

Biological and morphological parameters of *Dysdercus maurus* fed with low- or high-oil cotton seeds





Abstract – The objective of this work was to determine the biological and morphological parameters of *Dysdercus maurus* fed with cotton (*Gossypium hirsutum*) seeds with a high or low oil content, as well as to identify genotypes to be used in breeding programs as sources of resistance to this stink bug. The development, survival, and reproduction of the cotton stainer bug were determined in a completely randomized experimental design. The treatments consisted of the insect nymphs being fed with cotton seeds of the CNPA 2001-5581 (high oil content) or CNPA 2001-5087 (low content) genetic line. Survival, weight, and morphological parameters of the bug were determined. The survival of second- and third-instar nymphs and of the total nymph stage of *D. maurus* was lower with cotton seeds with a low oil content. The body length and head width of *D. maurus* adults were greater, but pronotum length and width were smaller and the females heavier with cotton seeds with a high oil content. Low-oil cotton genotypes can reduce populations of the stainer bug.

Index terms: *Gossypium hirsutum*, cotton stainer bug, Hemiptera, seed-sucking bug.

Parâmetros biológicos e morfológicos de *Dysdercus maurus* alimentado com sementes de algodão com baixo ou alto teor de óleo

Resumo – O objetivo deste trabalho foi determinar os parâmetros biológicos e morfológicos de *Dysdercus maurus* alimentado com sementes de algodão (*Gossypium hirsutum*) com alto ou baixo teor de óleo, bem como identificar genótipos a serem usados em programas de melhoramento como fontes de resistência a esse percevejo. O desenvolvimento, a sobrevivência e a reprodução do percevejo manchador de algodão foram determinados em delineamento experimental inteiramente casualizado. Os tratamentos consistiram de ninfas do inseto alimentadas com sementes de algodão da linhagem genética CNPA 2001-5581 (alto teor de óleo) ou CNPA 2001-5087 (baixo teor). Foram determinados a sobrevivência, o peso e os parâmetros morfológicos do percevejo. A sobrevivência das ninfas de segundo e terceiro ínstars e do estágio total de ninfas de *D. maurus* foi menor com sementes de algodão com baixo teor de óleo. O comprimento do corpo e a largura da cabeça dos adultos de *D. maurus* foram maiores, mas o comprimento e a largura do pronoto menores e as fêmeas mais pesadas com sementes de algodão com alto teor de óleo. Genótipos de algodão com baixo teor de óleo podem reduzir as populações do percevejo.

Termos para indexação: *Gossypium hirsutum*, percevejo manchador do algodão, Hemiptera, percevejo sugador de sementes.

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Introduction

Cotton (*Gossypium hirsutum* L.) is an important crop worldwide (Sharif et al., 2019), whose fibers are used in natural textiles, seed oil for human consumption and biofuels, and cottonseed meal for animal feed (He et al., 2013; Sharif et al., 2019). In Brazil, cotton contributes significantly to agricultural economy, being grown in all regions of the country, covering an area of 1,610,300 ha, with a production of 2,691,400 tons (IBGE, 2020). Brazil is the fourth largest cotton producer, the second largest exporter of cotton lint, and the fifth largest production park in the world, with the cotton production chain including 27,500 companies with 1.5 million direct and indirect jobs in 2020 (Araújo, 2020).

However, the crop can be attacked by several pests, among which stand out cotton stainers of the genus *Dysdercus* (Pyrrhocoridae), which are important seed-sucking bugs in several countries around the world (Shah, 2014; Saeed et al., 2016; Shafiq et al., 2020). *Dysdercus maurus* Distant, 1901 (Heteroptera: Pyrrhocoridae) is one of the most competitive species for food resources, with a wide variety of host plants and geographical distribution (Almeida & Gonçalves, 2007). This stinkbug is one of the most destructive cotton pests in Brazil (Jorge & Lomônaco, 2009), causing either direct damage, by destroying cotton boll tissue and mature seeds, making them unviable and wrinkled, with weight loss and reduced oil content, or indirect damage, by the inoculation of opportunistic microorganisms (bacteria and fungi), mainly staining cotton fibers (Salem et al., 2013; Sontakke et al., 2013; Azher et al., 2019).

