

Rapid Ability Adaptation of *Callosobruchus maculatus* to a Novel Host *Vigna unguiculata*

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ABSTRACT

Haouel-Hamdi, S., Labidi, M., Hedjal-Chebheb, M., Aouji, A., Boushah, E., and Mediouni-Ben Jemâa, J. 2018. Rapid ability adaptation of *Callosobruchus maculatus* to a novel host *Vigna unguiculata*. Tunisian Journal of Plant Protection 13 (si): 113-121.

In Tunisia, the cowpea seed beetle *Callosobruchus maculatus* is the major and economic insect pest of stored chickpea. This work aims to study the adaptive behavior of Tunisian strain of *C. maculatus* exclusively reared on chickpea for 5 years on a novel host, the cowpea *Vigna unguiculata*. The relative aspects of the host adaptation tests consist of the assessment of the reproductive parameters and the demographic traits of the insect over six months of storage period on chickpea and cowpea seeds. Two types of bioassays free-choice and no-choice were performed. Comparison of reproductive and demographic parameters for *C. maculatus* showed that under no-choice situation, chickpea was the preferred host of *C. maculatus* along the first two months of storage. However, under free-choice, the results revealed that from the first month of storage, the reproductive and demographic parameters of *C. maculatus* shifted in favor of cowpea. Thus, through this work, we have demonstrated the rapid adaptive potential of *C. maculatus* toward its original host and its ability to recognize and adapt to it over a short period.

Keywords: Adaptive behavior, *Callosobruchus maculatus*, chickpea, cowpea

Beetles of the genus *Callosobruchus* are major storage pests of chickpea crops and cause considerable economic losses worldwide (Sharma and Thakur 2014). The cowpea seed beetle *Callosobruchus maculatus* is the principal field-carry-over storage pest of pulses

including cowpea, chickpea, green gram, black gram, and red gram (Loganathan et al. 2011). The neonate larvae penetrate the grains causing serious damage such as grain weight loss, reduction in germination, weak seed viability, and nutritional low quality (Haouel-Hamdi et al. 2017a; Oke and Akintunde 2013).

It is well established that *C. maculatus* is among herbivorous insects characterized by the exploitation of novel food sources due to its rapid host diversification and its capacity of frequent

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host shifts (Fricke and Arnqvist 2007). Adaptation to one novel host may simultaneously affect an insect's performance on other hosts, including hosts that the population may never have encountered. For example, if a population has a means to detoxify a particular secondary compound in a novel host, it may be able to exploit closely related hosts that contain similar compounds (Agrawal 2000). Females of *C. maculatus* attach eggs to the surfaces of grain-legume seeds. Hatching larvae burrow into the seed directly beneath the oviposition site and complete development within a single seed. All suitable hosts for *C. maculatus* are in the subfamily Papilionoideae within the legume family Fabaceae. Within the Papilionoideae, most *C. maculatus* hosts belong to the tribe Phaseoleae, and the most severely infested crops are in the genus *Vigna* (Tuda et al. 2005). Lima et al. (2004) reported that alternation of hosts is an efficient strategy to avoid the development of *C. maculatus* populations.

Previous studies of Simmonds et al. (1989) reported that bruchids have already indicated that host size affects the number of oviposited eggs. Other factors that strongly affect egg laying are morphological parameters, such as texture (Johnson and Kistler 1987), chemical characteristics of the seed coat (Lale and Makoshi 2000), and nutritional quality (Janz and Nylin 1997). The texture of all the three host seeds was smooth, and probably only the relative quantity and/or quality of resource available inside the seeds was taken into account by females for deciding on the number of eggs oviposited on seeds (Cope and Fox 2003).

Therefore, this work aims to investigate population dynamics, reproductive parameters, and demographic traits of *C. maculatus* reared

on two host legumes: chickpea and cowpea and to study its rapid ability adaptation to a novel host.

MATERIALS AND METHODS

Insect rearing and seed material.

The mass rearing method of *C. maculatus* used has been described by Haouel-Hamdi et al. (2017b). The stock cultures of the cowpea seed beetle were maintained in glass bottles of 1 liter of volume in a growth chamber at $30 \pm 5^\circ\text{C}$ temperature, $65 \pm 5\%$ RH and 12:12 Light:Darkness photoperiod.

The strain of *C. maculatus* used for the experiments was isolated from chickpea infested seeds for five years. Ten mature couples of the flightless-form were transferred on seeds of two food legume hosts for one year by regular rearing: a chickpea (*Cicer arietinum*) Amdoun 1 variety and cowpea (*Vigna unguiculata*) Black eye variety. The study of the rapid ability adaptation was studied after three generations.

