Journal of Experimental Agriculture International

42(4): 82-87, 2020; Article no.JEAI.57294
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Susceptibility of Bagged Guavas to the Attack of Fruit Flies (Tephritidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author AR designed the study and wrote the first draft of the manuscript. Authors EMS, SBS and LRFL contributed to the maintenance of fruit-fly colonies and development of the experiments. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2020/v42i430502
Editor(s):
(1) Dr. Mirjana Bulatovic-Danilovich, West Virginia University, USA.
Reviewers:
(1) Mileny dos Santos de Souza, Brazil.
(2) Aba-Toumnou Lucie, University of Bangui, Central African Republic.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57294

Original Research Article

Received 20 March 2020
Accepted 26 May 2020
Published 01 June 2020

ABSTRACT

Fruit bagging is an important strategy to protect fruit against fruit fly (Tephritidae) infestation and pesticide contamination. In laboratory, we compared the forced infestation of guavas by *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) when wrapping them with non-woven fabric (NWF) bags. The combined repellence and protection effects of white, green, blue and red NWF bags were tested in comparison with un-bagged guavas. Bagged fruit with the NWF tissue stuck to the pericarp were separately exposed to both fruit fly species for 48 hours, with a ratio of 10 females per fruit inside of laboratory cages. In *C. capitata,* the number of pupae per fruit was significantly higher in the green NWF bags, and the infestation in the other colour bags were similar to the control group (non-bagged). In *A. fraterculus*, guavas with red NWF bags had significantly higher infestation (205.42 pupae per fruit) than fruit that received the other colour bags White and blue bags were less attractive for oviposition from both fruit fly species. The adherence of the NWF bags to the fruit surface causes egg-laying of fruit flies.

Keywords: Insecta; Tephritidae; non-woven fabric; mechanical control; colour attraction.

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1. INTRODUCTION

Although eight reported *Anastrepha* Schiner species and the medfly *Ceratitis capitata* (Wied.) are known to infest guava fruit [1], *Anastrepha fraterculus* (Wied.) and *C. capitata* are predominant in the state of São Paulo [2,3,4]. Generally, fruit growers spray insecticides to control fruit flies.

Detrimental effects caused by excessive use of pesticides include hazards to workers, decreased the food safety, environmental contamination, and a decline in the biodiversity of the agroecosystem [5]. New demands from consumers for the cultivation of crops without the use of chemical insecticides has motivated growers to return to the practice of bagging fruit [6].

Fruit bagging can protect fruit from the damages caused by birds, insects, pathogens and mechanical injuries [7]. Additionally, the use of bags may improve the yield [8] and the quality both before and after harvesting [9,10]. This technique of covering the fruit with different types of protective materials prevents fruit fly (Tephritidae) oviposition on suitable hosts [11, 12].

The production of fresh guavas is conducted by pruning and, consequently, the fruit are harvested all year long. Growers traditionally also use paper bags to protect fresh guavas against fruit flies in the state of São Paulo [12]. The wrapper should be placed when the guava is 2.3 cm diameter because is susceptible to fruit fly infestation in an early developmental stage [13]. Generally, the guava is wrapped with a wax paper bag or a non-woven fabric bags (NWF), with dimensions of 11.0 cm (width) per 15.0 cm (length), and 17.0 cm (width) per 24.0 cm (length), respectively.

For larger guavas, wax paper bags adhere to the fruit and can tear, leaving them vulnerable to fruit fly infestation. In the case of larger fruit wrapped with NWF bags, there is still the risk of fruit fly oviposition because the bag sticks the guava´s pericarp (peel).

The fruit fly females are attracted to and stimulated by the specific colour of their fruit hosts [14,15] because the insects prefer a certain hue [16]. Bagged fruits should not be attractive to fruit fly females.

NWF bags are available in white and many other colours to help growers protect their fruit. In this study, we evaluated in the laboratory how well guavas were protected when wrapped with different colours of NWF bags stuck on fruit and exposed to two fruit fly species. The objective of the present study was to evaluate the combined effects of repellence and protection in guavas by different colours of NWF bags against two fruit fly species in laboratory. With the results and discussions presented here, we hope to contribute to the management of fruit flies in commercial orchards.

