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Interactions between *Frankliniella occidentalis* (Insecta; Thysanoptera; Thripidae) damages and eleven pepper crop varieties' photosynthetic pigmentations

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Abstract – *Frankliniella occidentalis* Pergande (1895) (Thysanoptera ; Thripidae) is a polyphagous thrips species that may attack several plant species belonging to many botanical families. This study showed that pigmentation rates measured using a spectrophotometer of uninfected fruits and leaves showed that the varieties Jebli, Zumurut and PP1 are the richest in Chlorophylls (Ca and Cb), Carotenoids and Xanthophylls (C+X). These same varieties presented the highest damage degrees in fruits and leaves proving thus that the richest plant organs in pigmentations are more likely to be attacked by thrips. On the other hand, a decrease in pigmentations rates was observed in slightly and severely attacked plant organs when compared with intact ones. Generally, pigmentations rates in uninfected organs did not show significant differences with slightly attacked ones as for leaves and fruits. In fact, the maximum decrease in slightly infected leaves pigmentation amounts was observed especially for Carotenoids and Xanthophylls with 17.47%. Regarding fruits, the sucked chlorophylls amounts in slightly damaged fruits ranked between 0.55 and 24.37%. However, severely attacked organs showed a great reduction in pigmentations levels with significant differences when compared with uninfected organs.

Keywords: Chlorophylls, Xanthophylls, Carotenoids, pollen, phenological parameters.

1. Introduction

Frankliniella occidentalis Pergande (1895) (Thysanoptera, Thripidae), known also as the Western Flower Thrips (W.F.T.) or the Californian thrips, originates from the western United States and specifically from California. Since the sixties, 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). It is still considered as quarantine pest in Tunisia due to its ability of viruses' transmission (Belharrath et al. 1994). *F. occidentalis* is a thrips species that may attack and occur on a very large rank of botanical families including tree, vegetables, horticultural and ornamental species (Chau and Heinz 2006; González-Zamora and García-Mari 2003; Lewis 1973; Papadaki et al. 2008; Yudin et al. 1986). This thrips species may cause several damages generally during feeding of its different developmental stages. Generally, scares appear on leaves and white spots on the flowers' petals that soon 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). For instance, *F. occidentalis* on rose crop causes damages especially on flowers. They results in yellow spots and distortions of the attacked organ (Brun et al. 2004). Regarding pepper crop, the WFT may cause serious damages which leads to sever economic lost. Discoloration can be observed on attacked organs with appearance of necrosis and chlorosis. Leaves and fruits when attacked, they acquire a brownish silvery appearance and are deformed. Laying eggs can cause some damages expressed by a reaction of leaf

tissue surrounding the egg (Belharrath et al. 1994; Frantz and Mellinger 2009; Richard and Schalk 1991). On the other hand, the WFT sucks the cell host plant contents including pigments that contain such as Chlorophylls (Ca and Cb) or Carotenoids, causing thus the loss of the original color and acquisition of a silver one. According to Bournier (1983) and Alford (1991), this silver color corresponds to the empty cell of the attacked organ that will turn into brown and then dry and perforate.
This study aims to determine impact of pigmentations of several pepper crop varieties on *F. occidentalis* damage occurrence, and how thrips injury those varieties by evaluating their photosynthetic pigmentation rates in leaves and fruits.

2. Material et Methods

2.1. Experimental site

The present study was carried out in two pepper crop greenhouses situated in the Agricultural Support Station of Nebhena (35°45'02.58''N 10°49'20.47''E) belonging to the governorate of Monastir in the Eastern Central Coast of Tunisia from March 24th till June 09th 2011. Each greenhouse has an area of about 520 m².

2.2. Pepper crop varieties studied

Both greenhouses were formed by four rows that each of which is formed by two lines of pepper plants. The inter-row distance is about 1m. In the first greenhouse G1, only the two central rows concerned the study because planted varieties were marked in each plot. Each one of these rows was planted with five varieties of pepper crop; Zumurut, Jebli, Guebli, DRH 7461 and DRH 7462, forming thus five blocks in each row. Concerning the second greenhouse G2, only three rows was considered for this study. Each row was planted with six pepper crop varieties; PP1, PP2, PP3, PP4, Chargui and Starter, making each row formed by six blocks.