Free-choice and no-choice bioassays.

The free-choice bioassay aimed to evaluate the influence of the attractiveness of the food legume seeds used toward the cowpea seed beetle oviposition. The no-choice bioassay was carried out in order to assess the influence of each seed type on the oviposition without any interference by the other tested hosts.

Free-choice bioassay. The two-way device applied in this bioassay consisted of the following PVC parts:

1. A common release arena, got from a cylindrical box (11 cm diameter, 6 cm depth for 0.7 liter). Two circular holes (1 cm diameter), equidistant among them, were made on the sidewalls of this box, at 1 cm from its bottom;

2. Two oviposition arenas, got from smaller cylindrical boxes (5 cm diameter, 3 cm depth for 50 ml) and their intact lid. A circular hole (1 cm diameter) was made on the sidewall of each box at 3.5 cm from its bottom;
3. Two tubes (pipes 6 cm long and 1 cm for the external diameter) connected the release with the oviposition arenas.

Each oviposition arena was filled up 30 seeds of only one food legume hosts (chickpea and cowpea). No seed was placed in the common arena. Fifteen females and five males of *C. maculatus* adults (all emerged within the last 24 hours) were collected from the maintained culture and released in the common arena. They could freely come back from each oviposition arena and visit the other ones. The device was kept in a growth chamber at $30 \pm 5^\circ\text{C}$ and $65 \pm 5\%$ RH (Panzarino et al. 2012). The adults were removed just two days later, before first larval hatching, and the seeds of each arena were examined to count the eggs laid on their surfaces. The bioassay was thrice replicated with genotypes placed randomly into the device.

No-choice bioassay. The test was performed using glass bottles of 1 liter of volume. One bottle for each food legume host was filled up 30 seeds. Three females and one male of *C. maculatus* adults (all emerged within the last 24 hours) were released in each bottle according to the methods of Panzarino et al. (2012). Adults were collected from the maintained culture and three replicates per food legume host were performed. The bottles were kept in a growth chamber at $30 \pm 5^\circ\text{C}$ and $65 \pm 5\%$ RH (Haouel-Hamdi et al. 2017a) and the adults were removed just two days later,

before first larval hatching, and eggs were counted on the seeds of each genotype.

Reproductive parameters and demographic traits study.

The reproductive parameters and demographic traits were assessed according to free-choice and no-choice bioassays. The total number of eggs laid and the emergence rate were determined according to Haouel-Hamdi et al. (2017b).

The population growth parameters namely: the mean growth rate (MGR), the mortality rate of eggs (MRE), the mortality rate of larvae (MRL), the sex-ratio (SR), the net reproductive rate (R_0), the generation time (GT), and the intrinsic rate of increase (r) were calculated according to Haouel-Hamdi et al. (2017a).

Statistical analyses.

To analyze the possible effects of the rearing substrates (chickpea and cowpea) on all biological parameters (reproductive and demographic traits) of *C. maculatus*, statistical analyses were performed using SPSS statistical software version 20.0. All values given were the mean of three replications and were expressed as the mean \pm standard deviation. Significant differences between the mean values ($P \leq 0.05$) were determined by using Duncan's Multiple Range test. When necessary, data were transformed by common logarithm or square root to meet the assumptions of normality. Correlation analyses (Pearson's correlation coefficient) were established between demographic traits parameters and assays of choice, food host and generation.

RESULTS

Reproductive parameters.

Total number of laid eggs. The data recorded on total number of eggs laid

under free-choice and no-choice conditions by *C. maculatus* for three generations on different hosts are presented in Fig. 1.

