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Thrips in citrus orchards, emerging pests in Tunisia

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Dans plusieurs pays méditerranéens, les thrips sont des ravageurs des agrumes causant de graves cicatrices sur la surface du fruit. En Tunisie, le statut de ces insectes reste inconnu. Cette étude a porté sur l'inventaire des thrips sur agrumes, leur abondance, leur importance économique liée aux agrumes et leurs fluctuations saisonnières par rapport à la phénologie des plantes hôtes. L'étude a été réalisée dans 6 vergers d'orangers à Bizerte, Cap-Bon et Mornag, situés dans le Nord et dans des zones importantes de production d'agrumes. Les thrips ont été récoltés par 3 méthodes : échantillonnage des fleurs, du sol et par l'utilisation de pièges collants. L'estimation des dégâts de thrips a été réalisée sur les fruits mûrs/jeunes fruits. Treize genres et 14 espèces ont été observés, dont les plus abondantes étaient *Thrips major* (Uzel 1895), *Pezothrips kellyanus* (Bagnall 1916) et *Frankliniella occidentalis* (Pergande 1885) avec respectivement 90,0 %, 3,3 % et 2,6 % des thrips collectés. Les principaux dégâts observés étaient soit sous forme de marbrures argentés couvrant une surface plus ou moins étendue variant de 11 à 70 % des fruits soit des dégâts minimes sous forme d'anneau à la base des pédoncules, spécifique à *P. kellyanus*. Les vergers les plus infestées étaient à Bizerte avec 4 adultes/fleur, tandis qu'à Cap-Bon qui est la principale zone de production d'agrumes, le niveau était 10 fois plus faible. Les densités les plus élevées de larves et d'adultes de *T. major* ont été enregistrées au printemps, à la floraison et la chute des pétales, avec une tendance à se concentrer principalement sur le côté nord de la canopée. Ce travail contribue à améliorer les connaissances du statut des thrips sur agrumes en Tunisie. Ces ravageurs nécessitent de nouvelles recherches pour comprendre les facteurs favorisant leur multiplication et leur contrôle notamment au Cap-Bon.
Mots-clés: Thrips, agrumes, *Thrips major*, *Pezothrips kellyanus*, dégâts.

In several Mediterranean countries, thrips have emerged as pests of citrus that cause severe scarring of fruit surface. In Tunisia, the accurate status of these insects remains unknown. This research focused on the inventory of species living on citrus, their abundance, economic significance related to citrus and their seasonal fluctuations in relation to host plant phenology. The study was carried out in 6 orange orchards in Bizerte, Cap-Bon and Mornag, all located in the North and important citrus production areas. Thrips were collected using 3 methods: flower and soil samples and sticky traps. The estimate of thrips damage was achieved on mature and young fruits. Thirteen genus and 14 species were observed, the most abundant were *Thrips major* (Uzel 1895), *Pezothrips kellyanus* (Bagnall 1916) with and *Frankliniella occidentalis* (Pergande 1885) with with 90.0 %, 3.3 % and 2.6 % of the fauna's thrips respectively. The main damage observed were silvery marblings covering a surface more or less extended and varying from 11 to 70 % of fruits and at a lesser degree rings at the base of the peduncles, specific of *P. kellyanus.* The most infested orchards were in Bizerte with 4 adults/flower, while in Cap-Bon which is the main citrus production area; the level was 10 times lower. The monitoring of larvae and adults of *T. major* in soil, air and citrus organs showed that the highest densities were recorded in spring, at flowering and petal fall, with tendency to concentrate mostly on the northern side of canopy. At the end of spring and with the development of young fruit, the percentage of infested organs decreased. This work contributed to improve knowledge on thrips status in Tunisia. It pointed out that in Cap-Bon region as well as in Mornag, thrips populations were present in low levels while in Bizerte they were more abundant and mainly represented by *T. major* the most common species. These emerging pests need a regular survey to understand factors that increase their densities and further control to prevent outbreaks in Cap-Bon.
Keywords: Thrips, citrus, *Thrips major*, *Pezothrips kellyanus*, damage.