Cotton stainers predominantly feed on cotton seeds, which contain 14 to 25% lipids (Bellaloui & Turley, 2013; He et al., 2014, 2015; Bellaloui et al., 2015). Lipids, also called oils or fats, are the main reserve material of cotton seeds, and genetic improvement programs aim to increase their content (Carvalho et al., 2017). Lipids are generally present as glycerides (triglycerides) and unsaturated fatty acids, such as oleic (15%), linoleic (58%) and palmitic (26%) acids, besides others (1%) (Matthäus & Özcan, 2015). These compounds are essential for seed-sucking insects (Berezina, 2017), especially in periods of prolonged energy demand, which make seeds the preferred feeding sites, from which nutrients are more easily obtained (Panizzi, 2000; Visser et al., 2017). However,

seed suckers also obtain nutrients from other plant tissues, such as growing tips or flowers, in periods of seed scarcity, although, in general, these sources do not allow a complete nymph development or egg production (Panizzi, 2000).

Therefore, seed-sucking bugs can store reserves as lipids, whose content varies with the nutritional quality of genotypes (Panizzi et al., 2012). For this reason, in the present study, it was hypothesized that the biological performance of *D. maurus* should vary according to the oil content of cotton seeds, which requires further studies for the integrated management of this pest, in order to reduce its damage to cotton plants.

The objective of this work was to determine the biological and morphological parameters of *D. maurus* fed with cotton seeds with a high or low oil content, as well as to identify genotypes to be used in breeding programs as sources of resistance to this stink bug.

Materials and Methods

The study was conducted in an experimental field (7°13'31"S latitude, 35°54'18"W longitude) and in the Laboratory of Entomology of Embrapa Algodão, located in the municipality of Campina Grande, in the state of Paraíba, Brazil (7°13'32"S latitude, 35°54'19"W longitude).

Dysdercus maurus specimens were collected in the field in a 50 m² (10x5 m) experimental area, cultivated with the BRS Safira cotton cultivar, and were reared in the laboratory, at 25±2°C, 60±10% relative humidity, and 12-hour photophase (Mendes, 1964; Shah et al., 2016), for five generations on fresh cotton seeds without soaking in water. The used species was identified by Blöte's dichotomous key (Lima et al., 1962).

The CNPA 2001-5581 and CNPA 2001-5087 cotton genetic lines, with a high and low oil content, respectively, were cultivated in the field on March 15, 2019, in two areas of 81 m² (8.1x10 m) spaced at 0.90x0.20 m, leaving one plant per pit after thinning. The plants of each area were self-fertilized, and, after their formation at 142 days, bolls were collected to determine their seed oil content and to be supplied to the cotton stainer bug. The seed oil content of 50 bolls of each genetic line of cotton was determined by solvent extraction (AOCS, 2005) (Figure 1), and the remaining cotton seeds of each genetic line in the field

were also collected and offered to the cotton stainer bugs as food.

Per treatment, 100 fertilized *D. maurus* eggs were placed in a transparent plastic container (6 cm in diameter by 2 cm in height) with a lid.

After hatching, 80 first-instar *D. maurus* nymphs were separated and kept in groups of ten per plastic container; half were fed ad libitum with cotton seeds with a high oil content and water and the other half, with cotton seeds with a low oil content and water, from the first instar until the adult stage. The experimental design was completely randomized, with two treatments and 40 replicates. The treatments consisted of the cotton stainer bug nymphs being fed with either seeds of the CNPA 2001-5581 (high oil content) or CNPA 2001-5087 (low oil content) cotton lines. The insects were kept in climate chambers, with temperature of $25\pm1^{\circ}\text{C}$, relative humidity of $60\pm10\%$, and photoperiod of 12 hours, for 96 days. Water was supplied through a cotton ball kept in the central part of the base of the container. Seeds and cotton balls were changed every two days. *Dysdercus maurus* adults were sexed (Mendes, 1964), separated into couples, and mated in plastic containers, where they received cotton seeds and water until their death.

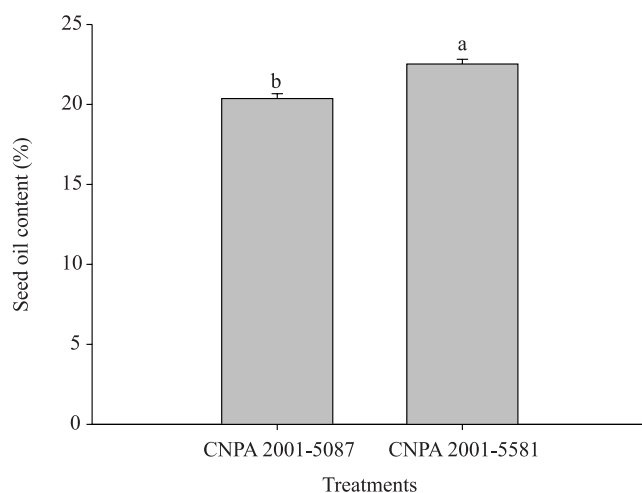


Figure 1. Oil content in cotton (*Gossypium hirsutum*) seeds of the CNPA 2001-5087 and CNPA 2001-5581 genetic lines, with a low and high oil content, respectively. Bars followed by the equal lowercase letters per treatment do not differ by Fisher's LSD test, at 5% probability.