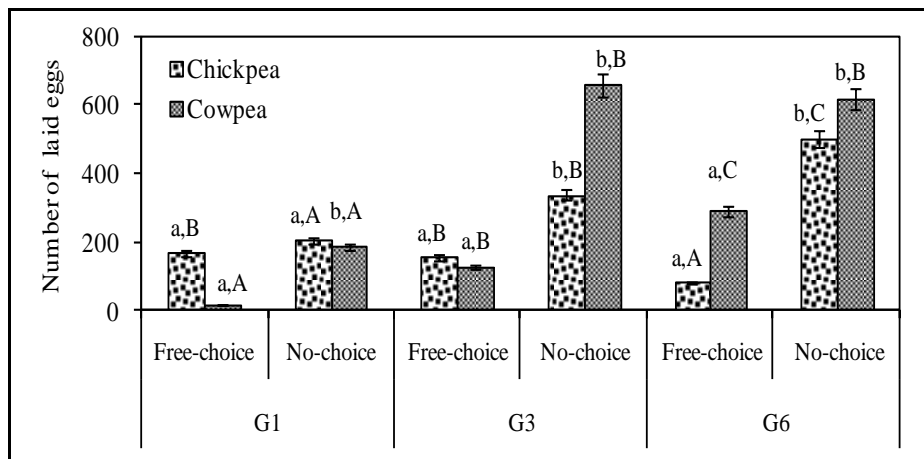


Fig. 1. Total number of laid eggs of *Callosobruchus maculatus* reared on different hosts for three generations under free-choice and no-choice assays. For each generation and each host, comparisons were made among free-choice and no-choice assays (lowercase letters) and for each assay (free-choice and no-choice) and each host comparisons were made among generations (uppercase letters). Bars having different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

For the first generation, under no-choice assays, females laid the highest number of eggs on chickpea, and the lowest number on cowpea. However, for the 3rd and 6th generation, under no-choice assays, females laid the highest number of eggs on cowpea, and the lowest number on chickpea (Fig. 1).

Results revealed that under free-choice condition, the number of eggs laid by *C. maculatus* on different food legume host ranged from 0 to 166, from 32 to 154 and from 18 to 290 for the 1st, 3rd and 6th generations, respectively. However, under no-choice condition, the number of eggs laid by *C. maculatus* on different food legume hosts ranged from 187 to 226, from 102 to 660, and from 164 to 616 for the 1st, 3rd and 6th generations, respectively.

In both bioassays (free-choice and no-choice), the total number of laid eggs was significantly dependent on host and generation number (For host $F = 29.84$, $P < 0.001$ and for generation $F = 28.49$, $P < 0.001$). Under free-choice condition, for the 1st generation, females laid significantly more eggs on chickpea (166 eggs) than on cowpea (12 eggs). For the 6th generation, the number of eggs laid was 290 eggs on cowpea against 81 eggs on chickpea (Fig. 1).

Emergence rate.

The data recorded on *C. maculatus* emergence rate under free-choice and no-choice conditions for 6 generations on different food hosts are presented in Fig. 2.

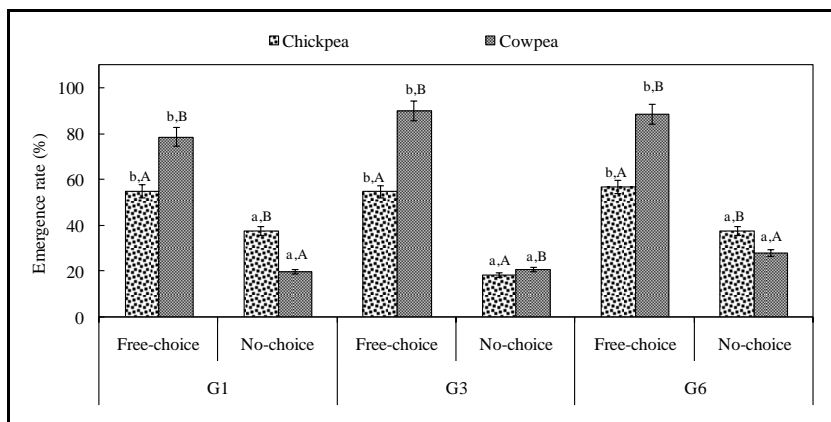


Fig. 2. Emergence rate (%) of *Callosobruchus maculatus* reared on various food hosts for the three generations under free-choice and no-choice assays. For each generation and each host, comparisons were made among free-choice and no-choice assays (lowercase letters) and for each assay (free-choice and no-choice) and each generation, comparisons were made among hosts (uppercase letters). Bars having different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Fig. 2 showed that under free-choice conditions, adults' emergence rate was higher than under no-choice condition for 6 generations. Maximum emergences were recorded on cowpea (78.61% for G1, 89.91% for G3 and 88.45% for G6) under free-choice condition but for the no-choice condition, maximum of emergences were observed on chickpea (37.66% for G1 and 37.61% for G6). In case of no-choice conditions, for the 1st generation, emergence rate of *C. maculatus* was higher when reared on chickpea (38%) than on cowpea (20%). In addition, for the 6th generation, the emergence rate was similar to the 1st generation when 38 and 28% were noted on chickpea and cowpea, respectively (Fig. 2).

When referring to the number of eggs laid and to the emergence rate under free-choice conditions, the most preferred host was cowpea followed by chickpea.

Demographic parameters.

