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Frankliniella occidentalis (Thysanoptera; Thripidae) sex-ratio evaluation in pepper crop greenhouses in relation with biotic and abiotic parameters

M. ELIMEM[1], A. HARBI[2], A. HAFSI[2], S. BEN OTHMEN[2], E. LIMEM-SELLEMI[3], B. MICHEL[4], B. CHERMITI[2]

[1] High School of Agriculture of Mograne (ESAM), Mograne, Zaghouane, University of Tunis, Tunisia

[2] Higher Institute of Agronomy of Chott-Mériem, 4042, Université de Sousse, Tunisia.

[3] General Directorate of Agricultural Protection, Ministry of Agriculture of Water Resources and Fisheries, 30, Alain Savary Street, 1002-Tunis le Belvédère, Tunisia

[4] French Agricultural Research Centre for International Development. Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Montpellier, France.

*Corresponding author: mohammed.elimem123@gmail.com

Abstract – This study was held in two experimental sites belonging to the Tunisian Eastern Central Coast. *Frankliniella occidentalis* Pergande 1895 (Thysanoptera; Thripidae) males and females counting during the study period using two sampling methods; blue sticky traps and sampling of flowers, showed that differences between both sexes tends gradually to rise approaching hot season with a continuous increase of females' number till the end of prospecting. These differences between both sexes affected the sex ratio recorded on flowers and blue sticky traps which tended to decrease progressively from 0.13 and 0.15 to 0.02 and 0.009 in pepper flowers in the biotope of Bekalta, from 0.11 and 0.14 to 0.08 and 0.06 in pepper flowers and 0.37 to 0.19 on blue sticky traps in the biotope of Moknine. Biotic and abiotic parameters in the greenhouses had an impact on sex-ratio tendency; more climatic conditions and pepper flowers' number improve and thrips density becomes higher, more sex-ratio tends to decrease. The apical stratum of pepper crop had the highest sex-ratio and males, females and total thrips number with often significant differences with the other strata (central and basal). Same as for blue sticky traps where sex-ratio was higher than in pepper flowers with significant differences.

Keywords: males, females, host plant phenology, climatic conditions, sampling.

1. Introduction

The Western Flower Thrips, known also as *Frankliniella oddidentalis* Pergande, 1895 (Thysanoptera, Thripidae), originates from the western United States and specifically from California. Its dispersion was limited since the sixties in the northwest of the United States, Canada and Mexico. Thereafter it has spread since 1970 to many countries in different continents such as Europe, Africa, Asia and Oceania (EOPP 2002; Lacasa et al. 1996). However, it is still considered as quarantine pest in Tunisia due to its ability of viruses' transmission (Belharrath et al. 1994). It is a species of thrips that may attack a very large rank of botanical families including trees, vegetables and horticultural and ornemental species (Yudin et al. 1986; González-Zamora and García-Mari 2003; Papadaki et al. 2008; Chau and Heinz 2006).

Damages of this thrips species occur generally during feeding of larvae and adults; scares are externalized on leaves and white spots appear on the petals of flowers, but soon they become brown, then dry and perforate. When scares affect flower buds they may prevent them of fully deploying. Sepals, in case of attack, become crimped and slightly discolored (Alford 1991; Brun et al. 2004).

Abiotic parameters such as temperature, relative humidity and photoperiod and biotic factors such as phenology of the host plant have a big influence on this pest development, its biotic potential, population evolution and increase, and even morphological and biological characteristics (Bournier 1983; Loomans and van Lenteren 1995; Guérineau 2003; Whittaker and Kirk 2004; Chaisuekul and Riley 2005; Fraval 2006; Elimem and Chermiti 2009; Elimem et al. 2011).

This work aims to study *F. occidentalis* sex-ratio evolution and variation in relation with climatic conditions and host plant phenology in two pepper crop greenhouses situated in different locations during 2009 and 2010 in Tunisia using two sampling methods, bleu sticky traps and pepper crop sampled flowers.

2. Materiel and Methods

2.1. Experimental sites

The study of sex-ratio and adults population evolution was carried under two 500 m² pepper crop greenhouses situated in different regions of the governorate of Monastir (Central Eastern coast of Tunisia). First greenhouse is located in the region of Moknine (35°37'45.39''N 10°55'57.41''E, elevation: 1m) during 2009, and the second one is located in the region of Bekalta (35°36'16.50''N 11°00'16.69''E, elevation: 6m) during 2010. Both greenhouses were formed by four rows of the Tunisian pepper variety "*Chargui*". The inter-row distance is about 1 m.

2.2. Adults' trapping

F. occidentalis adults' population monitoring was carried out only in the greenhouse situated in the region of Moknine using ten weekly renewed blue sticky traps (Koppert®) installed along the greenhouse between the pepper rows and suspended at a height of 30 cm above pepper plants. The distance between each trap is about 10 m. The blue sticky traps used were 25 cm long and 10 cm wide.

2.3. Sampling of flowers

Adults' population monitoring on the host plant flowers was done through a weekly flowers sampling in both greenhouses using same sampling method. In each greenhouse, the four rows have been divided into five blocks each, making thus a number of 20 sampling units. From each one, a pepper plant was selected randomly and from which three flowers were sampled from the different plant parts (upper part, medium part and lower part). Each sampled flower was placed in a plastic bag on which the numbers of sampling unit and the strata have been marked. It must be noted that flowers' sampling was done between 9:00 am and 11:00 am in both experimental sites.

2.4. Estimation of flowers' number

Flowers' number was estimated once a month during 2009 in the region of Moknine and 2010 in the region of Bekalta by marking ten pepper plants in each greenhouse that served to determine the evolution of flowers' number in each greenhouse.

2.5. Monitoring of environmental parameters

Environmental parameters, temperature and relative humidity, were weekly monitored with a thermohygrograph.

2.6. Statistical analysis

The statistical analyses were done by the statistical software program Graph Pad Prism version 5. This program was used for analysis of variance (ANOVA) and nonparametric tests to determine differences between sex-ratio on both sampling methods. Statistical tests were conducted at p= 5% level of signification (p=0.05).

