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Full Length Research Paper

Bioecology of broad bean bruchid *Bruchus rufimanus* Boh. (Coleoptera: Bruchidae) in a region of Kabylia in Algeria

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In this study, the conditions of colonisation of the broad bean *Vicia faba* L. by the adults of the Coleoptera Bruchidae *Bruchus rufimanus* Boh. were analysed in the region of Kabylia in Algeria. *B. rufimanus* adults began to colonise the *V. faba* cultures in February after termination of a larval and a reproductive diapause. Males appeared in February and had terminated their reproductive diapause. Females began to colonise the broad bean culture in March; they terminated their reproductive diapause after consumption of nectar and pollen of the host- plant flowers. The adult density depended on the abundance of the trophic resources at the beginning of adult colonisation phase. The females oviposited on the green pods as soon as they appeared on the plants and laid on these pods as long as they did not became mature. The first and the secondary larval instars developed in maturing seeds in the green pods. The last larval instars and the pupae developed in dry seeds after harvesting and storage in granaries. A high inter- individual variability in the duration of the post-embryonic develop-ment was observed in this study. The adaptive signification of this developmental heterogeneity was analysed in this study.

Key words: *Vicia faba*, *Bruchus rufimanus*, host plant colonisation, life cycle, larval and reproductive diapause, post-embryonic development.

INTRODUCTION

The broad bean *Bruchus rufimanus* BOH is a univoltine species developing in the seeds of *Vicia faba* L. The adults reproduce in the fields and the females lay eggs on the green pods of its host plant. The insects are in reproductive or in larval diapause when the host plant pods were not available. This bruchid cannot reproduce and cannot develop in the seed storage systems. Its boil-biological cycle differs from that of the polyvoltine bruchids *Acanthoscelides obtectus* or *Callosobruchus maculates* which are able to colonize a seed storage system; the successive generations causing high losses (Hoffmann et al., 1962). The *B. rufimanus* diapausing adults reach wintering sites after the broad bean fructification period and survive in protected sites, e.g. under bark of trees, in lichens or in the soil cavities (Speyer, 1951; Middlekauff, 1951; Huignard et al., 1990). Increasing temperature and changes in photoperiod in the beginning of spring favor the migration of adults from the wintering sites to the *V. faba* culture (Dupont and Huignard, 1990). A part of the *B. rufimanus* population survive dur-

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during winter in larval or pupal diapause inside the *V. faba* seeds. They terminate their diapause, finish their post-embryonic growth and emerge from the seeds when the seeds are sowed. The increase of the soil temperature and humidity are the main factors favoring the diapause termination (Franssen, 1956; Boughdad, 1996). The date apparition of first adults in bean's fields depends not only on climatic conditions but also on the host-plant development. Females may have obligatory alimentation period before oviposition. They feed on nectar produced by host plant or advertise extra-floral nectar glands and pollen of flowers (Chakir, 1998). According to Speyer (1951), *B. rufimanus* adults' alimentation is not necessary to eggs maturation. However Tran and Huignard, (1993) showed that pollen of *V. faba i*s not only a trophic supply but also a set of specific informations allowing reproductive diapause termination and gonad maturation. The importance of pollen consumption was also demonstrated in *Bruchus pisorum* (Pajni and Sood, 1975; Pescho and Van Houten, 1982) . Females become sexually mature when the first green pods appeared on the host plant and oviposit on the surface of these pods after a long exploration phase. The survival chances of the larvae depend on the egg location at the surface of the pods; the eggs, which are deposited on the pericarp recovering maturing seeds, have more chance to survive (Boughdad, 1994). In this study, the conditions of colonization of the broad bean cultures by the adults of *B. rufimanus* are analysed in the Kabylia region in Algeria and the reproduction and development of this beetles are followed.