Correlation analyses of demographic traits and assays of choice, food hosts and generation were recorded in Table 1. Results showed the importance of the type of bioassay (free-choice or no-choice) during the six generation. A highly significant and positive correlation was recorded between the assays of choice and sex-ratio ($r = 0.53$, $P < 0.001$) and generation time ($r = 0.46$, $P < 0.001$). Similarly, correlation studies were worked out between generation and mean growth rate, data showed a positive correlation ($r = 0.41$, $P < 0.001$). The correlation coefficient data indicated that when the generation increases the mean growth rate increases. However, a highly significant and negative correlation was observed between the assays of choice and mean growth rate ($r = -0.87$, $P < 0.001$), between generation and mortality rate of larvae ($r = -0.36$, $P < 0.001$) and between food legume hosts and intrinsic rate of increase ($r = -0.31$, $P = 0.008$).

Table 1. Correlation analyses between demographic traits of *Callosobruchus maculatus* and assays of choice, food host and generation

| Correlation | | MGR ^a | MRE ^a | MRL ^a | SR ^a | R ₀ ^a | GT ^a | r ^a |
|-------------------|---|------------------|------------------|------------------|-----------------|-----------------------------|-----------------|----------------|
| Assays of choice | r | -0.14 | -0.87** | -0.07 | 0.53** | 0.08 | 0.46** | 0.06 |
| | P | 0.25 | <0.001 | 0.59 | <0.001 | 0.50 | <0.001 | 0.63 |
| Food legume hosts | r | -0.09 | -0.06 | 0.18 | 0.15 | -0.21 | 0.08 | -0.31** |
| | P | 0.47 | 0.61 | 0.14 | 0.21 | 0.08 | 0.51 | 0.008 |
| Generation | r | 0.41** | 0.01 | -0.36** | 0.09 | -0.10 | -0.10 | 0.01 |
| | P | <0.001 | 0.95 | <0.001 | 0.45 | 0.41 | 0.39 | 0.91 |

^a MGR = Mean growth rate, MRE = Mortality rate of eggs, MRL = Mortality rate of larvae, SR = Sex-Ratio, R₀ = Net reproductive rate, GT = Generation time, r = Intrinsic rate of increase.

** Significant at $P \leq 0.01$.

DISCUSSION

Results of the current investigation pointed out that the *C. maculatus* biology was largely affected by the free-choice and no-choice bioassays conditions, food host and generation number. In fact, under no-choice assays, *C. maculatus* female were highly attracted by chickpea and cowpea seeds. However, they showed a strong preference for cowpea seeds in free-choice assay. The differences in attraction of *C. maculatus* female to the food host was investigated by Rees (2004) who indicated that the bruchids have varied preferences for different food legume varieties.

C. maculatus is primarily a pest of cowpea but has many alternative hosts among leguminous seeds (Haouel-Hamdi et al. 2017a). However, little is known about the mechanisms of host location and preference. Being a field-to-store pest suggests that dispersing individuals are guided by specific cues to their preferred hosts (Ajayi et al. 2015).

The habitual behaviors of this insect in the search for the oviposition and feeding sites, can also be influenced by the perception of the colors and the shape of the host seed (Nicole 2002). Moreover, this author showed that the color, and the shape of host seeds as well

as the chemicals they contain appear to play a very important role in the attraction of *C. maculatus* females to these host seeds. In this respect, Sankara et al. (2010) showed that *C. maculatus* females were able to recognize odors from their egg-laying substrates and to find their way to the sources of these odors. When these females have the choice between clean air and air containing seed odors, they are significantly more attracted to the smell of seeds. This would be explained by the volatile substances emitted by seeds. In addition, Ignacimuthu et al. (2000) confirmed that these chemicals would have an attractive effect on females of Bruchidae beetles in general and species of the genus *Callosobruchus* in particular.

Agosta (2008) indicated that in the free-choice test both the survival of offspring and the host seed size showed a positive relationship with the number of eggs laid on seeds. However, in the no-choice test, only the survival of offspring and the number of eggs laid showed a positive relationship, while there was a significant correlation between seed size and the number of eggs laid on the seed. Taken together, these results suggested that the females could choose appropriate hosts for their offspring both in free-

choice and no-choice host preference and survival of offspring.

The developmental plasticity is illustrated in this study by several biological indicators, which gave evidence of an important potential for a relatively rapid adaptation of *C. maculatus* on the three tested food legume hosts. In addition, according to Conord (2006) and Sankara et al. (2016), the preference of females of *C. maculatus* for *Vigna subterranea* and *Cajanus cajan* strains for the odor of cowpea seeds may be due to the difference in the chemical composition existing between used seeds of the host species. This difference in chemical composition is potentially the basis of specific olfactory discrimination by this insect in favor of cowpea. Indeed, Sankara et al. (2016) showed that *C. maculatus* females seem to remember the volatile signals from cowpea, the original host, even after three years of adaptation or development on the *Arachis hypogaea* and *C. cajan*. According to Sankara et al.