Biological observations were performed daily at 8:00 a.m. and 4:00 p.m. with the aid of a stereoscopic microscope.

The biological variables determined were: number of instars, development period and survival of each instar, development period and survival of total nymph stage, and number of nymphs that originated males or females. The periods of preoviposition, oviposition, postoviposition, longevity, fecundity, and the number and percentage of viable eggs were also evaluated.

The weight and biometrics of *D. maurus* were determined, respectively, using the AY220 precision analytical balance (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), with a decimal precision of 0.0001 g, and the EL224 stereomicroscope (BEL Equipamentos Analíticos LTDA., Piracicaba, SP, Brazil), with 20-fold magnification. Five females and six males per treatment were weighed one day after emergence, had their morphological variables obtained after their death, and were preserved for up to 24 hours in 70% alcohol and then mounted with an entomological pin on Styrofoam covered by graph paper.

Oil percentage in the cotton seeds and the development data of *D. maurus* were subjected to the analysis of variance (Anova), and means were compared by Fisher's LSD test, at 5% probability. Data of *D. maurus* weight and morphological parameters were subjected to the Anova, and means were compared by Tukey's test, also at 5% probability. Statistical analyses were performed using the Saeg statistical and genetic analysis system (Ribeiro Jr., 2001).

Results and Discussion

The survival of immature stages of *D. maurus* varied between treatments and insect development stages (Table 1). The survival of second- and third-instar nymphs and of the total nymph stage of *D. maurus* was lower when the insect was fed with cotton seeds with a low oil content. However, nymph hatching and survival in the fourth and fifth instar were higher with low- and high-oil cotton seeds, respectively. This suggests that surviving nymphs depend on the oil content in the cotton seed, because the consumption of seeds with a low oil content can affect the energy demand for the metabolic processes responsible for the survival of the insect (Panizzi et al., 2012). Survival

was lowest in the second and third instars and in the total nymph stage when cotton seeds with a low oil content were supplied. This higher mortality rate in the second and third instars partially agrees with the results obtained for the same species, but fed with *Chorisia speciosa* St. Hil. (Bombacaceae) seeds and with the IAC 22 cotton cultivar with a low oil seed content (Almeida & Gonçalves, 2007), as well as for *Dysdercus peruvianus* Guérin-Ménéville fed with seeds of this same cotton cultivar (Milano et al., 1999).

The cotton genotype also affected the development period (days) of *D. maurus* (Table 1). The periods of egg incubation, total nymph stage, egg to adult, and first and fourth instars were similar when the insect was fed with cotton seeds with a low or high oil content, but those of the second, third, and fifth instars differed between treatments. The duration of the second instar and of the third and fifth instars was longer for insects that were fed with cotton seeds with a low and high oil content, respectively. This shows that variations in the duration of the second, third, and fifth instars of *D. maurus* are related to the oil content of the cotton seeds. In general, when seed-sucking cotton stainer nymphs store more lipids, they accumulate less water in their bodies, which may increase metabolic water production to compensate for water deficiency, affecting their development period (Mishra et al., 2004; Sláma & Lukáš, 2016). However, this depends on juvenile hormone production and total metabolic lipid combustion in the diet, as reported for *Pyrrhocoris apterus* L. (Jindra et al., 2013; Vinokurov et al., 2014; Sláma & Lukáš, 2016). In the present study, the fifth instar of *D. maurus* always lasted longer than

the others in both cotton genotypes. This is common in Hemiptera, since intense changes occur when the insects are nearing maturity and a larger amount of nutrients is required for molting and to produce adults with a good reproductive potential (Panizzi et al., 2012).

The periods of preoviposition, oviposition, and postoviposition, as well as longevity and fecundity, were similar for *D. maurus* females fed with cotton seeds with either a high or low oil content, but the number of viable eggs and nymphs hatched were higher for those fed with high-oil cotton seeds (Table 2). The lower egg viability of *D. maurus* females fed with cotton seeds with a low oil content may be related to the lower energy value of this food (Sahayaraj et al., 2011). Seed-sucking insects spend energy constantly and cannot survive periods of food scarcity without accumulating reserves in periods of food abundance by feeding on seeds with a high oil content (Arrese & Soulages, 2010). Therefore, fat reserves have direct implications on the energy metabolism of insects (Bártů et al., 2010; Toprak et al., 2020), being the most important reserve used to meet their energy demand during metamorphosis, diapause, embryogenesis, and flight (Toprak et al., 2020).