3. Results and discussion

3.1. Monitoring climatic conditions of the experimental sites

Climatic conditions monitoring in the region of Moknine in 2009 revealed some variations throughout the study period. In fact, during the early observation period till the end of March, the highest average temperature (Figure 1) was 14.17°C, while the lowest average that was 11.70°C was registered on March 12th. Beyond this phase, the temperature tends to increase gradually over the next months to reach average values approaching 25°C reported on May 28th.

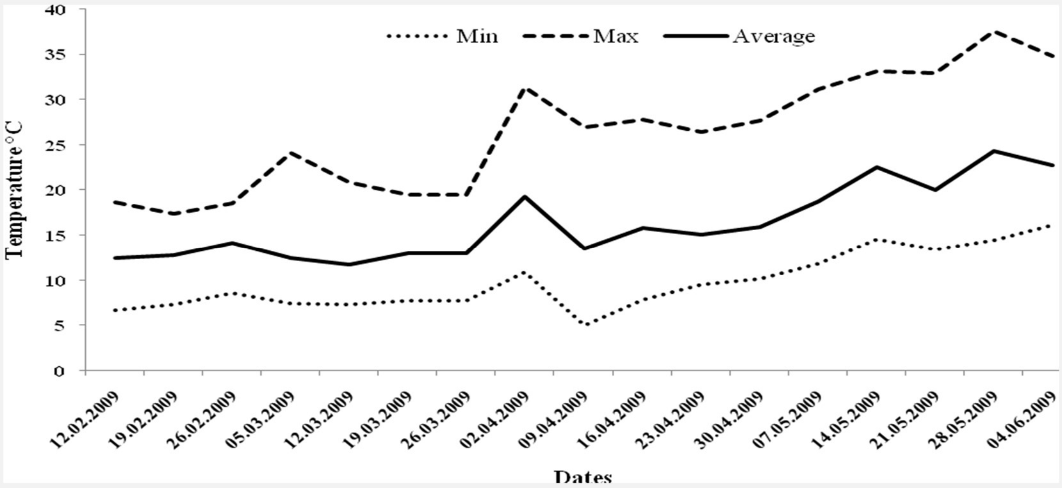

Figure 1. Temperature recorded in the greenhouse of Moknine in 2009.

Second monitored climatic condition is relative humidity (Figure 2) which average values reported were highly elevated since the beginning of prospecting till April 02nd, date from which it tended to decrease progressively without reaching very low average values, knowing that lowest average value was recorded on May 14th with 73.13%.

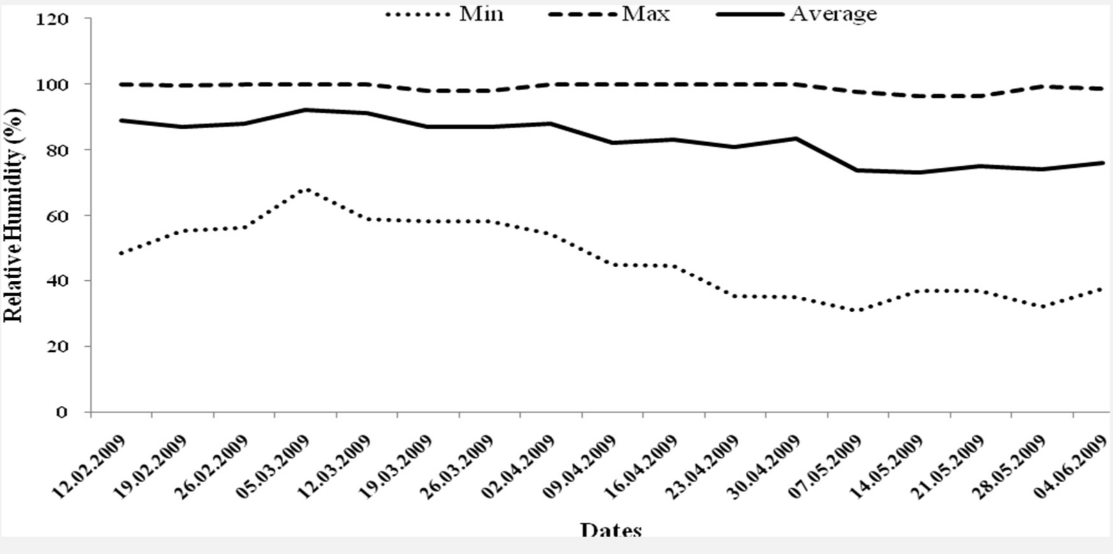

Figure 2. Relative humidity recorded in the greenhouse of Moknie in 2009.

Concerning the experimental site of Bekalta in 2010, temperature during the first period of observations increased slightly from average values of about 17.47°C on February 17th to reach 21.44°C on March 10th. Beyond that date, average temperature was more or less stable without showing huge variations with average values comprised between 19 and 21°C till February 7th where temperature had began to increase considerably to reach 26.53°C on May 05th (Figure. 3).

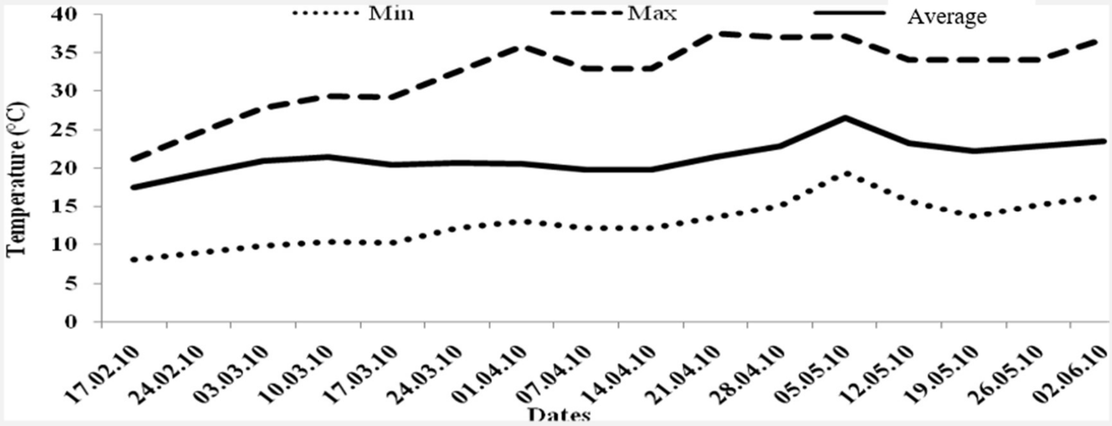

Figure 3. Temperature recorded the greenhouse of Bekalta in 2010.