1 INTRODUCTION

Thrips (Thysanoptera : Thripidae) are insects that feed on a large variety of plants. On citrus, numerous species of thrips has been recorded but few of them are considered as serious pests in different parts of the world (Lewis, 1997; Childers & Nakahara, 2006). Among these, *Pezothrips kellyanus* (Bagnall 1916) has emerged as an economic key pest of citrus fruits in New Zealand (Blank & Gill, 1997), southern Australia (Mound & Jackman, 1998), and some areas of the Mediterranean Basin such as Italy in Eastern Sicily (Conti *et al*., 2001) and Spain (Navarro *et al*., 2011) during the last decade. This species damage appeared as scabby, silvery scars on the rind that lower the fruit market grade and value (Navarro *et al*., 2008b; Conti *et al*., 2001).

In Tunisia until recently, very few information are available about biology and ecology of thrips on citrus, and thrips have not been considered as pests of citrus since they did not produce severe visible damage in citrus orchards. Their status is under change as silvery scars more or less extended, have been increasingly noticed on citrus fruits. In 2008, *Pezothrips kellyanus*, was first detected in Tunisia in citrus groves, and its damages were observed for the first time in 2010 (Trabelsi & Boulahia-Kheder, 2009; Belaam & Boulahia-Kheder, 2012). In addition to *P. kellyanus* other species phytophagous and predators were reported on citrus and weeds (Belaam & Boulahia-Kheder, 2012).

Therefore, the objectives of this research were: (a) to provide an inventory of thrips species in citrus orchards, (b) to confirm the presence of *P. kellyanus* and other economic species and assess their extension and damage (c) to study the distribution of thrips in the tree canopy, (d) and to study the dynamic of thrips in relation with the phenology of the host plant.

2 MATERIALS AND METHODS

Field observations were conducted in six orange orchards, three located in Cap-Bon (CB_1, CB_2 and CB_3), one in Mornag (M), and two in Sounine (Bizerte) (B_1 and B_2). This study focuses on Thomson variety.

Inventory of thrips on orange trees

Two techniques were used to collect thrips adults over 2 years: the beating of 40 branches per orchard on a white cloth of 50 cm × 50 cm from April 9th to June 8th 2011; and the sampling of one hundred of different vegetative organs (buds, flowers, fruits, leaves) per week per orchard, from March 26th to May 14th 2012. The thrips fallen on the cloth and collected from sampled organs were identified using the protocol Mound and Tree (2009). The identification of adult's thrips was confirmed by Cristina Navarro Campos from the Polytechnic University of Valencia in Spain according to several key uses (Palmer *et al*., 1989; Mound & Kibby, 1998; Moritz, 1994; Zu Strassen, 1996).

In addition to the identification of thrips, the counting of individuals found in organs has enabled to figure out the dynamics of larval and adult stages.

Assessment of thrips damages

The damages were estimated in 2 steps: first on the group of the six study orchards then on another larger group of 16 others citrus orchards in Cap-Bon to establish a more reliable assessment and clarify the status of thrips. The first assessment was achieved in November and December 2011 on 200 mature fruits by orchard directly before harvest (fruits diameter of ≈55 mm), then and in the same orchards in June 2012 on 300 young fruits per orchard (fruit diameter between 5-13 mm). The second estimate was conducted in July 2012 in 16 other orchards in the region of Cap-Bon of localities Menzeh, Intilaka, Zawit Ejdidi, Gobba, Hadaik, Brej, El Rammel, Dwela and Khriza at the rate of 300 fruitlets per orchard. The infestation rate of thrips was calculated by the following formula: Infestation rate = (number of fruits with thrips scars * 100) / total number of checked fruits.

Monitoring of thrips populations

Adult thrips monitoring

The adult thrips population was monitored by 10 cm x 30 cm white sticky traps made of polyvinyl chloride (PVC TROVIDUR®). Two traps per orchard were hung on the North side of the tree from mid-December until the end of June 2012 and were replaced every week by a new one. In the lab, thrips captured on each trap were counted under a binocular microscope. The dynamics of adult and larva thrips were also monitored by sampling 100 plant organs per week from March 26th to May 14th 2012. Individuals were counted under binocular microscope.