2.3. Thrips damages estimation

To evaluate the damages' rate of thrips pest, ten leaves were taken from each strata (upper one, medium one and lower one) making thus a total number of thirty leaves per variety. Concerning fruits, ten from each variety were sampled. Each vegetal organ sampled is considered infested when they represent thrips damages.

2.4. Pigmentations amounts

This parameter was estimated according to the method mentioned by Lichtenthaler (1987) that consists of extracting 0.1 g of fresh leaves and fruits that do not represent thrips damages. Then it must be mixed with 10 ml of acetone 80%. The obtained solution is then filtred through a filter paper and placer into Eppendorf tubes. These ones were placed in a centrifuge (Sigma®) at 13 000 rounds per minute during twenty minutes in the reason to remove and eliminated all plant debris. Then the solution was placed in a quartz curve in the reason to measure its absorbance using a spectrophotometer (PG Instruments®). The wavelength used was between 430 and 710 nm.
Pigmentations amounts ($\mu g.ml^{-1}$) were determined according to formulae of Lichtenthaler (1987).
Chlorophyll a (Ca) ($\mu g.ml^{-1}$) = $12.25\ A_{663.2} - 2.79\ A_{646.8}$
Chlorophyll b (Cb) ($\mu g.ml^{-1}$) = $21.5\ A_{646.8} - 5.1\ A_{663.2}$
Carotenoids and Xanthophylls $C_{(C+X)}$ ($\mu g.ml^{-1}$) = $1000\ A_{470} - (1.82\ Ca - 85.02\ Cb) / 198$
Knowing that Ca and Cb absorb light at wavelengths of 663.2 nm and 646.8 nm respectively and Carotenoïdes and Xanthophylls at 470 nm.
It must be noted that this part of the study interested only uninfected organs (leaves and fruits). For this, three vigorous plants of each variety were chosen since the beginning of the work and covered by muslin to prevent thrips infestation.

2.5. Impact of *F. occidentalis* on pigmentations amount in different plant organs parts

F. occidentalis impact on pigmentations amount in different plant organs was studied by comparing rates of each measured photosynthetic pigment in leaves and fruits with those of slightly and severely attacked ones from pepper plants not protected by muslin.
It should be noted that for the fruits of pepper, they were classified as uninfected and slightly attacked since no fruit was observed severely attacked in both greenhouses.

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2.6. Statistical analyses

The statistical analyses were done by the statistical software program SPSS 17 (Statistical Package for the Social Sciences version 17). This program was used for analysis of variance (ANOVA) and Duncan test to determine differences between different varieties. Statistical tests were conducted at a 5% level of signification ($p = 0.05$).

3. Results and discussion

3.1. Leaves and fruits damages estimation

Leaves damage estimation in greenhouse G1 (Fig. 1) showed that Guebli and Zumurut leaves are the most damaged with a respective percentage of about 71.11 and 67.77%. These percentages are significantly different from the other three varieties. Lowest percentages of damaged leaves were observed in DRH 7461 and DRH 7462 varieties. In the second greenhouse G2 the PP1 variety had the most damaged leaf with a percentage of 66.66%. Regarding fruits (Fig. 2), variety that has the most damaged fruits in the greenhouse G1 was Guebli with 5
8.88% followed by Zumurut variety (40%) while Jebli variety had the lowest percentage of damaged fruits. In the second greenhouse G2 the highest percentage of damaged fruits was recorded on PP1 (71.11%). This number was significantly different from the others noted for the rest of the varieties.

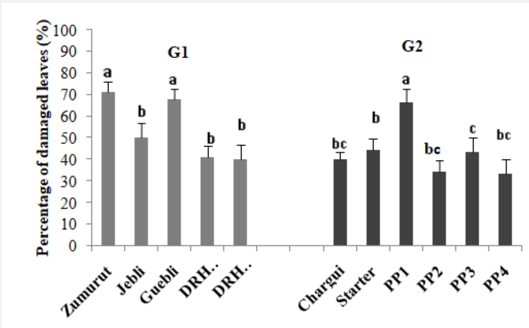

Figure 1. Percentage of damaged leaves by *F. occidentalis* in greenhouses G1 and G2. Means followed by different letters within each column are significantly different according to the Duncan test ($p = 0.05$)

