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Bioassay

Evaluation of artificial substrate for oviposition and embryonic development of *Xylocoris sordidus* (Reuter, 1871) (Hemiptera: Anthocoridae)

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Abstract. The use of predators of the family Anthocoridae in biological control programs depends on efficient methodologies for their mass rearing. Thus, this study aimed to evaluate the potential of an alternative substrate for oviposition and egg viability of the predator *Xylocoris sordidus* (Reuter, 1871) (Hemiptera: Anthocoridae). For this purpose, no-chance and double chance choice tests using natural (*Bidens pilosa* L.) and artificial substrates (cotton rolls) were made. In the double chance choice test, *X. sordidus* preferred the natural substrate for oviposition with about 12 times more eggs laid on the natural substrate. In the no-chance test, we observed no difference in the number of eggs laid on the substrates with means of 18.73 and 19.92 eggs for natural and artificial substrates respectively, however; egg viability was higher on cotton rolls with 85.5 and 99.8% for eggs laid on natural and artificial substrates respectively. The results demonstrated that cotton rolls substrate offers advantages for oviposition and egg viability, thus being strongly recommended for mass rearing of *X. sordidus.*

Keywords: Protocols rearing, cost-benefits, predator.

Predators of the family Anthocoridae (Hemiptera) can locate and feed on prey efficiently, and they can also survive in the near absence or dearth of prey by consuming alternative food sources, which may facilitate their permanence in fields (Burgio et al. 2004; Brito et al. 2009). This behavior makes them potential agents for the biological control of many agricultural pests, such as mites, thrips, aphids, psyllids and eggs, larvae or pupae of Lepidopteran pests (Xu et al. 2006; Mahmoudi-Dehpahni et al. 2020; Santos et al. 2020; Gharbi 2021).

The use of these predators in biological control programs depends on efficient methodologies for mass rearing in abiotics conditions in the laboratory prior to field release. The methodology for rearing must be cost and space-efficient while enabling the production of good quality individuals in a desirable quantity (Cohen 2015).

Model rearing methodologies for some species, such as *Orius* spp. and *Xylocoris* spp., depends on plant substrates for oviposition, as they are usually performed endophytically, with *Bidens pilosa* L. being widely used for this purpose (Mendes et al. 2005; Matsuyama et al. 2018; Santos et al. 2020). The use of plant material as oviposition substrate depends on its constant production in fields or greenhouses to maintain insect colonies in the laboratory (Rahman et al. 2009; Pascua et al. 2019).

Vieira et al. (2021) described a rearing methodology in which cotton rolls were used as an artificial oviposition substrate to optimize the rearing of some anthocorid species. However, the influence of this artificial substrate on the reproduction of these species has not been reported in the literature. Thus, the goal of this study was to evaluate the efficiency of cotton rolls as an artificial substrate in comparison with the natural substrate (*B. pilosa*) for oviposition and egg viability of *Xylocoris sordidus* (Reuter, 1871) (Hemiptera: Anthocoridae).

Forty-five *X. sordidus* couples with 24 h of adult stage were obtained from a colony kept in laboratory conditions (temperature = 25 ± 1 °C; relative humidity = 70% ± 10%; photoperiod = 12 h). The adults were being fed "*ad libitum*" with *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) eggs.

The substrates tested were bouquets of *B. pilosa* (10 cm) (natural substrate) and hydrophilic cotton rolls (10 cm) moistened with distilled water (alternative substrate). 30 couples of *X. sordidus* (15 for each treatment) were used for the no-choice test, and Petri dishes (15 cm in diameter) lined with filter paper to maintain humidity were used as arena with the oviposition substrates positioned in the center with *C. cephalonica* eggs as feed. 15 couples were used for the double chance choice test with the same arena type and with the two substrates positioned in the sides of the arena. For seven days, the substrates were daily changed, and *C. cephalonica* eggs were offered as feed in the center of the arena equidistant from the substrates. The eggs laid on both substrates were counted and then stored in Petri dishes until the emergence of the nymphs when the viability of the eggs was evaluated.

The number of eggs and percentage viability were submitted to the Kolmogorov and Bartlett tests (Proc UNIVARIATE) to verify the normality of the residuals and homogeneity of their variances, respectively. When the data meet these assumptions, analysis of variance (ANOVA) and Student's *t*-test (Proc UNIVARIATE) were used to compare the treatments ($P < 0.05$). Even when using the most adequate transformation, the egg viability data did not meet the requirements for ANOVA, thus we made comparisons using the Wilcoxon test (Proc NPAR1WAY). The frequency data from the double chance choice test were interpreted using the chi-square (χ^2) test (Proc FREQ), in which 1:1 was the null hypothesis, assumed if the female of *X. sordidus* had no preference for one substrate over the other. All data analyses were conducted using the SAS software (SAS Institute 2015).

