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Evaluation of *Frankliniella occidentalis* (Thysanoptera: Thripidae) sex ratio in crops by two monitoring methods

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Inside plastic greenhouses with carnations and spray-type carnations, it was noticed that, in blue sticky traps placed at the top of crops, more males than females of *Frankliniella occidentalis* (Thysanoptera: Thripidae) were captured, while, in flowers, more females than males were detected. Six hypotheses were experimentally explored to explain this. It was concluded that the colour of traps and the height of traps in relation to the crop affect the sex ratio of thrips caught: males are more attracted towards blue than females, and the ratio of males flying at the top of the crop is higher than in other height levels. It was also observed that after a pesticide treatment, sex ratio (males /females) in blue traps increases.

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INTRODUCTION

Frankliniella occidentalis (Thysanoptera: Thripidae) is a serious pest in vegetable and ornamental crops in Portugal.

This paper deals with this species sex ratio evaluation in crops. In greenhouses with carnations and spray-type carnations, by the end of winter, beginning of spring, when the number of *F. occidentalis* individuals was increasing, it was observed that its sex ratio in blue sticky traps, placed at the top of the crop, was completely different from the one detected in flowers by an extraction method (essays 1 and 2). In traps, most thrips were males, and in flowers most were females. Six hypothesis were experimentally explored, in order to explain these observations:

(1) males may be more difficultly extracted from the flowers than females by the extraction method used (effect of the extraction method)- essay 3;
(2) males may be more attracted to blue traps than females (effect of traps colour)- essay 4;
(3) there may be a higher number of males flying at the top of the crop than females (effect of traps vertical location)- essays 5A, 5B and 5C;
(4) males could be on other parts of the plants when flowers were sampled (effect of intra-plant distribution)- essay 6;

(5) females could be concentrated inside flowers by the time they were analysed (effect of the sampling hour)- essay 7;
(6) results of essays 1 and 2 may be due to structural aspects of the crop, like the plants height or the size or structure of the flowers (effect of the crop)- essay 8.

MATERIAL AND METHODS

Field and laboratory work

Essay 1 occurred from 11 February to 1 April, in a plastic greenhouse of 1000 m^2, with several varieties of carnations and spray-type carnations. Essay 2 (a repetition essay) occurred from 3 March to 19 May, one year later, in a plastic greenhouse of 2000 m^2, also with several varieties of those two crops. Greenhouses were located near Palmela, 50 Km south Lisbon, in Portugal.

Essays 3, 4, 5A, 5B, 6 and 7 were conducted in the same greenhouses indicated above. Essays 5C and 8 were developed in an 800 m^2 plastic greenhouse with strawberry plants, in the same geographic area. Field work of all these essays occurred in spring (March, April and May).

Thrips were collected from sticky traps and from vegetable material.

Sticky traps were acrylic plates (Plexiglas, 10x15 cm, 3 mm thick), coated by glue (Napvis) on both faces. The colours of the plates were: blue (no. 326), yellow (no. 566), and white (no. 199). In the laboratory, thrips captured in traps were taken off with the aid of drops of commercial petroleum spilled over the specimens, and acting for approximately 10 minutes to dissolve the glue. Slides for microscope observation were prepared.

A "shaking method" for removing thrips from flowers was developed: a white cotton cloth, trapped inside an embroidering frame, was placed under the flowers, which were shaken towards that white surface 3 times. Thrips fallen onto the cloth were collected with a moistened thin brush and put inside a tube with 70% alcohol. For each flower, this process was repeated 3 times. With this method, flowers remain in the crops, constituting a non-destructive sampling procedure.

A "washing method" was applied, in the laboratory, to the vegetable samples taken from the greenhouses: the vegetable material was dipped inside a recipient with water and some drops of detergent and it was agitated during approximately 2 minutes.

Liquids with thrips (alcohol or soapy solution) were filtered and thrips present on the filter paper were analysed.

Essays 1 and 2. Blue sticky traps were hung up at the top of the crop, regularly distributed through the area of the crop (12 traps in essay 1, and 24 in essay 2). Traps were replaced by new ones, every week. On the day of traps replacement, at sunset, 30 flowers were randomly chosen, and thrips were collected from them by the shaking method.

