Scientific Research Publishing

Advances in Entomology, 2018, 6, 160-169
http://www.scirp.org/journal/ae
ISSN Online: 2331-2017
ISSN Print: 2331-1991

Reproductive Potential of *Spermophagus niger* (Coleoptera: Chrysomelidae: Bruchinae: Amblycerini) Developing on the Seeds of Two Roselle Varieties in Burkina Faso

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How to cite this paper: Koussoubé, J.C., Ilboudo, Z., Waongo, A. and Sanon, A. (2018) Reproductive Potential of *Spermophagus niger* (Coleoptera: Chrysomelidae: Bruchinae: Amblycerini) Developing on the Seeds of Two Roselle Varieties in Burkina Faso. *Advances in Entomology*, **6**, 160-169.
https://doi.org/10.4236/ae.2018.62012

Received: March 28, 2018
Accepted: April 27, 2018
Published: April 30, 2018

Abstract

In West Africa and particularly in Burkina Faso, Roselle, *Hibiscus sabdariffa* L. (Malvaceae) is receiving increasing attention as a crop with potential for making great socio-economic impacts. The biology of *Spermophagus niger,* recently identified as the main insect pest in the storage of roselle seeds, is poorly known. The present study aimed at comparing the reproductive potential of this insect on the seeds of two roselle varieties *Altissima* and *Sabdariffa*, mainly grown in Burkina Faso to identify susceptible/resistant variety to the pest. The results obtained are the first complete data on life history traits of *S. niger* in laboratory conditions. Overall, they indicated that both seed varieties have similar susceptibility to *S. niger*. The main life history traits measured did not differ significantly on both varieties ($P > 0.05$). However, the first generation individuals from the *Altissima* seeds were significantly larger ($P = 0.0007$ and $P < 0.0001$ for males and females respectively) and weighed more ($P < 0.0001$) than those from the other roselle variety. This result suggests that individuals from the *Altissima* variety may have a higher seed-damaging potential than those developed in the *Sabdariffa* variety and this potential might increase over successive generations. The consequences of such findings are discussed with a view to improving the post-harvest storage of roselle seeds and reducing losses in West Africa.

Keywords

Hibiscus sabdariffa, Spermophagus niger, Life History, Post-Harvest Storage, Varietal Susceptibility, Seed Damage

1. Introduction

Roselle, *Hibiscus sabdariffa* L. is an herbaceous and perennial plant found in the tropical and subtropical areas of both hemispheres [1]. This plant species occurs in almost all West African countries specifically in tropical savannas of semi-arid areas [2] [3] where it is grown mainly for food needs [3] [4] [5] [6], therapeutic [7] [8], industrial and economic uses [9] [10]. In Burkina Faso, Roselle is booming with real economic, agronomic and environmental potentials [11]. Two main varieties, *Altissima* and *Sabdariffa*, are grown in all agro-ecological areas here. The *Sabdariffa* variety is grown for the production of calices, which are used in the preparation of *Bissap*, a high nutritional value and commercial drink [9] [10] [11] [12]. All the plant parts of the *Altissima* variety are used in the preparation of several sauces and other recipes or a locally popular meat substitute condiment called "Bikalaga" [5].

Despite a high socio-economic impact, roselle cultivation is faced with biotic constraints including attack by several insect pests [11] [13]. Plants are often attacked in fields by larvae of cotton bollworm, *Earias biplaga* and *Earias insulana* causing significant damage. Moreover, the larvae of *Podagrica* spp., attack roselle roots while adults cause damage to leaves and terminal buds [13]. During post-harvest storage, the seeds are also damaged by the larvae of *Spermophagus niger* (Coleoptera: Chrysomelidae: Bruchinae) developing inside [14] [15]. At the beginning of grain storage, infested seeds generally had only one insect emergence hole, with seed perforation rates ranging from 1.8% to 4% depending on their origin [15]. Strongly attacked seeds become unfit for consumption and their germination capacity decreases significantly. The identification of this insect as a pest of roselle seeds is relatively recent and very little data are available on its biology. However, the life cycle has the same stages as other Bruchines including the egg, 4 larval stages, a pupal stage all developing inside the seeds from which imago is obtained [15]. In this study, the reproductive potential of *S. niger* has been investigated using the seeds of the main roselle varieties (*Altissima* and *Sabdariffa*) available in Burkina Faso. The results will help to determine the susceptibility of each variety to the pest [16], which may contribute to better protection of the stored grain against the insect. Specifically, in laboratory conditions, the key life-history parameters of *S. niger* (lifespan, fecundity, larval survival, development time, intrinsic rate of natural increase, size and weight of the first generation insects) were measured, for the first time, and compared for the seeds of both roselle varieties.