Monitoring of pre-pupa stage

Because thrips pupate in the soil and to monitor their population in this habitat, soil samples were

collected in the 6 orchards. Four samples per orchard were taken twice a month from December 2011 to June 2012. An amount of 7.064 dm^3 of soil were sampled randomly under canopies. In the laboratory thrips were extracted by the Berlese funnel where thrips fall in tubes filled with alcohol 10 % for conservation, identification and counting.

Distribution of thrips in the tree canopy

Flower sampling was also used to study the concentration areas of thrips inside a tree. For that purpose, 20 flowers per tree: 10 from North and South orientations respectively were sampled on 5 randomly selected trees per orchard.

Statistical Analysis

Results of distribution of thrips in the tree canopy from this study were analysed by GLM procedure with L means / pdiff option of SAS.

3 RESULTS

Identification of thrips on orange trees

A total amount of 1261 individuals of thrips were collected in the 3 localities of Bizerte, Cap-Bon and Mornag by beating and flower sampling during all the period of study and were composed of 11 genera and 14 species (**Figure 1**). Among these species, ten belong to the suborder Terebrantia: *Thrips mareoticus* (Priesner,1932), *T. angusticeps* (Uzel,1895), *T. tabaci* (Lindeman,1889), *T. major* (Uzel,1895), *Frankliniella occidentalis* (Pergande,1895), *Stenothrips graminum* (Uzel,1895), , *Pezothrips kellyanus* (Bagnall,1916), *Limothrips cerealium* (Haliday,1836), *Melanthrips fuscus* (Sulzer,1776) and *Aeolothrips intermedius* (Bagnall, 1934) and four belong to Tubulifera: *Haplothrips* sp., *Hoplothrips corticus* (Degeer,1773) , *Liothrips* sp. and *Karnyothrips* sp.

Frequency and density of thrips

The most common species was *T. major* with 90 % of the total number. *P. kellyanus* and *F. occidentalis* species were very little represented. All the other species were quite rare (**Table 1**).

Dynamic of thrips

The (**Figure 2**) shows a significant disparity between the 6 orchards in the level of adult thrips population. The orchards of Bizerte show significant peaks exceeding 300 and 75 individuals / trap / week, respectively. The density of the thrips was very low in the other 4 orchards

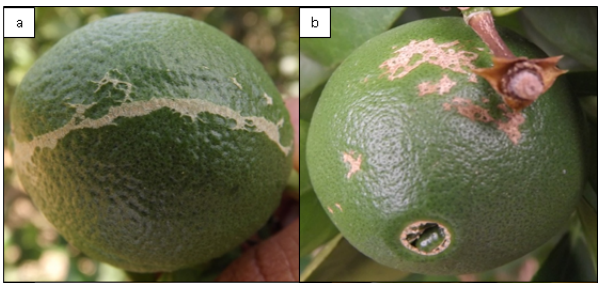

Figure 3: Depreciation of the cortex of citrus fruits caused by various factors; a: Wind effect; b: Contact between fruit and other plant organ.

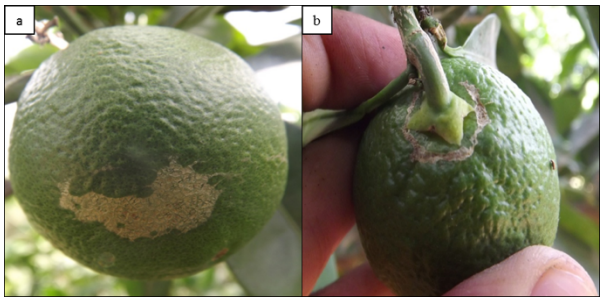

Figure 4a: Marbling caused by thrips on young citrus fruits; **4b**: Ring caused by thrips at the base of peduncle.

Assessment of thrips damages

Some scars very similar to those of thrips were observed on fruits in the three areas, ranging from 20 to 45 % of fruit skin area. These scars should be caused by mechanical, chemical or biotic factors. The wind seems to be one of the abiotic agents causing significant damages and can easily be confused with thrips marblings (**Figure 3a**). The rubbing of young fruit against the branches, twigs and leaves resulted also in the development of superficial stripes and scars on fruits (**Figure 3b**).