Essay 3. In each of 6 sampling dates, at sunset, 20 flowers were randomly chosen and the shaking method was applied to them. Fallen thrips were collected and, afterwards, each flower was cut and put individually in alcohol 50%. These flowers were later submitted to the washing method.

Essay 4. 12 yellow traps and 12 white traps were randomly distributed over the area of the crop, and, at a distance of 50 cm from each one, a blue trap was placed. So, each pair of colours (yellow- blue and white-blue) had 12 blocks of traps. All traps were hung up at the top of the crop. One week later, traps were removed.

Essays 5A and 5B. In each essay, 10 blocks, each with 4 blue traps hung up at different height levels, were randomly distributed, and stayed there for one week. In Essay A, level 1 corresponded to the top of the crop; level 2 was inside the crop, immediately below level 1; level 3 was 30 cm above level 1, and level 4 was 30 cm above the 3. In essay B, levels 1 and 2 were

the same as in essay A, but level 3 was 30 cm below the 2, and level 4 was 30 cm below the 3. Laterally, the distance between traps in each block was of 50 cm.

Essay 5C. Blue sticky traps were distributed randomly in 10 blocks, each with 3 traps, placed at 3 different height levels over the crop. Level 1 corresponded to the top of the crop; level 2 was 30 cm higher than level 1, and level 3 was 30 cm higher than level 2. Laterally, the distance between traps inside each block was of 50 cm. At the end of 5 days, traps were replaced by new ones, for two times, constituting two sampling dates.

Essay 6. A total of 60 plants were chosen randomly in different sampling dates. From each one, one flower bud and 5 leaves were collected randomly, at sunset. Buds were transported in alcohol 50% and leaves in plastic bags. In the laboratory, they were submitted to the washing method.

Essay 7. In each of 5 sampling dates, 30 flowers were randomly chosen and submitted to the shaking method in the middle of the morning, at 11:00 h, and, in each of 5 other sampling dates, the same occurred in the middle of the afternoon, at 15:30 h.

Essay 8. 10 blue sticky traps were randomly placed at the top of the crop and, 5 days later, were replaced by new ones. This occurred 6 times. At the day of traps replacement, at sunset, 15 flowers, 30 leaves (in the first two samples and 50 leaves afterwards) and 30 fruits were collected. Leaves and fruits were randomly chosen. Flowers were chosen randomly for one hour and those with thrips were collected; by the end of that period of time, the number of flowers was completed with flowers chosen randomly with or without thrips. This procedure was followed because thrips density was still too low by the time of sampling collection. Leaves and fruits were transported to the laboratory inside plastic bags, and flowers, in alcohol 50%. Later, they were submitted to the washing method.

Statistical analysis

Thrips analysed belong to *F. occidentalis*. Statistical tests were conducted at a 5% level of significance. Sex ratio was analysed as the males/ females ratio, except when no females were captured; in this situation, the ratio analysed was females/ males.

Essays 1 and 2. In each essay, mean sex ratios in traps and flowers were compared by the paired- sample t test. Data were previously transformed by the square root of (x+0.5).

Essay 3. The number of fallen males and females (removed from the flowers by the shaking method) was determined, in each sample. The total number of thrips previously inside the flowers was also calculated (by the sum of those fallen by the shaking method plus those still found in the flowers by the washing method). The mean rates (%) of fallen males and females were compared by the paired- sample t test.

Essay 4. Mean sex ratios in blue and yellow traps and in blue and white traps were compared by the Wilcoxon paired- sample test.

For each pair of traps, the total number of males captured was calculated, in each block. This number was divided in two (the expected number of males in each trap of the block, if the males were equally attracted to both colours). The mean value of expected males in blue traps was compared with the one of males, in fact, captured by the blue traps, using the paired- sample t test. Data were previously transformed by log (x). The same analysis was performed for females.

In relation to the blue traps of the pairs blue-yellow and blue-white, the deviations of males and females captured from the expected values were calculated (the number

captured in traps subtracted by the expected referred above). Comparison between males and females deviations was done by the Wicoxon paired- sample test.