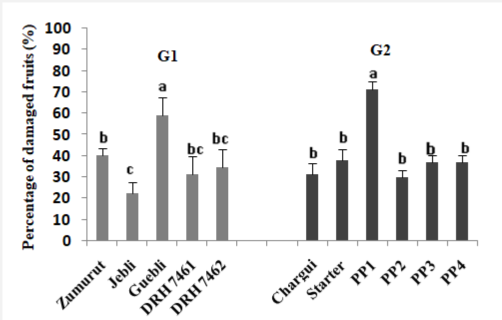

Figure 2. Percentage of damaged fruits by F. occidentalis in greenhouses G1 and G2. Means followed by different letters within each column are significantly different according to the Duncan test (p = 0.05).

3.2. Pigmentation rate assessment in leaves and fruits

3.2.1. Pigmentation rate assessment in pepper leaves

In the greenhouse G1**,** the pigmentation assessment mentions that Zumurut and Guebli leaves have the highest rate of Ca, Cb and Xanthophylls and Carotenoids (Fig. 3). Indeed significant differences were recorded between varieties. In the second greenhouse G2, PP1 variety displays a significant difference with the others; actually it contains the highest amounts of Ca, Cb and Xanthophylls and Carotenoids. Then come PP2, PP3 and PP4. Varieties that contain the lowest pigmentations rate are Chargui and Starter (Fig. 4).

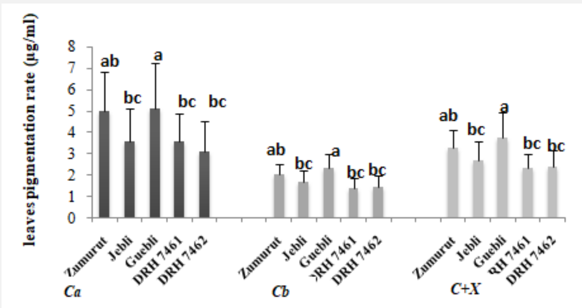

Figure 3. Pigments content Chlorophyll a (Ca), Chlorophyll b (Cb) and Xanthophylls (C + X) of leaves in greenhouse G1.

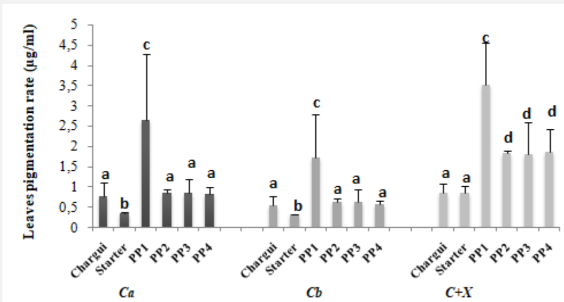

Figure 4. Pigment content Chlorophyll a (Ca), Chlorophyll b (Cb) and Xanthophylls (C + X) of leaves of greenhouse G2.

3.2.2. Pigmentation rate assessment in pepper fruits

In greenhouse G1, Guebli fruit's had the highest level of Ca, Cb and Xanthophylls and Carotenoids significantly from other varieties (Fig. 5). In the second greenhouse G2 PP1 fruits showed a significant difference with other varieties (Fig. 6). Actually, it contains the highest amount of all pigmentations. Chargui and Starter hybrids have the lowest amount of pigmentation.

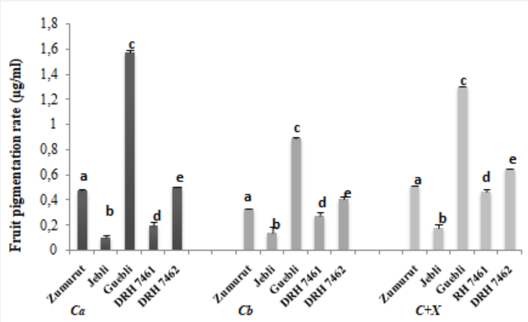

Figure 5. Pigment content Chlorophyll a (Ca), Chlorophyll b (Cb) and Xanthophylls (C + X) of fruits in the greenhouse G1.

