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Report on *Spermophagus niger* Motschulsky, 1866 (Coleoptera: Chrysomelidae: Bruchinae: Amblycerini) infesting the seeds of roselle, *Hibiscus sabdariffa* L. (Malvaceae) during post-harvest storage in Burkina Faso

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ABSTRACT

This paper reports the presence and characteristics of the bruchid *Spermophagus niger* Motschulsky as a major pest of roselle (*Hibiscus sabdariffa* L.) seeds during post-harvest storage in Burkina Faso. Samples of roselle seeds collected in farmers granaries at three locations in Burkina Faso at the beginning of crop storage were brought to the laboratory and held for 2 months. All seed samples were infested with varying levels of *S. niger,* though samples from Tougan and Dedougou were more highly infested than those from Ouagadougou. Infested seeds generally had only one insect emergence hole, with seed perforation rates ranging from 1.8% to 4%. Insect rearing in the laboratory provided an opportunity for a clearer discrimination of sexual dimorphism and a better morphological description of the species. Males were smaller and weighed less than females. Post-embryonic development, which took place entirely within the seed, included four larval instars and one pupal stage; these stages are common in the subfamily Bruchinae. Our preliminary observations will enable a better understanding of this previously little-known insect pest. Furthermore, these results offer baseline data for further into appropriate post-harvest management of roselle in West Africa.

1. Introduction

In West Africa, roselle, *Hibiscus sabdariffa* L. (Malvaceae), is mainly cultivated in savanna and semi-arid areas (Schippers, 2000). Formerly grown over a small area, roselle is receiving increasing attention as a crop with potential for socio-economic impacts (Sanou et al., 2005). Roselle is increasingly being appreciated for its various uses, including the use of calyces to produce valuable food products such as liquors, jellies, beverages, and jams (Amin et al., 2008). Roselle calyces are therefore the object of trade between various African and European countries (McClintock and El Tahir, 2004; Egharevba and Law- Ogbomo, 2007), representing a notable source of income for roselle producers. In Burkina Faso, roselle leaves and calyces are also used to prepare local sauces. In addition, the seeds are subjected to solid-state fermentation to produce a meat substitute condiment that is consumed in Burkina Faso, where it is known as *Bi-kalaga* (Bengaly et al., 2006) and in many other West African countries as well (Atta et al., 2013). Roselle seeds are also a valuable food resource owing to their protein, calorie, and fat content as well as the substantial amount of fiber and valuable micronutrients (Akanbi et al., 2009). Roselle calyces are also rich in vitamin C, and contain nine times the amount in vitamin C as citrus (Amin et al., 2008). Calyces are infused to prepare a popular drink called *Bissap* that is consumed throughout West Africa.

Roselle plays an important role in household food economies in West Africa. However, production of roselle and post-harvest preservation of seeds are subjected to biotic and abiotic constraints. In Burkina Faso, several insect orders are causing damage to roselle crops (Sanou et al., 2005). Moreover, a bruchid species identified as *Spermophagus niger* Motschulsky 1866 has been

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described as infesting seeds in post-harvest storage (Amadou et al., 2016; Koussoube et al., 2016), and is responsible for major losses in storage. This paper describes for the first time the infestation of roselle seeds by this beetle species. Insect rearing under laboratory conditions enabled us to gain information on *S. niger* morphology, post-embryonic instars, seed infestation levels and damage to seeds.

2. Materials and methods

2.1. Collecting samples of roselle seeds and determining the level of infestation by S. niger

In December 2009, five batches of 500 g roselle seeds were collected from newly harvested crops from farmers production at each of the three following locations: Tougan (W 3°4′10″/N 13°04′21″), Dedougou (W 3°27′36″/N 12°27′47″) and around Ouagadougou (W 1°31′05″/N 12°21′58″). These regions are known as major producers of roselle in Burkina Faso. The seed batches were taken to the laboratory and immediately placed in Plexiglas boxes (18 × 11 × 4 cm) and monitored for 60 days. During this monitoring, insects that emerged from the seeds were collected from each seed batch and then counted. Insects were then divided, with fifty individuals placed into jars containing 70% alcohol and carefully labeled. The other portion of the insects were introduced into breeding boxes (10 × 8 × 3 cm), each containing 50 g of roselle seeds and fifty individual insects. The breeding insects were maintained at 32 ± 0.1 °C and 36% ± 1% r. h. After 60 days of monitoring, the 500 g-seed batches from each location were examined again to determine their damage status. Then, one-hundred seeds were randomly taken from each batch to count the perforated seeds.

