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Effect of trap bait and colours, and number of entry holes in monitoring of Drosophilidae (Diptera) species in a fig orchard

Bir incir bahçesinde Drosophilidae (Diptera) türlerinin izlenmesinde tuzak cezbedicisi, tuzak rengi ve giriş delik sayısının etkisi

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ABSTRACT

The study's objective was to evaluate how the capture rate of bottle traps is influenced by their color, number of entry holes, and bait liquids.For this purpose, yellow, blue, green, red, black, white, and transparent (colourless) traps were tested. Apple cider vinegar, grape vinegar, white wine, yeast, and water (control) were used as bait liquids. The number of entry holes was 2, 4, 6, and 8 holes per trap on the side of the bottle traps. The experiments were conducted in a randomised block design with three replicates in a fig orchard (variety Bursa Siyahı) with 437 trees in Aydın province from September 2018 to March 2019. In total, 48 traps were mounted on the experimental trees (1 trap per 1 tree), and counting of the drosophilid individuals in the traps was performed weekly. Red-coloured traps attracted the highest number of drosophilid individuals, followed by yellow-coloured. Regarding different baits, grape vinegar attracted the most drosophilid individuals, followed by white wine. Concerning the number of trap entry holes, the highest number of individuals were caught in traps with the highest number of holes, 8. In all traps, *Zaprionus tuberculatus* Malloch, 1932 was the species caught in the highest numbers, followed by *Drosophila subobscura* Collin, 1936 and *Drosophila suzukii* (Matsumura, 1931). *Z. tuberculatus* was mostly caught in traps containing grape vinegar, while *D. subobscura* and *D. suzukii* were mainly caught in traps containing white wine. In our study, the red-coloured traps containing vinegar with 8 entry holes were the most effective in monitoring drosophilid populations.

INTRODUCTION

Drosophilidae members are minute and fragile flies represented by highly numerous species. Brake and Bachli (2008) stated that the family Drosophilidae comprises 3950 valid species and is distributed worldwide throughout many biogeographic regions. Many drosophilid species inhabit and consume fermenting and decaying organic materials (Atkinson 1977, Schmitz et al. 2007). Unlike other common drosophilid species, *D. suzukii* is a pest on ripening fruits whose females can insert their highly sclerotised serrated ovipositor into the intact fruit to lay eggs (Walsh et al. 2011). It is an invasive pest species reported in many countries and introduced into many others (Calabria et al. 2012, Depra et al. 2014, Hauser 2011, Kinjo et al. 2014, Lee et al. 2011). Since the first appearance of *D. suzukii* in Turkey (Orhan et al. 2014), the presence of this pest has been reported in many regions, and many studies have been performed in the country. Tozlu et al. (2018) studied the bacterial composition isolated from *D. suzukii*, and the hypersensitivity reaction of these bacteria was determined. In addition, population dynamics (Zengin and Karaca 2019), population development in vineyards (Kasap and Özdamar 2019), its parasitoids (Kaçar 2020), its spread and hosts (Özbek-Çatal et al. 2021), population dynamics and damage in cherry and nectar orchards (Arıdıcı-Kara and Ulusoy 2022) were studied. Moreover, an international project (including Türkiye) on the biology, ecology and control of *D. suzukii* for IPM studies was carried out (Sanches-Ramos et al. 2022).

Recently, the other drosophilid species, *Zaprionus indianus* (Gupta) 1970, has been reported as a serious pest in Turkey (Özbek Çatal et al. 2019). *Z. indianus* has recently introduced into many countries (Commar et al. 2012). Drosophilids are common flies in fruit-growing areas, especially where a mixed culture of fruit trees is available. Their populations can reach high levels where environmental conditions such as climate and availability of breeding sites are favourable for them in Turkey. In three orchards in Aydın (Çakmar village), a total of 11 drosophid species was shown using yellow-banded grape vinegar traps (Başpınar et al. 2022). Drosophilids are flies that typically feed on ripe fruits and have hosts from plants other than agricultural products. They are polyphagous and have numerous generations per year, which makes their control difficult (Bieńkowski and Orlova-Bienkowskaja 2020, Kenis et al. 2016, Lee et al. 2015, Wang et al. 2022). Additionally, there is also insufficient information about the natural enemies of drosophilids (Walsh et al. 2011).