Assessment of thrips damage

Two types of symptoms were observed: a more or less developed silver silver marbling or a ring at the peduncle base which is specific to *P. kellyanus* (**Figures 4a-4b**).

On mature fruits, the ring depreciation, likely caused by *P. kellyanus*, was detected only in Bizerte (B1, B2) and Mornag (M) with no more than 2 % of damaged fruits. In Cap-Bon, no similar damage was observed. The marblings were found in all orchards of the 3 regions with a percentage of 49 %, 52 % and 62 % in Bizerte, Mornag and Cap-Bon respectively.

On young fruits, the larger estimation achieved on July 2012 in Cap-Bon and showed that thrips damage reported from half of the orchards, were

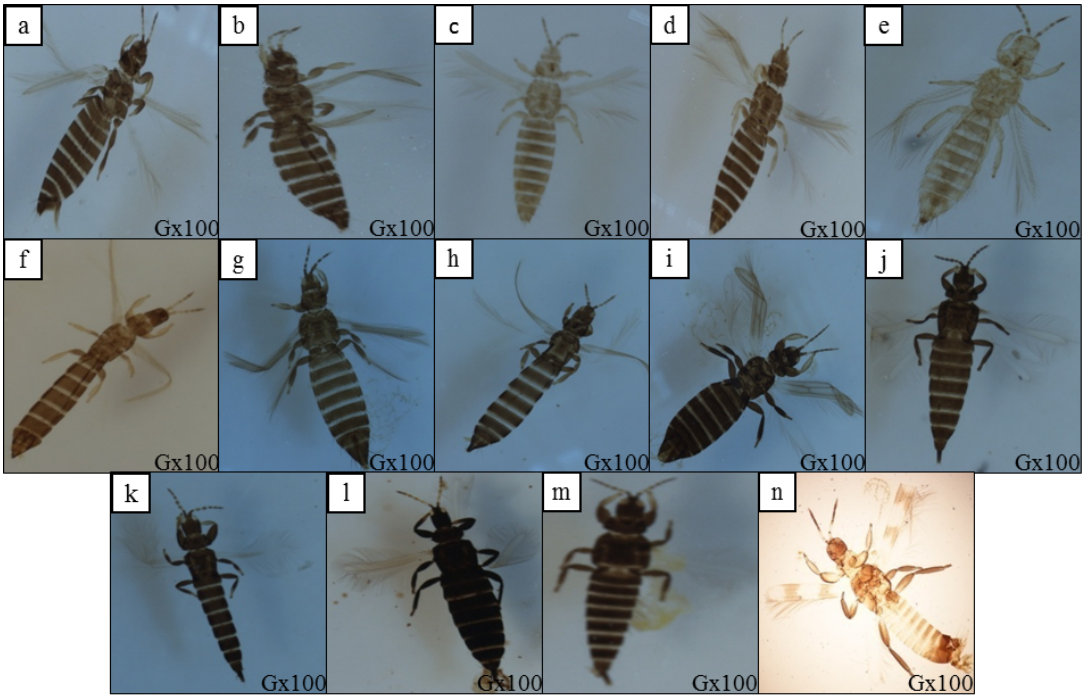

Figure 1: Thrips species collected from citrus in the areas of Cap-Bon, Bizerte and Mornag (G × 100): a : *Thrips mareoticus*, b *: T. angusticeps*, c : *T. tabaci* , d : *T. major*, e : *Frankliniella occidentalis*, f : *Stenothrips graminum*, g : *Pezothrips kellyanus*, h : *Limothrips cerealium*, i : *Haplothrips* sp., j : *Hoplothrips corticus*, k : *Liothrips* sp., l : *Karnyothrips* sp., m : *Melanthrips fuscus* and n : *Aeolothrips intermedius.*

Table 1: Diversity and abundance of thrips species collected in 6 orange orchards of Cap-Bon, Mornag and Bizerte.