Concerning average relative humidity, it was comprised between 60 to 65% from February 17th till March 07th. Then, it decreased considerably to reach average values of about 26.06% on April 07th and it had been stabilized at values comprised between 37 and 40% during the rest of the observation period. On the other hand, maximum value of relative humidity was observed on March 10th 2010 with 72.57% while lowest one was noted on May 19th 2010 with 17.14% (Figure 4).

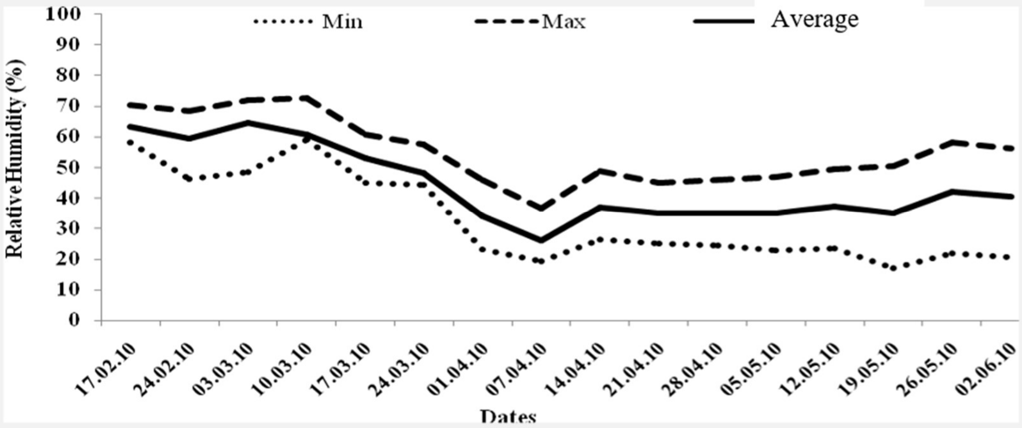

Figure 4. Relative humidity recorded in the greenhouse of Bekalta in 2010.

Photoperiod data was taken from the National Institute of Meteorology of Tunisia, and it showed that in the governorate of Monastir, this parameter never stopped to get longer till the end of the study period with 276 and 303 hours/month respectively on May and June during 2009 and 2010 (Table 1).

Table 1. Temporal evolution of the Photoperiod (in hours) during the different months of both study periods during 2009 and 2010 in the governorate of Monastir (NIMT* 2010).

Months	February	March	April	May	June
Photoperiod (Hours/month)	179	208	228	276	303

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3.2. Flowers' number estimation

Flowers' number in both greenhouses showed an obvious increase. In fact, flowers' number in the greenhouse of Moknine increased from average values of about 3.08 and 2.5 respectively during February and March 2009 to 8.03 and 10.75 flowers per plant respectively on April and June 2009. Concerning the greenhouse of Bekalta, flowers' number augmented from mean values of about 1.83 and 3.91 flowers per plant respectively on February and March 2010 to 5.08 and 8.33 flowers per plant respectively on April and May 2010 (Figure 5).

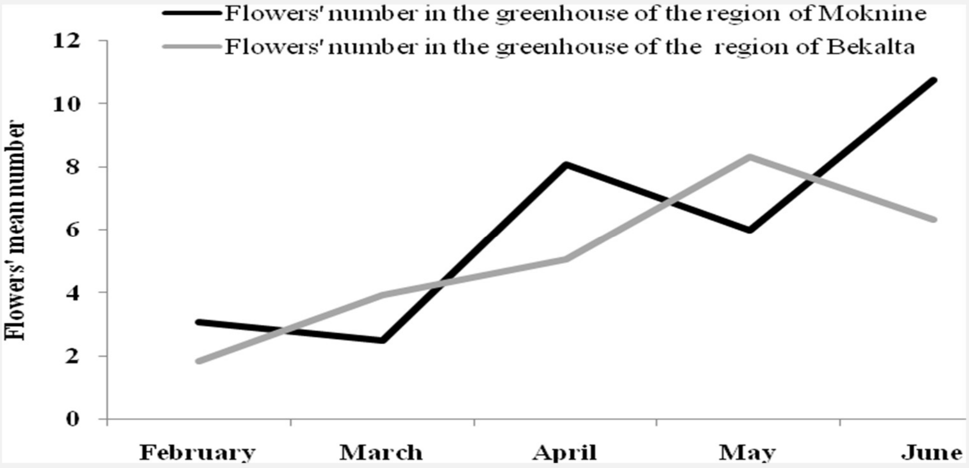

Figure 5. Pepper crop flowers' number estimation during 2009 in the region of Moknine and 2010 in the region of Bekalta.

3.3. *F. occidentalis* adults' population evolution in both greenhouses

Adults' monitoring in flowers and on blue sticky traps in both experimental sites showed that females' number was higher than males' number. However, differences between both sexes was lower during early observations, but over time, females exceeded highly males' number.

In fact, females' number in the greenhouse of Moknine (Figure 6) varied between 4.65 and 10.45 females per flower respectively on February 26th and March 19th 2009 during cold season, while males' number was about 0.75 and 1 male per flower respectively on February 26th and March 12th 2009. Approaching hot season, females' number reached values of about 15.48, 14.28 and 13.98 females per flower respectively on April 09th, May 28th and June 04th 2009 with a maximum value of 18.13 females per flower on April 30th 2009. Concerning males' number, it ranged between 1.01, 0.9 and 0.66 males per flower respectively on April 09th, May 28th and June 04th 2009 with a maximum value recorded on April 16th 2009 that was 1.35 males per flower.