Monitoring the pest population is the first step to determining the time of insecticide application (Ekström and Ekbom 2011). The bait traps are effective tools for monitoring and controlling *D. suzukii* (Cha et al. 2018). However, many factors affect the capture efficiency of food bait traps used for monitoring and mass trapping.

These factors include the type of trap, some physical features such as trap colour, size, shape, the kind and quality of the lure, the position and number of entry holes on the traps and the site and location where the traps are placed (Basoalto et al. 2013, Lee et al. 2012, Renkema et al. 2014). Trap efficacy also depends on ambient temperature and phenological stages of plants; thus, different attractants are suitable to be applied in different phenological periods, localities and hosts (Tonina et al. 2017).

Recently, many studies have focused on developing more effective species-specific and economic traps for drosophilids (Basoalto et al. 2013, Lee et al. 2012, Renkema et al. 2014).

Therefore, it is essential to determine the effectiveness of traps in pest management. Our study aimed to compare the efficacy of different trap baits and colours and the number of entry holes in monitoring drosophilids.

MATERIALS AND METHODS

Site, preparation of traps, and protocols

The study was conducted in a fig orchard (variety Bursa Siyahı) in size of 2 ha with 437 trees in Aydın province, (37°45'25.0"N, 27°46'49.1"E) from September 2018 to March 2019. The efficacy of trap colour, the number of holes for the entry of drosophilids on the side of the bottle trap, and the attractiveness of different liquid baits were studied in separate trials. Transparent plastic bottles of 500 ml were used as bottle traps. They were perforated with holes (0.25 cm in diameter) placed in the upper quarter of the bottle as entries for drosophilids, and 100 ml of liquid bait was added into the traps. The bottle traps were placed in the orchard in the canopy of trees at a 1.5-2.0 metres above the ground on the south side of the trees and replaced with new ones every week. Fly samples were separated and counted under the stereomicroscope. They were then preserved in vials with 70% alcohol and stored in the fridge for identification. Misshaped or incomplete individuals were omitted from the samples.

Experiments

Three successional experiments were conducted at the same orchard to compare the efficacy of different trap baits and colours and the number of entry holes in monitoring drosophilids. First, in the colour experiment, seven different colours (yellow, blue, green, red, black, white, colourless (transparent) (Table 1) were implemented, keeping the bait grape vinegar and the number of 4 entry holes constant. Paint cards were cut in 4 cm width bands and stuck in the surrounding middle of the bottle. In the following bait experiment, five different liquid baits were used (apple cider, vinegar, grape vinegar, white vine, yeast, and water) (Table 2), keeping the red colour and the number of 4 entry holes constant. Finally, in the hole number experiment, four different entry hole numbers (2, 4, 6, 8) per trap on the side of plastic bottles were tested, keeping grape vinegar as bait and the red colour constant.

Table 1. Characteristics of the paint card colours stuck on the traps

Paint card colour	Colour analysis[1]		
	L* value	a* value	b* value
Yellow	78.90	14.66	80.43
Blue	32.63	1.16	-29.42
Green	59.88	-24.40	45.73
Red	44.12	49.56	25.07
Black	25.31	0.04	-0.31
White	92.25	0.27	0.99
Transparent (=clear)	-	-	-

[1]Paint card colours were analysed with a DOHO DR-10 colourmeter

Table 2. Bait liquids in the traps

Bait liquids	Trade Mark
Apple cider vinegar	KlikyaTM with an acidity of 4-5%
Grape vinegar	TarişTM with an acidity of 4-5%
White wine	DimitriTM with the alcohol of 13%
Yeast	PakmayaTM ingredient is *Saccharomyces cerevisiae*
Water (as control)	Tap water

Experiments were set up in a randomised complete block design with three replicates. Experimental plots were placed in three rows, and each row was represented for each replication, omitting one row between replicates. Traps were placed individually on every other tree in the rows. During the trial period, 16 traps were placed on the experimental tree in each row. Trials were conducted on 48 trees in three replications in total.

