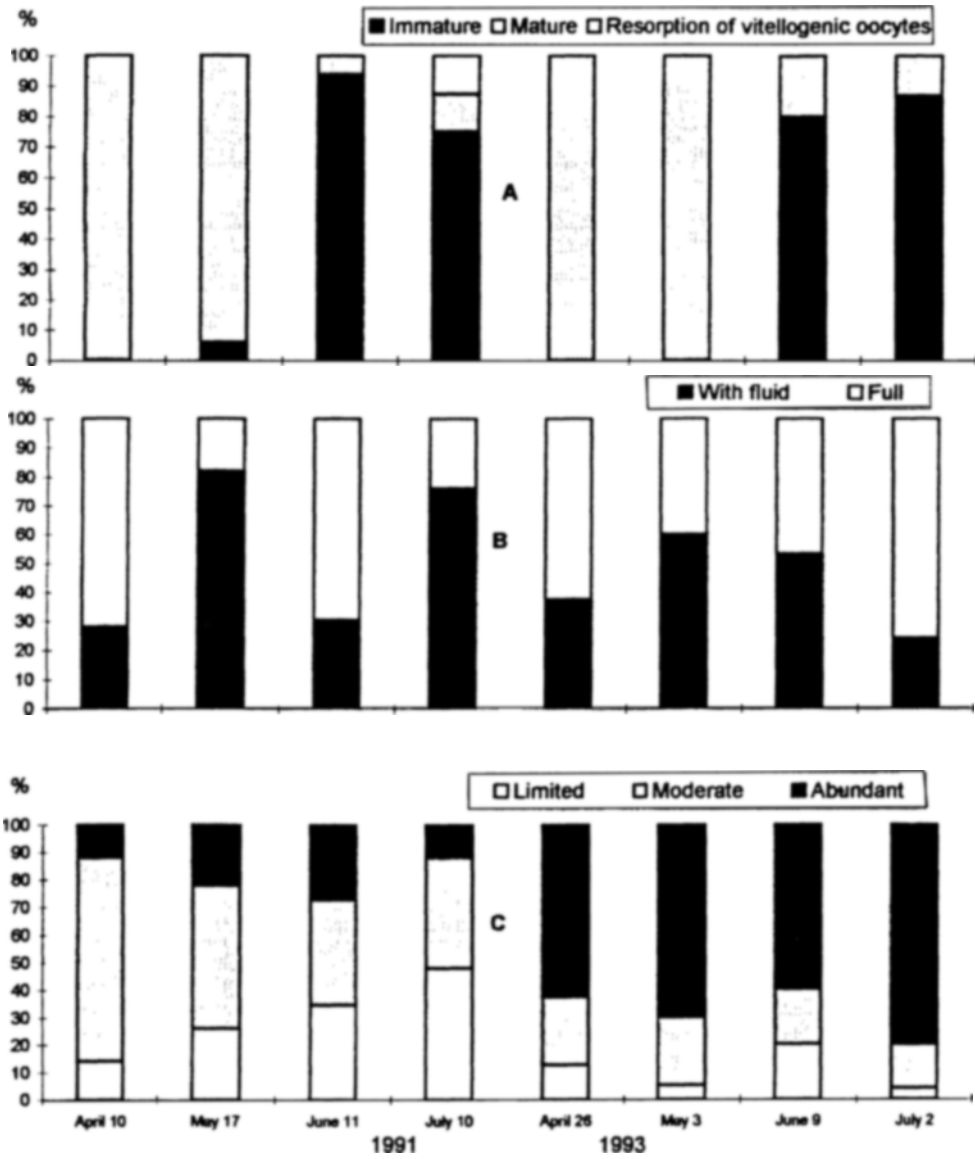


Fig. 1. Dissections results of *Coccinella septempunctata* adults collected from the plain of Kopais, mid spring - mid summer, 1991 and 1993. A: Maturity of ovaries. B: Status of mid-guts. C: Fat body reserves.

