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Oviposition preference of the neotropical brown stink bug *Euschistus heros* on artificial substrates of different colors

Preferência de oviposição do percevejo marrom *Euschistus heros* em substratos artificiais de diferentes cores

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ABSTRACT

*This study aimed to evaluate the oviposition preference of the Neotropical brown stink bug, **Euschistus heros** (F.) on artificial substrates of different colors (felt woven). For this, ten pairs of stink bugs were transferred into plastic pots. Each pot contained seven felts of 6x20 cm (100% polyester-atoxic) of different colors (white, black, blue, green, red, yellow and brown). The pots were evaluated daily for three weeks (21 days) being counted the number of eggs, clutches and eggs per clutch for each color of felt. The choice test was set in a completely randomized design. Data obtained was submitted to the analysis of variance and means were compared by Scott-Knott test at 5% probability. During the evaluation were produced 7074 eggs distributed in 977 clutches, accounting a mean of 7.06±0.67 eggs per clutch. Results indicated that females laid a significantly greater number of eggs (2380 and 1686) and a greater number of clutches (319 and 233) on the yellow and white felts, respectively, than on the remaining substrates. The substrates in color red, black and brown were the least preferred, and the colors blue and green were intermediate when compared with others. Therefore, the substrates colored yellow and white are suggested to be used in rearing **E. heros** in the laboratory.*

Key words: *behavior, ecology, egg laying, insect pest, insect rearing.*

RESUMO

*Este estudo teve como objetivo avaliar a preferência para oviposição do percevejo marrom **Euschistus heros** (F.) em substratos artificiais de diferentes cores (feltros). Para isso, dez casais de percevejos foram transferidos para potes plásticos que continham sete feltros de 6x20 cm (100% poliéster atóxico) de cores diferentes (branco, preto, azul, verde, vermelho, amarelo e marrom). Os potes foram avaliados diariamente durante três semanas (21 dias), sendo contado o número de ovos, posturas e de ovos por postura para cada cor de feltro. O teste de preferência foi montado em um delineamento experimental inteiramente casualizado. Os dados obtidos foram submetidos à análise de variância e as médias comparadas pelo teste* Scott-Knott *a 5% de probabilidade. Durante a avaliação, foram produzidos 7.074 ovos, distribuídos em 977 posturas, contabilizando uma média de 7,06±0,67 ovos por posturas. Os resultados indicaram que as fêmeas ovipositaram um número significativamente maior de ovos (2380 e 1686) e de posturas (319 e 233) sobre os substratos de cores amarela e branca, respectivamente, do que nos demais substratos coloridos. Os feltros das cores vermelha, preta e marrom foram os menos preferidos, e as cores azul e verde foram intermediários, quando comparados aos demais. Assim, os substratos de cor amarela e branca são sugeridos para utilização na produção de ovos e criação de **E. heros** em laboratório.*

Palavras-chave: *comportamento, ecologia, oviposição, inseto-praga, criação de insetos.*

INTRODUCTION

Many species of insect pests have been reported attacking soybean, ***Glycine max*** (L.) Merrill (***Fabaceae***), and among the most important is the Neotropical brown stink bug, ***Euschistus heros*** (F.) (Heteroptera: ***Pentatomidae***) (PANIZZI et al., 2012). The impact of feeding this species can cause significant losses in yield, quality and germination potential of grains (PANIZZI & SLANSKY-JÚNIOR, 1985; PANIZZI et al., 2000a; CORRÊA-FERREIRA & AZEVEDO, 2002; SANTOS, 2008). Several studies have been performed with ***E. heros***

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on its biology, ecology and behavior in order to develop effective control tactics (SILVA et al., 2011; PANIZZI, 2013). Thus, rearing and maintaining phytophagous stink bugs in the laboratory is important to allow the conduction of biology tests, resistant strains tests, efficacy of insecticides, use in biological control programs with parasitoids, identification of pheromones, among others (CORRÊA-FERREIRA, 2002; PEREZ & CORRÊA-FERREIRA, 2004; GODOY et al., 2005; SILVA et al., 2008; SILVA et al., 2011).