The body of *D. maurus* adults was longer and their head wider when fed with cotton seeds with a high oil content, while their body was longer and pronotum wider, with low-oil cotton seeds (Table 3). The abdominal width and antenna length of the stainer bug were greater for females fed with cotton with a high and low oil seed content; however, head length and interocular distance did not differ between treatments

Table 1. Survival and development period (days, mean±standard error) of the egg stage, of the first, second, third, fourth and fifth instars, and of the egg and total nymph stage of *Dysdercus maurus* (Hemiptera: Pyrrhocoridae) fed with seeds from cotton (*Gossypium hirsutum*) lines with a low (CNPA 2001-5087) or high (CNPA 2001-5581) oil content at 25±1°C, 60±10% relative humidity, and 12-hour photophase⁽¹⁾.

Stage	Low oil content			High oil content		
	Survival (%)	Period (days)	N ⁽²⁾	Survival (%)	Period (days)	N ⁽²⁾
Egg	100.0	5.00±0.00A	40	100.0	5.00±0.00A	40
First instar	90.0	4.00±0.00A	36	90.0	4.00±0.00A	36
Second instar	30.6	5.00±0.39A	11	91.7	3.00±0.00B	33
Third instar	45.5	4.00±0.00B	5	96.9	5.91±0.35A	32
Fourth instar	100.0	4.80±0.18A	5	96.9	6.29±0.39A	31
Fifth instar	100.0	8.00±0.00B	5	96.8	9.70±0.27A	30
Total nymph stage	12.5	26.60±0.22A	5	75.0	28.77±0.49A	30

⁽¹⁾Means followed by equal letters, uppercase in the lines, do not differ by Fisher's LSD test, at 5% probability. ⁽²⁾N, number of individuals.

or sexes. Conversely, the interaction between the weight of *D. maurus* adults with treatments and gender was significant (Figure 2), indicating variation in this parameter with cotton seed type and insect sex. *Dysdercus maurus* females, fed with cotton seeds with a high oil content, were heavier than those fed with a low oil content and than males from both treatments. Moreover, the weight of *D. maurus* females fed with cotton seeds with a low oil content was similar to that of males fed with either seed type. Therefore, food nutritional quality affects the morphology and weight of cotton stainer bugs, as reported for *Dysdercus koenigii* Fabricius, which showed higher biometric

parameters when fed with the MNH-886 cotton seed cultivar (higher oil content) than with *Abelmoschus esculentus* L. (lower oil content) (Naqqash et al., 2014). The body of *Dysdercus nigrofasciatus* Stål adults was larger when fed with cotton seeds with a high oil content than with seeds of *Dombeya emarginata* AE Bruce (Malvaceae) with a low oil content (Pearson, 1958). A larger body size and heavier weight increase the chances of the insect surviving during periods with limited or no food, facilitating its dispersal and fecundity (Panizzi et al., 2012; Sturm, 2016). Larger females of *Dysdercus andreae* (Linnaeus), *Dysdercus bimaculatus* Stål, *Dysdercus fasciatus* Signoret, *Dysdercus mimus* Blöte, *Dysdercus mimulus* Hussey, *D. nigrofasciatus*, *Dysdercus supersticiosus* Fabricius, and *Dysdercus suturellus* (Herrich-Schäffer) laid a greater number of eggs in a shorter time than the smaller ones (Derr et al., 1981).

The survival of second- and third-instar nymphs and of the total nymph stage (Table 1), as well as the number of viable eggs per *D. maurus* female (Table 2), were lower with cotton seeds with a low oil content. Since seed-sucking insects typically do not use other foods, the low-oil cotton varieties can reduce populations of the bug in areas with a history of its occurrence. This is important because it is possible to obtain, via conventional breeding, productive cotton varieties with a high fiber quality and a low oil content (Khan et al., 2010). This shows the need to select cotton lines with a low seed oil content in breeding programs for plant resistance to the cotton stainer bug.

Table 2. Reproductive characteristics (mean±standard error) of *Dysdercus maurus* (Hemiptera: Pyrrhocoridae) fed with seeds from cotton (*Gossypium hirsutum*) lines with a low (CNPA 2001-5087) or high (CNPA 2001-5581) oil content at 25±1°C, 60±10% relative humidity, and 12-hour photophase⁽¹⁾.

