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Yellow or purple: African fig fly (*Zaprionus indianus* Gupta, 1970) capture using colored baits

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ABSTRACT. *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) is an invasive species of African origin with its first record in Brazil in 1999. The species quickly dispersed throughout Brazil and produced significant impacts on fig culture. It is currently considered a potential pest of berries, which justifies the investigation of techniques that enable their capture. In this sense, the objective of this study is to evaluate the attractiveness of bait colors in *Z. indianus* capturing from locations with different degrees of urbanization. For this, traps with yellow and purple baits were used and the relative abundance in the capture in areas with different degrees of urbanization was evaluated. Our results indicated a higher *Z. indianus* capture in environments with a high degree of urbanization using the purple. The capture using attractants is highly effective, and we noticed that for places where there are populations with large numbers of individuals, the visual stimulus inside the bait is a variable of great attractiveness for this species, mainly by purple baits.

Keywords: African fig fly; bioinvader; cultivar pests; drosophilid traps; insect capture

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INTRODUCTION

Zaprionus indianus Gupta, 1970 (Diptera: Drosophilidae) is a bioinvader species known as the African fig fly (AFF) (Gupta, 1970; Bragard *et al.*, 2022) that was recorded in Brazil for the first time in 1999. The AFF presents approximately 3mm long, yellowish body with two white bands interspersed by black longitudinal bands from head to chest. This species belongs to the globally distributed family Drosophilidae, characterized by small flies, grouped taxonomically into 73 genera (Vilela & Goñi, 2015).

One possible introduction scenario of the AFF in the Neotropical region was proposed in our previous studies (Galego & Carareto, 2007; Galego & Carareto, 2010). According to this hypothesis, *Z. indianus* arrived in Brazil by the Maritime fruit transportation between Africa and Brazil and the rapid spread of Brazilian territory by road transportation. From the AFF introduction, in about six years, the species was found in several countries of the Neotropical and Neartic regions, including Brazil (Commar *et al.*, 2012), Uruguay (Goñi *et al.*, 2001), Argentina (Lavagnino *et al.*, 2008; Lavagnino *et al.*, 2020), Paraguay (Benítez Díaz, 2015), Mexico (Markow & O'grady, 2008; Lasa & Tadeo, 2015), the United States (van der Linde *et al.*, 2006; Joshi *et al.*, 2014) and Canada (Renkema *et al.*, 2013).

The AFF, besides being a successful bioinvader, is a primary pest of fig orchards and a potential one of soft fruit cultivars (Joshi *et al.*, 2014; Bernardi *et al.*, 2017; Pfeiffer *et al.*, 2019; Bragard *et al.*, 2022). The potential pest and its high ability to invade new areas could be related to the biological characteristics of *Z. indianus*, which include the polyphagous feeding niche (van der Linde *et al.*, 2006; Commar *et al.*, 2012) and their capacity for survival and reproduction in environments beyond those of their biogeographical origin (Mata *et al.*, 2010; Mata *et al.*, 2015) and in areas with different urbanization degrees, which indicates the ecological versatility of the AFF, being present both in

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areas with high and medium urbanization degrees and in natural environments (Ferreira & Tidon, 2005).

The pest potential of *Z. indianus* justifies studies that optimize its management and control. Several forms of *Z. indianus* capture have already been tested, and the proposal by Cruz-Esteban (2021) focused in the trap colors, and the investigation was carried out in blackberry cultivars at different levels of ripening in Mexico. Traps of different colors containing Drosophilidae lures were used in *Z. indianus* capture. These traps had seven different characteristics: externally painted in black+red (i), green+purple (ii), green+yellow (iii), red+black (iv), or yellow+green (v), and transparent traps with yellow (vi) or purple (vii) color cards. The transparent traps containing the yellow card presented the highest capture of the AFF.

This result may indicate that the yellow color reflected the specific intensity that could be attractive for the captured specimens. Another reason could have been the highest luminosity degree of yellow color, which could be similar to the natural habitat where the fruit colors contrast with the environment and it can be a variable to be considered when the attraction is the focus (Cruz-Esteban, 2021). It demonstrates that what would attract the species is not necessarily the color of the trap itself, but the visual stimulus that the color of the bait provides. Furthermore, other *Z. indianus* capture techniques should be performed to optimize the AFF management in contexts where it would be a pest.