Essays 5A and 5B. Sex ratios in traps were analysed by a two factor ANOVA for randomised block design, and means were compared by the Tukey test. Data were previously transformed by log (x+0.5) for essay A and log (x) for B.

Essay 5C. Sex ratio was analysed by Friedman analysis of variance by ranks (randomised block data), followed by a nonparametric test for multiple comparisons (Zar, 1984).

Essay 7. Mean sex ratios detected in flowers at 11.00 h, and at 15:30 h were compared by the t test. The overall mean sex ratio from the samples taken at these two sampling hours (joined together) was compared with the one obtained in essay 2, in flowers, at sunset (at approximately 21:00 h), also by the t test.

Essay 8. Mean sex ratios in traps and in flowers were compared by the paired-sample t test.

Software used was Minitab (Minitab, Inc. 1994) and Statistica (Statsoft, Inc. 1993).

RESULTS

All data presented concern to *F. occidentalis*.

For essay 1 and 2, in every sampling dates, sex ratio (male/ female) was always higher than 1 in traps and lower than 1 in flowers (Table 1). There were significant differences in the mean sex ratios in flowers and traps in both essays. It was also observed that after a pesticide treatment there was a visible increase in the sex ratio in traps.

In relation to essay 3, the rates of fallen males and females by the shaking method are presented, for each sample, in table 2. The mean rate of fallen males was 83.0% (± 7.0) and of fallen females was 83.7% (± 4.6), and there was no significant difference between them.

In essay 4, the comparison of the sex ratios in blue and yellow traps (pair 1) and in blue and white traps (pair 2) revealed significant differences (Table 3).

The mean number of males and females captured in blue traps was higher than the ones expected if both colours in pair 1 or in pair 2 attracted equally *F. occidentalis* (Table 4). Differences were significant in 3 of the 4 comparisons.

The deviations of males captured in blue traps (in relation to the expected number) were significantly higher in both pairs of colours than the deviations of females (Table 5).

In essays 5A, 5B and 5C, it was at the top of the crop (level 1) that the highest ratio of males was observed. Comparisons between the height levels are presented in tables 6 and 7. There were differences in the sex ratio between levels.

In relation to essay 6, in the 300 leaves sampled, 2 females and 1 male were collected, and in the 60 flower buds, 15 females and 9 males were collected. These data correspond to a sex ratio (male/ female) of 0.5 in leaves and 0.6 in flower buds.

There was no significant difference between the mean sex ratios detected in flowers at 11:00 h and at 15:30 h, in essay 7 (Table 8). There was also no significant difference between the overall mean sex ratio (the two sampling periods joined together) (0.22± 0.06) and the one observed also in flowers, at sunset, at approximately 21:00 h (table 1- essay 2).

Finally, in essay 8, there was a significant difference between the mean sex ratio in traps and the one in flowers (Table 9). Males were predominant in traps, and females in flowers. In leaves, 2 females and 1 male were detected, and, in fruits, no thrips were observed.

Table 1. **Sex ratio (males/females) of *F. occidentalis* in blue traps and in carnation and spray-type carnation flowers (essays 1 and 2)**

Essay 1			Essay 2		
Sample	Traps	Flowers	Sample	Traps	Flowers
1	7.0	0	1	2.8	0.2
2*	1.3	0	2*	3.6	0.2
3	3.0	0	3	7.1	0.3
4*	1.1	0.3	4	3.0	0.3
5	70.0	0.7	5	1.6	0.4
6	17.9	0.8	6	1.3	0.3
7	9.9	0.4	7	1.3	0.2
			8	1.3	0.5
			9	1.1	0.3
			10	1.1	0.2
Mean	15.7	0.31	Mean	2.42	0.29

the asterisk indicates a pesticide treatment in the crop; standard deviations (by order of means presentation):24.65, 0.34, 1.88, 0.10.

Table 2. **Rate (%) of fallen males and females of *F. occidentalis*, dislodged from carnation and spray-type carnation flowers by the shaking method (essay 3)**

Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
92.3	88.5	87.5	80.0	75.0	88.5	87.0	86.1	76.4	77.6	79.7	81.7

Table 3. **Mean sex ratio (females/ males) of *F. occidentalis* in the two pairs of coloured traps (in essay 4)**

Blue	Yellow	Blue	White
0.36	0.23	0.29	0.03

mean values are indicated just as a reference (the statistic test used was a non-parametric one); standard deviations: 0.47, 0.31, 0.55, 0.05, respectively.

