

Effect of host seed species and seed coat on the biological parameters of *Callosobruchus maculatus*

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Abstract

In order to preserve legume seeds in storage and establish an effective, health and environmental control strategy against food legume pests, we studied the biology and development cycle of an insect *Callosobruchus maculatus*. as an example of pests that cause significant economic and health damage. This work focuses on the study of biological parameters such as female fecundity, egg fertility and larval development (emergence rate and cycle time) of *Callosobruchus maculatus* grown on seed cotyledons (whole and shelled) of five types of legumes; *Phaseolus vulgaris* (bean), *Vigna unguiculata* (cowpea), *Cicer arietinum* (chickpea), *Vicia faba* (bean) and *Pisum sativum* (dry pea).

The results show that female fecundity is higher in the presence of whole seeds of *V. unguiculata*, *C. arietinum* and *P. vulgaris* than *V. faba* and *P. sativum* seeds, where fertility is low. Also, the emergence rate of *C. maculatus* is higher on their natural hosts (*V. unguiculata* and *C. arietinum*) than seeds not belonging to their food spectrum. In the case of *P. vulgaris*, all the larvae die in the first stage, whereas in the case of *P. sativum* and *V. faba* a small number of larvae are able to begin their post-embryonic development which lasts longer than in the natural hosts where the duration is very short.

On the other hand, dehulled seeds show that the seed coat causes a significant reduction in the emergence rate and a delay in the development of survivors in *V. faba* seeds, whereas in other seeds the seed coat only influences the life cycle length for *P. sativum*.

Keywords: *Callosobruchus maculatus*, legumes, seed coat, host seed species

1. Introduction

Endowed with a special ability to exploit nitrogen gas in an unlimited quantity in N₂ form in the ambient air, legumes are the exception in the plant world. They stand out from other plants grown mainly by their nitrogen diet, while providing mainly carbohydrates (source of metabolic energy) and proteins (sources of constitutive elements and regulators) but also a varied range depending on the species of other elements. (Lipids, fiber, minerals, vitamins) for the feeding of humans and animals. Also, in addition to their high dietary wealth, legume production interacts with other biogeochemical cycles such as those related to phosphorus or xenobiotics.

However, huge quantities of dry legumes are lost each year due to phytophagous insects FAO (2006). Among the phytophagous insects that have settled on the cultivated plants, thus becoming "harmful" insects, the phytophagous beetles of the family Brachia occupy a place of choice. The chickpea bream *Callosobruchus maculatus* (F. 1775)

In Morocco, the main types of legumes are beans, chickpeas, dry peas, lentils and beans, but there is a decrease in quality because of reduced germination potential. As a result, the amounts of production as well as the nutritional value during storage by different pests are altered ^[1]. The best-known example is chickpea (*Cicer arietinum*), which is attacked by a serious pest named *Callosobruchus*

maculatus, which requires protection by synthetic insecticides every year ^[2, 3].

These post-harvest losses caused by this pest are now a food security problem. The study of a possible eradication of this problem is one of the major answers expected from the scientific community especially in the North African context. While some sparse work ^[1, 2, 3, 4] investigated this scourge in its phytosanitary, environmental and ecological aspect, the fact remains that no work has corroborated the exhaustive study of the reproductive cycle of *Callosobruchus maculatus* and its ecology. This pest presents by its bitterness a serious threat for a whole national economy. Hence the relevance of this research works.

The objective of this work is to follow the biological reproduction cycle of *C. maculatus* while determining the effects of this development on the degradation of the food substrate of legumes. It has been necessary to study several biological parameters (fertility, fertility, larval development and life cycle duration) of *C. maculatus* on the seeds of several legumes in order to develop strategies for the protection of the environment.

2. Materials and methods

2.1. Plant material

The legume seeds studied in this work are bean (*Phaseolus*

vulgaris), cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*), bean (*Vicia faba*) and pea (*Pisum sativum*). The seeds were collected during the month of June 2017 from the Saïs region of northern Morocco. *Cicer arietinum* (L) plants were mowed at the physiological maturity of the seeds and then dried in the field for 10 to 30 days. Threshing was accomplished with a stationary threshing machine.

The Saïs region, which is part of the Great Saïs Plain, is located in central Morocco (33 ° N / 4 ° W) between the cities of Fez and Meknes. With an area of 2200 km², this plain has a high agricultural potential thanks to the presence of fertile clay-limestone soils and shallow and relatively abundant groundwater aquifers. The climate of Saïs is semi-arid with an average annual rainfall of 510 mm [5]

These characteristics explain why agriculture was traditionally turned towards rainfed crops (cereals, pulses, fodder) and ruminant (ovine, bovine) farming, valorizing crop residues [6].