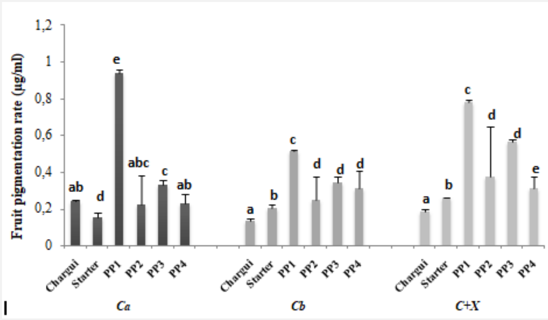

Figure 6. Pigment content Chlorophyll a (Ca), Chlorophyll b (Cb) and Xanthophylls (C + X) of fruits in the greenhouse G2

3.3. *F. occidentalis* Impact on pigmentations richness in pepper leaves and fruits of studied varieties

Obtained results show that there is not a significant difference between uninfected leaves and the slightly attacked by the WFT in both experimental greenhouses, except the case of PP2 variety (Tables 1 and 2). Moreover, the pigmentations contents especially Carotenoids and Xanthophylls in the slightly infested leaves showed a reductions with a maximum amount of 17.47% on Starter hybrid in the second greenhouse (Tables 1 and 2). As for the severely attacked leaves by *F. occidentalis*, they have suffered a great reduction in their levels of pigmentations compared to uninfected leaves and with highly significant differences for all varieties in both greenhouses. The reduction rate of pigmentation levels having been sucked by thrips from severely damaged leaves recorded a maximum of 61.88% of chlorophylls Cb on Starter variety in the second greenhouse and a minimum of 13.65% of Carotenoids and xanthophylls on DHR 7462 variety in the first greenhouse G1 (Table 1 and 2).

Pepper fruits were not severely attacked. Comparison was done only for lightly infested fruits with uninfected ones. Indeed, this comparison didn't reveal a significant difference in both greenhouses (Table 3 and 4). The sucked pigment percentage of slightly damaged fruits ranged between a minimum of 0.55% Cb chlorophyll recorded on PP1 variety in the first greenhouse and a maximum of 24.37% Ca chlorophyll recorded for Jebli variety in the second greenhouse (Table 3 and 4).

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Table 1. *F. occidentalis* impact on pigment rate of chilli leaves in greenhouse G1 (Ca: Chlorophyll a, Cb: Chlorophyll b, C + X: Carotenoids and Xanthophylls). (At each line the values followed by the same letters are not significantly different according to Duncan's test at p ≤ 0.05).

Pigmentation		**Ca**			**Cb**			**C+X**	
Severity of the attack	**Sain**	**Slightly attacked**	**Severely attacked**	**Sain**	**Slightly attacked**	**Severely attacked**	**Sain**	**Slightly attacked**	**Severyly attacked**
Zumurut	4,984±1,838 a	4,527±1,957 a	2,349±0,684 b	2,043±0,486 a	1,885±0,642 a	1,202±0,524 b	3,319±0,831 a	3,142±0,744 a	2,715 ±0,347 b
Jebli	3,609±1,525 a	3,478±0,983 a	2,003±0,473 b	1,701±0,559 a	1,683±0,584 a	1,149±0,436 b	2,725±0,858 a	2,533±0,524 a	2,016±0,333 b
Guebli	5,148±2,084 a	4,786±1,673 a	2,835±0,935 b	2,341±0,671 a	2,163±0,530 a	1,437±0,633 b	3,779±1,158 a	3,473±0,622 ab	2,846±0,676 b
DRH 7461	3,595±1,317 a	3,402±1,689 a	2,337±0,464 b	1,412±0,458 a	1,345±0,660 a	0,770±0,473 b	2,351±0,663 a	2,140±0,448 a	1,582±0,256 b
DRH 7462	3,125±1,407 a	2,903±1,752 a	1,681±0,628 b	1,455±0,521 a	1,284±0,583 ab	0,864±0,514 b	2,401±0,800 a	2,337±0,833 a	2,074±0,460 a

Table 2. *F. occidentalis* impact on pigment rate of chilli leaves in greenhouse G2 (Ca: Chlorophyll a, Cb: Chlorophyll b, C + X: Carotenoids and Xanthophylls). (At each line the values followed by the same letters are not significantly different according to Duncan's test at p ≤ 0.05).

