Monitoring the activity and biology of pest *Bruchus rufimanus* (Coleoptera: Chrysomelidae) in beans crops

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**ABSTRACT**

The main objective of this study is the Population dynamics evaluation of broad bean bruchid *Bruchus rufimanus* (Coleoptera: Chrysomelidae) in crops beans of *Vicia faba* within the El Asnam’s region (Algeria). A weekly monitoring of *B. rufimanus* was made during breeding diapauses in the laboratory and during their colonization in the parcel of variety of Séville and then a comparative study had been implemented to characterize the total haemolymphatic protein levels during diapause and the periods of reproductive activity. The obtained results demonstrated that after the diapause period, the colonization of the field by adults of *B. rufimanus* coincides with the period of full bloom of the host plant which represents a trophic source necessary for the reproductive activity. 54 beetles were captured from which 28 were males. Oviposition covering a period of one month and 15 days, with a total of 168 eggs being counted, of which 72 were placed on the low layer pods. The total hemolymphatic protein content of *B. rufimanus* was found a high concentration during diapause and varying between 46 to 85.8 μg/μl. High levels of protein were probably due to the stock of proteins during the larval life of this pest. Protein levels had decreased significantly in April, reflecting a recovery in the activity and colonization of bean fields for food, sexual partnership, spawning and other activities. However, throughout the month of May, the protein concentration increased due to the pollen supply provided by the host plant and weeds present in the agrosystem.

**Key words:** Broad bean, *Bruchus rufimanus*, Diapauses, Oviposition, Total protein.

**INTRODUCTION**

Broad bean is an important legume crop used as a major source of dietary protein for subsistence farmers (Tekalign et al., 2016). Due to their symbiotic nitrogen fixation, this crop can reach potential yields without any nitrogen (Sarkar et al., 2017). Generally, farmers revealed strong preferences for varieties with superior quality, resistance to diseases and pests, and also better adaptation to abiotic stresses (Yadav et al., 2017). *Bruchus rufimanus* is a monovoltine species. It causes both quantitative and qualitative damage to seeds (Keszthely et al., 2018). Reproductive activity of *B. rufimanus*, unlike polyvoltine bruchid, is limited to the period of vegetation and fruiting of the bean (Medjdoub et al., 2015). Outside this period, the insect is in a state of imaginal diapause, which is characterized not only by cessation of reproductive activity, but also by a complex of physiological anatomical and biochemical changes. The colonization of cultures by *B. rufimanus* adults is done from overwintering sites such as woods, tree bark, lichens, and soil crevices or from seeds containing live bees at the time of sowing. (Khelfane-Goucem and Medjdoub-Bensaad, 2016). Dupont and Huignard (1990) mentioned that temperature increase is a major factor that allows the disappearance of adults from overwintering sites. The start of reproductive activity for *B. rufimanus* is conditioned to pollen consumption, particularly in females (Tran and Huignard, 1992). Oviposition in bruchids is induced by the setting of the host plant. Females begin to lay eggs as soon as the first pods appear at the first floral stage of the plant, when daytime temperatures are above 15°C (Medjdoub-Bensaad et al., 2007). The concentration of total haemolymphatic proteins of *B. rufimanus* seems to change according the amount of fat; which presents a high concentration of insects in diapause. However, it has lower concentration for adults that become sexually active (Chakir, 1998). The objectives of this study were to establish an effective control and to reduce the population densities of this pest by monitoring the dynamics and biology of its populations during the reproductive activity under the predefined conditions of our agrosystem.

**MATERIALS AND METHODS**

The study was conducted in the region of El Asnam 38°18’44”Nord, 4°04’30” Est at 440m of altitude, during the agricultural campaign 2014/2015. The observations had been made in a plot of faba bean Seville variety; the experimental plot had an area of 100 m². The seeds used in the present study were obtained from previous harvests (2013 harvest). These ones didn’t receive any chemical treatment and often contained beetles. Seeding took place on the 24th of...
of December 2014. The total protein was measured on the haemolymph of diapausing *B. rufimanus* adults removed from the dry seeds harvested the previous year and those in reproductive activity caught in the bean fields. The beetles were sexed and then frozen at -15°C.