Research shows that ***E. heros*** can be easily maintained in laboratory conditions, resulting in high rates of survival and fecundity (PARRA, 1992; SILVA et al., 2008). However, the major challenge to rear stink bugs in the laboratory is to provide adequate substrates of oviposition to concentrate postures and reduce the time of handling during the maintenance of colonies. Studies using artificial oviposition substrates with different species of stink bugs show that these insects can lay eggs on paper towel hanging inside cages (SHEARER & JONES, 1996), in voil (BUNDY & McPHERSON, 2000), on plastic structures that mimic soybean leaves (PANIZZI et al., 2000b) on cheesecloth stretched treated with chemical extract of soybean pods (PANIZZI et al., 2004), on wool yarn, (SILVA & PANIZZI, 2008), and also on cotton balls (SILVA & PANIZZI, 2007). All these artificial substrates have proved to be appropriate for oviposition of several pentatomids species, but not evaluated the color of the substrates as attractive characteristics to stink bugs. In this context, the present study aimed to verify the oviposition preference by ***E. heros*** on artificial substrates of different colors (felts), with the purpose of improving the rearing technique of this stink bug in the laboratory.

MATERIAL AND METHODS

The ***E. heros*** colony was established in the laboratory from insects adults field collected. Adult stink bugs were placed in cages (40cmx40cmx60cm) for nine days to reach sexual maturation (COSTA et al., 1998). After this time, ten pairs of ***E. heros*** were transferred into plastic pots of 8L. Each pot contained seven felts of 6x20cm (100% polyester-atoxic) of different colors: white, black, blue, green, red, yellow and brown. The Treatments were replicated five times (five pots with seven different colors). The sexing of stink bugs was done according BORGES et al. (2006). The diet used to feed the stink bugs was composed of green bean, soybean, peanut (COSTA et al., 1998) and sunflower seeds. The pots were evaluated daily for three weeks (21 days) being counted the number of eggs, clutches and eggs per clutch for each color of felt. The choice test was set in a completely randomized design. Data obtained was submitted to Shapiro-Wilk test (normality test) and how it not presented normal distribution, the values were compared by Kruskal-Wallis test (nonparametric test) at 5% probability using the software Statistica.

RESULTS AND DISCUSSION

All colors of felt were used by ***E. heros*** for oviposition, and during evaluation were obtained 7074 eggs distributed in 977 clutches, accounting for an average of 7.06±0.67 eggs per clutch (Figure 1 a - h and Table 1). The colors of felt that had higher number of eggs were yellow (2380) and white (1686), with statistical differences between them and also among the blue (840) and green (797) colors. Red (470), black (463) and brown (438) felts had the lowest amounts of eggs. This same pattern was observed for the number of clutches. Regarding the average number of eggs per clutch, there was no significant differences between the colors. These results show that ***E. heros*** have oviposition preference between artificial substrates to lay its eggs, and that colored yellow and white were preferred (Table 1, Figures 1 i - k).

In general, there is not a clear relation of the substrate color (natural or artificial) with the insect fecundity. Most studies relate the effects of colors with photo-tactical behavior (movement in response to light stimuli) (DREW et al., 2003). The few studies of attractiveness with colors were performed with Lepidoptera, Diptera, Hymenoptera and Coleoptera, e.g., (KATSOYANNOS et al., 1985; PEITSCH et al., 1992; CHITTKA et al., 1992; AZEREDO, 2006; SANTOS et al., 2008; GREGORIO et al., 2010). McINNIS (1989) was one of the few that studied the influence of color on fecundity of tephritid (Diptera) and observed a significantly smaller number of eggs in blue substrates compared to blacks and reds. To our knowledgement, there are no studies that relate the color of the artificial substrate with the oviposition of stink bugs.

According to SILVEIRA-NETO et al. (1976), the insects have 100% response at wavelengths around 365nm, a value within the range of ultraviolet light. However, LANDOLT et al. (1988) report that the insects are generally attracted by the portion preferably yellow-green (500-600nm) of the light spectrum, and this wavelength is typical of green foliage and mature fruits. This characteristic has been related by RODRIGUES-NETTO et al. (2002) who