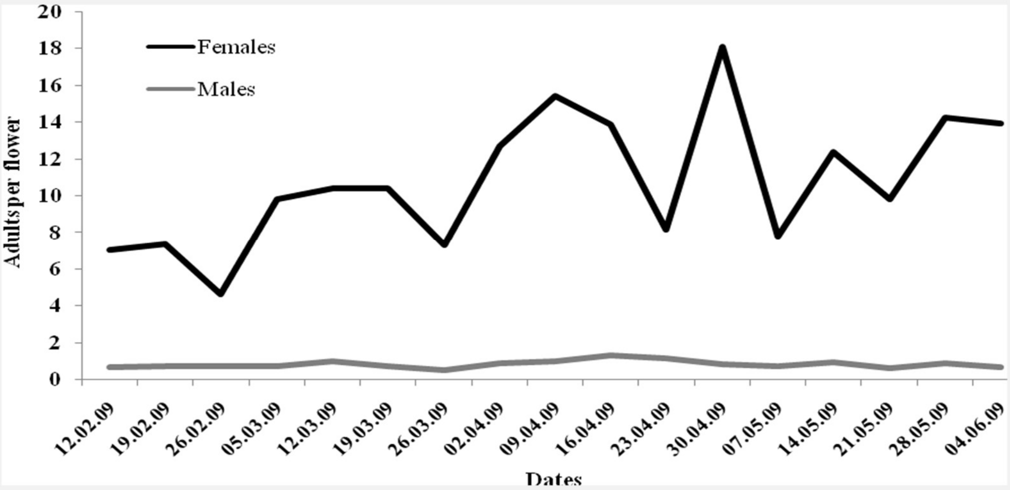

Figure 6. Mean number's evolution of *F. occidentalis* males and females per flower in a pepper crop greenhouse in the region of Moknine in 2009.

Concerning the greenhouse of Bekalta (Figure 7), females' mean number during cold season varied between 3.15, 3.05 and 0.95 females per flower respectively on February 24th, March 10th and March 17th 2010 with a maximum value of about 3.55 on March 24th 2010 while males' number was very low without exceeding a maximum value of 0.33 males per flower on February 24th 2010. Approaching hot season, females' mean number began to increase considerably from an average of about 0.8 females per flower on April 21st 2010 to 8.1 and 8.5 females per flower respectively on March 12th and 19th 2010. Males also showed an increase during the same period, however it was lowest than females' mean number increase. In fact, it rose from 0.066 males per flower on April 21st 2010 to 0.46 and 0.81 males per flower respectively on March 12th and 19th 2010.

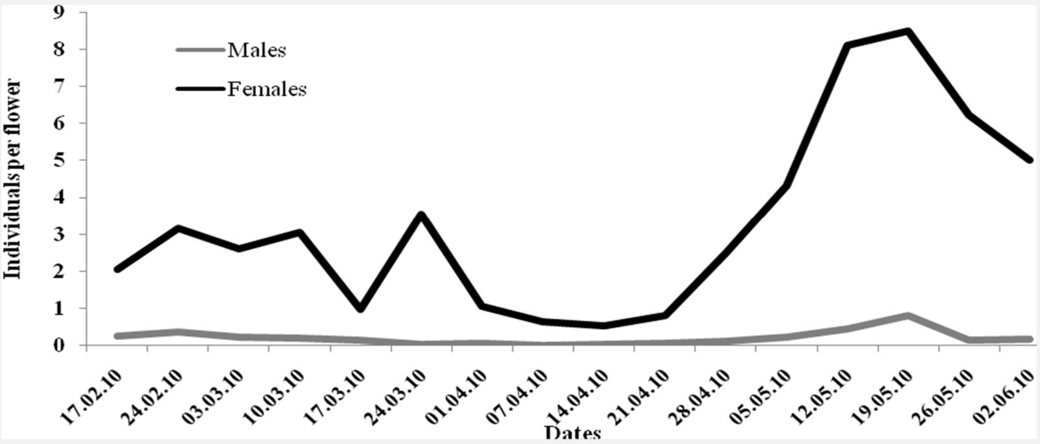

Figure7. Mean number's evolution of *F. occidentalis* males and females per flower in a pepper crop greenhouse in the region of Bekalta in 2010.

Concerning adults population on the blue sticky traps installed in the greenhouse of the region of Moknine during 2009, females and males' numbers showed almost same evolution than on flowers only that values recorded was higher (Figure 8). In fact, females' number varied between 300.9 and 701.4 females per trap respectively on February 19th and March 19th 2009. It increased approaching hot season from an average value of 854.7 females per trap on April 2nd 2009 to 2455.2 on April 23rd 2009. Beyond that date females' number did not show big variations and was comprised between 2002.8 females per trap on May 14th 2009 and 2223.2 females per trap recorded on June 04th 2009. On the other hand, males' number on blue sticky traps was lower than females with average values between 100.7 on February 12th 2009 and 309.6 males per trap on April 02nd 2009 during cold season. Throughout the period approaching hot season, it showed a maximum value of about 443.1 males per trap on April 23rd 2009 and has been stabilized at average values comprised between 397.6 on May 14th 2009 and 385.4 males per trap on June 04th 2009.

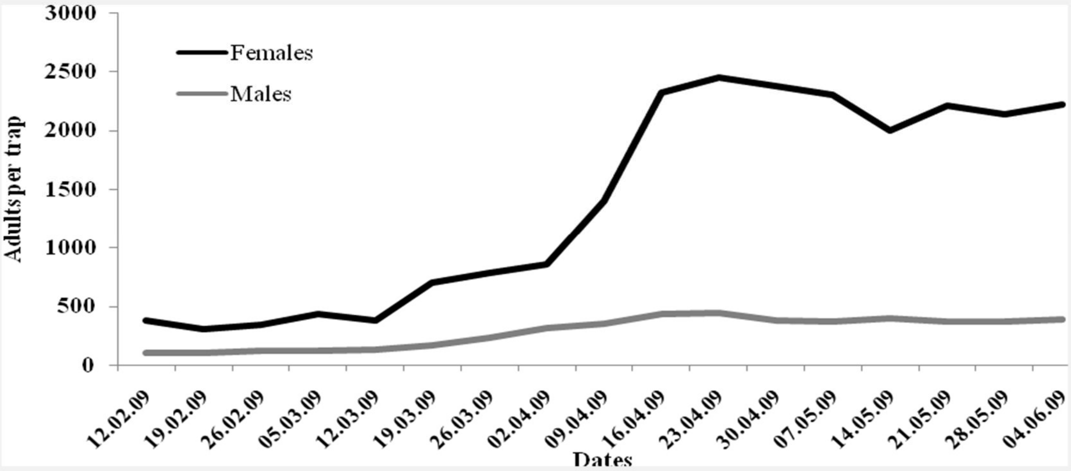

Figure 8. Mean number's evolution of *F. occidentalis* males and females per trap in a pepper crop greenhouse in the region of Moknine in 2009.

