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ECOPHYSIOLOGY OF THE REPRODUCTIVE ACTIVITY OF Adalia bipunctata L. (COL., COCCINELLIDAE)

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Abstract

The reproductive strategy of Adalia bipunctata L. in Belgium is established by field and laboratory observations and by complementary histophysiological studies. A. bipunctata is a multivoltine species having 3 to 4 generations through late spring and summer. In autumn, adults undergo reproductive diapause until next spring season. At this time, adults emerge from hibernation sites and reproduction is restored. The seminal vesicles of male adults entering in diapause are filled up with sperm but spermatogenesis is stopped. Only a small number of prospermatogonia remain in the testicles. The oogenesis of adult females is going on but the vitellogenesis does not take place, the growing oocytes being resorbed in the ovariole. In the laboratory, after a diapause of 2 months, the adults still stay in quiescence if the hivernal conditions are maintained. But, if the postdiapause adults are reared in spring conditions (20° ; 18 L/6 D), the reproduction takes place immediately. The " hivernal"sperm is able to fertilize females which lay eggs rapidly . If favorable conditions persists, spermatogenesis and ovarian maturity are fully resumed. The ability of Adalia bipunctata to give quickly a new off-spring at the emergence coupled with an intense gonadic activity are valuable quality to control aphids.

Introduction

The Coccinellid beetle Adalia bipunctata has a wide distribution range throughout the Holarctic regions. It is counted as depending upon aphids of trees or bushy vegetations. Moreover, this ladybird was recorded as being the most abundant one in orchards which seems to be it's typical habitat (IPERTI, 1965; HODEK, 1973; HONEK & REJMANEK ,1982; RADMAN & LOVELEI, 1982 ; BRAKEFIELD ,1984). However, A. bipunctata become very common on herbaceous vegetations in some countries. Nettles patchs bean and cereal fields are typical habitats for this ladybird in Great-Britain (BANKS, 1955 ; DEAN, 1982) .The life cycle of Adalia bipunctata in Belgium is closely related to the one observed in Great-Britain. At the end of september, the ladybirds migrate towards their dormancy sites where they will stay untill the end of april. They hibernate in small size aggregations in bark crevices of tree rows, in window-frames or behind anything on house frontages .All the sites choosen are generally oriented to the South or the South-east (HEMPTINNE, 1985). In the beginning of may, just after the emergence, they visit bushy vegetations (willows, hazeltrees, ..) or orchards where they are feeding upon pollen and microarthropods lyke Psylla mali Först (Hom.

Table I : Sampling of A.bipunctata for histological studies

Month	Nb of Adalia collected	Origin of Adalia
August	10	Broad bean fields
September	10	Broad bean fields
November	6	Broad bean fields
January	30	Dormancy sites
February	30	Dormancy sites
March	30	Dormancy sites
April	29	Dormancy sites
May	19	Orchards
June	11	Orchards

For an easier comprehension, we characterize the ovary by the development stage of the oocytes in the ovarioles and we use a numeric scale ranging from 1 to 5 (fig. 1) :

1 : differenciated oocyte
2 : growing oocyte
3 : full-grown oocyte
4 : vitellogenesis
5 : chorionated oocyte in the lateral oviduct