2. Material and Methods

2.1. Experimental Conditions

All experiments were conducted in fluctuating conditions in the Laboratory of Fundamental and Applied Entomology, University Ouaga I Professor Joseph Ki-Zerbo. The temperature and humidity variations were recorded daily throughout the study period using a thermohygrometer placed on the bench.

The average temperature ranged 25.5˚C - 33.8˚C and the average relative humidity varied between 21% and 55% during the study.

2.2. *S. niger* Origin and Rearing

S. niger adults used for this study were collected from several samples of roselle seeds purchased in Ouagadougou market before the experiments started. Insects emerging from the seeds and identified using the previously described morphological criteria [15], were selected to obtain a laboratory strain reared for several generations on the seeds of the *Altissima* variety. For continuous rearing, batches of 10 pairs of newly emerging *S. niger* were put in Plexiglas boxes (17 × 11 × 4 cm) with 50 g of healthy seeds of the *Altissima* variety for 24 hours. After insect removal, infested seed batches were placed in rearing conditions until the emergence of adults used for the experiments.

2.3. Roselle Seed Varieties Used

Roselle Seeds from *Sabdariffa* and *Altissima* varieties were used to measure the life-history parameters of *S. niger*. These seeds were obtained from samples bought in a market in Ouagadougou. Brought to the laboratory seeds of each variety were sorted and stored in a freezer at −18˚C at least two weeks before the experiments. This procedure allowed the elimination of any initial infestation.

Five batches of 150 seeds of each roselle variety were then checked for morphological characteristics such as seed texture, color, average diameter and weight. The texture was determined by touching the seeds between fingers and the diameter was measured by a digital caliper for each of the sampled seeds. Finally, each batch of 150 seeds was weighed using a precision weighing scale.

2.4. Determining the Life History Parameters of *S. niger*

The same experimental procedure was used for each roselle variety tested. Twenty Petri dishes were selected each containing 10 seeds of the tested variety. The dishes were then individually infested by introducing one pair of newly emerged *S. niger*. Every day the seeds were renewed and those of the day before were kept in experimental conditions and followed until the emergence of the first generation of insects. This procedure was repeated until the death of the male and female in each petri dish. Seven days after infestation, seeds from each Petri dish were observed under a binocular microscope to count the eggs laid on seeds and hatched eggs. Daily monitoring of infested seeds recorded the emerging males and females of the first generation.

From emerging insects, 50 individuals (25 males and 25 females) from each variety were individually weighed and their size measured using a binocular microscope. The following parameters were then determined from the data collected in insects developing on both roselle varieties from the twenty Petri dishes:

- Lifespan of males and females (L)

- Number of eggs laid per female (N)
- Larval survival rate (S) determined as the percentage of insects emerged relative to the total number of hatched eggs
- Development duration (T): is the mean time between egg laying and the emergence of adults
- Sex-ratio considered as the % of males or females among the first generation of emerging insects
- The intrinsic rate of natural increase (r) is a more synthetic parameter that incorporates the above cited ones. It is estimated using the following formula [17]:

$r = \frac{\ln\ln(NS)}{T + 1/2L}$; With ln = Napierian logarithm

In practical terms, since the intrinsic rate of natural increase of a given insect population is proportional to the susceptibility of the host plant, this variable is therefore useful for comparing the susceptibility of both roselle varieties.