3.4. *F. occidentalis* sex-ratio evaluation

F. occidentalis sex-ratio was evaluated according to the formula mentioned by Ramade (2003) and Mateus et *al*. (2003): Sex-ratio = Number of males / Number of females.

According to the obtained results using both sampling methods and in both experimental sites, sex-ratio evolution was characterized by an obvious decline throughout the study periods in spite of some variations.

Concerning sex-ratio evolution in pepper flowers in the region of Bekalta during 2010 (Figure 9), it was comprised between 0.06, 0.15 and 0.04 respectively on February 17th, March 03rd and March 17th 2010. However, it began to decrease since March 24th reaching thus mean value of about 0.009. During the rest of the study period sex-ratio evolution was marked by a continuous decrease approaching hot season though it showed some variations between 0.083, 0.04, 0.008 and 0.05 respectively on April 21st, April 28th, May 19th and May 26th 2010. On the other hand, it must be noted that the coefficient of determination (R^2) of the polynomial tendency curve of order 2 is of about 0.207.

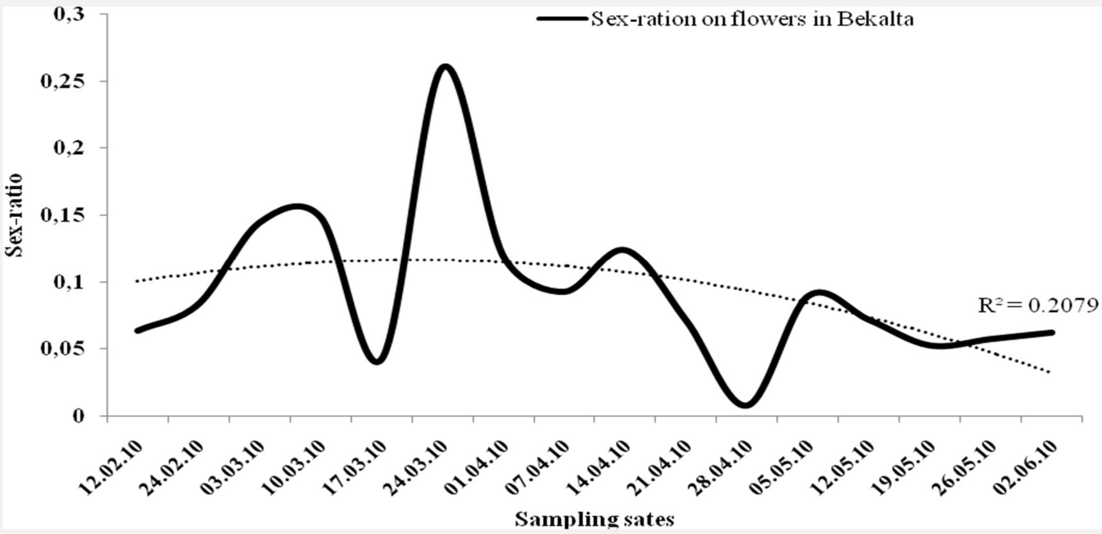

Figure 9. *F. occidentalis* sex-ratio evolution in pepper crop flowers in the region of Bekalta during 2010.

In the greenhouse of Moknine during 2009, sex-ratio evolution in flowers showed some variations throughout the study period. In fact, sex-ratio mean values ranged between 0.111, 0.147 and 0.144 respectively on February 12th, 19th and 26th 2009. Beyond those dates, this parameter decreased to 0.08 and 0.06 respectively on March 19th and April 09th 2009. Approaching the hot season, sex-ratio evolution continued its decrease till reaching mean value of about 0.04 on June 04th 2009. However, some variations were recorded showing thus two peaks of about 0.17 and 0.11 respectively on April 23rd and May 14th 2009. On the other hand, the coefficient of determination (R^2) of the polynomial tendency curve of order 2 was higher than in the region of Bekalta during 2010 with a value of about 0.221 (Figure 10).

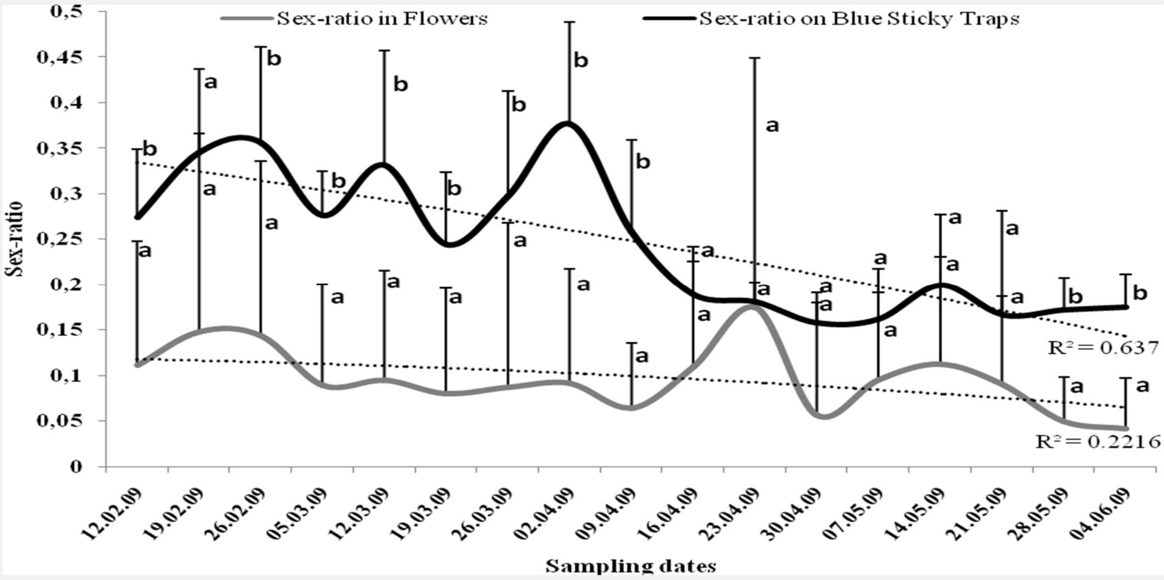

Figure 10. Evolution of *F. occidentalis* sex ratio on flowers and blue sticky traps in a pepper crop greenhouse in the region of Moknine in 2009 (Means followed by the same letter are not significantly different *p*=0.05).