2.2. Bruchus breeding

The species studied is *Callosobruchus maculatus*, obtained from a sample of chickpeas from a stock in the city of Fez. It is maintained by mass culture at the laboratory level in glass jars on chickpea seeds (*Cicer arietinum*). The jars are maintained at a temperature of 26 ± 1 ° C, a relative humidity of 70 ± 5% and a photoperiod of 14h (light) / 10h (dark) for several successive generations.

2.3. Study of biological parameters.

For the fecundity experiments (fig 3), 10 g of each type of seed were placed in 10 cm diameter mesh petri dishes. Then 5 pairs of *C. maculatus* insects not exceeding 24 h of age. The assembly is placed in a culture chamber at a temperature of 26 ± 1 ° C, a relative humidity of 70 ± 5% and a photoperiod of 14h (light) / 10h (darkness). The tests were carried out on shelled and unshelled seeds.

During the experiment, determination of the duration of the incubation, the different larval stages and pupation is done by regular observations using a binocular loupe; in addition the opening of the chickpea seeds is essential to observe the hidden stages of *C. maculatus* within them.

After 13 days, the count of eggs laid (hatched and unhatched) on the seeds is done under a binocular magnifier at x40 magnification.

Fertility is defined as the percentage of hatched eggs relative to eggs laid per female. After counting the eggs laid, the hatching rate is calculated as follows:

Egg hatching rate (%) = number of eggs hatched / number of eggs laid) × 100

From day 26 to day 45, adult individuals are removed from boxes and enumerated as they emerge. The viability rate is calculated by the following formula:

Larval viability rate (%) = (number of emerged adults / number of eggs hatched) × 100

This study also consists of determining the sex ratio that corresponds to the ratio between the number of male offspring and the number of female offspring.

2.4. Statistical analysis of the data.

We used the statistical software SPSS for Windows® (version 21.0). The data were subjected to a bidirectional analysis of variance (ANOVA) to determine the difference between the extreme values of the group. Fisher's Least Significant Difference (LSD) test was used to separate significant from non-significant means at $\alpha = 0.05$.

3. Results & Discussion

3.1. Effect of host seed species

Following the developmental cycle of *C. maculatus*, we have identified four stages of development: egg, larva, nymph and imago. After copulation, the female lay transparent eggs on the seeds or on the walls of Petri dishes, the eggs are ovoid. The average incubation time of eggs is 7 days. For some lots, we found that a large number of eggs are placed on the inner walls of petri dishes, a phenomenon that we have not been able to explain now. These eggs abort and die in their entirety. Statistically, the ANOVA revealed that there is a significant difference in mean fertility ($p < 0.001$) on all seeds, except for the mean between *P. vulgaris* and *C. arietinum* where the difference is non-significant (Table 1). This indicates that the egg-laying substrate has an effect on the number of eggs laid. The highest fecundity rate was recorded on seeds of *V. unguiculata*.

The average fertility of *C. maculatus* for the different egg-laying substrates used ranges from 93.95±2.76% to 78.5±3.54% (Table 1). The difference between the fertility rate observed on seeds of *C. arietinum*, *V. unguiculata* and *P. vulgaris* is not significant. However, it decreased significantly on seeds of *Vicia faba* and *Pisum sativum*.

The number of imaginal output allows to have an idea about the rate of contamination. For the number of exit holes corresponding to emergences, we did not take into account intra-cotyledonary mortality. The average emergence rate of adults ranges from 0% to 66.65±3.32 %. The contamination rate of *V. unguiculata* and *C. arietinum* seeds is very important, ranging from 66.65±3.32 % to 43.7±2.69 % respectively. On the other hand, the contamination rate is low for the seeds of *P. sativum* and *V. faba* with values of 22.2±2.55% and 9.8±2.4 % respectively. On the other hand, on the seeds of *P. vulgaris*, although eggs were enumerated, no emergence of adult insects was observed. Statistically, the analysis of variance "ANOVA" shows that the difference is very significant ($p \leq 0.001$) between all the mean values of emergences.

The larvae of *C. maculatus* are found to grow on four types of legume seeds among the five studied (Table 1). On the seeds of *V. unguiculata*, the development phase (average duration of development of the egg to the adult) lasts on average 30 ± 1 day. On the seeds of *C. arietinum*, the development time of the egg to the adult is on average 29 ± 1 day against 50.33±1.53 days for the seeds of *V. faba* and 47±1days on *P. sativum*. On *P. vulgaris*, postembryonic development is interrupted and death occurs during the 1st instar stage.