Species	Number of individuals	%
Thrips major	1136	90.0
Pezothrips kellyanus	41	3.3
Frankliniella occidentalis	33	2.6
Thrips angusticeps	11	0.9
Thrips tabaci	7	0.6
Limothrips cerealium	6	0.5
Thrips mareoticus	5	0.4
Haplothrips sp.	5	0.4
Aeolothrips intermedius	5	0.4
Melanthrips fuscus	5	0.4
Liothrips sp. *Stenothrips graminum* *Hoplothrips corticus* *Karnyothrips* sp.	7	0.5
TOTAL	**1261**	**100**

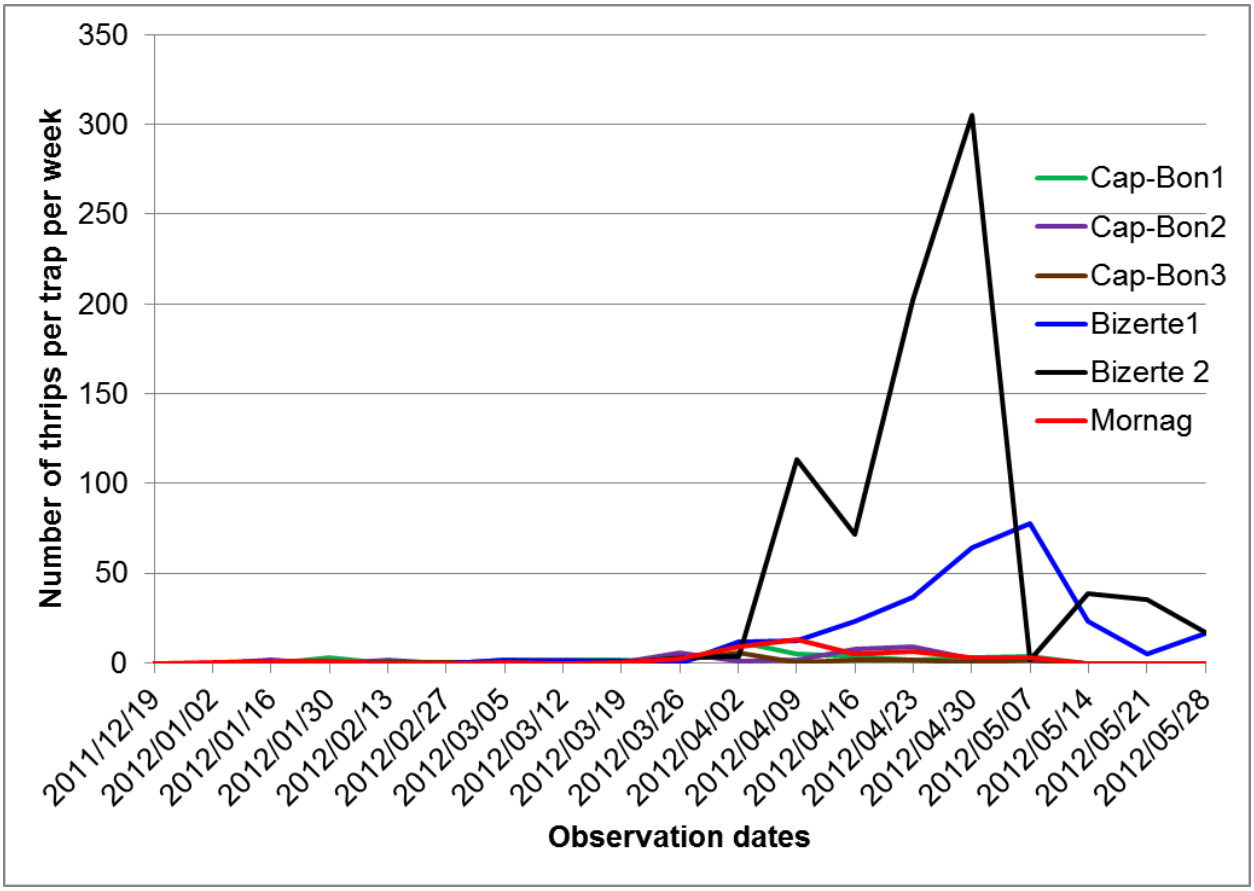

Figure 2: Dynamic of thrips adults in 6 citrus orchards of Cap-Bon, Bizerte and Mornag.