MATERIALS AND METHODS

The study was realized in the broad bean culture zone at the east of the city of Tizi Ouzou in the Kabylia region (Algeria) at 36°42' NL. The host plant phenology, the *B. rufimanus* adult density and the number of eggs laid on pods were followed from 8 December 2003 to 30 may 2004 in a broad bean culture of a local winter bean variety. The time intervals between to observation phases were 4 days during the flowering period (in relation with the rapid appearance of inflorescences on the stem of *V. faba*) and 7 days during fructification phases. The observations were made in a plot (area; 100m^2, slop 20%) located in a culture of a traditional winter variety of *V. faba* L. The seeds were collected in 2002 and were stored in the absence of chemical treatment. They were sowed in December 8th 2003. The flowering and fructification phases were followed on one stem of 10 sampled plants every 7 days from February 22nd to April 25th 2003. Observations are done on the high part of the stem with young leaves and inflorescences and on the low part of the stem with different pod steps. The temporal variations of the number of inflorescences and of the length of the pods (in cm) were estimated at the two levels of the sampled stems.

B. rufimanus adult density

Early in the morning when temperatures were lower than 15°C, the adults were captured in the flowers, leaves and between leave cornets every 4 days. The insects did not present any locomotive activity when temperatures were lower than 15°C on the plants and were easily captured (Franssen, 1956). Adults are researched manually. The sex of the captured insects was determined and some of them are dissected under binocular microscope, in order to estimate the different development states of their reproductive organs as defined by Tran and Huignard (1993). In sexually active males, the accessory glands presented an important secretory activity and their lumen contained abundant secretions. Spermatozoa gathered in spermatodesms were present at the base of the testis and in the seminal vesicles. In the diapausing males, the lumen of the accessory glands was empty, the testis and the seminal vesicles contained a low number of spermatodesms. In the sexually active females, the different ovogenesis phases were observed in the ovarioles and mature oocytes were present in their vitellarium. In the diapausing females, the ovarioles were reduced to their germarium.

Number of eggs deposited on sampled pods

Young green pods were sampled on the stems 7th and 29th March. These pods were observed each week during their maturation phase and the number of new eggs deposited on the pods between two observation phases was determined. The young eggs were easily recognized from the old ones because they contained abundant vitellus under the chorion or 1th instar larvae before their penetration in the pod. The older eggs were empty and were reduced to the chorion and contained fragments of pericarp crushed by the larvae during the penetration phase.

Post-embryonic development

Ten pods are collected each two weeks from the beginning of the egg-laying phase on green pods to the pod maturation phase. The mature pods were harvested and shelled in order to follow the bruchid development. The seeds were opened under binocular microscope with a scalpel; the number of insects present within the seeds and the state of their development (different larval instars, pupae or adults) were estimated. The number of emerging adults from stored seeds was also determined.

RESULTS

V. faba flowering and fructification phases

The broad bean stems began to produce flowers at the end of February with a limited number of inflorescences on the stems. The mean number of inflorescences per stem increased progressively in March reach a maximal value during the last week of March and decreased in April. There was not much inflorescence on the stems from the 3rd week of April (Table 1). During the flowering period, each inflorescence produced 8 to 12 flowers. Fructification period began in March, the green pods were small (3 cm of length), their length progressively increased (16.5 cm in May). Then, the pods became dry and black when mature. The first mature pods appeared on the stems in May. The last inflorescences appeared at the beginning of April on the high level of the stems and were mature at the end of May or the beginning of June (Table 2).

Table 1. Temporal variations of the number of inflorescences produced by the samples stems (m + sem).

Dates	22/2	29/2	3/3	7/3	11/3	15/3	21/3	25/3	29/3	4/4	8/4	11/4	15/4	19/4	25/4
No inflorescences	0	1	1.8	2.6	3.5	4.3	5.0	6.2	6.9	4.1	2.8	2.0	1.7	0.7	0
(sem)	0	0.6	0.6	0.7	0.7	0.8	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0

Table 2. Temporal variations of length in cm of pods during the fructification periods at two levels of the broad bean stems (m + sem).