Table 4. **Mean number of captured and expected males and females in blue traps of both pairs of coloured traps (essay 4)**

Individuals	Pair 1 (Blue/ Yellow)		Pair 2 (Blue/ White)	
	Captured	Expected	Captured	Expected
Males	1.13	0.98	1.12a	1.03a
Females	0.54	0.44	0.45	0.33

means followed by "a" are not significantly different; means presented are those of transformed data; standard deviations (by order of presentation of males means and females means): 0.37, 0.35, 0.46, 0.40, 0.52, 0.45, 0.30, 0.25.

Table 5. **Mean deviations of males and females of *F. occidentalis* captured by blue traps in both pairs of colours (essay 4)**

Pair 1 (Blue/ Yellow)		Pair 2 (Blue/ White)	
Males	Females	Males	Females
6.71	2.13	3.92	1.08

standard deviations: 12.10, 3.10, 4.73, 1.43, respectively.

Table 6. **Mean sex ratio (male/female) of *F. occidentalis* in blue traps placed at different height levels (essays 5A and 5B)**

Essay	Level 1	Level 2	Level 3	Level 4
A	0.40 a	0.24 a, b	0.27 a, b	0.09 b
B	0.18 a	–0.22 c	0.04 a, b	–0.12 b, c

level 1 corresponds to the top of the crop; means presented are those of transformed data; in each essay, values followed by the same letter are not significantly different; Error MS: 0.19 and 0.15 respectively.

Table 7. **Mean sex ratio (female/ male) of *F. occidentalis* in blue traps placed at different height levels (essay 5C)**

Height levels	Sample 1	Sample 2
1	0.03	0.05
2	0.20	0.32
3	0.57	0.79

level 1 corresponds to the top of the crop; mean values are indicated as a reference (the statistic test used was a non-parametric one); standard deviations by the order of means presentation (per column): 0.59, 0.22, 0.03, 0.89, 0.15, 0.03.

Table 8. **Sex ratio (male/ female) of *F. occidentalis* in carnation and spray-type carnation flowers at two sampling hours (essay 7)**

Hours	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Mean
11:00	0.3	0.3	0.2	0.2	0.2	0.24
15:30	0.3	0.1	0.2	0.2	0.2	0.20

standards deviations: 0.05 and 0.07, respectively.

Table 9. **Sex ratio (female/ male) of *F. occidentalis* in blue traps and in flowers of strawberry plants (essay 8)**

Sample	Traps	Flowers
1	0.0	4.0
2	0.1	6.5
3	0.1	4.5
4	0.1	4.5
5	0.1	6.0
6	0.1	0.2
Mean	0.08	4.28

standard deviations: 0.44 and 2.22, respectively.

DISCUSSION AND CONCLUSIONS

In this paper, the authors try to explain the observations made in essays 1 and 2: the sex ratio of *F. occidentalis* obtained by the two monitoring methods used were significantly different- with blue sticky traps, more males than females were detected in the crop (carnations and spray-type carnations), but samples taken in flowers by a shaking method indicated the presence of more females than males. HIGGINS (1992) and TERRY & KELLY (1993) also observed, in other crops, that the proportion of males of this species was higher in traps (yellow ones) than in samples of vegetable material.

Having in mind the importance of sex ratio evaluation for crop protection (see review in MATEUS, 1998), six hypothesis were experimentally explored.

It was not detected a significant difference between the rate of males and females extracted from flowers by the shaking method (essay 3).

It was noticed that the colour of traps attracts males and females differently (essay 4): there were significant differences in the sex ratio observed in blue traps when compared with yellow or white traps. Furthermore, the number of males and females captured in blue traps was significantly higher (in 3 of the 4 cases studied) than the expected one if they were equally attracted to both colours of the pairs of traps (blue- yellow or blue- white). Additionally, the mean deviations of males in relation to those expected values were significantly higher than those of females.