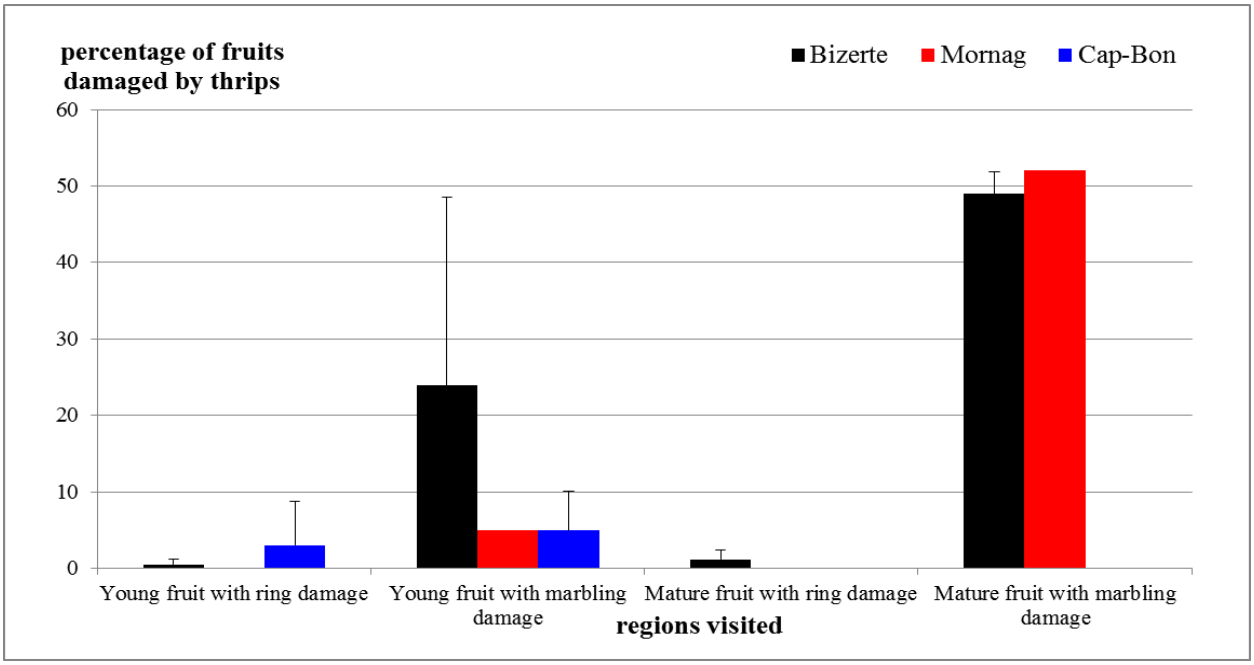

Figure 5: Thrips damage on young and mature citrus fruits in Cap-Bon, Bizerte, and Mornag.