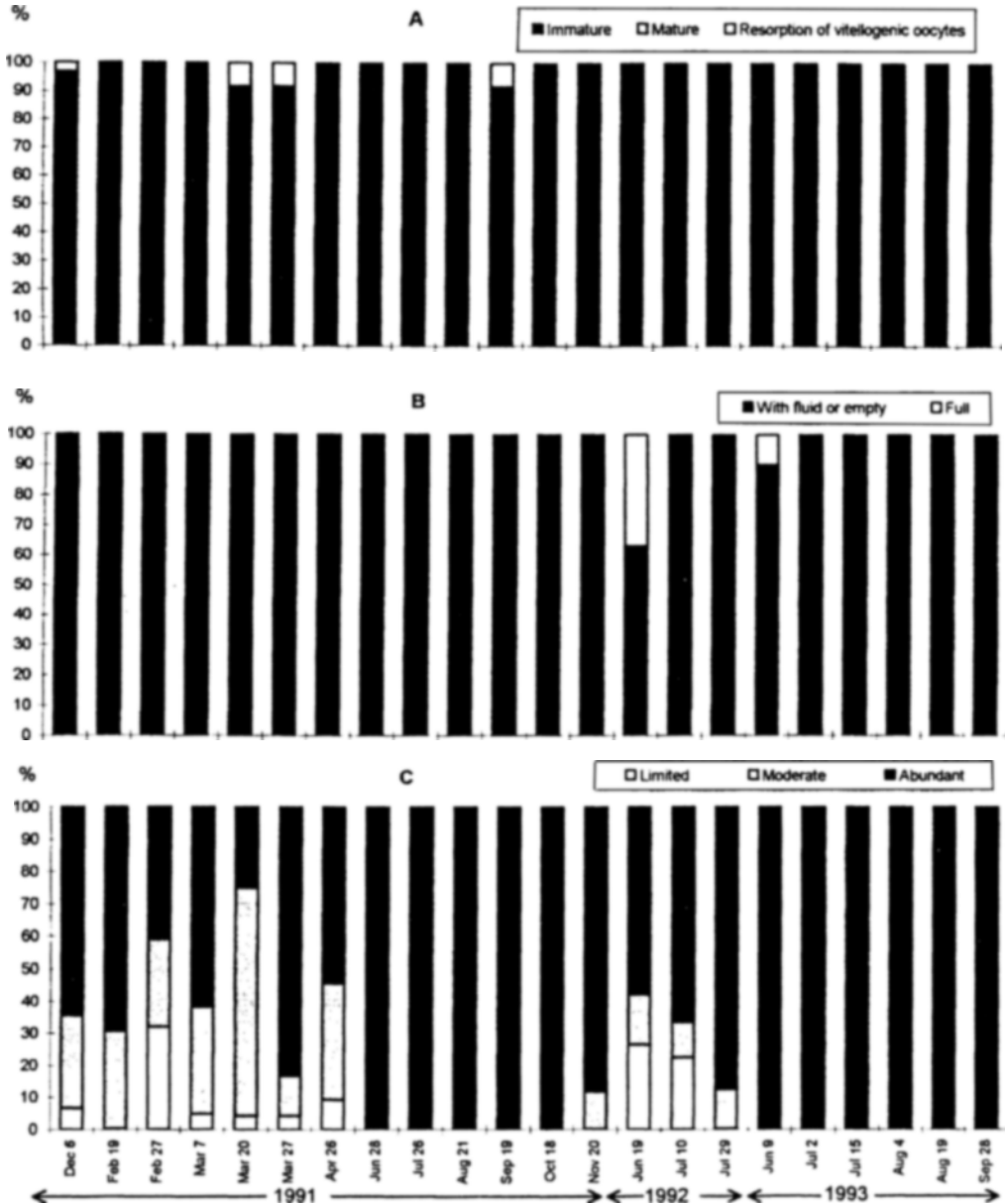


Fig. 2. Dissections results of *Coccinella septempunctata* adults collected from the summit of Mount Kitheron throughout the year 1990-1991 and during the summer months of 1992 and 1993. A: Maturity of ovaries. B: Status of mid-guts. C: Fat body reserves.