Concerning sex-ratio evolution on blue sticky traps in the region of Moknine during 2009, it showed also an obvious decrease throughout the study period and approaching hot season. In fact, sex-ratio evolution was marked by some variations during February and March. Mean values varied between 0.274, 0.356, 0.276, 0.331, 0.244 and 0.376 respectively on February 12th and 26th, March 05th, 12th and 19th and April 02nd 2009. Beyond the last date, sex-ratio decreased considerably to average values of about 0.189 and 0.158 respectively on April 04th and 30th, and it continued to decrease slowly approaching the hot season showing just one peak on May 05th 2009 with an average value of about 0.199. On the other hand, the coefficient of determination (R^2) of the polynomial tendency curve of order 2 was higher than in flowers in the region of Bekalta and the region of Moknine with a value of 0.637, showing thus that sex-ratio on blue sticky traps has a higher tendency to decrease than on flowers (Figure 10).

Sex-ratio values registered during all the study period on the blue sticky traps were always higher than those recorded on flowers in the region of Moknine in 2009 and even in the region of Bekalta in 2010. In fact, the statistical analysis of variance (ANOVA) and the non-parametric tests at $p = 5\%$ showed that in the majority of the sampling dates, significant differences were noted between sex-ratio on blue sticky traps and sex-ratio in pepper flowers in the region of Moknine in 2009, except February 12th and from April 16th to May 14th 2009.

Evaluation of sex-ratio depending on different host plant strata (apical, central, basal) revealed according to the table 2 that in the region of Bekalta, the highest sex-ratio value was observed in the apical strata with significant differences with central and basal strata. Regarding the experimental site of Moknine, in spite of no significant differences between the three strata, the apical one had the highest cumulative value of sex-ratio.

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Table 2. Comparison between mean cumulative sex-ratio values encountered in the different host plant strata in both experimental sites (Values followed by the same letter in each line are not significantly different at $p \leq 0.05$).

Experimental site	Apical strata	Central strata	Basal strata
Region of Moknine	0.092 ± 0.030 b	0.083 ± 0.040 b	0.075 ± 0.032 b
Region of Bekalta	0,130±0,115 a	0,078±0,050 b	0,071±0,042 b

Cumulative values of *F. occidentalis* males, females and total adults number were the highest in the apical strata in both experimental sites and with significant differences with the central and basal strata (Table 3).

Table 3. Cumulative values of males, females and total adults in each strata in both experimental sites (Values followed by the same letter in each line are not significantly different at $p \leq 0.05$).

		Males			Females			Total adults		
		Apical strata	Central strata	Basal strata	Apical strata	Central strata	Basal strata	Apical strata	Central strata	Basal strata
Region of Moknine	Mean values	1.308 ± 0.354 a	0.723 ± 0.227 b	0.508 ± 0.155 c	15.173 ± 5.464 a	9.702 ± 3.304 b	7.573 ± 2.994 b	16,482 ± 5,660 a	10,426 ± 3,116 b	8,082 ± 3,032 b
	%	51.479 ± 5.009 a	28.341 ± 4.612 b	20.179 ± 4.944 c	46.739 ± 4.604 a	30.039 ± 3.304 b	23.221 ± 3.993 c	47,052 ± 0,303 a	29,957 ± 3,021 b	22,990 ± 3,807 c
Region of Bekalta	Mean values	1,184 ± 0,639 a	0,637 ± 0,387 b	0,484 ± 0,313 b	13,640 ± 9,354 a	9,784 ± 7,157 ab	7,940 ± 4,956 b	14,690 ± 10,052 a	10,303 ± 7,606 ab	8,387 ± 5,265 b
	%	52,071 ± 11,731 a	26,719 ± 8,680 b	21,208 ± 8,253 b	43,992 ± 4,654 a	30,863 ± 2,653 b	25,143 ± 3,958 c	45,269 ± 5,498 a	29,797 ± 3,658 b	24,932 ± 3,578 c

Obtained results concerning adults' evolution in flowers and on blue sticky traps in both experimental sites showed that in general differences between both sexes were lower during early observations in cold season when *F. occidentalis* populations were lower. However, mean number of females exceeded highly males' number and became more abundant approaching hot season and when *F. occidentalis* populations increased considerably. In fact, those results concords with those mentioned by Higgins and Myers (1992); *F. occidentalis* males tend to be more prevalent at low population densities whereas females are typically more abundant at higher densities. It must be noted that the Western Flower Thrips have a haplo-diploid breeding system, which means that the female develops from fertilized eggs and males from unfertilized eggs (Moritz et al. 2004). Unmated females can produce males or sons parthenogenetically, meaning thus without mating, whereas females must be mated in order to produce additional females or daughters (Bryan and Smith 1956; Higgins and Myers 1992; Heming 1995; Mound 1996; Moritz 1997). On the other hand, Higgins and Myers (1992) cited that increasing population densities of *F. occidentalis* in greenhouses enhances the probability of females encountering and mating with males immediately after emerging from pupal stage, and that high population densities create an age structure consisting of young and fecund females producing a pre-dominance of daughters.

Obtained results concerning sex-ratio evolution showed an obvious variation throughout the study periods in both experimental sites and using both sampling methods. Sex-ratio values were higher during cold season and tend to decrease approaching hot season. This may be explained that differences between both sexes during first period of observations were lower, and that females' numbers tended to increase approaching hot season. According to Bournier (1983), Higgins and Myers (1992), Steiner and Goodwin (2005), Fraval (2006) and Elimem and Chermiti (2009) several environmental factors may contribute directly or indirectly to obtain those variations of sex-ratio evolution. Among factors that may influence sex-ratio and even the pest reproduction ways are temperature, relative humidity, photoperiod and phenology of the host plant. Those factors may act independently or in interaction.