Z. indianus is considered a potential pest of berries, in which pest alert occurrences were recorded in several cultivars in different locations, including outside Brazil (Bragard *et al.*, 2022). The AFF caused substantial damage to the fig and berries cultivars in Brazil. Thus, the implementation of management techniques that intensify this insect capture becomes necessary and the use of traps with higher attractiveness may constitute an alternative in the population control of *Z. indianus* in cultivars with this drosophilid infestation. In this sense, the goal of this study was to evaluate the bait´s color luring in the AFF capture from localities with different urbanization degrees and, therefore, to propose a strategy for the pest population management.

MATERIALS AND METHODS

Study area and AFF collections. The *Z. indianus* collections were carried out in three areas with varying degrees of urbanization in Uberaba (MG). The urbanization criteria adopted here was proposed by Ruszczyk (1986) and adaptations for AFF collections according to Gottschalk *et al*. (2007) and Garcia *et al*. (2008).

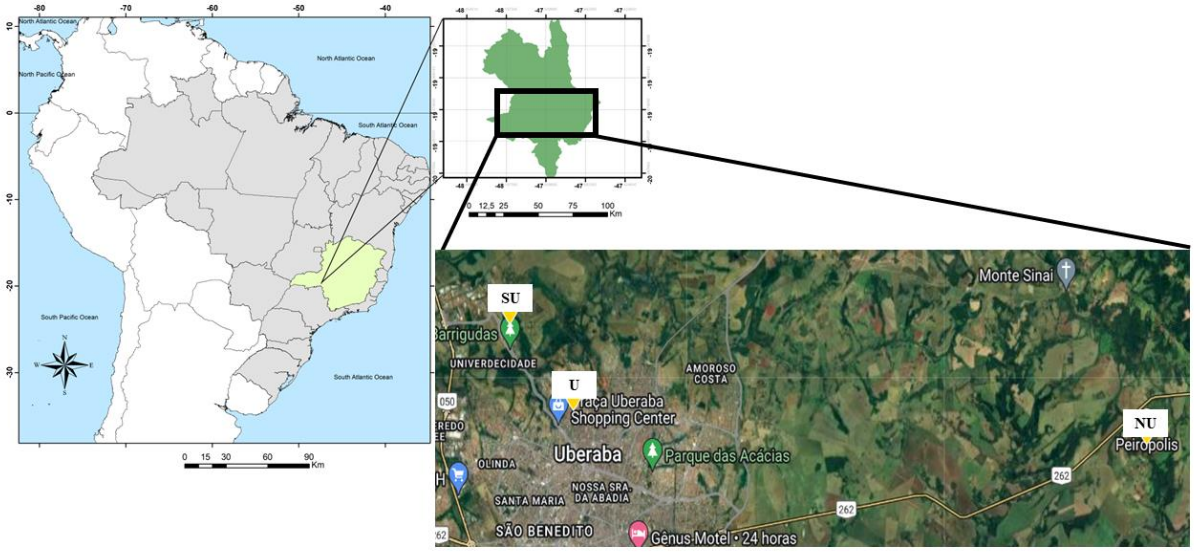

Fig. 1. The collection areas in Uberaba, Minas Gerais, Brazil, where *Z. indianus* was sampled using color baits. In the highlight, view of the urbanization degrees of the areas: SU: semi-urbanized, Univerdecidade-ICENE; U: urbanized, Praça do Quartel; NU: non-urban, Peirópolis (rural district).

The sampled areas (Fig. 1) were Praça do Quartel (-19.734; -47.939), urbanized area (U); ICENE-Univerde (-19.717; -47.959), semi-urbanized (SU); and the rural district of Peirópolis (-19.744; -47.743), a non-urban area (NU).

Experimental design. The collections were carried out between August and September 2021, and each of them used a transect of 100 meters, with three collection points, separated by a distance of 25 meters, where were deposited closed traps with banana-yeast baits (Tidon *et al*., 2003; Penariol & Madi-Ravazzi, 2013), and placed approximately 1.70 m from the ground in the sampled points (Fig. 2).

