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Short Communication

Differential preference of colored surface in *Tribolium castaneum* (Herbst)

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Abstract

Insects show color preferences mostly to those which resembles the color of foliage, flower or even their hosts. In the present study observations were made to determine vision towards different colored surfaces in young (second instar), and advanced (fourth instar) larvae, and adults of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The larva and adult beetles showed significant color preferences when given a choice between white (control) and colored surfaces at 24- and 48-h exposures. The second instar larvae were more attracted by yellow and pink than green surfaces. The fourth instar larvae did not show any significant preference between white and colored surfaces at 24-h exposure, but avoided red and pink surfaces (P>0.05) and had a marginal choice for black (P<0.05). The adults avoided green significantly at both exposure times and pink at 48-h exposure, but was moderately attracted by black (P<0.05) at both exposure times.

Key words: color preference; *Tribolium castaneum*

Introduction

Insect traps are used either for population sampling or for management of pest species. For collecting cropfield insects sticky traps and baited traps are used. The sticky traps are often made attractive by using pheromones in it, or by using different colore and shape traps (Hoback *et al*., 1999). Different insect families showed preferences for different trap colores. Hoback *et al*. (1999) provided a list of insect families with their preferences for different color traps. Members of the same family may prefer more than one color, for example in case of Curculionidae (Cross *et al*., 1976), even color preference may differ at species level (Capinera and Walmsley, 1978). Lobdell *et al.* (2005) observed that the egg parasitoid *Trichogramma ostriniae* showed differential responses to egg color or the hosts as well as the background color in a Petri dish arena while searching for the host.

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Studies on the cropland insects demonstrated the preferences of many insects for yellow color, as the peak color reflectance of plants is the yellow band at 50-560 nm, so this color may act as a super-normal foliage stimulant to herbivorous insects (Prokopy and Owens, 1983). Sawflies showed least preference to dark colors like blue (Anderbrant *et al*., 1989). It is reported that most Hymenoptera have color receptors in the green, blue and ultra-violet range, with a few species also being able to perceive red (Peitsch *et al.,* 1992).

The previous studies revealed that the insects are attracted to colors, which are close to their food color. Numbers of attractant and repellent tests were carried for the stored product insect pests as part of their management program. Most of these researches involved addition of different insecticides, hormones, pheromones, plant extracts, etc., to their food. However, color preference in stored product insects remains poorly studied. The objective of this study was to determine whether *Tribolium castaneum*, the red flour beetle can differentiate colors?

The differential preferences for colored surfaces were studied for young (2nd instar) and advance (4th instar) larvae and adults of *T. castaneum***.**

Materials and Methods

Stages of Tribolium castaneum used

About 200 adults of *T. castaneum* (Herbst) beetles were collected from the stock culture, which has been maintained in the Stored Product Insect Laboratory, Department of Zoology, Rajshahi University, since 1985 without any loss of fitness. The adults were kept in a beaker and provided with 10 g of standard food (Park and Frank, 1948). After 24 h the subculture was sieved through 500 and 250 µm mesh to separate the adults and the eggs respectively from the food. The newly hatched larvae were collected from these eggs and provided with standard food and reared in beakers, covering the mouth with fine cloth to restrict escape of the larvae. A few similar subculture of the beetles were established during subsequent days. The subcultures were kept at 30 ± 1 °C in an incubator without controlling light and humidity. The food was changed after every three days to avoid contamination by the larvae (Park, 1935).

The second and fourth instar larvae were normally obtained on the third and ninth days respectively after hatching of the eggs (Mondal, 1984). The adults emerged 21-22 days after hatching. In this experiment, 30 larvae of second or fourth instars, or adults of 48-h age were used for each color test. Each of these experiments was replicated three times.

Table 1 Distribution of larvae and adults of *T. castaneum* in different colored surfaces after 24 h exposure (N = 90)