2. MATERIALS AND METHODS

The experiment of forced infestation by two fruit fly species was conducted at the Instituto Biológico Campinas, State of São Paulo (SP), Brazil. *Ceratitis capitata* (12 day-old) and *A. fraterculus* (16 day-old) mature females were obtained from colonies maintained in our laboratory since 1993. Both species were reared as described in earlier study [17]. Experiments were undertaken in a room at 23.0º - 26.9ºC and 53–70% RH.

Mature-green guava cultivar Tailandesa (red pulp), bagged at 2.0 cm with butter paper bag and without previous fruit fly infestation, were harvested from an unsprayed orchard in the Campinas municipality (SP). In laboratory, the fruit were cleaned and divided randomly into two groups of 24 fruit. The mean fresh fruit weight (g) was measured using an analytical balance (Balmak ELC 6/15/30). We use a calliper to measure the fruit diameter and length.

The experiments were conducted using white, green, blue and red NWF bags (17.0 cm width per 24.0 cm length) in comparison with un-bagged guava (five treatments and 12 replications). Hunter a, b and L parameters were measured in a sample of coloured bags using a HunterLab MiniScan XE plus in the reflection mode.

We bagged the fruit so that a significant part of the NWF bag would adhere to the fruit peel. Wrapped guavas were randomly placed on the infestation cage floor (100 × 100 × 100 cm) with a minimum of 12.5 cm of distance between fruit, preventing the successive disposal of bags of the same color. To avoid competitive advantage under same cage, all color bags were exposed to infestation by each fruit fly species separately

(choice test) for 48 hours, at a ratio of 10 females per fruit inside of two infestation cages (24 fruit per cage). Twelve non-bagged fruits were used as control and were exposed in a separate cage from the bagged fruit, using the same ratio of 10 adult females per fruit.

After infestation, the guavas were separated and kept in circular plastic containers, 15 cm in diameter (1.00 litre), containing approximately 1.5 cm of vermiculite substrate. The containers were capped with a voile bound with an elastic. We evaluated the recovered pupae and adults approximately 18 and 30 days after infestation for *C. capitata* and, 20 and 40 days after infestation for *A. fraterculus*, respectively.

We consider each fruit to be one replication. The level of infestation by the two species and for the various bag colours were measured by the number of pupae and adults produced per fruit and were compared using a two-way analysis of variance (two-way ANOVA), which tested the interaction between the two factors, followed by the Tukey post-hoc test for multiple comparisons of the main factors (The SAS System for Windows, version 9.2). Due to the absence of normal distribution, the data were transformed into ranks in the analysis [18]. The standard errors of the means were calculated with Microsoft Excel (Microsoft Professional Plus 2016).

3. RESULTS AND DISCUSSION

The means of the diameter, length and weight of the tested guava were 5.36 cm (4.6 – 5.9), 5.68 cm (4.6 – 6.9) and 165.9 g (132 – 204), respectively. The hue angles of the NWF bags tested during fruit fly infestation are described in Table 1.

After analysis with a two-away ANOVA of the number of pupae and adults of each fruit fly species obtained per fruit, there were interactions between species and bag colours for pupae ($F = 9.00$; $P < 0.001$) and adults ($F = 5.46$; $P < 0.001$). In *C. capitata* (Table 2), the number of pupae per fruit was significantly higher in green NWF bags, and the infestation level of the other bag colours was similar to the control (non bagged) (Fig. 1).

In *A. fraterculus* (Table 2), the infestation of guavas with red NWF bags was significantly more pronounced than other colours and, in fact exhibited the highest infestation value of the entire experiment (205.42 pupae per fruit). White and blue bags were slightly repellent to oviposition of *A. fraterculus* (Fig. 1).