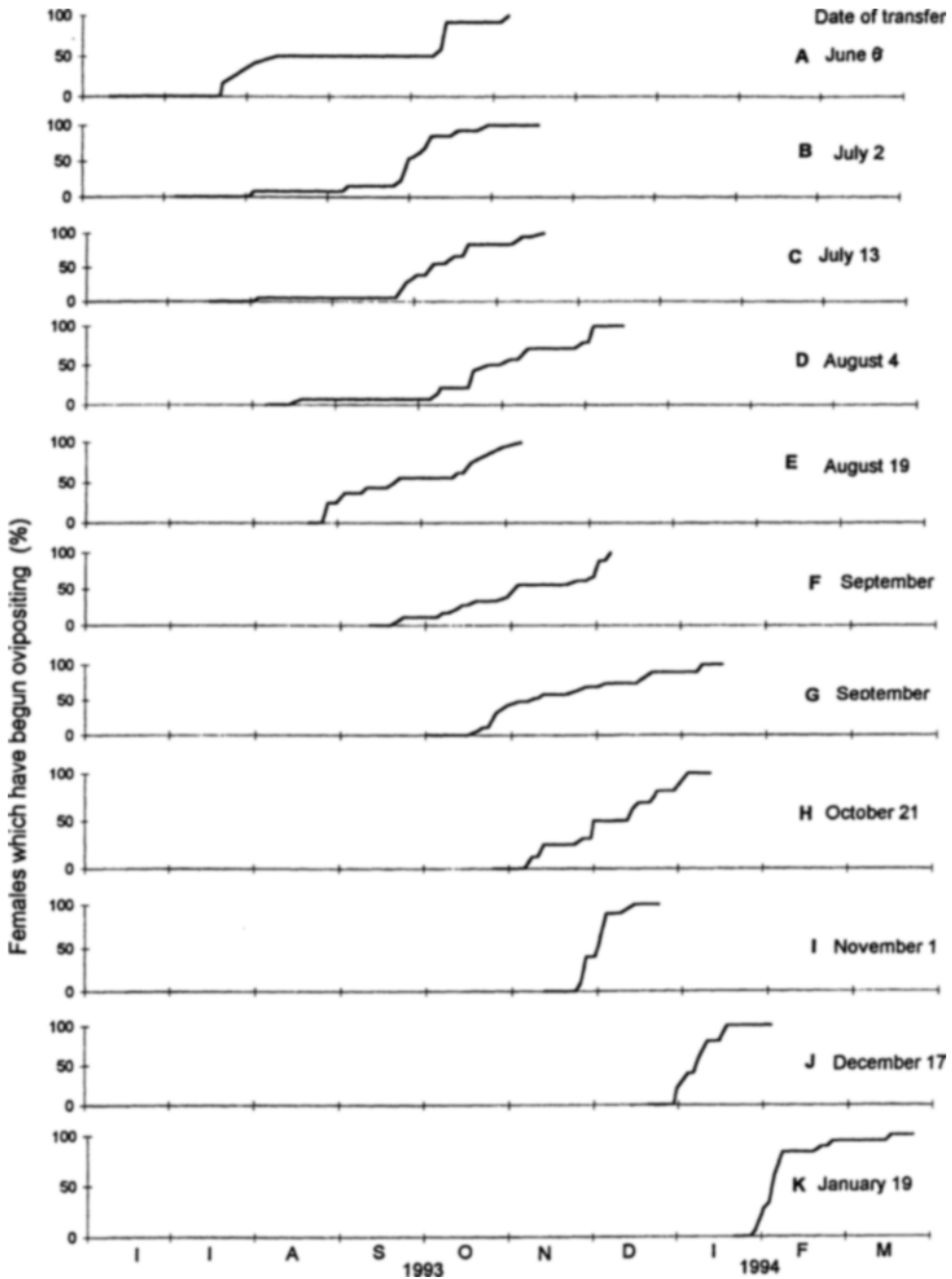


Fig. 3. Preoviposition periods of females in batches of *Coccinella septempunctata* collected from the summit of Mount Kitheron (1993-1994) and transferred to 25°C temperature, 16 hrs light/24 hrs and presence of aphids in the laboratory, during their aestivo-hibernation.

TABLE 1

Median, minimal and maximal values, for the duration of preoviposition period of females of *Coccinella septempunctata* collected from the summit of Mount Kitheron (1993-1994) and transferred to 25°C temperature, 16 hrs light 24 hrs, and presence of aphids in the laboratory, during their aestivo-hibernation.