2.5. Statistical Analysis

Data were submitted to analysis of variance (ANOVA) using SAS 9.1 software. When this analysis revealed significant differences, means were separated by the Newman-Keuls test. Discrimination was made at the probability level of 5%.

3. Results

3.1. Seed Characteristics of Both Roselle Varieties

Seeds of *Altissima* variety are significantly wider and weigh more than the *Sabdariffa* variety (ANOVA; $P < 0.0001$). However, the texture and color do not vary among seeds of both varieties being rough and grey (Table 1).

3.2. Comparison of *S. niger* Growth Parameters on Both Roselle Varieties

3.2.1. Insect Lifespan

There was no significant difference between the values of lifespan obtained for *S. niger* females (Table 2) whatever the roselle variety used for development. Females lived 7.20 ± 0.86 days and 7.60 ± 1.05 days on seeds of *Sabdariffa* and *Altissima* variety respectively. However, males lived significantly longer than females on both variety seeds (9.20 ± 0.86 days, $P = 0.0002$ and 10.93 ± 2.54 days, $P<0.0001$ respectively for *Sabdariffa* and *Altissima*) (Figure 1).

3.2.2. Number of Eggs Laid per Female

The average number of eggs laid by a female during its life did not vary according to roselle variety (Table 2). A daily monitoring of this parameter showed that whatever the variety, the number of eggs laid was higher on the first day before gradually decreasing. More than 90 percent of eggs are laid during the first 6 days of the female life regardless of the variety (Figure 2).

Table 1. Variations in mean size, weight of 150 seeds and some physical characteristics of the seeds of both roselle varieties used.

Varieties	Diameter (±SD mm)	Weight (±SD mg)	Texture	Color
Sabdariffa	2.20 ± 0.19a	24.68 ± 3.08a	Rough	Grey
Altissima	2.48 ± 0.22b	37.86 ± 5.46b	Rough	Grey
ANOVA	68.89	440.02		-
P	<0.0001	<0.0001		-

Means (±SD) within column followed by different letters are significantly different according to the Newman-Keuls multiple comparison test. P < 0.05.

Table 2. Main growth parameters of *S. niger* developing on the seeds of both roselle varieties used for insect development.

Roselle varieties	Female lifespan (days)	Number of eggs laid per female	Larval survival (%)	Development duration (days)	Intrinsic rate of natural increase (r) (per day)
Sabdariffa	7.20 ± 0.86a	51.00 ± 8.57a	69.54 ± 21.81a	34.00 ± 0.73a	0.088 ± 0.01a
Altissima	7.60 ± 1.05a	50.06 ± 8.73a	74.53 ± 13.49a	34.09 ± 0.47a	0.090 ± 0.00a
ANOVA	1.29	0.09	0.57	0.15	0.33
P	0.237	0.770	0.458	0.700	0.570

Means (±SD) within column followed by different letters are significantly different according to the Newman-Keuls multiple comparison test. P < 0.05.

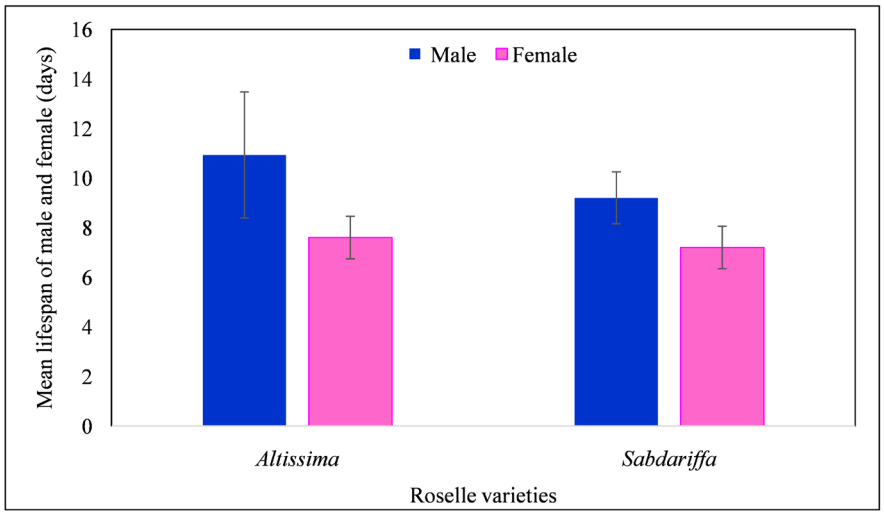

Figure 1. Lifespan of the adult males and females of *S. niger* on both roselle varieties.