Significant differences in the infesting species were detected between bags of different colours. Guavas in green and blue bags showed significantly larger infestation of medflies than *A. fraterculus*. The opposite situation occurred in red bags where the *A. fraterculus* infestation was nearly double that of *C. capitata* and was statistically similar to the non- bagged guavas. Although the number of recovered adults is an intrinsic variable, the statistical analysis of the responses of fruit flies to bag colours for this stage was similar to that of pupae (Table 2).

The heaviest fruit infestation occurs under conditions of high fruit fly density and the number of eggs per oviposition varied according to the host fruit and the size fruit [19]. In our study, although the number of eggs per cluster was not evaluated, the red bags stimulated the *A. fraterculus* oviposition (Table 2) because this species commonly lay one egg per puncture [20], while in *C. capitata* the number of eggs per cluster varies from 1.7 to 8.2 [19].

Green light was less attractive than red (relative to a 400 nm standard) for *Glossina morsitans morsitans* Westw. males and *Musca domestica* L. females (Diptera) in laboratory [21]. Yellow sticky-coated boards hanged in olive and apricot orchards capture more *C. capitata* than orange, green, red and grey traps. In olive, green sticky-coated boards captured six times more flies than the red ones [22]. Hemispheres stick traps painted either black, blue, or sap green captured the fewest *Bactrocera cucurbitae* (Coquillet) (Tephritidae) females, while the yellow, white and orange were the most attractive [23].

Table 1. Hue angle (º), *L*, *a* and *b* values of the four colours of non-woven fabric (NWF) bags

NWF bag	Hue	*L*	*A*	*B*
1. White	123.79	92.38	-0.19	0.57
2. Green	172.49	74.58	-37.22	5.44
3. Blue	230.87	75.77	-18.89	-22.68
4. Red	27.25	47.03	53.94	29.87