2.2. Morphological identification of S. niger adults

2.2.1. Sex identification

All the collected insects proved to belong to the same species. After initial identification, the insect were thoroughly inspected under a binocular microscope to identify the most representative morphological traits. Using the insects that emerged in the rearing boxes, we first tried to detect any apparent sexual dimorphism between adults. The most critical morphological criteria were then identified. To confirm this initial identification, insects of each identified sex were placed for 24 h in Petri dishes, each containing ten roselle seeds that were free of any infestation. The insects were then removed from the boxes and individuals who laid eggs on the seeds were classified as females. The experiment was replicated 50 times to allow us to successfully distinguish males and females. After this, we were able to give precise and sex-specific morphological descriptions.

Twenty-five individuals of each sex were randomly selected from first-generation insects that emerged in rearing boxes. Size was measured from the head to the pygidium using a binocular microscope equipped with millimeter graph paper. Weight was determined using an OHAUS Analytical Standard microbalance.

2.3. Monitoring post-embryonic developmental instars

To determine post-embryonic development, we conducted an experiment in which we infested healthy roselle seeds with newly emerging *S. niger* mating pairs each day for 20 days. Every day, three pairs of insects were introduced into one of 10 separate Petri dishes, each containing 10 g roselle seeds, for 24 h. Starting on the date of the first egg hatching in each series of infested seeds, five seeds were dissected every 2 days to observe any developmental instars that occurred until the emergence of adults. This method allowed us to identify the major post-embryonic developmental instars of *S. niger*. We took measurements (length and width) and made descriptions of the major developmental instars based on the most representative individuals of each instar. Ten individuals of each post-embryonic developmental instar were selected for these measurements.

2.4. Data analysis

The recorded quantitative data were first subjected to analysis of variance (ANOVA) using SAS software version 9.1 (SAS, 2003). When significant differences were observed, means were separated using Fisher's LSD test at the 5% probability level.

3. Results

3.1. Adult insect morphology

The collected insects were morphologically identified as *Spermophagus niger* according to the general description provided by Borowiec (1991). The examined specimens were mainly dark brown in color and had a body covered with bristles that often formed distinct bands on the abdomen. The head, which faced downwards, appeared sharp, with 11 brown, thread-like antennal articles. The convex and clearly visible pronotum had sharp lateral edges and extended back from the head. Elytra, which were similar in males and females, were short with rounded posterior margins and never covered the pygidium. The legs were also dark brown, with strong tibia and femora in the hind legs. Sharp dorsolateral carinas were also present on hind tibia.

The distinction between male and female specimens was not obvious. Here, for the first time, we report reliable morphological criteria for distinguishing specimens by sex. First, the pygidium is curved in males but straight in females. Moreover, in the ventral view, the end of the pygidium is visible in the male but hardly noticeable in the female. Sexual dimorphism was also confirmed by body size measurements. Males tended to be significantly smaller and weighed less than females (Table 1).

3.2. Characterization of insect damage

All collected seed batches of roselle exhibited the emergence of *S. niger* individuals at varying levels of infestation and damage. The batches from Tougan and Dedougou were more infested than those from Ouagadougou (Table 2). Seed damage included the removal of internal reserves by larvae feeding during their development and perforation of seeds by adult insects once they were fully developed. Thus, 1.8%–4% of the seeds examined at the end of monitoring were perforated (Table 2). Infested seeds presented regular emergence holes and were covered with many eggs laid by newly emerging females. However, usually only one emerging hole was observed per seed.

Table 1
Mean (±SD) body size and weight of males and females *S. niger* emerging from roselle seeds. Min-Max represented minimal and maximal measurements.

	Body Size (mm)		Weight (mg)	
	Mean (±SD)	Min – Max	Mean (±SD)	Min – Max
Male (n = 25)	3.54 ± 0.16	3.4–3.8	5.16 ± 0.51	4.2–5.9
Female (n = 25)	3.76 ± 0.18	3.5–4.0	5.57 ± 0.58	4.0–6.3
ANOVA	20.37		6.65	
P	<0.0001		0.0130	