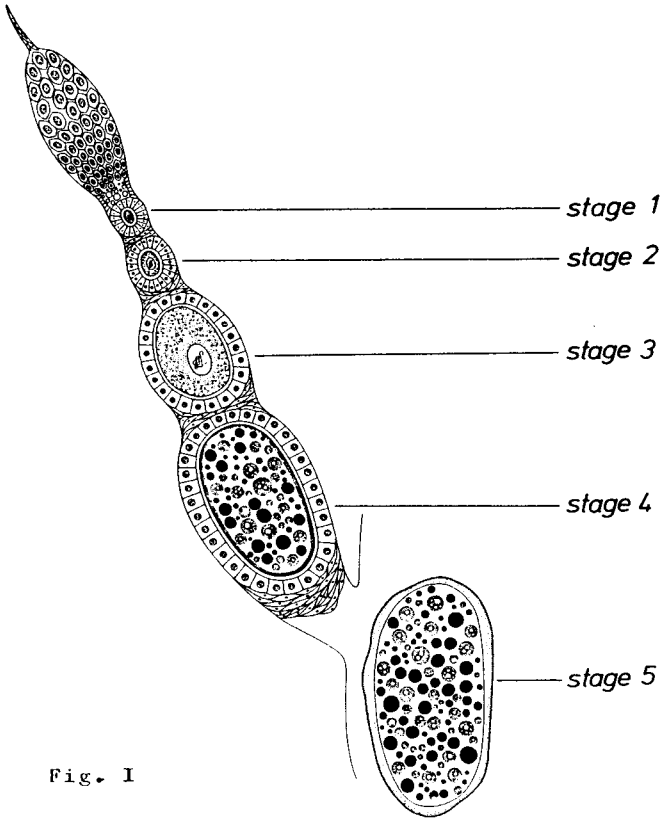

Fig. I

Psyllidae).Afterward, they are found in herbaceous vegetations, nettles, wheat, maize fields and finally several kinds of wild plants (Heracleum sphondylium L., Tanasetum vulgare L.).
This paper deals with histological observations of gonads realized to improve the knowledge of Adalia bipunctata ecology.

Material and methods

Histological methods.
After the elytra and legs were cut, the beetles were fixed in Bouin-Hollande fluid for 24 hours and then embedded in paraffin wax.Serial sections of 7 m were made and stained with the trichrome stain (Iron hematoxylin of Heidenhain, phloxine,light-green).
The sampling method of the ladybirds used for histology are described in Table 1. However, additional informations are needed for the ladybirds collected in autumn. On the 2 august 1985 ,they were collected as nymphs in broad beans (Vicia faba L.) fields and put outdoor in a cage under natural conditions of temperature and light. After the emergence, adults were fed with an excess of Aphis fabae .

Results

Histophysiological observations (table 2 and 3).

In autumn, the adults from the cage only laid few eggs that never hatched. Moreover,ladybird's activity decreased from the end of august to late september when aggregative behaviour took place night and day. At this period, most of the ovaries are in stage 1 or 2 (fig.1). In fact,ovaries show an unceasing activity but oocytes are resorbed at the beginning of stage 2 (Pl.1A)(43% of stage 2 oocytes are degenerative one). The spermatheca of all the females are empty.
The seminal vesicles of the males are always filled up with sperm and of great volume.Complete spermatogenesis takes place in all the testicles.(Pl. 1B).However it begins to end in september. At this time, we observe the degeneration of all the differentiated spermatogonia and the follicular invasion by testicular fluid(Pl. IC). The situation is quite stable in january and february.
The ovaries look like those prepared in autumn.In the males, the spermatogenesis is now completely stopped. The testicles are very characteristic with,at the distal top, a cluster of prospermatogonia and ,at the proximal part, some remaining abnormal spermatozoa (Pl.ID). In march,the frequency of stage 2 oocytes begins to increase while spermatogonial mitotic activity starts to recover. However, these gonadic changes become more obvious in april (chi-squared independent tests have shown,table 2).At this time, neither vitellogenesis nor spermiogenesis are observed.(Pl.IE). As the number of filled up spermatheca rise in march and april,it looks as thought mating can occur in dormancy sites before dispersal. The fitness of the sperm from "hibernating" males to fertilize females is discussed below.
The spermatogenesis and oogenesis of ladybirds caught in may in the orchards are fully resumed. In our samples, 64 % of the females show vitellogenesis stage and some of them have already laid eggs although these first laying is not very large (Pl.IF-G). Histological preparations indicate that many vitellogenesis are abnormal and abortive (Pl. IH). As others still unpublished data suggest it, this is probably in relation with alternative foods the ladybirds only find. Although the first generation is not very important and difficult to observe in the nature, it will migrate with overwintering adults towards herbaceous vegetations where 2 or 3 additive generations will develop during the summer.