Life stage	**Colored surface**	**Percentage distribution (Number)**		**X^2-values (df = 2)**
		Control (white)	**Color**	
Second instar larvae	Green	31.11 (28)	68.89 (62)	7.14*
	Red	52.23 (47)	47.77 (43)	0.09
	Blue	35.55 (32)	64.45 (58)	4.18
	Chocolate	46.67 (42)	53.33 (48)	0.22
	Yellow	23.33 (21)	76.67 (69)	14.22***
	Pink	21.11 (19)	78.89 (71)	16.69***
	Black	47.77 (43)	52.33 (47)	0.11
Fourth instar larvae	Green	56.66 (57)	43.34 (39)	0.89
	Red	50.00 (45)	50.00 (45)	0
	Blue	38.88 (35)	61.12 (55)	0.50
	Chocolate	35.55 (32)	64.45 (58)	4.18
	Yellow	48.88 (44)	51.12 (46)	0.02
	Pink	62.22 (56)	37.78 (34)	2.99
	Black	43.33 (39)	56.67 (51)	0.89
Adults	Green	71.11 (64)	28.89 (26)	8.91*
	Red	42.22 (38)	57.88 (52)	1.24
	Blue	40.00 (36)	60.00 (54)	2.00
	Chocolate	57.78 (52)	42.22 (38)	1.21
	Yellow	37.78 (34)	62.22 (56)	2.99
	Pink	57.78 (52)	42.22 (38)	1.21
	Black	31.11 (28)	68.89 (62)	7.14*

*P<0.05; ***P<0.001

Color choice test

These tests were conducted in choice chamber. A choice chamber was made in glass Petri dish of 9 cm diameter. A straight line was drawn through the middle of the Petri dish. Colored poster papers were used for the test, and white paper as the control. The papers of each color were first cut into a circle of 9 cm diameter, and then cut into two equal halves. At the outer surface of the Petri dish color paper was pasted on one half and white paper was pasted on the other half. Similarly, these choice chambers were made for each color.

The color used in this experiment were green, red, blue, chocolate, yellow, pink and black.

Experimentation

At the middle of the each choice chamber, 30 of either second or fourth instar larvae, or adults were released. The Petri dish was covered with lid, and kept undisturbed at room temperature (20-22 °C). No food was given to them. After 24 h the number of larvae or adults at colored and white parts of the choice chamber were recorded, and the insects were kept undisturbed for another 24 h. Similarly, the number of insects in colored and control halves were recorded after 48 h.

Table 2 Distribution of larvae and adults of *T. castaneum* in different colored surfaces after 48 h exposure (N = 90)

Life stage	**Colored surface**	**Percentage distribution (Number)**		**X^2-values (df = 2)**
		Control (white)	**Color**	
Second instar larvae	Green	15.55 (14)	84.45 (76)	23.73***
	Red	34.44 (31)	65.56 (59)	4.48
	Blue	33.33 (30)	67.67 (60)	5.56
	Chocolate	61.11 (55)	38.89 (35)	2.47
	Yellow	24.44 (22)	75.56 (68)	13.07***
	Pink	17.78 (16)	82.22 (74)	20.76***
	Black	45.55 (41)	54.45 (49)	0.39
Fourth instar larvae	Green	43.33 (39)	56.67 (51)	0.89
	Red	75.55 (68)	24.45 (22)	13.06***
	Blue	46.66 (42)	53.33 (48)	0.22
	Chocolate	56.66 (61)	43.34 (39)	0.89
	Yellow	43.33 (39)	56.67 (51)	0.89
	Pink	75.55 (68)	24.45 (22)	13.06***
	Black	28.89 (26)	71.11 (64)	8.91*
Adults	Green	81.11 (73)	18.89 (17)	19.36***
	Red	48.89 (44)	51.11 (46)	0.02
	Blue	33.33 (30)	66.67 (60)	5.56
	Chocolate	43.33 (39)	56.67 (51)	0.89
	Yellow	50.00 (45)	50.00 (45)	0
	Pink	67.78 (61)	32.22 (29)	6.32*
	Black	28.89 (26)	71.11 (64)	8.91*

*P<0.05; ***P<0.001

Statistical analysis

The distribution of larvae and adults on each colored surface was tested using X^2-test. ANOVA was done to examine the effect of the exposure period and stages of *T. castaneum* on the choice of different colors.

Results and Discussion

There is difference for color preference among the larvae and adults of *T. castaneum.* The second instar larvae were mostly attracted to yellow and pink colors ($P<0.001$) at both 24- and 48-h exposure (Tables 1, 2).

Choice test for the fourth instar larvae showed no preference for any one of the tested colors at 24-h exposure (Table 1). At 48-h exposure, the fourth instar larvae preferred the white color (control) more than red and pink ($P<0.001$) (Table 2), and showed a little attraction towards black ($P<0.05$) (Table 2).