Pigmentation		**Ca**			**Cb**			**C+X**	
Severity of the attack	**Sain**	**Slightly attacked**	**Severely attacked**	**Sain**	**Slightly attacked**	**Severely attacked**	**Sain**	**Slightly attacked**	**Severely attacked**
Chargui	0,786 ± 0,237 a	0,725 ± 0,186 ab	0,613 ± 0,125 b	0,547 ± 0,237 a	0,502 ± 0,297 a	0,286 ± 0,125 b	0,866 ± 0,330 a	0,712 ± 0,472 a	0,282 ± 0,174 b
Starter	0,363 ± 0,008 a	0,327 ± 0,043 a	0,293 ± 0,013 b	0,326 ± 0,008 a	0,286 ± 0,014 a	0,124 ± 0,045 b	0,846 ± 0,015 a	0,775 ± 0,756 a	0,376 ± 0,276 b
PP1	2,639 ± 1,084 a	2,414 ± 0,763 a	1,466 ± 0,433 b	1,718 ± 1,084 a	1,661 ± 0,770 a	0,863 ± 0,640 b	3,516 ± 1,646 a	3,142 ± 1,278 a	1,763 ± 1,178 b
PP2	0,872 ± 0,074 a	0,824 ± 0,124 a	0,634 ± 0,062 b	0,643 ± 0,074 a	0,624 ± 0,134 a	0,337 ± 0,134 b	1,846 ± 0,064 a	1,632 ± 0,126 b	1,142 ± 0,096 c
PP3	0,866 ± 0,305 a	0,813 ± 0,534 a	0,683 ± 0,115 b	0,646 ± 0,305 a	0,637 ± 0,237 a	0,376 ± 0,214 b	1,812 ± 0,337 a	1,775 ± 0,478 a	1,298 ± 0,224 b
PP4	0,840 ± 0,095 a	0,831 ± 0,108 a	0,714 ± 0,065 b	0,575 ± 0,095 a	0,519 ± 0,115 a	0,279 ± 0,047 b	1,860 ± 0,152 a	1,696 ± 0,286 a	0,925 ± 0,301 b

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Table 3. *F. occidentalis* impact on pigment rate of chilli fruit in greenhouse G1 (Ca: Chlorophyll a, Cb: Chlorophyll b, C + X: Carotenoids and Xanthophylls). (At each line the values followed by the same letters are not significantly different according to Duncan's test at p ≤ 0.05).

Pigmentation	Ca		Cb		C+X	
Hybrids	Sain	Slightly attacked	Sain	Slightly attacked	Sain	Slightly attacked
Zumurut	0,472±0,008 a	0,453±0,126 a	0,332±0,003 a	0,329±0,012 a	0,517±0,001 a	0,498±0,018 a
Jebli	0,097±0,026 a	0,073±0,037 a	0,142±0,046 a	0,133±0,053 a	0,173±0,030 a	0,152±0,047 a
Guebli	1,576±0,017 a	1,493±0,096 a	0,889±0,007 a	0,867±0,034 a	1,301±0,003 a	1,283±0,027 a
DRH 7461	0,196±0,028 a	0,189±0,031 a	0,274±0,031 a	0,261±0,043 a	0,466±0,016 a	0,458±0,034 a
DRH 7462	0,502±0,001 a	0,463±0,084 a	0,410±0,015 a	0,402±0,024 a	0,646±0,000 a	0,637±0,005 a

Table 4. *F. occidentalis* impact on pigment rate of chilli fruit in greenhouse G2 (Ca: Chlorophyll a, Cb: Chlorophyll b, C + X: Carotenoids and Xanthophylls). (At each line the values followed by the same letters are not significantly different according to Duncan's test at p ≤ 0.05).

Pigmentation	Ca		Cb		C+X	
Severity of the attack	sain	Slightly attacked	sain	Slightly attacked	sain	Slightly attacked
Chargui	0,243±0,006 a	0,231±0,016 a	0,132±0,011 a	0,128 ± 0,023 a	0,185±0,012 a	0,176 ± 0,024 a
Starter	0,153±0,022 a	0,143±0,031 a	0,200±0,019 a	0,184 ± 0,021 a	0,258±0,002 a	0,235 ± 0,034 a
PP1	0,941±0,015 a	0,927±0,022 a	0,513±0,007 a	0,510 ± 0,014 a	0,779±0,014 a	0,753 ± 0,172 a
PP2	0,222±0,155 a	0,204±0,173 a	0,247±0,129 a	0,242 ± 0,184 a	0,375±0,027 a	0,361 ± 0,183 a
PP3	0,332±0,020 a	0,318±0,037 a	0,339±0,034 a	0,333 ± 0,042 a	0,566±0,007 a	0,542 ± 0,017 a
PP4	0,225±0,053 a	0,216±0,082 a	0,312±0,092 a	0,308 ± 0,102 a	0,314±0,060 a	0,296 ± 0,122 a