Alternating between these two modes of reproduction affects the WFT sex-ratio leading to important variations. The change of sex-ratio reflects the change of *F. occidentalis* reproduction mode change. Correlation between biotic and abiotic factors with sex-ratio evolution throughout the study period showed that with the improvement of those factors in favor of development of the WFT population, the sex-ratio decreases. In fact, the more average temperatures increase (Fig. 11), extends of the photoperiod (Fig. 12) and more flowers number becomes more important, more sex-ratio tends to decline (Fig. 13). However, it must be noted that the lowest values of the sex-ratio is corresponding to period during which relative humidity was low (Fig. 14).

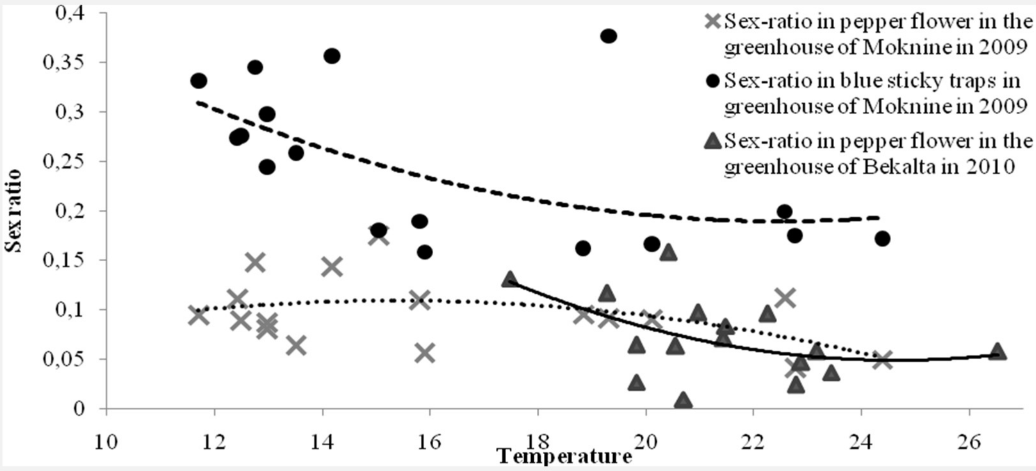

Figure 11. Correlation between sex ratio and temperature in the greenhouse of Moknine in 2009 and Bekalta in 2010.

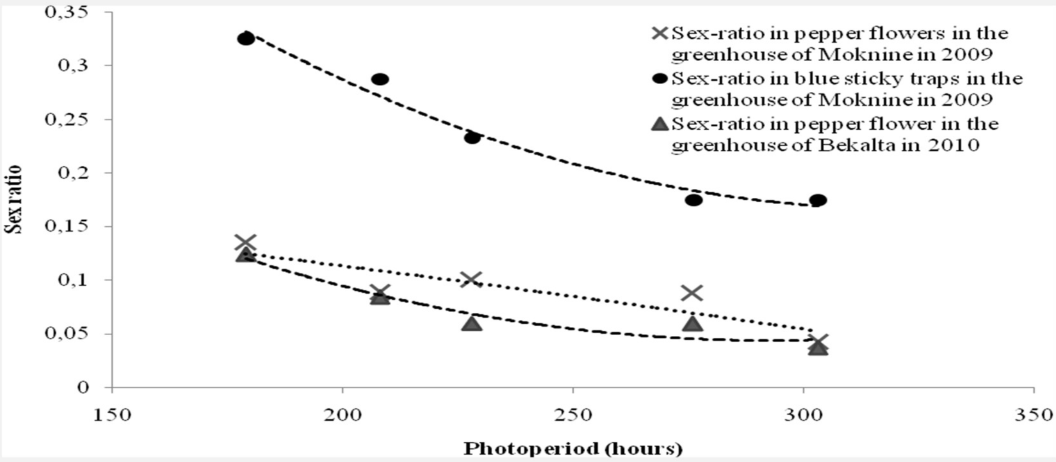

Figure 12. Correlation between sex ratio and photoperiod in the greenhouse of Moknine in 2009 and Bekalta in 2010.

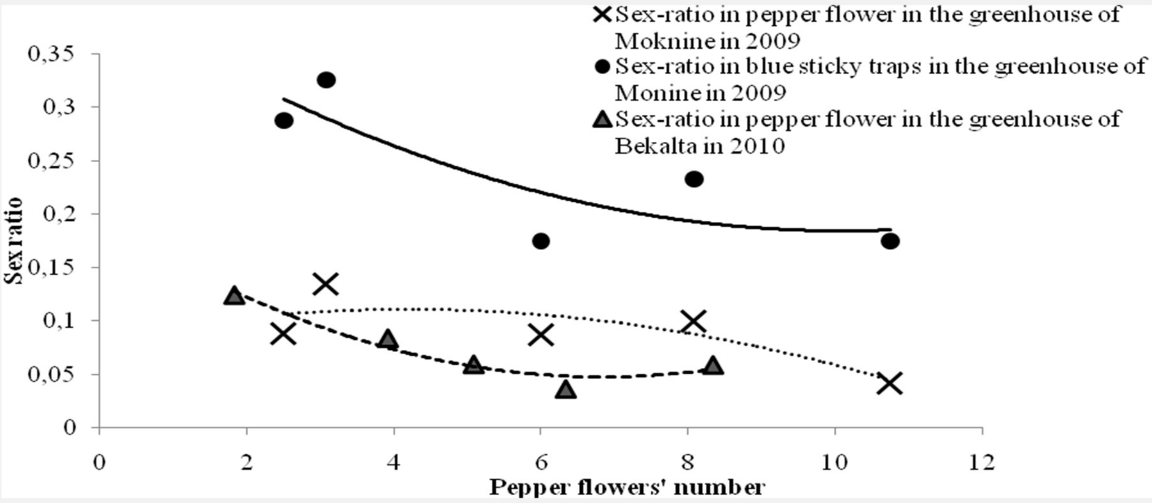

Figure 13. Correlation between sex ratio and pepper flowers' number in the greenhouse of Moknine in 2009 and Bekalta in 2010.