3.2.3. Larval Survival during Post Embryonic Development

The larval survival rates were 69.54% ± 21.81% and 74.53% ± 13.49% respectively on seeds of *Sabdariffa* and *Altissima* varieties. The seed varieties did not affect larval survival (P > 0.05; Table 2).

3.2.4. Development Duration

The development from egg to adult lasted 34.00 ± 0.73 days and 34.09 ± 0.47

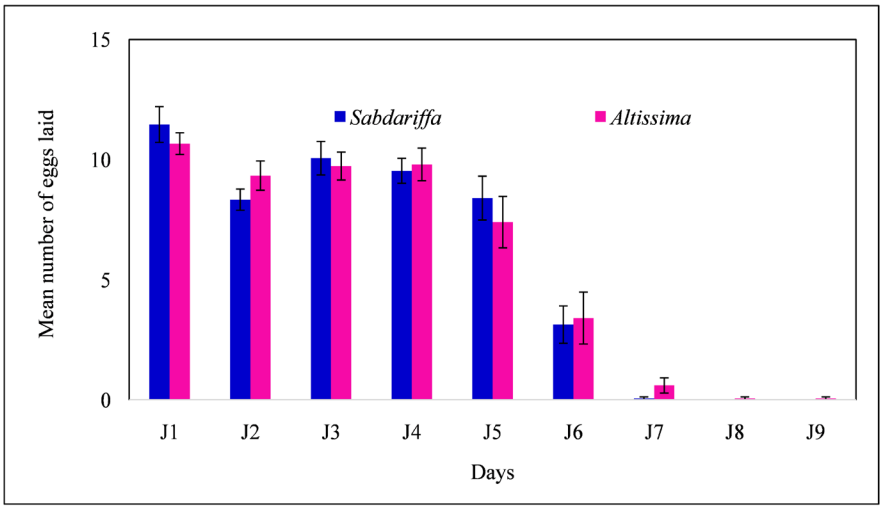

Figure 2. Evolution of the daily oviposition of a *S. niger* female on seeds of both roselle varieties.

days respectively on the seeds of *Sabdariffa* and *Altissima* varieties. No significant difference was noticed between development duration in relation with roselle seed varieties (Table 2). Similarly, the development duration did not significantly differ between males and females (P = 0.096 and P = 0.321 respectively for insects developing on *Sabdariffa* and *Altissima* seeds).

3.2.5. Intrinsic Rate of Natural Increase

The intrinsic rate of natural increase was relatively low (0.088 ± 0.01 and 0.090 ± 0.00 individuals per day respectively on *Sabdariffa* and *Altissima* seeds varieties) but similar for insects developing on the seeds of both roselle varieties (P > 0.05; Table 2).

3.3. Effects of Roselle Varieties on First Generation Insects

A female *S. niger* produced on average 30.73 ± 11.98 and 32.53 ± 8.45 individuals when development occurred respectively on *Sabdariffa* and *Altissima* varieties. There was no variety effect on the mean number of emerging insects (P > 0.05; Table 3). The sex ratio was 68% - 70% female bias whatever the seed variety considered. Insects from the seeds of *Altissima* variety had significantly larger size and weighed more than those from *Sabdariffa* variety (P < 0.05; Table 3).