Data were transformed by using square root transformation to provide homogenous variances. Then the General Linear Model procedure in the SPSS statistical program was used to fit a linear model for each data set to determine the substantial effect of the treatment groups. After significant effects were identified, differences between treatment means were considered significant at 0.05 based on the Tukey adjustment type I error rate.

RESULTS

Considering the overall species composition, seven species belonging to the family Drosophilidae were detected in the trap trials in the Black variety fig orchard. *Zaprionus tuberculatus* Malloch, 1932 was the most abundant species in terms of the number of individuals caught in the traps, followed by *Drosophila subobscura* Collin, 1936, *Drosophila suzukii* (Matsumura, 1931), *Hirtodrosophila confusa* (Staeger, 1844), *Drosophila melanogaster* Meigen, 1830, *Drosophila busckii* Coquillett, 1901 and *Drosophila immigrans* Sturtevant, 1921 (Table 3, 4 and 6). The number of flies captured in the traps varied according to trap type. In the trap colour trials, the highest number of drosophilids was found in red traps with 804 individuals in sum (Table 3). Yellow-coloured traps ranked second in attractiveness with 683 individuals, followed by white with 582 individuals, transparent with 559 individuals, green with 483 individuals and blue with 419 individuals. The black colour was the least attractive to 265 individuals compared to the others. When considering the catch results by species, many drosophilid species were attracted to the red colour. *Z. tuberculatus* was the most common species caught in the red traps, with a mean of 202.00±56.89 individuals. *D. subobscura* and *D. suzukii* were more attracted to yellow traps (69.00±6.11 and 8.33±2.91 individuals, respectively) than the red ones. *D. subobscura* and *D. suzukii* were more attracted to yellow traps (69.00 ± 6.11 and 8.33 ± 2.91 individuals, respectively) than red ones. In addition, *H. confusa* was caught more in black and transparent traps than in any other coloured traps, with 5.33±0.89 and 5.00±3.10 individuals, respectively. However, there was no statistically significant difference between the effectiveness of different colours (Tukey test, X>0.05).

In the bait trials, grape vinegar traps made the highest number of drosophilid catches with 4379 individuals. It was followed by white wine with 4208 individuals and apple cider vinegar with 1818 individuals. While 869 drosophilid individuals were caught in traps containing the yeast, no drosophilid fly was detected in control traps containing tap water (Table 4). In the bait trials, *Z. tuberculatus* was the most numerous species, with 9975 individuals in sum, followed by *D. subobscura* with 1047 individuals and *D. suzukii* with 197 individuals. Considering the weekly catches by species, statistically significant differences were found in the effectiveness of different baits (Tukey test, $P<0.05$). The most common species, *Z. tuberculatus* was

Table 3. The mean number of the sum of drosophilid individuals captured per trap with different colours in the study period (individuals/trap/study period)