In the no-choice test we observed similar *X. sordidus* oviposition levels in both substrates ($F_{1, 26} = 6.98$; $P = 0{,}0838$) and higher egg viability in the hydrophilic cotton rolls compared to *B. pilosa* bouquets ($Z = 1.8004$; $P = 0.0359$) (Tab. 1).

When both substrates were offered at the same time to couples, there was greater preference for *B. pilosa* bouquets than for hydrophilic cotton rolls ($\chi^2 = 76.21$; GL = 1; $P < 0.0001$). For a total of 177 eggs laid,

163 eggs were found on the *B. pilosa* substrate while only 14 eggs were found on the hydrophilic cotton substrate. Egg means were 12.5 on the *B. pilosa* substrate and 4 on the hydrophilic cotton roll substrate (Fig. 1).

Table 1. Number and viability of eggs laid by *Xylocoris sordidus* (Reuter, 1871) on different oviposition substrates in the non-choice test.

Substrate	Eggs		
	Total per substrate	Means[1]	Viability (%)[2]
Bidens pilosa L.	256	18.73 ± 1.54 a	85,51 ± 12,1 b
Hydrophilic cotton rolls	262	19.92 ± 1.77 a	99.85 ± 0.36 a

[1]Means (± SE) within a column followed by different letters are statistically different (Student-Newman-Keus Test, $P < 0.005$). [2]Means (± SE) within a column followed by different letters are statistically different (Wilcoxon Test, $P < 0.005$).

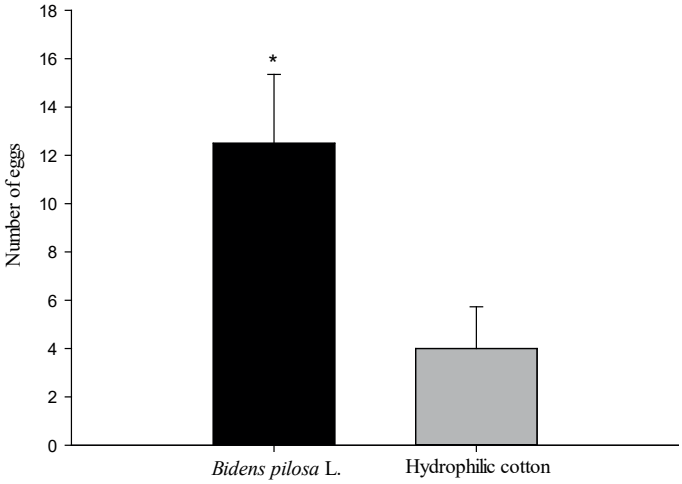

Figure 1. Means of eggs laid by *Xylocoris sordidus* (Reuter, 1971) on different oviposition substrates in the double chance-choice test, (*) = statistically significant.

We observed the presence of the *X. sordidus* couples more often on the bouquets in comparison with the hydrophilic cotton rolls. This behavior could be due to the fact that the natural substrate offers more protective locations than the hydrophilic cotton rolls, and insects from the family Anthocoridae sometimes complement their diet by feeding on plant resources (Armer et al. 1998; Lundgren et al. 2009; Seagraves et al. 2011).

Cotton rolls have key benefits when used as an oviposition substrate. Cotton rolls for insect rearing can be supplied consistently throughout the year, excluding the need for cultivation and collection of natural substrates, which are seasonal, variable and pose the risk of bringing pathogens from the field to the laboratory colony (Vieira et al. 2021). In addition, cotton rolls can provide and retain better humidity for eggs, which is another important factor for eggs viability (Tawfik et al. 1986; Santos et al. 2020). Our results demonstrated that the cotton rolls offer quantitative and qualitative advantages as shown by the high number of eggs laid and greater egg viability of *X. sordidus* compared with the natural substrate, thus the cotton roll oviposition substrate for mass rearing of *X. sordidus* is strongly recommended.

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Author's Contributions

Pinto, MMD: Data collection, statistical analysis, methodology, writing; Santos, ILP: Data collection, methodology; Barros, TN: Data collection, methodology; Dias, LEO: Data collection, methodology; De Bortoli SA: Supervision, writing, review.

Conflict of Interest Statement

There is no conflict of interest.

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