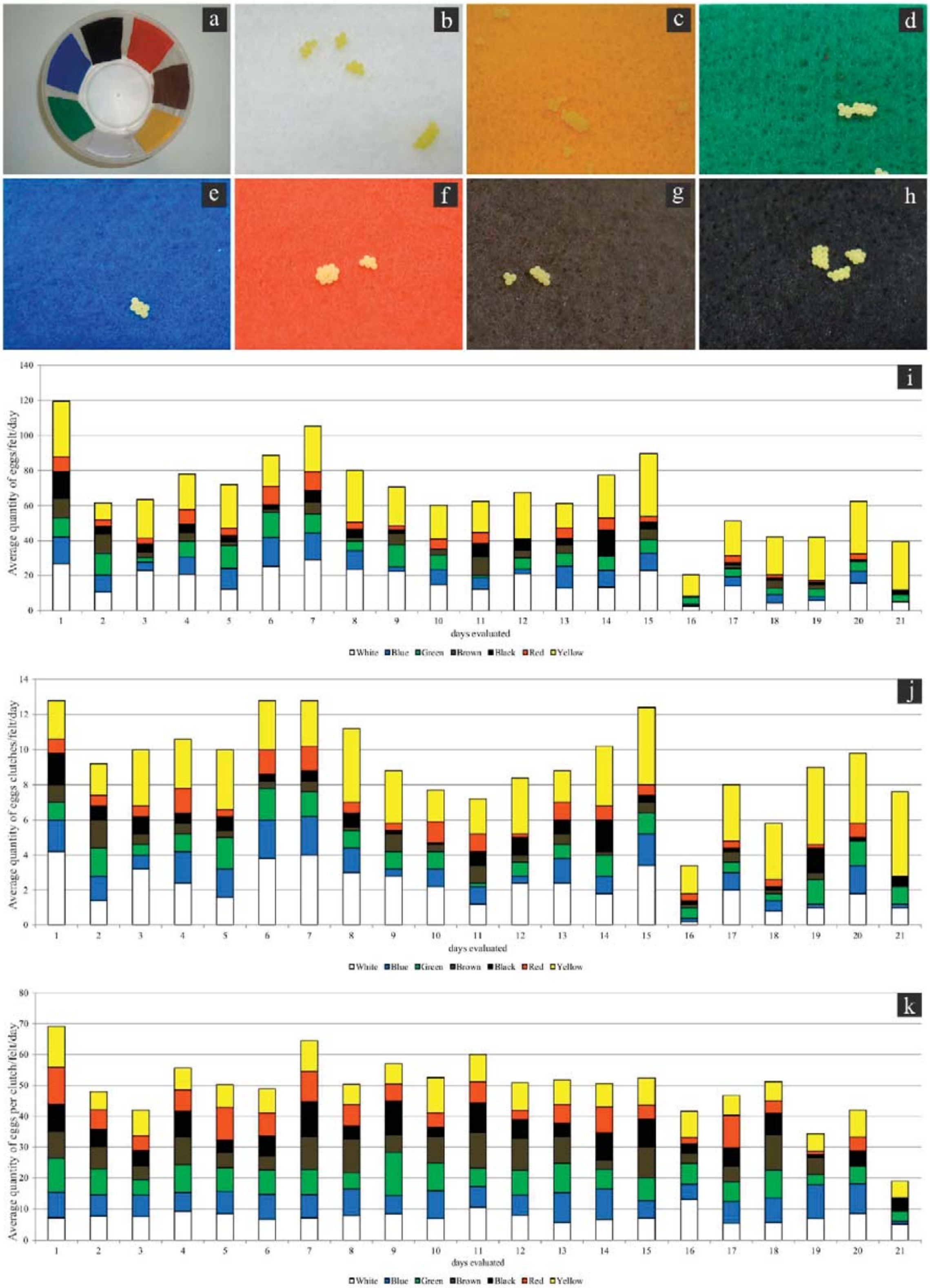

Figure 1 - Colours used for experiment. Pot with all the felts (a), white (b), yellow (c), green (d), blue (e), red (f), brown (g) and black (h). Quantity (average) of eggs (i), clutches (j) and eggs per clutch (k) laid by ***Euchistus heros*** on artificial substrates (felt) of different colors in the laboratory. Tangará da Serra/MT. Brazil, 2011.

Table 1 - Fecundity (average±SE[1] and total values*) of ***Euschistus heros*** on artificial substrates (felts) of different colors tested in rearing conditions in the laboratory. Tangará da Serra/MT. Brazil, 2013.