Variable	Low oil content	High oil content
Preoviposition (days)	7.50±0.67a	10.54±1.28a
Oviposition (days)	27.50±7.35a	36.38±4.17a
Postoviposition (days)	9.67±1.58a	19.31±7.59a
Longevity (days)	44.67±8.84a	66.23±9.43a
Fecundity	353.00±82.61a	372.62±54.63a
Number of viable eggs	51.17±17.37b	227.00±34.57a
Hatching (%)	16.23±3.66b	59.79±5.06a

⁽¹⁾Means followed by the equal letters, lowercase in the lines, do not differ by Fisher's LSD test, at 5% probability.

Table 3. Morphological characteristics (mean ± standard error) of *Dysdercus maurus* (Hemiptera: Pyrrhocoridae) adults fed with seeds from cotton (*Gossypium hirsutum*) lines with a low (CNPA 2001-5087) or high (CNPA 2001-5581) oil content at 25±1°C, 60±10% relative humidity, and 12-hour photophase⁽¹⁾.

Morphological characteristic (mm)	Low oil content		High oil content	
	Male	Female	Male	Female
Body	15.67±0.33bA	15.00±0.55bA	16.67±0.76aA	17.20±0.20aA
Abdominal width	4.00±0.00aB	4.60±0.24aA	3.67±0.33aB	5.00±0.00aA
Head length	1.50±0.22aA	1.60±0.24aA	1.33±0.21aA	2.00±0.00aA
Head width ⁽²⁾	1.00±0.00bA	1.20±0.20bA	1.67±0.21aA	2.00±0.00aA
Interocular distance ⁽³⁾	1.83±0.17aA	2.00±0.00aA	2.00±0.00aA	2.00±0.00aA
Antenna length	7.67±0.21aB	8.20±0.20aA	7.83±0.31aB	8.40±0.24aA
Pronotum length	1.33±0.21aA	1.60±0.24aA	1.00±0.00bA	1.00±0.00bA
Pronotum width ⁽⁴⁾	2.33±0.21aA	2.80±0.37aA	2.00±0.00bA	2.00±0.00bA

⁽¹⁾Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ by Tukey's test, at 5% probability. ⁽²⁾Between the outer edges of the eyes. ⁽³⁾Between the inner edges of the eyes. ⁽⁴⁾At the humerus level.

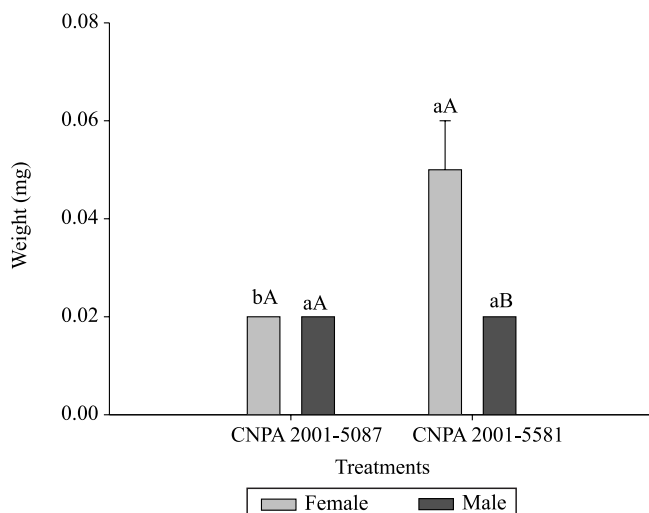


Figure 2. Weight of females and males of *Dysdercus maurus* (Hemiptera: Pyrrhocoridae) fed with seeds from cotton (*Gossypium hirsutum*) lines with a low (CNPA 2001-5087) or high (CNPA 2001-5581) oil content at 25±1°C, 60±10% relative humidity, and 12-hour photophase. Bars followed by equal letters, lowercase per treatment or uppercase per genus, do not differ by Tukey's test, at 5% probability.

Conclusions

1. The survival of second- and third-instar nymphs and of the total nymph stage of *Dysdercus maurus* is lower when the stainer bug is fed with cotton seeds with a low oil content.

2. The body length and head width of *D. maurus* adults are greater when the insect is fed with cotton seeds with a high oil content, but the length and width of the pronotum are smaller.

3. The weight of *D. maurus* females is greater when they are fed with cotton seeds with a high oil content, but that of the males is similar between those fed with seeds with either a high or low oil content.

4. Cotton genotypes with a low oil content can reduce cotton stainer bug populations.

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