Dates	07/3	15/3	21/3	29/3	04/4	11/4	19/4	25/4	02/5	09/5	16/5	23/5
Low level	0	3	6	9	11	12	13.5	14.3	16.3	16.5	16.5	-
	0	0.9	0.9	0.9	1.0	1.0	1.0	0.9	0.9	0.9	0.9	
High level	0	0	0	0	3.2	5.5	7.3	10.5	13.1	14	14.5	14.5
	0	0	0	0	1.0	1.0	0.8	1.0	1.1	1.1	1.1	1.1

Table 3. Temporal evolution of mean number of adults of *B. rufimanus* captured and analysis of their sex-ratio (males number / total number of captured insects).

Dates	8/2	15/2	22/2	29/2	7/3	15/3	21/3	29/3	4/4	11/4	19/4	25/4	2/5	9/5	16/5
Males	0	1	3	5	7	11	12	27	22	19	10	5	4	2	0
Females	0	0	0	0	3	7	15	33	33	27	19	13	10	8	4
Sex-ratio	0	1	1	1	0.7	0.61	0.44	0.45	0.40	0.41	0.34	0.27	0.28	0.20	0

Estimation of *B. rufimanus* adult density

The adults colonized the broad bean culture during the host plant vegetation phase. The first adult was captured in February 15th, when the photoperiod was of 11: 13h LD and the mean temperature reached 19°C. During this month, a limited number of males were captured in the broad bean field. The first females were captured in Mar-ch when the first inflorescences appeared on the host-plant. However, the sex ratio remained male-dominant until March 14th. The captured males were in reproductive diapause in February and became sexually active in March (Table 3). The number of captured adults progres-sively reached a maximal value in March when the photo-period was of 12h 30: 11h30 LD. Since March 21st, the sex- ratio was female-dominant. The captured females had terminated their diapause at this period and mature oocytes were observed in the ovarioles and in the lateral oviducts. These females were able to mate with the males in the culture and to lay eggs on the green pods which appeared on the stems. The number of captured adults remained high despite the end of the flowering phase. At this period, the adults were observed on the flowers of wild plant species and consumed the pollen of these plants. The numbers of adults captured in the bro-ad bean culture progressively decreased from April 19th to May 16th. This decrease of the number of adults could be due to death of the bruchids after the reproduction phase or to their migration towards plots where the broad bean varieties were still in flowering phase.

Number of eggs deposited on the pods

The first pods maturing on the plants were progressively pushed out at the base of the stems while the new flowers and then the green pods appeared at the apex of the plant. The bruchid egg-laying activity was followed on two samples of pods appearing on the broad beans 7th March and 29th March (Table 4).

Pods sampled 7th March: 50% of these pods had received a mean number of 2.6 eggs 15th March. During the growth of the pods and their progressive ripening, the percentage of pods bearing eggs and the number of eggs increased over time. The number of eggs reached a ma-ximal value in April 4th and then decreased until 9th May. The pods did not receive eggs from 16th May when they began to dry out and became mature.

Pods sampled 29th March: 80% of these pods received a mean number of 3.2 eggs in April 4th. The number of eggs deposited on the pods between two observation phases remained important during the two following weeks and then decreased. The eggs were deposited on these pods until 16th May.

In April, the mean number of eggs deposited on the two samples of pods did not significantly differ (f=0.21,

Table 4. Temporal evolution of mean number of laid eggs and percentages of pods with eggs on pods sampled on the stems 7th March (A) and on pods sampled 29th March (B).