**Observations in the field:** The phenology of the plant was followed from the period of the vegetation until the period of the harvest. The phases of flowering and fruiting are followed on 10 stems per plant each week. The capture of *B. rufimanus* adults in the field was usually done in the morning, as the temperature exceeds 15°C, corresponding to the threshold of the imaginary activity temperature of the insect. Beetles were caught manually on all parts of *V. faba* plant on 10 direct-approach stems. The adults of *B. rufimanus* caught in the plot were enumerated and sexed using a binocular microscope.

**IN THE LABORATORY**

**Monitoring the egg-laying activity of *B. rufimanus* females:** The laying period of *B. rufimanus* on *V. faba* pods occurs throughout the fruiting phase of the host plant. On each observation date of the beginning of the spawning at harvest, ten pods formed the first inflorescences, located at the base of the stems (old pods) and ten pods formed late from the last inflorescences located at the top of the stems (young pods), were taken at random on ten bean stems by browsing the entire plot of study. The sampled pods were then brought back to the laboratory for further measured and observation using binocular microscope to count the eggs deposited on their pericarp.

**Haemolymph collection and total protein essay:** Extraction of the haemolymph was used on 10 males and 10 females during the diapause period from October 2014 to March 2015 for the beetles in diapauses obtained from previous harvests and during the months of April and May for the beetles sexually active. The frozen beetles were crushed in a mortar with a pestle in 10ml of methanol; then centrifuged; the mixture was then refrigerated. To determine the concentration of proteins, we had opted to use the Bradford method which was a spectrophotometric analysis using a standard range of bovine serum albumin (BSA). The absorption measurements, at 595 nm, were carried out on a JENWAY 6850 UV/VIS spectrophotometer.

Whether there was a significant difference in pod infestation or not between the two strata of the Seville variety, as well as the total protein ratio according to sex and time, we used the analysis of variance (ANOVA) test at 5% and the test of Newman and Keuls. This analysis was carried out by a statistical software (STAT-BOX) version 6.3.

**RESULTS AND DISCUSSION**

**Temporal estimation of the number of adults of *B. rufimanus* caught in the study plot:** Fig 1 showed that the first beetles were captured on the 1st of April with 13 individuals, at a photoperiod of 10.7h and a mean diurnal temperature of 18.1°C, which coincides with the period of full bloom, which serves as trophic substrate. Atakan (2012) reported that coleopteran insect species were recorded mainly at the flowering stage of plants. Similar results were obtained by Haftom *et al.* (2014) that flowering period had a significant effect on the abundance of insect visitors. The presence of pollen and nectar of *V. faba* inflorescences triggered after certain latency the emergence of reproductive diapause of *B. rufimanus*. This number increases to reach a peak of 16 beetles, noted in April 7th. Titouhi *et al.* (2015) indicated that the peak of adult activity of the bean’s Cole was noted in April, corresponding to the full flowering period of the host plant. The number of beetles decreased to 5 individuals captured on April 28th and only 4 individuals were observed on May 5th. From May 12th, a total absence of beetles was noted in the study plot. A total of 54 adults were counted of which 28 were males.

**Mean time course of eggs laid by female *B. rufimanus* in the study plot:** The egg laying by females of *B. rufimanus* started on April 14th for low layer pods (Fig 2a) with 0.3±0.48 eggs/pods where 2 out of 10 pods were infested, a maximum of 2.5±1.7 eggs/pods deposited on 80% of pods sampled was recorded on May 12th. For young pods, oviposition started one week after that of older pods (Fig 2b) observed on 21st April, when a maximum of 2.6±1.52 eggs/pods were recorded on 90% of the eggs. At the end of May, when the pods mature, their pericarp didn’t receive any more eggs, which probably corresponds to the end of *B. rufimanus* reproductive activity. The monitoring of the laying period showed that the eggs were deposited on old pods and young pods as soon as they were available. The laying activity on *V. faba* pods was spread over a period of one month and 15 days; when we counted a total of 168 eggs, of which 72 were issued on low stratum pods. According to (Medjoudou-Bensaad *et al.*, 2011) in the Tizi-Ouzou’ region the laying activity spread over a period of one month to two and a half months. In the same vein, Franssen (1955) indicated that the duration of spawning was 17 days in 1951 and 4 days in 1954. It spaned the whole fruiting period of pods and it finished with the hardening of the pericarp and the blackening of the latter corresponding to the period of seed collection.