Table 1: Effect of host change of *C. maculatus* breeding parameters

	<i>Vigna unguiculata</i>	<i>Cicer arietinum</i>	<i>Phaseolus vulgaris</i>	<i>Vicia faba</i>	<i>Pisum sativum</i>	LSD _{0.05}
Fecundity	51.33±3.05 ^a	39.33±53 ^b	39±4 ^b	29.33±2.52 ^c	15±2 ^d	1.88
Fertility	90.35±1.2 ^a	93.95±2.76 ^a	91.25±2.47 ^a	78.5±3.54 ^b	82.2±2.97 ^c	3.71
Emergence	66.65±3.32 ^a	43.7±2.69 ^b	0	9.8±2.4 ^c	22.2±2.55 ^d	10.1
Duration of life	30±1 ^a	29±1 ^a	-	50.33±1.53 ^b	47±1 ^b	7.14

cycle					
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Data are means ± SEM of three replications In the same line, letters of similar alphabets are not statistically significant (P < 0.05 for LSD).

3.2. Influence of the husk seed coat on the biology of *C. maculatus*.

Females of *C. maculatus* show a fecundity that varies according to the nutrient substrate (Table 1). The seed coat of *C. arietinum*, *V. unguiculata* and *P. vulgaris* has no effect on fertility, but the number of eggs laid on peeled seeds of *V. faba* and *P. sativum* is significantly higher than the number of eggs laid on unpeeled seeds (fig 1).

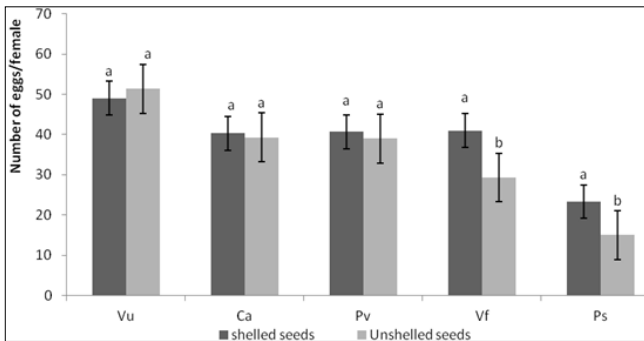


Fig 1: Tegment effect on fecundity

VU: *Vigna unguiculata*, CA: *Cicer arietinum*, PV: *Phaseolus vulgaris*, VF: *Vicia faba*, PS: *Pisum sativum*

In the same column, letters of similar alphabets are not statistically significant (P < 0.05 for LSD).

For the emergence rate, no insect exceeds the first larval stage; all larvae die on the seed coat or on the shelled seeds of *P. vulgaris*.

Of the whole *V. faba* seeds only 9.8% of the individuals managed to achieve larval development. The shelled seeds allow 53.27% of the brushes complete their development. The rest of the population knows a variable mortality, according to the stage of development (fig 2). These results are similar to those observed in natural hosts of *C. Maculatus* (cowpea and chickpea) which is confirmed by statistical tests that indicate the difference and highly significant (p≤0.001). In the case of *P. sativum* seeds, with or without integument, the emergence rate is lower (22.66% and 16.66%) and the observed differences are not significant.

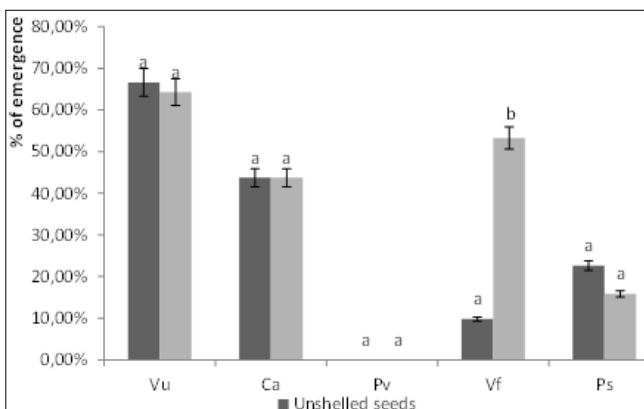


Fig 2: Tegment effect on emergence rate

VU: *Vigna unguiculata*, CA: *Cicer arietinum*, PV: *Phaseolus vulgaris*, VF: *Vicia faba*, PS: *Pisum sativum*

In the same column, letters of similar alphabets are not

statistically significant (P < 0.05 for LSD).