Fig. 2. Closed traps for *Z. indianus* capture containing banana-yeast baits colored with food dyes: a. bait with purple dye; b. bait without dye (control); c. bait with yellow dye

Thus, each collection area and type of bait constituted an analysis group and were considered nine groups: UC: urbanized area with control bait; UY: urbanized area with yellow bait; UP: urbanized area with purple bait; SUC: semi-urbanized area with control bait; SUY: semi-urbanized area with yellow bait; SUP: semi-urbanized area with purple bait; NUC: non-urban area with control bait; NUY: non-urban area with yellow bait; and NUP: non-urban area with purple bait (Table 1).

Table 1. Bait conditions, for each closed trap, for *Z. indianus* capturing

Type of bait	Mashed banana	Yeast (*Saccharomyces cerevisiae*)	Distilled water	Dye food
C	100 g	5 g	5 ml	0 ml
Y	100 g	5 g	4 ml	1 ml (yellow dye food)
P	100 g	5 g	4 ml	1 ml (purple dye food)

Notes: C: control without dye; Y: yellow food dye; P: purple food dye

Data analysis. The AFF individuals collected were screened and counted according to relative and absolute abundance, as well as the proportion of males (Nm) and females (Nf). For the screening of *Z. indianus* from the other Drosophilidae, the morphological characteristics were considered the yellow color, the two silver-white stripes bordered in black along the head and four stripes along the mesonotum and two on the scutellum (Yuzuki & Tidon, 2020). The comparison between the abundances of *Z. indianus* concerning the proportion of the other species was performed employing proportion comparisons Minitab 19. Chi-square with Yates correction [$\chi2=((O-E)-0.5)2)/E$] compared the proportion of males and females.

RESULTS AND DISCUSSION

The *Z. indianus* and other drosophilids captured using color baits varied between the areas (Table 2). Considering all collections, the proportion of *Z. indianus* and the other drosophilid species was the same (1:1), which indicates a dominance of the AFF, expected result because this species is abundant in Brazilian savannah-cerrado (Chaves & Tidon, 2010; Mata & Tidon, 2013; Lasa & Tadeo, 2015; Cavalcanti *et al.*, 2022; Rios *et al.*, 2022), biome where Uberaba is localized. The urbanized area (U) presented the highest number of individuals collected (3.082 individuals), and *Z. indianus* was the most frequent species (2.242 individuals, relative abundance of 73%), while semi-urbanized and non-urban areas showed low capture of *Z. indianus*, respectively 366 e 177 individuals.

Table 2. Absolute and relative (in brackets) abundance of *Z. indianus* and other drosophilids in each collected area

Bait	Species	SU	U	NU	Total
Purple	*Z. indianus*	138 (40%)	1136 (75%)	71 (58%)	1345 (68%)
	other drosophilids	211 (60%)	375 (25%)	51 (42%)	637 (32%)
	Total	350 (100%)	1511 (100%)	122 (100%)	1983 (100%)
Yellow	*Z. indianus*	100 (34%)	682 (67%)	27 (22%)	809 (56%)
	other drosophilids	198 (66%)	343 (33%)	96 (78%)	637 (44%)
	Total	298 (100%)	1025 (100%)	123 (100%)	1446 (100%)
Control	*Z. indianus*	98 (25%)	424 (78%)	79 (44%)	601 (54%)
	other drosophilids	289 (75%)	122 (22%)	100 (56%)	511 (46%)
	Total	387 (100%)	546 (100%)	179 (100%)	1112 (100%)
Total	*Z. indianus*	336 (32%)	2242 (73%)	177 (42%)	2755 (61%)
	other drosophilids	698 (68%)	840 (27%)	247 (58%)	1785 (59%)
	Total	1034 (100%)	3082 (100%)	424 (100%)	4540 (100%)

Notes: (SU, U and NU) and color bait (purple, yellow and control without dye). SU: semi-urbanized; U: urbanized; NU: non-urban

The purple baits showed the highest *Z. indianus* capture (1345 individuals), mainly in U area, where 1136 flies (84%) were collected. Control and yellow baits captured 809 and 601 individuals, respectively. In addition, the yellow bait in the NU area presented the lowest AFF capture, with only 27 individuals in three traps. Moreover, the attractivity of *Z. indianus* for the color baits depends on number of flies in the environment, whereas in the U area, the purple bait where more attractive to species, in SU and NU the same did not occur. The yellow and the control baits showed low capture of *Z. indianus* in SU and NU areas.