(2012), in the case of a three-dimensional device, the insects of *V. subterranea* and *C. cajan* strains express their preference for *Arachis hypogaea* and *C. cajan*, suggesting that the ability to discriminate of *C. maculatus* led to different decision making in the case where the hosts are visible. Indeed, Nicole (2002) and Yang et al. (2006) showed that in the olfactometric studies based on olfactory stimuli and the other on the combination of two stimuli (visual and olfactory), *C. maculatus*, a cosmopolitan pest in storage is known for its ability to recognize its host.

Our results are in accordance with Wasserman and Futuyama (1981) who found a positive response to selection for ovipositional preferences after eleven generations of selection in the same species and Messina et al. (2009) who also demonstrated a change in oviposition preferences in seed beetle lines switched to new hosts.

RESUME

Haouel-Hamdi S., Labidi M., Hedjal-Chebheb M., Aouji A., Boushah E. et Mediouni-Ben Jemâa J. 2018. Adaptation rapide de *Callosobruchus maculatus* à un nouvel hôte *Vigna unguiculata*. Tunisian Journal of Plant Protection 13 (si): 113-121.

En Tunisie, la bruche du niébé *Callosobruchus maculatus* est un ravageur majeur d'importance économique du pois chiche conservé. Ce travail a pour but d'étudier le pouvoir adaptatif des souches tunisiennes de *C. maculatus* élevées exclusivement sur pois chiche pendant 5 ans à un nouvel hôte le niébé *Vigna unguiculata*. Les aspects relatifs du test d'adaptation au nouvel hôte consistent à l'évaluation des paramètres reproductifs et des traits démographiques durant six mois de stockage sur des graines de pois chiche et de niébé. Deux tests de choix et de non choix ont été effectués. La comparaison des paramètres reproductifs et démographiques de l'insecte a montré qu'en situation de non choix, le pois chiche constituait l'hôte préféré de *C. maculatus* durant les deux premiers mois de stockage. Cependant, en situation de libre choix, les résultats ont révélé qu'à partir du premier mois de stockage, les paramètres biologiques et démographiques de *C. maculatus* étaient en faveur du niébé. Ainsi, ce travail a montré la dynamique adaptative de cette bruche par rapport à son hôte d'origine et son aptitude à le reconnaître et à s'y adapter au cours d'une courte durée.

Mots clés: *Callosobruchus maculatus*, niébé, pois chiche, pouvoir adaptatif

حوال-حمدي، سمية ومريم العبيدي ومريم حجال-شبهاب وعلي عوجي وأمنة بوضيح وجودة مديوني-بن جماعة. 2018. التكيف السريع لخنفساء حب اللوبيا الجنوبية *Callosobruchus maculatus* على عائل جديد *Vigna unguiculata*. **Tunisian Journal of Plant Protection 13 (si): 113-121.**

في تونس، تعتبر خنفساء حب اللوبيا الجنوبية *Callosobruchus maculatus* واحدا من أكثر الآفات الضارة على الحمص أثناء الخزن. يهدف هذا العمل إلى دراسة القدرة على التكيف لمجتمعات خنفساء حب اللوبيا الجنوبية التي نمت حصريا على العائل الأصلي الحمص لمدة 5 سنوات وتكيفها على عائل جديد وهو اللوبيا الجنوبية. وتتألف الجوانب النسبية لاختبار التكيف مع العائل من متابعة الصفات الإنجابية والديموغرافية مدة ستة أشهر من التخزين للحمص واللوبيا الجنوبية. تم إجراء اختبارين للاختيار وعدم الاختيار للعائل من قبل الخنفساء. أثبتت مقارنة الصفات الإنجابية والديموغرافية للحشرة أنه في حالة عدم الاختيار كان الحمص العائل المحبذ للحشرة على طول الشهرين الأولين من التخزين. لكن في حالة الاختيار، أثبتت النتائج أنه منذ الشهر الأول من التخزين كانت الصفات الإنجابية والديموغرافية لصالح اللوبيا الجنوبية. هكذا، من خلال هذا العمل أثبتنا ديناميكية التكيف لهذه الحشرة فيما يتعلق بالعائل الأصلي وقدرتها على التعرف والتكيف معها في فترة قصيرة من الزمن.

كلمات مفتاحية: حمص، خنفساء حب اللوبيا الجنوبية، قدرة على التكيف، لوبيا جنوبية

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