![Fig 1: Adult density of *B. rufimanus* captured by the number of inflorescences in the *Vicia faba* plot.](image-url)
According to the analysis of variance (ANOVA) the level of the stratum didn’t have a significant effect on the preference of the females to lay their eggs ($F=3.24$, $df=1$, $p=0.13$). However, the weekly sampling period had a highly significant effect on $B. rufimanus$ spawning rate ($F=8.54$, $df=5$, $p=0.01$). According to Newman and Keuls the highest number of eggs laid on the pods was marked in the second week. While the lowest number of eggs laid on the pods was observed in the first week.

**Total haemolymphatic proteinemia in $B. rufimanus$ adults in diapause:** the total hemolymphatic protein content of adults of $B. rufimanus$ at emergence in October was $78.5\pm0.16\mu g/\mu l$ and $76.2\pm0.11\mu g/\mu l$, for females and males respectively. These rates increased considerably in February for both sexes, which was around $85.8\pm0.005\mu g/\mu l$ and $94.6\pm0.31\mu g/\mu l$, respectively for females and males. It should be noted a drop in hemolymphatic protein levels in March when the concentration was only $40.8\pm0.09\mu g/\mu l$ and $41.1\pm0.15\mu g/\mu l$ for males and females respectively. This was due perhaps to the beginning of the post-diapause process allowing the preparation of reproduction. Tran (1992) reported this reduction to a change in the metabolism of adults who apparently use proteins and carbohydrates for their survival and for reproduction (Fig 3). The results obtained on the rate of proteins of adults of $B. rufimanus$ showed that these were more important during the six months of diapause for both sexes. These results were agreed with Hamani-Aoudjit and Medjdoub-Bensaad (2015) who suggested that the protein concentration was high at the emergence of the beetles in the region of Haizer in October to March. This can be explained that adults of $B. rufimanus$ possessed accumulated reserves certainly at the larval stage. In addition, Rivero et al. (2001) reported that in insects with multiple larval and adult life stages, nutritional resources were allocated to growth during the larval stage (s), while adults invest mostly in reproduction.

Between the two sexes there was not a difference in the protein concentration according to variance analysis ($F=0.34$, $df=1$, $p=0.58$). However, the period had a very highly significant effect on the concentration of proteins in the hemolymph ($F = 46.7$, $df = 7$, $p = 0.00008$). February, it was classified in group (A) by the Newman and Keuls test, because the protein store was more important at that month. After that it was followed by the months of October, November and December which were classified in class (B), while the month of January was classified in group (C). It was followed in group D by the month of March.

**Total haemolymphatic proteinemia in $B. rufimanus$ adults in sexual activity:** Fig 4 showed that during the reproductive activity period, in April, the concentration of total proteins in the haemolymph of females and males of $B. rufimanus$ caught in the V. faba parcel appeared low compared with the rates recorded during the diapause period, and was only $23.8 \pm 0.04\mu g/\mu l$ and $25.4\pm0.1\mu g/\mu l$ respectively. This reduction had to be related to the recovery of adult activity (displacement, research of the host plant, sexual partner, flight muscles ...). For Chakir (1998), the
reduction of total protein concentration of bruchids that were caught in April, would be related to the recovery of the activity. In May, these rates increased to reach 78.8±0.04 μg/μl for females and 68.8±0.27 μg/μl for males. This may be due to the result of consumption of pollen and other food by the beetles in the agro-ecosystem. Tran (1992) noted that in the field-grown adults of *B. rufimanus*, haemolymphatic protein concentrations remained elevated and maintained throughout reproductive activity. This author attributed this maintenance to the contribution of the pollen diet rich in proteins, sugars and vitamins.

According to the Newman and Keuls test, the month of April was classified in group E; while May was classified in group B.

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**CONCLUSION**

*B. rufimanus* is a monovoltine species, its life cycle is closely related to that of its *V. faba* host plant. The colonization of the adults of *B. rufimanus* depend the daytime temperature, the photophase and the phenology of the host plant. The latter, plays a decisive role in the population dynamics of *B. rufimanus* and leads to a finer adjustment between spawning and pod formation. To control this pest must, given the profitability of the crop, start with less expensive cultivation techniques, it will therefore be necessary to consider an appropriate control technique, including the use of bio-pesticides that aim to reduce the population densities of this pest to an economically acceptable level.