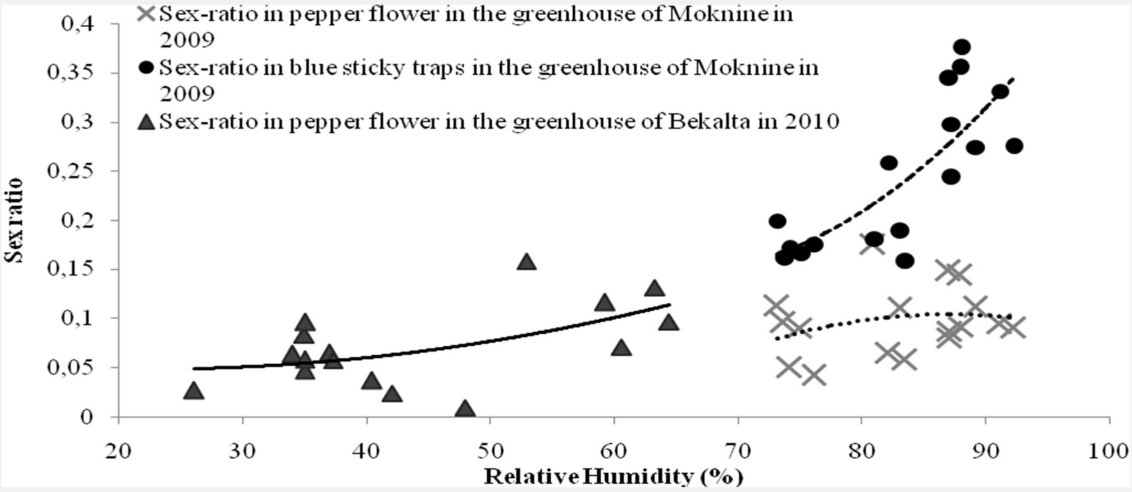

Figure 14. Correlation between sex ratio and relative humidity in the greenhouse of Moknine in 2009 and Bekalta in 2010.

In fact, according to Bournier (1983), Higgins and Myers (1992), Steiner and Goodwin (2005), Fraval (2006) and Elimem and Chermiti (2009) environmental conditions and flowers' number of the host plant may influence the population's density which has an impact on the sex-ratio evolution consequently. In both experimental sites highest thrips population was observed during hot season; period during which increase of temperature, decrease of relative humidity, day-light prolongation and high flowers' number were registered. Loomans and van Lenteren (1995), Stacey and Fellows (2002), Chaisuekul and Riley (2005) and Fraval (2006) noted that high temperatures allow *F. occidentalis* to develop rapidly and improve the population development rate and its biological potential and that the optimal thermal conditions are between 20 and 25°C. Indeed, the duration of embryonic development, and the period of pre-eggs laying become shorter at 20°C (Loomans and van Lenteren 1995). On the other hand, Bournier (1983), Loomans and van Lenteren (1995), Whittaker and Kirk (2004) and Chaisuekul and Riley (2005) mentioned that longer day length improves thrips development and also egg laying activity and that number of eggs deposed becomes higher at longer photoperiods. Moreover, it must be noted also that Western Flower Thrips development is favored by dry environments (Bournier 1983; Guérineau 2003). The obtained results are consistent with those mentioned by Elimem (2011) and Elimem and Chermiti (2009) who revealed that in Tunisian conditions highest thrips number was observed when mean temperature ranged between 15 and 25°C and that high relative humidity prevent thrips to develop and increase. Concerning flowers' role in *F. occidentalis* proliferation, Elimem and Chermiti (2009) showed that in rose crop greenhouse abundance and development of *F. occidentalis* varied depending on the host plant phenology. In fact, number of adult thrips was relatively low when

flowers were absent. However, since the first appearance of flowers on the host plant, thrips number increased. That was observed during this study when the highest thrips number was observed when flowers' number in the pepper crop greenhouses became higher. According to Gerin et al. (1999), flowers' petals are the preferential egg-laying site for the Western Flower Thrips and that this activity and the number of thrips are higher in flowering plants. *F. occidentalis* is a thrips species developing especially in flowers and on flowering plants due to its pollen consumption (EOPP 2002; González-Zamora and Garcia-Mari 2003). Moreover, Hulshof and Vänninen (2002) and Fraval (2006) mentioned that many thrips species such as *F. occidentalis* live in flowers and consumes pollen that improves their fertility and egg-laying activity, and that their development is better when flowers are rich in pollen. It is therefore clear that host plant phenology plays a key role in the development and progression of *F. occidentalis* population and consequently on its sex-ratio evolution.
It was observed also during this study that sex-ratio on blue sticky traps is higher than in flowers with significant differences in the majority of sampling dates according to the statistical analysis at $p = 0.05$. Mateus et al. (2003) showed that males were predominant in traps and females in flowers. The traps color and the height of traps in relation to the crop affect the sex-ratio of *F. occidentalis* caught. Males are more attracted towards blue than females, and the ratios of males flying at the top of the crop and at a height of 30 cm above plants are higher than in other height levels such as 60 cm above plants, making thus their capture by traps easier (30 cm above pepper plants in this work). During this study, males' number was not higher than females' number on blue sticky traps. However, males' proportion on traps was higher than males' proportion in flowers making thus sex-ratio on blue sticky traps higher than in flowers. Flowers' sampling during this study was done between 9:00 am and 11:00 am in both experimental sites which normally may have an effect on sex-ratio due to the activity of the pest. However, according to Mateus et al. (2003) and in relation to the time of flowers' sampling (in the middle of the morning, in the middle of the afternoon and in the middle of the end of the afternoon), there are no significant differences in the sex-ratios detected during those three sampling periods. Therefore, the authors added that it was not proved that the time of flowers' sampling has an effect on sex-ratio determination.

4. Conclusion

Frankliniella occidentalis sex-ratio evaluation using two sampling methods showed that approchnig hot season differences between both sexes tend to increase with a rising of females number affecting thus the sex-ratio which decrease gradually. These results show that biotic and abiotic parameters have an impact on thrips sex-ratio; temperature, relative humidity, photoperiod and flowers number. In fact, these results may help to improve different ways of control of the western flower thrips in greenhouses.

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