The AFF capture results indicate the bait color had relevance only in the highly urbanized place, where the *Z. indianus* abundance was higher than in the other areas. Therefore, using purple baits in cultivars with *Z. indianus* infestation could be an alternative to pest control. Our results differ from the study by Cruz-Esteban (2021), in which the trap with the yellow visual stimulus obtained the highest abundance of the AFF capture, followed by the trap with the purple visual stimulus. However, in the U area, the colored traps collected considerably more flies than the trap without dye (control). It can be inferred that in places of high *Z. indianus* abundance, the visual stimulus inside the bait showed more attractiveness. Other studies obtained divergent results, such as that of Rice *et al*. (2016), which obtained largest capture for traps with black and red colors, and that of Kirkpatrick *et al*. (2015), which obtained better results for traps with fluorescent red color. In these two studies, the traps that had the purple color and the yellow color did not show bigger attractiveness for drosophilids.

The U area also showed a higher proportion of males in all baits (Table 2), 65% of males and 35% of females in the trap with purple bait; 64% of males and 36% of females in the trap with yellow bait; and 65% of males and 35% of females in the trap with the bait without dye. The yellow bait trap in NU area obtained the lowest number of captured males, 19% of males and 81% of females. Cruz-Esteban (2021) obtained divergent results, with a higher proportion of females in all types of traps tested. The study by Kirkpatrick *et al*. (2015), obtained similar responses for males and females, pointing out that the choice of males may have been given to optimizing the since reproduction at oviposition sites is a reported phenomenon for drosophilids (Spieth, 1974; Markow & O'grady, 2008).

Table 2. Percentage (%) of males and females of *Z. indianus* captured in each area and color of the baits

Bait	*Z. indianus*	SU	U	NU
Purple	Males (M)	46.0	65.0	61.0
	Females (F)	54.0	35.0	39.0
	M/F	0.85	1.85***	1.56
Yellow	Males (M)	48.0	64.0	19.0
	Females (F)	52.0	36.0	81.0
	M/F	0.92	1.77***	0.23**
Control	Males (M)	44.0	65.0	52.0
	Females (F)	56.0	35.0	48.0
	M/F	0.78	1.85***	1.08

Notes: $^{**}p<0.01$; $^{***}p<0.001$. The numbers bolded indicate the significate values in Chi-square comparisons. SU: semi-urbanized; U: urbanized; NU: non-urban

The use of trap features with visual stimuli could determine their efficacy for drosophilid capture in monitoring surveys, especially for capture in attraction at a short distance, and the color has been most frequently visual characteristic used for traps (Lasa *et al.*, 2020). In fact, red, purple, orange and combinations of black and white were attractive to *Drosophila repleta* (Hottel *et al.*, 2015), and red traps with a black horizontal stripe were effective for trapping *Drosophila suzukii* (Basoalto *et al*., 2013; Rice *et al*., 2016; Lasa *et al*., 2017). Lasa *et al.* (2020) captured more individuals of *Z. indianus* in the orange trap with brown circles around the access hole, which could mimic the fruit damaged by birds.

On the other hand, the visual purple stimulus might emulate some fruit colors that *Z. indianus* feeds, as the case of blueberries, blackberries and figs (Lasa *et al*., 2020), what seems to be the case of the purple bait's attractiveness in environment with high demography of *Z. indianus.* The background could be yet a critical factor in attractivity. In fact, purple on a green background (green+purple) was more attractive than red on a black background (black+red), black on a red background (red black) or blue on a yellow background (yellow+blue) in the capture of *D. suzukii* (Little *et al.*, 2019). The environment where the traps have been placed shows this green background, which would attract more *Z. indianus* individuals to traps. The visual stimulus, in this case, could be a useful trap-lure combination for field testing in *Z. indianus*-infested crops and the population control of this species in avoiding potential economic losses in cultivars infested by the species using a low-cost tool that does not contaminate the environments.

CONCLUSION

The AFF is a bioinvader species and is considered a potential pest of berries, with a pest alert in several cultivars. In Brazil, the species caused economic damage to the cultivars of figs and other red fruits, demonstrating the need to develop forms of population control for this insect. The capture using attractants is highly effective, and we noticed that for places where there are populations with large numbers of individuals, the visual stimulus inside the bait is a variable of great attractiveness for this species, mainly by purple baits. In addition, more studies are needed to analyze the traps of high attractiveness in places with great potential for *Zaprionus indianus* infestation.

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