4. Discussion

For the first time, the biology of *S. niger* was studied using two varieties of roselle, the host plant in Burkina Faso. Overall, the results show that this insect is able to complete its life cycle inside the seeds of both roselle varieties without significant difference in varietal effect. These results confirm the preliminary observations indicating the presence of a Bruchine on a Malvaceae species as host plant in West Africa [14] [15]. Indeed, Bruchines are oligophagous specialist insects developing mainly on legumes, sometimes on Combretaceae and

Table 3. Characteristics of the S. niger first generation individuals obtained from the seeds of both roselle varieties.

Varieties	Mean number of emerging insects			Mean size (mm)		Mean weight (mg)	
	Males	Females	Sex ratio	Males	Females	Males	Females
Sabdariffa	14.93 ± 6.88a	15.80 ± 6.09a	0.48 ± 0.10a	3.66 ± 0.21a	3.58 ± 0.29a	5.76 ± 0.87a	6.07 ± 0.93a
Altissima	15.53 ± 5.22a	17.00 ± 4.50a	0.47 ± 0.06a	3.84 ± 0.16b	3.97 ± 0.19b	6.88 ± 0.52b	7.60 ± 0.62b
ANOVA	0.07	0.38	0.06	12.73	37.64	35.79	55.01
P	0.789	0.545	0.804	0.0007	<0.0001	<0.0001	<0.0001

Means (±SD) within column followed by different letters are significantly different according to the Newman-Keuls multiple comparison test. $P < 0.05$.

more rarely on other plant families including Malvaceae [15] [18]. Key life history traits such as adult lifespan, female fecundity, development duration, larval survival and finally the rate of natural increase were not influenced by variety even though the seeds of both varieties differed in size and weight. Certainly, the precise chemical composition of these seed varieties remains to be determined but we can assume that they offer on average the same potential for development to this pest.

However, some biological parameters are to be in-depth analyzed in comparison with those of other Bruchines or even other storage insect pests. This is the case for adult lifespan, which, while remaining short, was significantly longer in males. This result seems to be common in several insect species [19] [20] [21]. The shorter lifespan of females is usually correlated with greater energy expenditure due to their reproductive activity (vitellogenesis and egg production) [22] [23]. Contrary to expectations, the number of eggs laid per female was similar on both varieties of roselle although the seeds were significantly different in size (**Table 1**). Some results from previous studies have shown that oviposition is related to the size of host seed in several storage insect pests [24] [25] [26]. Chemicals present on the surface of the host seeds such as fatty acids and alkanes are involved in the induction of egg laying in *Callosobruchus maculatus* females [27]. In addition, the texture of the seeds can also influence egg-laying [28]. The chemical composition of the seed coat of both varieties is so far unknown, but interestingly it may be noted that they have the same rough texture and the same color, which could explain our results. However, in *C. maculatus* developing on several cowpea and chickpea varieties female egg laying was not related to host size or its texture [29]. For *S. niger*, the only parameter significantly influenced by the host seed variety is the size and weight of emerging insects of the first generation. Not surprisingly, insects from the larger seeds of the *Altissima* variety were larger and weighed more than those from the smaller seed of the *Sabdariffa* variety. This result suggests that insects of the second generation might have different life history traits depending on the variety on which they developed. Previous studies have shown that insect size especially that of females influences their life history traits [30] [31] [32] [33]. Specifically, bigger-size females could lay more eggs, producing bigger number of offspring, and so be

more damaging. This hypothesis assumes that insects developing from the seeds of the *Altissima* variety will have better fitness after several generations of development. Therefore, their potential for damaging the seeds could be greater compared to insects developing only on the seeds of the *Sabdariffa* variety. More precise studies are needed to confirm this hypothesis or not, but the increasing socio-economic importance of roselle, whatever the variety, deserves that effective seed storage solutions be deployed rapidly. The triple bagging technology a hermetic storage method that has proven effective in stored *H. sabdariffa* grain in Niger [34] could be recommended for roselle producers.

Acknowledgements

This research was conducted as part of the preparation of a PhD dissertation at the Université Ouaga I Pr Joseph KI-ZERBO, Burkina Faso. The authors are grateful to the authorities of the Ministry of Higher Education, Scientific Research and Innovation of Burkina Faso for a scholarship awarded to K J-C and for supporting his research.

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