Drosophilid species	Mean ±SE* (minimum-maximum numbers) number of flies							
	Yellow	Blue	Green	Red	Black	White	Transparent	Sum
Drosophila busckii	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.67±0.33 (0-1)	0.33±0.33 (0-1)	0.00	0.00	0.33±0.33 (0-1)	6
Drosophila immigrans	0.00	1.33±0.89 (0-3)	0.00	0.00	0.00	0.00	0.33±0.33 (0-1)	5
Drosophila melanogaster	0.00	0.67±0.33 (0-1)	1.00±0.58 (0-2)	0.00	0.33±0.33 (0-1)	1.33±0.89 (0-3)	0.33±0.33 (0-1)	11
Drosophila subobscura	69.00±6.11 (57-77)	44.67±9.13 (31-62)	52.00±2.52 (49-57)	54.67±10.17 (36-71)	38.00±20.52 (16-79)	45.00±2.90 (42-49)	56.33±2.91 (51-61)	1079
Drosophila suzukii	8.33±2.91 (3-13)	6.00±1.00 (4-7)	3.33±1.33 (2-6)	7.67±0.67 (7-9)	5.00±2.52 (2-10)	6.00±2.10 (3-10)	5.33±2.60 (1-10)	125
Hirtodrosophila confusa	3.00±1.00 (2-5)	1.67±1.20 (0-4)	2.33±0.33 (2-3)	3.33±2.40 (0-8)	5.33±0.89 (4-7)	1.67±3.33 (1-2)	5.00±3.10 (1-11)	67
Zaprionus tuberculatus	147.00±41.80 (67-208)	85.00±5.03 (75-91)	101.67±41.29 (55-184)	202.00±56.89 (89-270)	39.67±2.33 (36-44)	140.15±14.15 (116-165)	118.67±35.38 (48-157)	2502
Sum	683	419	483	804	265	582	559	3795

* SE: Standard error

most attracted by grape vinegar traps with 92.22±32.68 individuals/trap/week, followed by apple cider vinegar and white wine, respectively. *D. subobscura*, the second common species, was most attracted by white wine at 13.71±3.47 individuals/trap/week, followed by grape vinegar, apple cider vinegar and yeast, respectively (Table 5) (Tukey test, $P<0.05$). *D. suzukii*, the third common species, was most attracted by white wine with 1.76±0.54 individuals/trap/week, followed by grape vinegar, apple cider vinegar and yeast; however, the differences in attractiveness among the baits were not statistically significant (Table 5) (Tukey tests, $P>0.05$).

When the capture efficiency of traps with different numbers of entry holes was analysed, the number of drosophilids captured rose with the number of entry holes and traps with 8 holes provided the most captures (Table 6). The sum

Table 4. Mean of the sum of drosophilid individuals captured in different bait traps (individuals/trap/study period)

Drosophilid species	Mean ±SE* (minimum-maximum numbers) number of flies					
	Apple cider vinegar	Grape vinegar	White wine	Yeast	Water	Sum
Drosophila busckii	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.00±0.00	0.00±0.00	0.00±0.00	2
Drosophila immigrans	1.00±0.00 (1-1)	0.00±0.00	0.33±0.33 (0-1)	0.00±0.00	0.00±0.00	4
Drosophila melanogaster	0.33±0.33 (0-1)	1.67±0.61 (0-4)	1.00±0.58 (0-2)	0.00±0.00	0.00±0.00	5
Drosophila subobscura	87.67±7.33 (95-73)	116.67±19.74 (94-156)	205.67±20.10 (179-245)	24.00±13.50 (10-51)	0.00±0.00	1047
Drosophila suzukii	12.33±4.84 (7-22)	20.33±9.35 (10-39)	26.33±5.61 (18-37)	6.67±1.76 (4-10)	0.00±0.00	197
Hirtodrosophila confusa	2.00±0.58 (1-3)	9.00±0.58 (8-10)	3.66±1.10 (1-8)	0.00	0.00±0.00	44
Zaprionus tuberculatus	502.33±240.98 (147-962)	1398.33±613.67 (177-2114)	1165.67±527.00 (413-2181)	259.00±113.29 (82-470)	0.00±0.00	9975
Sum	1818	4379	4208	869	0	11274

* SE: Standard error

Table 5. The mean number of drosophilids captured weekly per trap with different bait in the study period (individuals/trap/week)

Drosophilid species	Mean* ±SE** number of flies in a trap Trap lure			
	Apple cider vinegar	Grape vinegar	White wine	Yeast
Drosophila subobscura	5.44±1.15b	7.78±1.88b	13.71±3.47a	1.60±0.62b
Drosophila suzukii	0.82±0.24a	1.36±0.62a	1.76±0.54a	0.44±0.23a
Zaprionus tuberculatus	33.49±15.20b	92.22±32.68a	17.71±24.70b	17.27±8.01b