Color	Eggs	Clutches	Eggs per Clutch[ns]
Yellow	22.67 ± 1.42 a[2] (2380)*	3.04 ± 0.21 a (319)	7.94 ± 0.42
White	16.06 ±1.70 a (1686)	2.22 ± 0.24 a (233)	7.52 ± 0.40
Blue	8.00 ± 1.07 b (840)	1.35 ± 0.25 b (119)	7.48 ± 0.46
Green	7.59 ± 0.83 bc (797)	1.04 ± 0.09 b (109)	7.38 ± 0.54
Red	4.48 ± 0.70 bc (470)	0.70 ± 0.10 bc (70)	7.25 ± 0.76
Black	4.41 ± 0.88 c (463)	0.67 ± 0.11 bc (73)	6.12 ± 0.66
Brown	4.17 ± 0.75 c (438)	0.51 ± 0.09 c (54)	5.75 ± 1.12
p	<0,0001	<0,0001	0.178
H	195.522	199.633	8.921

[1]Standard error (SE); [2]Average followed by same letter in the column do not differ by Kruskal-wallis test at 5%; [ns]Not significant

observed higher efficiency of yellow traps to attract tephritid (Diptera). Moreover, DREW et al. (2003) suggest that the visual responses of some insect species may reflect the genetic propensity of these organisms to be attracted by colors similar to those of their host plants. This can explain the results found in yellow felt, which presented the highest number of eggs and clutches, even though the green color has not shown good results for oviposition.

Other factor to be considered is the egg mass coloration. In the case of ***E. heros*** the yellow egg mass (COSTA et al., 1998) match with the yellow felt, and this could be analyzed as an attempt to “camouflage” (hide) the eggs from possible threats (see Figure 1 c).

In the case of the high preference of ***E. heros*** to lay its eggs on the white felt, we can consider the physical principles of reflection of the colors, because according to SOUZA et al. (2008) the color white reflects all other colors, and in this way, it can be assumed that stink bugs are attracted to white felt because it allow a greater range of wavelengths of light. This is reinforced comparing the results of felts black and brown, that presented the smaller amounts of clutches and eggs (Table 1). Black absorbs all other colors, thus not reflecting any wavelength, and so the insect may be less attracted to the substrate of this color.

CHAPMAN (1998) reports that various orders of insects (Odonata, Blattodea, Orthoptera, Hemiptera, Thysanoptera, Neuroptera, Hymenoptera, Diptera, Coleoptera and Lepidoptera) can discriminate light of different wavelengths due to the presence of specific cells (retinula cells) and photopigments with the maximum sensitivity to light. Insects generally have a visual pigment with a maximum absorption in the green range of the spectrum (490-540 nm at most) that extends frequently below 400 nm (ultraviolet) to 600 nm (orange). This author still reports that for most insects, long wavelengths (red) do not stimulate the eyes, since this color has a wavelength greater than 600 nm. Considering this characteristic it can be observed in this paper, that the felt red are among the colored substrates that showed lower amount of eggs and clutches (Table 1).

Several conclusions from different studies are apparently contradictory, due to types of experimentation, relationship between colors tested and diversity of these in each test. However, as reported by BRISCOE & CHITTKA (2001) the form that insects see the colors can not be comprehended without understanding the life history of these organisms.

Thus, the authors suggest that new studies are necessary, using for example, felts of color violet (380-440mn) to verify if higher oviposition occurs in this color due it’s closer reflectivity to ultraviolet spectrum. Furthermore, it can be tested the synergistic effect of the color of the artificial substrate with specific chemical attractive (aggregation pheromones, for example) to ***E. heros*** (RENWICK, 1989; MORAES, et al., 2008; BORGES et al., 2010) as well as utilize soybean plants extracts (PANIZZI et al., 2004) on artificial substrates to verify whether these compounds can further optimize the eggs production.

CONCLUSION

In conclusion, this study demonstrates that the use of yellow and white colors were most preferred for ***E. heros*** oviposition than other colors. However, further studies are needed to examine the oviposition of the Neotropical brown stink bug on colored artificial substrates with no choice, to verify if the pattern presented in this paper is maintained, since in large-scale rearing, commonly just one color is used.

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