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In fact, the study of the relationship between the different levels of leaves and fruits pigments with the percentage of damage assessed for the different varieties showed that more the variety has a rich organs in different pigmentations, it is the more likely to be attacked by *F. occidentalis* and present damages (Figs. 7, 8, 9 and 10).

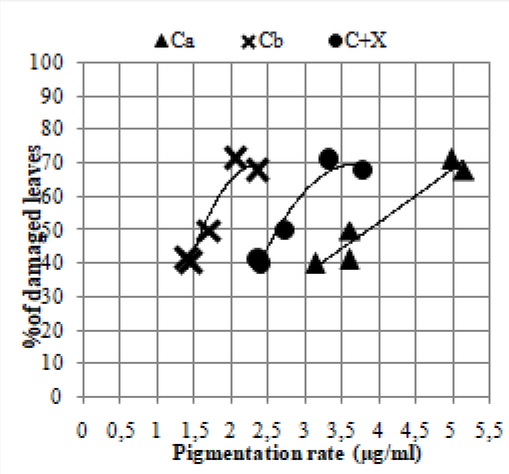

Figure.9. The correlation between the %of damadged leaves and the pigmentation rate in the G1 Ca : Chlorophylle a, Cb : Chlorophylle b, C+X : Caroténoïdes et Xanthophylles

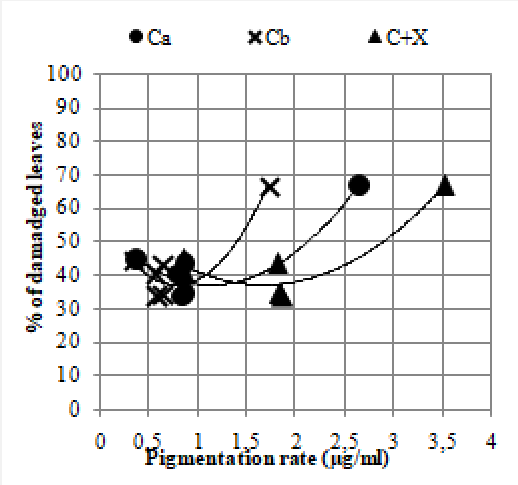

Figure 10. The correlation between the %of damadged leaves and the pigmentation rate in the G2 .Ca : Chlorophylle a, Cb : Chlorophylle b, C+X : Caroténoïdes et Xanthophylles

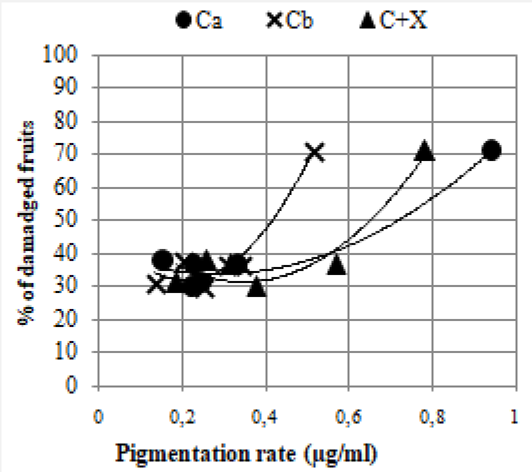

Figure.11. The correlation between the % of damadged fruits and the pigmentation rate in the G1 Ca : Chlorophylle a, Cb : Chlorophylle b, C+X : Caroténoïdes et Xanthophylles