The adults showed no preference for colored or white surfaces except black ($P<0.05$) at both exposure periods (Tables 1, 2). Total avoidance to green surface was observed. During 48 h exposure the adults avoided pink whereas at 24-h exposure the distributions in white and pink colors were similar (Tables 1, 2).

The present results revealed that advance larvae and adults showed no preferences for colored surface. Moreover, they avoided pink, red and green. White (control) was more preferred by the fourth instar larvae and the adults than the second instar larvae.

Table 3 Preference or avoidance to colored surfaces by larvae and adults of *T. castaneum*

Colors	Preference/avoidance of beetles at two exposures					
	2nd instar larva		4th instar larva		Adults	
	24-h	48-h	24-h	48-h	24-h	48-h
Green	MP	SP	NC	NC	MA	SA
Red	NC	NC	NC	SA	NC	NC
Blue	NC	NC	NC	NC	NC	NC
Chocolate	NC	NC	NC	NC	NC	NC
Yellow	SP	SP	NC	NC	NC	NC
Pink	SP	SP	NC	SA	NC	MP
Black	NC	NC	NC	MP	MP	MP

SP = strongly preferred ($P<0.001$); MP = moderately preferred ($P<0.05$); SA = strongly avoided; MA = moderately avoided; NC = no choice

ANOVA showed that there is no significant difference of color choice at different exposure periods ($P>0.05$, $F= 5.58$) by the larvae and the adults. The second instar larvae showed preference for green, yellow and pink colors, but had no significant choice for black. However, black was marginally preferred over white by the fourth instar larvae and adults (Table 3).

Generally, a wide range of insects exhibit attraction towards yellow color (Borror *et al.,* 1989). The coleopteran families like Chrysomelidae, Coccinellidae, Curculionidae and Scarabaeidae showed preference for yellow traps ($P<0.05$) as stated by Flemming *et al*. (1940), Cross *et al.* (1976), Dominick (1976) and Dowell and Cherry (1981). These beetles also showed choice for green and white traps. However, in the present investigation except young larvae, *T. castaneum* showed no choice for yellow color.

An experiment on the farmland sawflies showed that all the collected species showed strong responses to colored traps, especially to yellow (Barker *et al*., 1997). Moreover, the hymenopteran egg parasitoids of the genus *Trichogramma* showed preference for yellow and white, representing an adaptive preference for the egg color of the primary hosts (Pak and de Jong, 1987; Lobdell *et al*., 2005). Furthermore, Romies *et al*. (1998) observed that naturally occurring *Trichogramma* in sorghum fields in India were poorly attracted to yellow stick-traps as compared to white and green traps.

Khalil (1991) found that adult *T. confusum* attracted by red in a narrow space (45 ×1 cm) with a color area of 7×1 cm; while in a wide space (100×4 cm) having a color area of 16×4 cm, they were attracted by blue color. The author observed that *Sitophilous oryzae* adults were attracted by green and black, and these responses were greatly affected by the exposure period. However, in the present experiment the adult *T. castaneum* no definite choice for either blue or red color was observed. Normally, it is expected that there is no possibility of differential preference to colors between different species of an insect in the same habitat. However, different sawfly species showed significance difference between color choices (Barker *et al*., 1997).

In the present study no food was given in the choice chambers, which might be the reasons that the advanced larvae and the adults might be distributed evenly on white (control) and colored surfaces for the search of food at short exposure. Moreover, they preferred dark colors, as a moderate choice for the black surface, for a site of refuge. The fourth instar larvae showed a strong avoidance to red and pink colors at 48-h post-exposure. The adults totally avoided green, and moderately pink. But the second instar larvae showed strong attraction to green and pink colors along with the yellow. Hence, it can be said that the younger larvae are attracted to green, yellow and pink colors, and equally distributed on dark colors. Longer exposure period provided to fourth instar larvae and adults gave them sufficient time for a good selection of a suitable color. Color preference may change during the insect's life time. For example, the pre-reproductive adult anthomyiid flies were attracted to yellow colored traps, but reproductive flies were attracted to purple color (Jenkins and Roques, 1993); similarly tephritid flies are attracted to yellow color prior to maturation and red color during oviposition (Kring, 1970).

It can be concluded that for *Tribolium* control in the grain stores, green, red and pink grain bags could be used to keep away the older larvae and adults, or the wall and surface of the stores may be painted with these colors. Use of colored bags or colored surfaces may give better result in integrated pest management programs for the control of *Tribolium*.

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