Table 2 : Gonad evolution of Adalia bipunctata females

	Ovary (*)					Spermatheca		Nb observ.
Month	1	2	3	4	5	Empty	Filled	
August	1	4	0	0	0	5	0	5
September	3	2	1	0	0	6	0	6
November	4	1	0	0	0	5	0	5
January	11	6 [a b c]	0	0	0	16	1	17
February	8	6	0	0	0	14	0	14
March	4	15	0	0	0	17	2	19
April	2	12	0	0	0	9	5	14
May	0	0	2	2	7	0	11	11
June	0	3	1	0	0	0	4	4

(*) : 1,2,3,4,5 : oocyte stage
Chi-squared independent tests : a: $\chi^2_{obs} = 0,19$
b: $\chi^2_{obs} = 7,85$ ($P < 0,01$)
c: $\chi^2_{obs} = 12,82$ ($P < 0,001$)

Table 3 : Gonad evolution of Adalia bipunctata males

	Testicle			Seminal vesicles		Nb observ.
Month	1	2	3	Empty	Filled	
August	0	0	5	1	4	5
September	0	0	4	0	4	4
November	0	0	1	0	1	1
January	13	0	0	0	13	13
February	16	0	0	0	16	16
March	10	1	0	0	11	11
April	2	13	0	0	15	15
May	0	0	8	0	8	8
June	0	0	7	0	7	7

1: inactive prospermatogonia
2: recovering of mitotic activity in spermatogonia
3: completely resumed spermatogenesis

Table 4 : Male ability to fertilize females with "winter sperm"

Mating period / Diet	2days			3 days			4 days		
	A	Po	St	A	Po	St	A	Po	St
Egg laying	++	-+	--	-+	++	--	++	++	--
Fecundation	++	--	--	+-	+-	-o	++	++	--
Egg hatching	++	--	--	--	+-	-o	++	++	--

A :aphids Po : pollen St : starvation
+ : yes ; - : no ; o : death

Sperm quality at the end of hibernation (table 4)

At the time of mating in dormancy sites,it is expected females would be fertilized by the sperm produced before dormancy. This hypothesis was checked by the following experiment.
Males collected in March in dormancy sites were mated with 6 days old virgin females from the laboratory to test the fertilization ability of "winter" sperm. Before the beginning of the experiment, lonely females were fed with an excess of Acyrthosiphum pisum. Males and females were kept together at 25°C and 18H of light with aphids or pollen or without any food during 2 or 3 or 4 days. Two replications were made for each treatment. Then the males are removed and prepared for histology while egg laying and fertility are observed during 10 days. Females which do not laid fertile eggs were dissected to check the contents of the spermatheca.

Observations of the behaviour of the couples of ladybirds show that in all cases copulation occurs. But ,as pointed in table 4, egg laying and hatching only occur when the ladybirds are fed whatever the lenght of mating period. In those treatment(table 4), the negative results are due to the lack of female to develop the ovaries even if they are fertilized.
Histological observations indicate an intense reactivation of the testicles (Pl.Ii). Gonadic activity though being yet observed in starved males,is more consistent in the fed ones with pollen advantage over aphids. We do not observe spermiogenesis in any case. Thus it is obvious that only the sperm produced before dormancy is able to fertilize females at the end of hibernation.

Discussion

Gonad observation by the way of dissections or "in toto" preparations is a priceless source of information to understand Insect life cycle.This approach has been used,for example, in the case of Semiadalia undecimnotata (HODEK & LANDA, 1971; ROLLEY, 1972), A.bipunctata , Coccinella septempunctata and Propylea quatuordecim-punctata (EL HARIRI , 1966). However, serial histophysiological observations are more accurate and give a more dynamic picture of ecophysiology than the former technics do. In Belgium, the ovaries of hibernating A.bipunctata show an unceasing activity although all the oocytes are resorbed at the beginning of growing stage. EL HARIRI (1966) thought they remained dormant. On the other hand, testicles are completely inactive while the seminal vesicles are filled up with spermatozoa of preceding spermatogenesis.
Mating behaviour occurs before leaving the dormancy sites just as it was observed in Great-Britain (BANKS, 1955). We demonstrate the fertility of "winter " sperm.
In spring, A.bipunctata can be quickly reactivated as soon as favorable environmental conditions are gathered. At this period of the year,alternative food,specially pollen, are very important not only to survive but also to produce the first off-spring.Moreover,in the sperm quality experiment,spermatogenesis seems more developed in males fed with pollen than in those fed with aphids. This fact is interesting for the males can rapidly fertilize several females before they migrate to oviposition sites.
Moreover the females are able to develop eggs with alternative food and give rise to a new generation which could fly to cereal fields when aphids are present.Our observations do not agreed with those of HAGEN (1962) and HODEK (1973) for whom alternative food contributes only to build-up reserves, but they corroborate the results of SMITH(1960) on Coleomegilla lengi which can normally lay eggs on pollen.