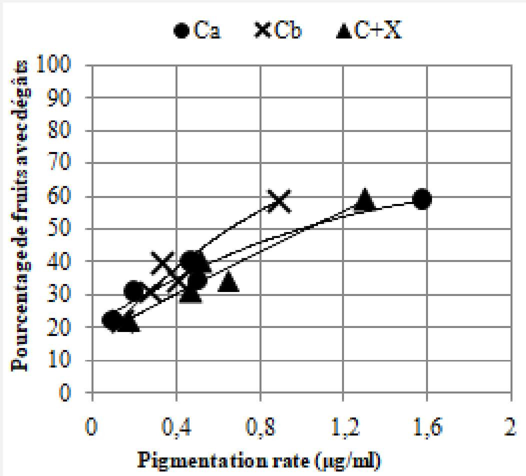

Figure.12. The correlation between the % of damadged fruits and the pigmentation rate in the G2 Ca : Chlorophylle a, Cb : Chlorophylle b, C+X : Caroténoïdes et Xanthophylles

According to Alston and Drost (2008) and Dai *et al*. (2009), thrips suck the contents of plant cells including pigments that contain, such as chlorophylls, carotenoids and xanthophylls, causing the appearance of damage to the plant organ which results by the loss of its original color and the acquisition of a silver color. Abdel-Rahman et al. (2008), indicate that thrips seek generally for the richest leaves in pigments. Alston and Drost (2008) reported that thrips prefer to feed on the mesophilic of young leaves which is even rich in pigmentation. On the other hand, the same authors confirm that thrips are attracted by the reflection of light in the visible range, which also depends on their concentration, in general, in pigmentations such as chlorophylls. In fact, this is consistent with the obtained results in this study, where the most attacked varieties are those that have the highest amount of photosynthetic pigments.
Obtained results regarding impact of *F. occidentalis* on its host plant are consistent with those cited by Dai et al. (2009), where photosynthetic pigments rate reduction were arrive to levels between 50 and 60% in the case of *Thrips tabaci*. According to Mao et al. (2007), leaves Chlorophylls are one of the

most important factors in determining photosynthesis rate. Tranavičienė et al. (2008) and Vatavu et al. (2012) indicate that this biogenetic process can be altered as well as his intensity by the variation of photosynthetic pigments rate of the chloroplasts as a result of fertilization impact or to numerous environmental factors. Moreover, fungal diseases such as mildew are also capable to reduce this mechanism by reducing the photosynthetic pigments up to 34% for slightly damaged leaves and 62% for the severely damaged ones (Mandal et al. 2009). Insects are also responsible for photosynthesis perturbation. Indeed, adults and larvae thrips feed on cells of plant organs such as leaves and fruits. The suction of cell contents causes a reduction of chlorophylls in leaves as well as the acquisition of a silver color in the attacked areas (Alston and Drost 2008). In fact, Dai et al. (2009) mentioned in their work the case of *T. tabaci* where their results are consistent with results of our study. In fact, they mentioned that damages caused by *T. tabaci* may affect the plants anatomical characteristics as well as its photosynthetic activity and pigmentation richness. They suggest that reduction of photosynthetic activity is due to a decrease of Chlorophylls rate particularly Chlorophyll Ca which is more directly involved in this process determination. On the other hand, they indicated that no significant differences were found between free leaves and the slightly attacked ones. However, highly significant differences were noted in the case of severely damaged leaves by *T. tabaci*, which is comparable to pepper leaves infested with *F. occidentalis* in the case of this study.
Furthermore, Dai et al. (2009) and Maxwell and Johnson (2000) point out that the reaction center of the PSII (photosystem II) closes and reduces gradually leading to a decline in the rate of transported electron and even to a deterioration of the thylacoïdal membrane of chloroplasts, when damages caused by *T. tabaci* are important. Indeed, molecules as Ca and Cb chlorophylls as well as carotenoids, form a collecting antenna that absorbs photons and transmits energy to the reaction centers of PSII. Then, reduction rates of these pigmentations contribute to a disturbance at PSII reaction center, and therefore to a reduction of photosynthesis (Dai et al. 2009).

4. Conclusion

The levels of pigmentations that have been analyzed in leaves and flowers have shown that the more the plant organ is rich in chlorophylls (Ca and Cb) as well as Xanthophylls, the more likely it is to be attacked by thrips. In the same context, correlations have shown that the higher the pigmentation rate, so the higher the percentage of plant damaged organs. All the more, the highest percentages of *F. occidentalis* populations are found in the richest pollen flowers. Furthermore, we can note that the pigmentation levels in attacked organs decrease substantially compared to those recorded on healthy plants. Indeed, the lowest chlorophyll and Xanthophyll levels were recorded on the highly infested organs.

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