* Means within a row followed by the different letters are significantly different (Tukey test, P<0.05). The means for the first three species and Hirtodrosophila confusa, as well as water as a control bait in Table 4, were not implemented in statistics in this table since the number of overall catches was very low. **SE: Standard error

Table 6. Mean of the sum of drosophilid individuals captured per grape vinegar trap with different numbers of entry holes (individuals/trap/study period)

Drosophilid species	Mean ±SE* (minimum-maximum numbers) number of flies				
	2	4	6	8	Sum
Drosophila busckii	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.67±0.33 (0-1)	5
Drosophila immigrans	0.00±0.00	0.67±0.67 (0-2)	0.67±0.67 (0-2)	0.67±0.67 (0-2)	6
Drosophila melanogaster	1.33±0.90 (0-3)	7.00±6.51 (0-20)	0.33±0.33 (0-1)	1.67±0.33 (1-2)	12
Drosophila subobscura	38.00±10.01 (23-57)	73.00±10.26 (53-87)	73.67±8.74 (63-91)	87.67±14.34 (59-103)	817
Drosophila suzukii	8.33±2.03 (5-12)	10.33±2.91 (5-15)	12.00±3.21 (7-18)	13.67±1.76 (11-17)	133
Hirtodrosophila confusa	2.33±0.88 (1-4)	6.67±4.70 (1-16)	6.33±5.84 (0-18)	7.67±3.67 (4-15)	69
Zaprionus tuberculatus	263.67±111.15 (135-485)	323.67±138.10 (167-599)	247.67±84.36 (140-414)	404.33±115.37 (179-560)	3482
Sum	942	1010	1024	1548	4524

* SE: Standard error

of the number of drosophilids was 1548 in the traps with 8 entrance holes. When the numbers were analysed, there were no statistical differences in the attractiveness of the entrance hole numbers (Tukey test; P>0.05). *Z. tuberculatus* was the most numerous drosophilid in the traps, with 3482 individuals, followed by *D. subobscura* and *D. suzukii* with 817 and 133 individuals (Table 6).

DISCUSSION

In recent years, drosophilids such as *D. suzukii* and *Z. indianus*, which are invasive species that damage many soft-textured fruits, have started to attract attention as agricultural pests with their spread in many countries with high agricultural potential (EPPO 2023). The fact that both of these drosophilid species have become critical pests in several fruits, which play an important role in world trade, encourages studies on their control. Within the framework of detecting, monitoring or mass trapping of the drosophilids, traps have gained importance, and their implementations are becoming widespread (Harmon et al. 2019, Joshi et al. 2014, Lee et al. 2013, Özbek-Çatal et al. 2019, Rodriguez-Saona et al. 2020). Trap optimisation is essential for the effective use of traps. For this reason, it is crucial to know the factors affecting the traps' capture efficiency to reduce the catch variation and get more effective results.

Within this context, Basoalto et al. (2013) studied the trapping efficiency of trap colour, volume and number and width of entry holes. According to the average number of flies landing on coloured cards, red and black, as well as burgundy, were the most effective colours for capturing *D. suzukii*. In laboratory studies, green and blue colours were found to be less attractive. However, in our study, red was the most effective colour for capturing Drosophilidae species in terms of total numerical value. When we evaluated the drosophilids separately by species, it was found that the red colour attracted *Z. tuberculatus* very effectively. On the other hand, yellow-coloured traps attracted more *D. subobscura* and *D. suzukii*. Lee et al. (2013) stated that the yellow colour was the most effective in attracting *D. suzukii* compared to the other colours. Still, there was no statistical difference between yellow and red, similar to the results of the present study. The transparent traps seem to be less effective in terms of the number of individuals captured in total.