Date of observations	7/3	15/3	21/3	29/3	4/4	11/4	19/4	25/4	2/5	9/5	16/5	23/5
A: No eggs(m ± sem)	0	2.6	4.8	7.4	8.6	6.3	5.4	4.3	2.5	1.8	0	0
	0	1.2	1.3	1.1	1.1	1.2	1.3	1.9	1.3	1.2	0	0
A: % of pods with eggs	0	50	80	100	100	90	70	60	50	40	0	0
B: No eggs (m±sem)					3.2	7.2	6.2	5.7	4.3	2.7	1.9	0
					1.1	1.0	1,3	1.3	1.4	1.2	1.1	
B: % pods with eggs					80	100	100	80	60	60	50	0

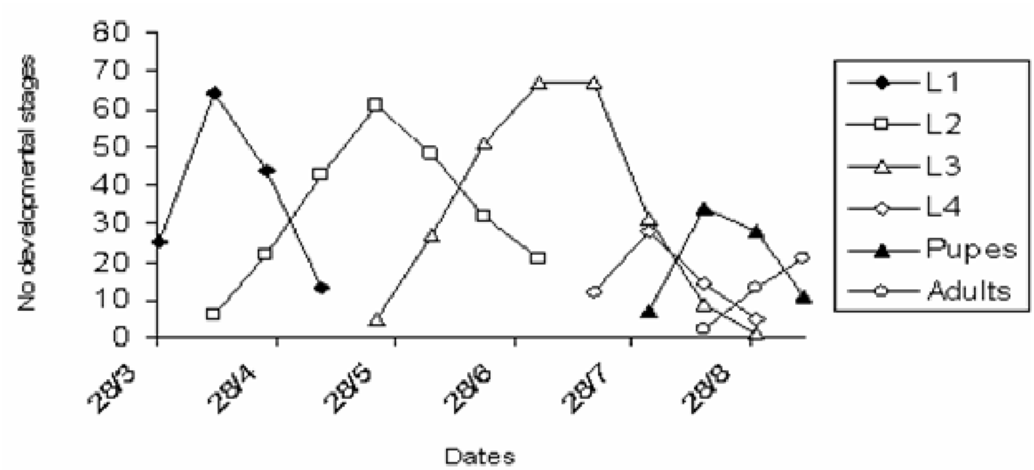

Figure 1. Post-embryonic development *B.rufimanus* inside *V. faba* seeds.Analysis of the number of insects developing in the seeds at each observation date

P=0.66); the females emitted their eggs on the pods regardless of their length and their level on the plants.

The females were captured at the end of the egg-laying period in the broad bean cultures and then dissected. The exhaustion of the reserves in the fat body and the absence of mature oocytes in the ovarioles and the lateral oviducts was one of the main factors explaining the reduction and then the end of the reproductive activity in May.

Post-embryonic development

B. rufimanus larval and pupal development was followed during 7 months from March to September (Figure 1). The eggs deposited on the pods at the end of March gave rise to L_1 larvae after a 10 day embryonic growth. This larval instar was observed within the seeds from March 22nd to May 2nd. L_2 larvae were found in the seeds during 13 weeks from April 11th to May 27th.The first L_3 larvae appeared in the seeds in May 23rd and this larval instar was present during 14 weeks. There was an imp-ortant heterogeneity in the duration of the post-embryonic development at these two larval instars. L_4 were found in the seeds during 8 weeks from July 11th to August 29th and the first pupae appeared in August 1st. Adult's emergence from seeds began since August 13th.

DISCUSSION

B. rufimanus was a univoltine species in the region of Kabylia as in the other mediterranean countries where this species is present. The reproduction of this bruchid was synchronized with the flowering and fructification cycles of its host-plant. A weak number of males started to colonise a crop even if broad bean plants were in a vegetative phase. These *B. rufimanus* adults probably came from hivernation sites like lichens and bark of trees or soil cavities (Larson and Hinman, 1931; Franssen, 1956) or from stored seeds containing larvae or pupae which terminated their diapause and gave rise to adults. When the temperatures became higher than 15°C (Hoffmann et al., 1962), the insects leave the hivernation sites and migrate towards the broad bean cultures. Such insect migrations from wintering sites to food supplies or to the host plants had been observed in ladybird species

(Hagen, 1962; Hodek, 1981) and in other bruchid species like *Bruchus dentipes* (Tahan and Hariri, 1981), *Bruchus pisorum* (Pajni and Sood, 1975; Pesho and Van Houten, 1982), *Bruchidius atrolineatus* (Germain et al., 1985) and *C. maculatus* (Zannou et al., 2003).