The larval duration varies according to the nutrient substrates (Table 2). With *V. faba* and *P. sativum* seeds, larval development in shelled seeds is faster than that in wholes.

Table 2: Comparison of larval development time on shelled and whole seeds

Food substrate	Unshelled seeds	Hulled seeds
<i>Vigna unguiculata</i>	30±1	28±2
<i>Cicer arietinum</i>	29±1	30±2
<i>Phaseolus vulgaris</i>	-	-
<i>Vicia faba</i>	50±3	38±3
<i>Pisum sativum</i>	48±3	40±3

Data are means ± SEM of three replications

VU: *Vigna unguiculata*, CA: *Cicer arietinum*, PV: *Phaseolus vulgaris*, VF: *Vicia faba*, PS: *Pisum sativum*

The extent of losses caused by *C. maculatus* (F.) on chickpea (*Cicer arietinum* L.) during storage, but also on other legumes of economic importance, suggests the existence of complex relationships between insect and these host plants. The chemical composition of seeds plays an important role in the adaptation of host-host relationships, and this composition is also at the basis of the maintenance or variation of food spectra [7]. The diet of insects of the family Bruchidae is characterized by a degree of specialization because their larvae are found in nature only in the seeds of a small number of host plants [8, 9].

The results of our study revealed that the choice of food spectra by insects and the maintenance of this choice is directly related to the plant's defense system. The significant variation in fecundity and emergence rate obtained in the presence of various legume seeds confirms the idea of a seed defense system against insects. These results are similar to those reported by Ouedraogo [10] who reported that female fecundity and life cycle length of *Caryedon serratus* (a species of the same family of *C. maculatus* that develops on the seeds of peanut) varies from one plant species to another. Southgate [11] showed that 90% of the insects of the family Bruchidae lay and grow at the expense of legumes.

Also, according to Ouedraogo [12] the insects of the species *Caryedon serratus* (groundnut bush) lay their eggs on all the fruits tested with a significant difference in the distribution of the spawning according to the host plants. On their part Delobel [13] shows that among 3 of the 6 species of *Caryedon*, female fertility is higher in the presence of their natural hosts than seeds not belonging to their food spectrum.

Seed teguments are also a form of defense against insect pests. The results obtained show that the presence of integument causes a significant reduction in emergence rate and delays the development of survivors of *V. faba* seeds. As a result, the integument of the seeds tested constitutes an impassable barrier which influences the life cycle of *C. maculatus*. In the same sense Boughdad [14] report that the failure of postembryonic development can be attributed to the integument. The leguminous seed coat of legumes, rich in tannins and lignin, is often presented as a chemical or mechanical barrier against the penetration of larvae of

unsuitable bruchids.

Thus, Stamopoulo and Huignard ^[15] reports that in *Acanthoscelides obtectus* (bean weevils) the dry extracts of *P. vulgaris* teguments do not allow the development of most larvae. The causes of this mortality, which occurs at different larval stages, remain hypothetical. It is possible that the integument represents a chemical barrier, having an important function at various periods of larval life.

In the seeds of *P. sativum* the integument only influences the life cycle, so the low rate of emergence observed due to other factors remains to be evaluated. These factors are mainly related to the biochemical composition of the seed itself.

On the seeds of *P. vulgaris* whether or not husked; the results have been shown to be very effective, even if the fecundity of the females is very high but the life cycle of *C. maculatus* is interrupted at the 1st larval stage, this can be explained by the toxicity of the chemical composition of the bean seeds, to the larvae of the insect. This is mentioned by Angharad ^[16] who reports that lectins purified from the seeds of *P. vulgaris* are found to be toxic to the development of *C. maculatus* larvae. Also, Silva ^[17] reported that the presence of phaseolin (*Phaseolus vulgaris* protein) in the seeds of *P. vulgaris* is detrimental to the development of *C. maculatus*.

4. Conclusions

The knowledge of the biology of the insect studied and the host-insect relationship as well as some defense factor of the plant against these enemies in general and especially insects, is an obligatory step in the search for alternative methods of protection seeds.

Our work revealed the existence of a toxic effect exerted by all the seeds studied, this effect differs from one seed to another by generating a total annihilation in the case of bean seeds and a small reduction in case of cowpea. This is due to the mechanical and / or biochemical action of the integument and other parts of the seed.

The identification of the molecules responsible for this biocidal effect may be one of the effective biological control solutions against this pest.

5. References

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