Collection (date)	Females collected (No)	Duration of preoviposition (No of days)				
		Median	Mean	SD	Minimum	Maximum
9/V1, 1993	20	63	91	41	43	149
2/VII, 1993	20	90	89	21	32	138
13/VII, 1993	20	86	88	23	21	127
4/VIII, 1993	20	82	87	29	14	132
19/VIII, 1993	20	32	38	26	8	78
9/IX, 1993	20	54	57	26	11	87
30/IX, 1993	20	30	49	27	18	106
21/X, 1993	20	40	47	20	18	82
1/XI, 1993	20	29	30	6	23	51
17/XII, 1993	20	21	21	7	13	44
19/I, 1994	20	16	20	12	9	66

DISCUSSION

The summer dormancy of *C. septempunctata* in Greece is a long day — and high temperature — induced diapause. Supporting this conclusion are the dissection results (fig. 2) showing reproductive immaturity in all females, cessation of alimentary activity and the presence of abundant fat body reserves in the great majority of adults during the summer months. Also, the duration of the preoviposition period of females collected from the same location and transferred to the laboratory (fig. 3; table 1) was much longer in the summer than in the winter months.

Further evidence is provided: 1) by the marked tendency of *C. septempunctata* adults to form aggregations during the period between late July and early September noticed in 1st, 2nd and 3rd generation (fig. 3) adults reared together in 1990 in plexiglass cages outside the Kifissia, Athens laboratory; and 2) by the long duration of preoviposition periods noticed during July and August 1994 in portions of 1st, 2nd and 3rd generation *C. septempunctata* females reared in male-female pairs in vials outside the laboratory (Katsoyannos *et al.*, in press).

The winter dormancy of *C. septempunctata* in central Greece is of the type of 91 quiescence. The durations of preoviposition periods in winter are less than one-quarter of the durations of equivalent periods noticed in females transferred during the summer diapause. Similar preoviposition results were obtained in France after transferring *C. septempunctata* adults from two areas of the Alps (near Digne) into optimal breeding conditions of 25°C constant temperature and 18 hrs light per 24 hrs (Hodek *et al.*, 1977). Also, the presence of ovarian activity in the form of resorption of vitellogenic oocytes in some of the dissected females and the decrease in proportion of dissected adults with abundant reserves of fat body indicate quiescence.

Additional evidence to support this conclusion is provided by Hodek *et al.* (1989), who found that *C. septempunctata* adults collected in November in northern Greece appeared

non-responsive to photoperiod; their metabolic rate increased considerably after they were transferred from outdoors to laboratory short-day conditions, suggesting that their photoperiodic sensitivity had already been lost.

RÉSUMÉ

Diapause estivale et quiescence hivernale de *Coccinella septempunctata* (Col. Coccinellidae) en Grèce

Coccinella septempunctata est une espèce multivoltine en Grèce. Le développement actif de tous les stades de développement se déroule au cours du printemps dans les régions basses. Au cours du reste de l'année, les adultes en grande majorité se trouvent en estivo-hivernation aux sommets des montagnes. L'estivo-hivernation des adultes de *C. septempunctata* provenant du sommet du Mont Kitheron en Grèce centrale passe, successivement, d'une période de diapause, au cours des mois de juillet et août, qui se termine progressivement entre la fin août et la fin octobre, à une période de quiescence hivernale, à partir du mois de novembre jusqu'au mois de mars de l'année suivante.

Au cours de la diapause estivale, l'arrêt du développement ovarien a été mis en évidence à travers l'immaturation des ovaires de toutes les femelles et par l'absence complète de signes de régression. De plus, les adultes ont été trouvés avec beaucoup de réserves de graisses et la période de préoviposition pour 50 % des femelles transférées dans des conditions favorables a été de 90 jours au début de juillet et de 82 jours au début août. Pendant la période de quiescence hivernale, l'arrêt du développement ovarien a été caractérisé dans la plupart des échantillons, par l'immaturation des ovaires de toutes les femelles et dans quelques échantillons, par la présence de signes de régression chez quelques femelles. En hiver, les adultes ont été trouvés avec des réserves de graisses en quantités différentes et la période de préoviposition moyenne des femelles transférées dans des conditions favorables a été de 29 jours au début novembre et de 16 jours à la mi-janvier.

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