A part of *B. rufimanus* males had terminated their diapause before the colonisation of the broad bean cultures as previously observed in the “region Centre” in France (Dupont, 1990; Tran and Huignard, 1993). In this country, a 16:8 h LD photoperiod was required for male diapause termination. In the Kabylia region, the *B. rufimanus* males terminated their diapause when photoperiod was 12:12h LD. This difference in photoperiod sensitivity is probably due to the adaptation of the *B. rufimanus* population of Kabylia to the specific climatic conditions of its ecosystem and to local variations of the photoperiod as in many insect species exhibiting a large geographical area (Danks, 1987). When the males terminated their diapause in absence of external stimulations, Tran and Huignard (1993) supposed that this physiological change could be due to a horotelic process as defined by Hodek (1983) which caused a progressive decrease of the diapause intensity and then the induction of reproductive organ development. For the other part of the male bruchid population and for the females, changes in the photoperiod and presence of the host plant flowers were required for diapause termination. The colonization of the *V. faba* culture by *B. rufimanus* adults depended on the plant flower availability; the pollen consumption allowing the insect nutrition and induced female reproduction. When important trophic supplies were available on a stem, the females could be attracted by these resources, foraged for a long period on this stem and could have higher chances of encounter the pods available and to lay eggs on these pods. A clear relationship between adult density and flower availability was observed at the beginning of our study. However, adults continued to be present in the culture after the end of the flowering phase; the females consuming pollen produced by the other plants of the ecosystem. There was not a clear relationship in our study; between egg-laying activity and pod density; the different levels of pods being equally contaminated by the females regardless of their level and their number. The decrease of the egg- laying activity on the pods over time could be due to two main factors: 1. The changes of the pod texture during their maturation phase. When the females had discovered the pods, they explored the surface of the pericarpe with their antenna and their ovipositor and only oviposited on green pods. They avoided the pods when they began to dry and became black. 2. The end of the *V. faba* flowering period causing an important reduction of the quantities of nectar and pollen. This reduction of the trophic supplies influenced the female reproductive activity. The adults captured at this period, had a limited quantity of reserves in their fat body and their ovarioles did not mature eggs at this period.

The analysis of the post-embryonic development clearly demonstrated that the *B. rufimanus* population presented in the ecosystem of Kabylia two strategies of growth. A part of the population developed without larval diapause and the adults began to emerge from the seeds at the end of August. These adults must survive during the end of autumn and in winter in reproductive diapause. They search for wintering sites such as the barks of the trees, the lichens and soil cavities for their protection against the adverse climatic conditions. The other part of the population present an important slowing of their growth at the second and the third larval instars which correspond to a larval diapause. The growth ended at the end of winter or the beginning of spring and the adults must survive during a short period in the fields before the host-plant flowering. Such heterogeneity of the duration of the development was also observed in *Megastigmus spermotrophus* populations developing in pine seeds (Roux et al., 1997) and *Curculio elephas* populations exploiting chestnuts (Menu and Debouzie, 1993). This developmental strategy with early adult emergences or with late adult emergences after 1 to 2 year- larval diapause allowed the survival of insect populations in an unpredictably changing environment (Menu et al., 2000). In *B. rufimanus*, the adult survival chances in the wintering sites depend on the winter climatic conditions; high humidity or low temperatures probably causing an important mortality. The larval diapause in a relatively protected environment within the seeds increased the insect population survival chances and represented an insurance against adverse climatic conditions. These survival strategies explain the importance of the *B. rufimanus* populations in the *V. faba* cultures in the agrosystems of Kabylia in Algeria and the importance, the seed losses observed every year.

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