Literature on *F. occidentalis* does not offer conclusive results about males and females reactions towards colours. Matteson *et al.* (1992) observed very small differences in male and female "spectral efficiency", and field experiments with traps of different colours showed differences in male and female attraction towards colours, but not in a consistent way (VERNON & GILLESPIE, 1990; MATTESON & TERRY, 1992; HIGGINS, 1992 and MATEUS *et al.*, 1997).

It was observed that the position of traps in relation to the crops (height level) affects the sex ratio determination (essays 5A, 5B, and 5C). It was also noticed a higher number of males than females flying at the top of the crop than in any of the other height levels tested. The crops here studied were carnations, spray-type carnations and strawberries. An effect of traps location on sex ratio evaluation was also observed in cucumber by GILLESPIE & VERNON (1990). It

would be interesting to study, in the future, this effect in relation to the position of flowers in the canopy of crops, since *F. occidentalis* is highly attracted towards flowers.

It was not proved that, when flowers were sampled, males were predominantly located in other parts of the plants (essay 6): in leaves and in flower buds, sex ratio was not deviated towards males. This result is supported by ROSENHEIM *et al.* (1990) and HIGGINS (1992), who observed, in vegetable crops, that the ratio of males was higher in flowers than in other parts of the plants.

In relation to the time of flowers sampling (essay 7), there were no significant differences in the sex ratios detected in the three sampling periods (in the middle of the morning, in the middle of the afternoon and in the end of the afternoon) and so, it was not proved that the time of flowers sampling has an effect on sex ratio determination. This conclusion is supported by CHYZIK *et al.* (1995) and KOGEL (1997), who also did not detect differences in the presence of males and females in flowers, at different hours of the day.

To test the hypothesis of a possible effect of crop structure, or flower structure or dimension, in the observations under study, strawberry crop was chosen, because it is very different in those parameters from carnations and spray type carnations (essay 8). Results indicate that sex ratio in traps and in the flowers were deviated in the same way in the three crops, which means that this hypothesis did not receive approval.

So, in conclusion, the deviation of sex ratio towards males in blue sticky traps placed at the top of the crop, while it was deviated towards females in the crops are supported by two hypothesis: (1) males are more attracted towards blue traps than females; and (2) the ratio of males flying is higher at the top of the crop.

An additional observation was made during field work: the influence of pesticide application on the sex ratio obtained in traps. This must be taken into account when studying this parameter or using it for evaluating the population dynamics.

In the future, it should be interesting to explore if males have a higher flight activity in relation to females, being, consequently, more likely captured by traps. In favour of this hypothesis is the work of TERRY & SCHNEIDER (1993), who observed that males of *F. occidentalis* may inseminate several virgin females in a period of 1 or 2 hours and that females have periods of several days in which they reject males in their attempt to copulate. This suggests a strong competition between males, looking for receptive females, possibly accompanied by a higher flight activity. Furthermore, BRYAN and SMITH (1956) observed that females of this species are relatively inactive during a period of time immediately before oviposition.

The evaluation of sex ratio for any purpose, biological studies or crop protection, must always be accompanied by the indication of the monitoring method used.

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RESUMEN

MATEUS C., J. ARAÚJO, A. MEXIA. 2003. Evaluacion de la proporción de sexos de *Frankliniella occidentalis* (Thysanoptera: Thripidae) en cultivos mediante dos métodos de muestreo. *Bol. San. Veg. Plagas*, **29**: 191-199.

En invernaderos de plástico con claveles estándar y claveles mini se observó que en las trampas adhesivas azules localizadas en la parte superior del cultivo se capturaron más machos de *Frankliniella occidentalis* (Thysanoptera: Thripidae) que hembras, mientras que en las flores se detectaron más hembras que machos. Para explicarlo se estudiaron experimentalmente seis hipótesis. Se concluyó que el color y la altura de las trampas con relación al cultivo afecta a la proporción de sexos: los machos son más atraídos hacia el color azul que las hembras, y hay una mayor proporción de machos que vuelan a la parte superior del cultivo (con relación a otros niveles del cultivo). También se observó que, después de un tratamiento plaguicida, se incrementa la proporción de sexos (machos / hembras) en las trampas azules.

Palabras clave: *Frankliniella occidentalis*, Thysanoptera, proporción de sexos.

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