Another factor affecting trap efficiency is the trap volume and entry area in the traps. In the trials with plastic jars, colour, number of entry holes, and the ratio of entry area on the bottle were compared concerning the capture efficacy of the traps, and red and black colours and larger entry areas provided more effective capture. Additionally, it was found that transparent traps followed them concerning the number of flies captured (Basoalto et al. 2013). In the present study, similar results were obtained relating to the ranking of the effect of transparent traps concerning the colour, and transparent traps followed the other colours found to be more effective in the experiment (Table 3). Controversially, however, fewer drosophilids were captured in the present study using black traps. The effect of different attractant lures in the traps could be a reason for these different trial results.

Traps with more number and larger diameter of entry holes were found to be more effective in capturing *D. suzukii* (Basoalto et al. 2013). From the results of the study, it can be said that the total number of drosophilids captured increased with the number of trap holes, and the most effective results were obtained in traps with 8 holes. In other words, increasing the number of entry holes on the trap increases the probability of entry by drosophilids.

The results in the present study indicated that the red-coloured traps with grape vinegar and 8 holes were the most effective traps for monitoring and surveillance studies of drosophilids in all respects. However, the yellow-coloured traps with wine and eight entry holes seem to be the most promising for *D. suzukii* in monitoring and mass trapping. Wine traps were the most attractive concerning the number of *D. suzukii* flies in the traps. Grape vinegar, being second in terms of trap efficiency by only a very small margin, may be preferred instead of wine, depending on the price of wine, and whether it will increase the costs of the traps in some countries. Many studies are required to develop more effective traps for catching pest flies. Drosophilid flies have a wide range of habitats in which they live, differ greatly in their feeding habits, and are affected by specific environmental factors that affect the efficiency of the trap. Our results may contribute to the development of more effective and more species-selective traps for different drosophilid pests.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışmanın amacı, farklı cezbedici besin, renk ve delik sayısı içeren tuzakların drosophilidleri yakalama etkinliklerinin belirlenmesidir. Bunun için renk olarak sarı, mavi, yeşil, kırmızı, siyah, beyaz ve şeffaf (renksiz) tuzaklar denemeye alınmıştır. Cezbedici sıvı olarak, elma sirkesi, üzüm sirkesi, beyaz şarap, maya ve su (kontrol) kullanılmıştır. Ayrıca, 2, 4, 6 ve 8 adet/tuzak giriş deliği olan tuzaklar değerlendirilmiştir. Tesadüf blokları deneme deseninde 3 tekerrürlü olarak Aydın ilindeki Bursa Siyahı çeşiti 437 ağaç içeren bir incir bahçesinde Eylül 2018 - Mart 2019 tarihleri arasında yürütülmüştür. Toplam 48 ağaç üzerine (1 tuzak/ağaç) tuzak asılmış ve sayımlar haftalık olarak yapılmıştır. Sonuç olarak, en çok drosophilid bireyini sayısal olarak toplamda kırmızı renkli tuzaklar cezbetmiş, bunu sarı renk izlemiştir. Mavi, yeşil ve şeffaf tuzaklar etkisiz bulunmuştur. Cezbedici olarak ise, üzüm sirkesi içeren tuzaklarda en çok drosophilid bireyi yakalanmış, bunu beyaz şarap içeren tuzaklar izlemiştir. Tuzak giriş deliği sayısı bakımından en çok yakalanma 8 delikli tuzaklarda sağlanmıştır. Tüm tuzaklarda toplamda en fazla *Zaprionus tuberculatus* Malloch, 1932 yakalanmış, onu *Drosophila subobscura* Collin, 1936 ve *Drosophila suzukii* (Matsumura, 1931) takip etmiştir. *Z. tuberculatus* en çok üzüm sirkesi içeren tuzaklarda, *D. subobscura* ve *D. suzukii* beyaz şarap içeren tuzaklarda yakalanmıştır. Drosophilidlerin

popülasyonlarının izlenmesinde içerisinde sirke bulunan, kırmızı renkli ve 8 delikli tuzakların en etkili sonucu verdiği gözlenmiştir.

Anahtar kelimeler: meyve sinekleri, tuzak, cezbedici, *Ficus carica*

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