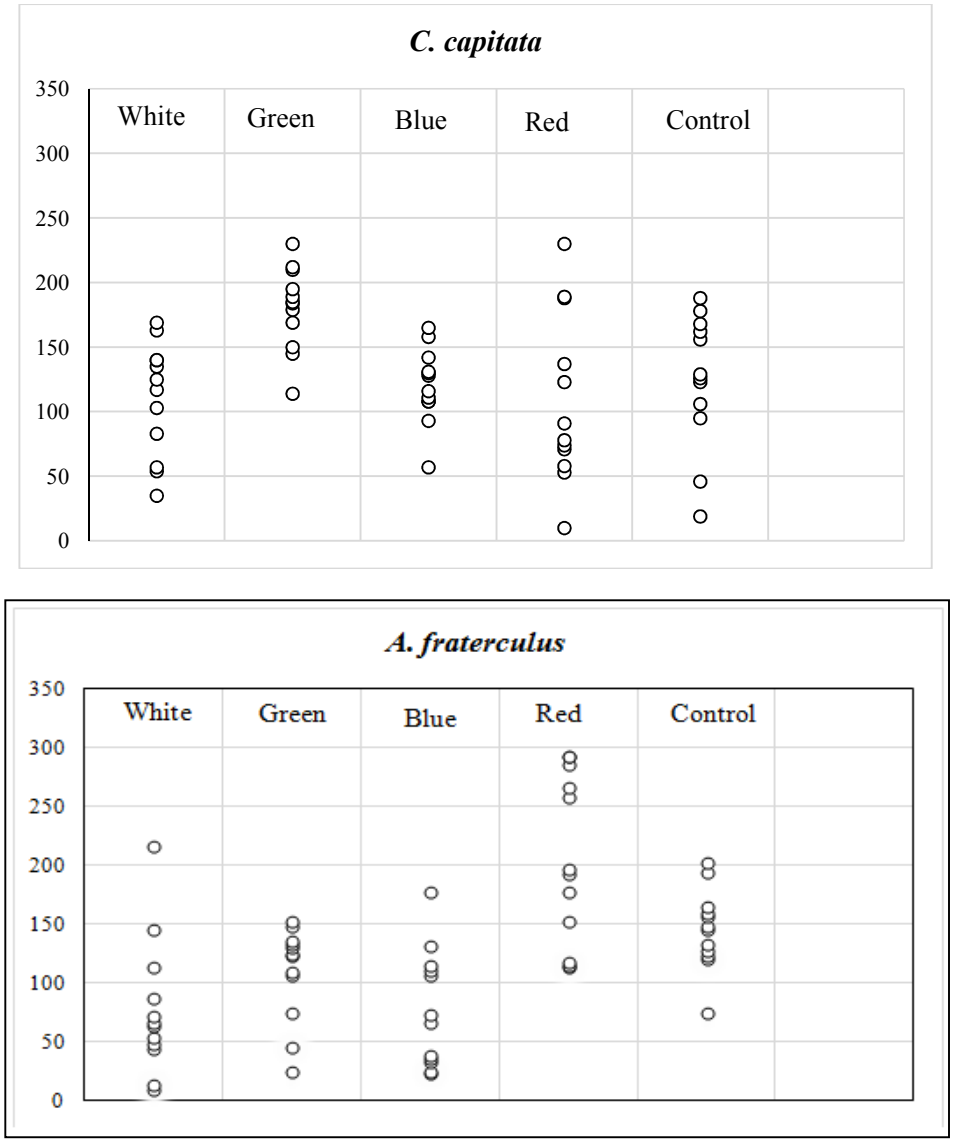

Fig. 1. Number of recovered pupae of *Ceratitis capitata* and *Anastrepha fraterculus* per fruit (n = 12) after infestation of bagged guavas from different colours in the laboratory

Table 2. Mean numbers ± SE of pupae and adults per guava bagged with the four colours and exposed to two fruit fly species (Tephritidae) in the laboratory

Treatment	Pupa		Adult	
	C. capitata	*A. fraterculus*	*C. capitata*	*A. fraterculus*
NWF white	110.08 ± 12.17bA	77.92 ± 16.07cA	102.42 ± 12.72bA	56.00 ± 9.86bB
NWF green	180.17 ± 8.89aA	109.08 ± 11.10bcB	163.83 ± 9.99aA	97.17 ± 10.59abB
NWF blue	120.58 ± 8.04bA	78.17 ± 13.79cB	111.42 ± 9.51bA	69.50 ± 11.61bB
NWF red	108.50 ± 18.21bB	205.42 ± 19.77aA	87.75 ± 15.05bA	127.17 ± 17.28aA
Control (nonbagged)	124.67 ± 14.36bA	145.75 ± 9.95abA	118.42 ± 12.90abA	129.08 ± 10.03aA

Means within columns followed by the same lower case letter are not significantly different (ANOVA – Tukey´s test, P<0.05). Means within rows followed by the same upper case letter in each time are not significantly different (ANOVA – Tukey´s test, P<0.05)

In our study, both species showed preference to red-green colors because *C. capitata* and *A. fraterculus* females detected primary responses to the hues from green and red bags, respectively (Fig. 1). White and blue NWF were less attractive for both species and should be tested in the field on fruit hosts suitable to co-infestation [24]. In some species, it has been found that color hue seems to be important during fruit finding [23].

Undoubtedly, bagging fruit is an important technique in reducing pest infestation and fruit contamination by pesticides [12,25,26]. Bagging fruits might be more effective mean of preventing fruit fly damage than insecticides [27] and these practices can support synergies with other components of IPM [28].

The colour of bags should be less attractive to the specific Tephritids. Besides, the adherence of NWF to the fruit surface causes egg-laying, because the female can perforate concomitant the NWF and the epidermis of the fruit at the same time. NWF double-layer should be developed to protect the fruit against tephritid infestation. We recommend special attention for choosing NWF during IPM activities.

4. CONCLUSION

The present study showed that the choosing a bag color depends on the local fruit fly diversity, because the attraction response is species-dependent. White and blue NWF bags can be tested in the field to confirm the slight oviposition deterrent effect for hosts of both *A. fraterculus* and *C. capitata*.

ACKNOWLEDGEMENT

We thank Dr. Juliana Sanches de Laurentiz (Instituto Agronômico de Campinas, SP) for assistance during measure of colour parameters of bags.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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(Acessed in 28 March 2020)
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Peer-review history:
The peer review history for this paper can be accessed here:
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