The ability of Adalia bipunctata to give quickly a new off-spring at the emergence coupled with an intense gonadic activity are valuable quality to control aphids. This can be confirmed with our field's data where we observe the efficacity of Adalia in Belgium

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Planche I - Legends

A : Ovary from an autumnal female.
In the vitellarium stage 2 degenerating oocyte.
ooc. : oocyte ; ov. : ovariole

B : Testicle of an autumnal male.
In the follicles all the stages of spermatogenesis are present.The seminal vesicle is filled up with sperm.
f. : follicle ; sg.p. : prospermatogonia; spz. : spermatozoa
v.s. : seminal vesicle

C : Testicle of a young hibernating male.
The differentiated germ cells degenerate; only the prospermatogonia are intact at the apex of the follicles which begin to be filled up with follicular fluid.
f. : follicle; fl. : follicular fluid; sg.p. : prospermatogonia; spz. : spermatozoa; v.s. : seminal vesicle

D : Testicle of a hibernating male.
The follicles are empty to the exclusion of prospermatogonia but the seminal vesicles are filledup with sperm.
f. : follicle; fl. : follicular fluid; sg.p. :prospermatogonia; v.s. : seminal vesicle

E : Ovary of a female in spring (april).
The oocytes begin to differentiate. One can see three oocytes in the vitellarium.
ooc. : oocyte; ov. : ovariole; ovd. : oviduct

F : Ovary of a female nourished with pollen which lays eggs.
One can observe all the developing stages of oocytes.
ooc. 5 : chorionated oocyte in the lateral oviduct (ovd.)

G : Magnification of the ovariole seen in F .
g. :germarium; ooc. 3 : full-grown oocyte; ooc. 4 : oocyte with vitellus

H : Ovary of a female nourished with pollen.
Some oocytes are degenerated.
3d. : full-grown oocyte degenerated ; 4d. : oocyte with vitellus begins to degenerate.

I : Testicle of an emerging male.
The spermatogenesis is reinitiated but no sperm is still formed. The sperm in the seminal vesicle is "winter "sperm.
c. :calice; f. :follicle; sp. :spermiduct; spc. :spermatocytes; v.s. : seminal vesicle


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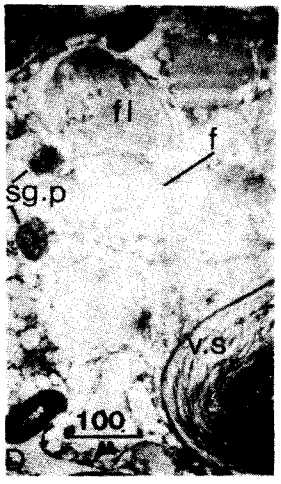
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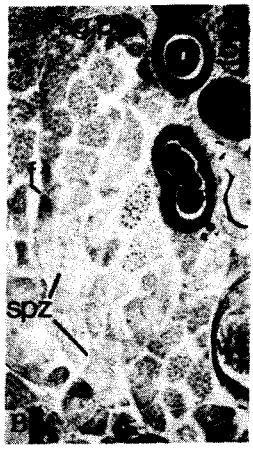
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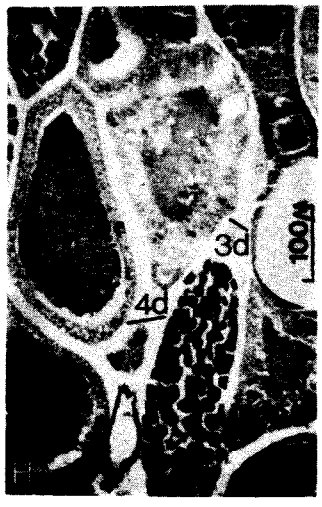
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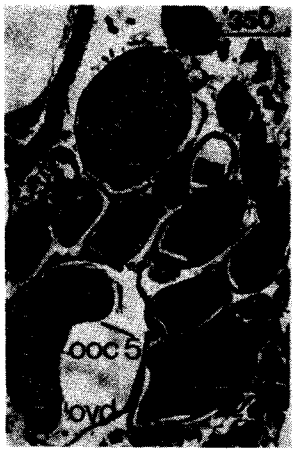
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