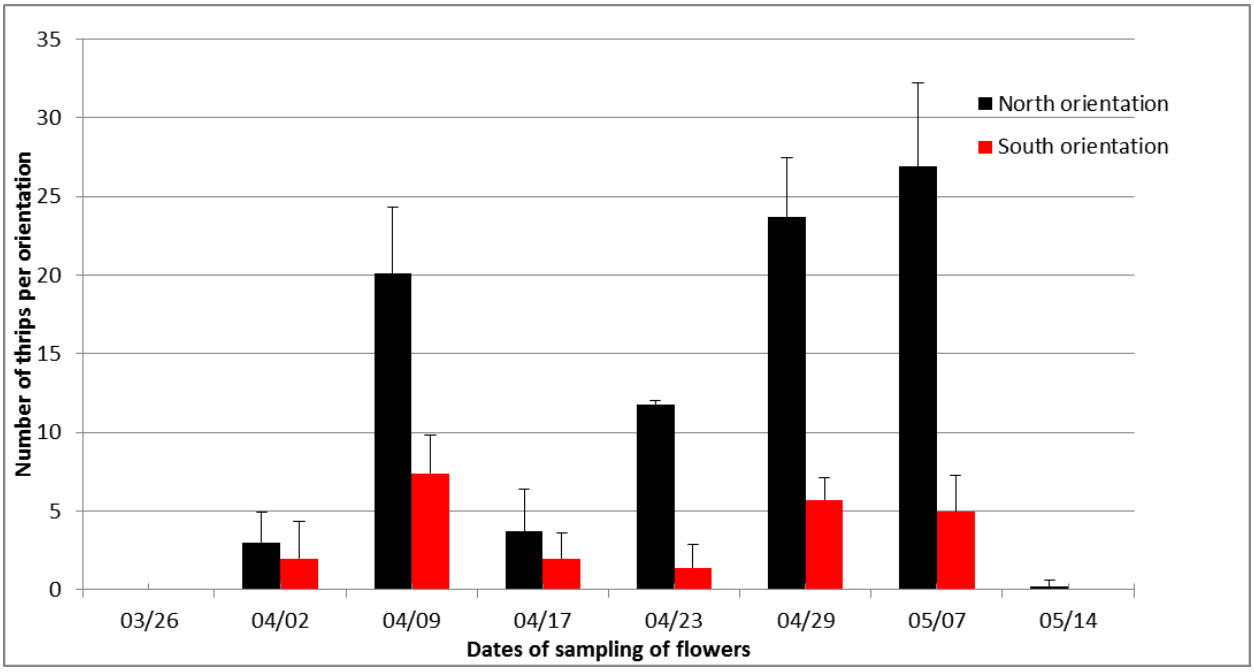

Figure 6: Distribution of thrips inside a tree canopy in orchards of Bizerte.

almost 20 % caused by *P. kellyanus* and ranged from 1 to 30 % of marblings (**Figure 5**).
This estimate has not showed any synchronism between the damage on young fruit and mature fruits for ring damage while for marbling damage the results were comparable.

Distribution of thrips inside a tree canopy
In both orchards B1 and B2, thrips distribution inside the canopy (North, South) was not uniform. The average number of adults per flower sampled was higher at the North side than at the South (**Figure 6**).

Distribution of thrips inside a tree canopy
In both orchards B1 and B2, thrips distribution inside the canopy (North, South) was not uniform. The average number of adults per flower sampled was higher at the North side than at the South (**Figure 6**).
Correlations between the density of thrips and its orientation on tree ($p = 0.0019 < 0.05$) and the evolution of this density in time ($p = 0.0004 < 0.05$) were observed.

Population fluctuation of thrips in Bizerte
Since *T. major* was the most abundant species in the B1 and B2 orchards, the study of the dynamic of thrips focuses on this species.
The adult population of *T. major* showed 2 generations: the first during the first decade of April and the second in the end of April-early May.
In both orchards, the first generation was the most significant with 70 and 200 individuals/100 flowers for B1 and B2 respectively. This generation occurred at the flowering of Citrus. The second generation, more discreet (50 and 120 individuals / 100 flowers respectively for orchards B1 and B2), occurred during petal fall. These two adult peaks were followed by a larval development which density was comparable in both orchards. The larval appears at petal fall – early fruit set, susceptible stages to the thrips attack (**Figures 7-8**).

The pre-pupae density in the soil showed a peak in orchard B_2 which exceeded 100 pre-pupae during the stage of petal fall-young fruit, while it was very low in B_1 (less than 16 insects per 7 dm^3 of soil). The dynamic of pre-pupae in the soil evolved in a similar initial peak of 20 pre-pupae by 7 dm^3 for the two orchards while the numbers of adults were not comparable at spring (**Figures 6-7**). This can be explained either by bad soil sampling or because adults were not all accused of nymphs.

4 DISCUSSION AND CONCLUSIONS

This research is a contribution to citrus thrips inventory and knowledge of species abundance,

and a study of population dynamic in three regions of Tunisia: Bizerte Cap-Bon and Mornag. With regard to biodiversity of thrips in citrus orchards, it was composed of 11 genera and 14

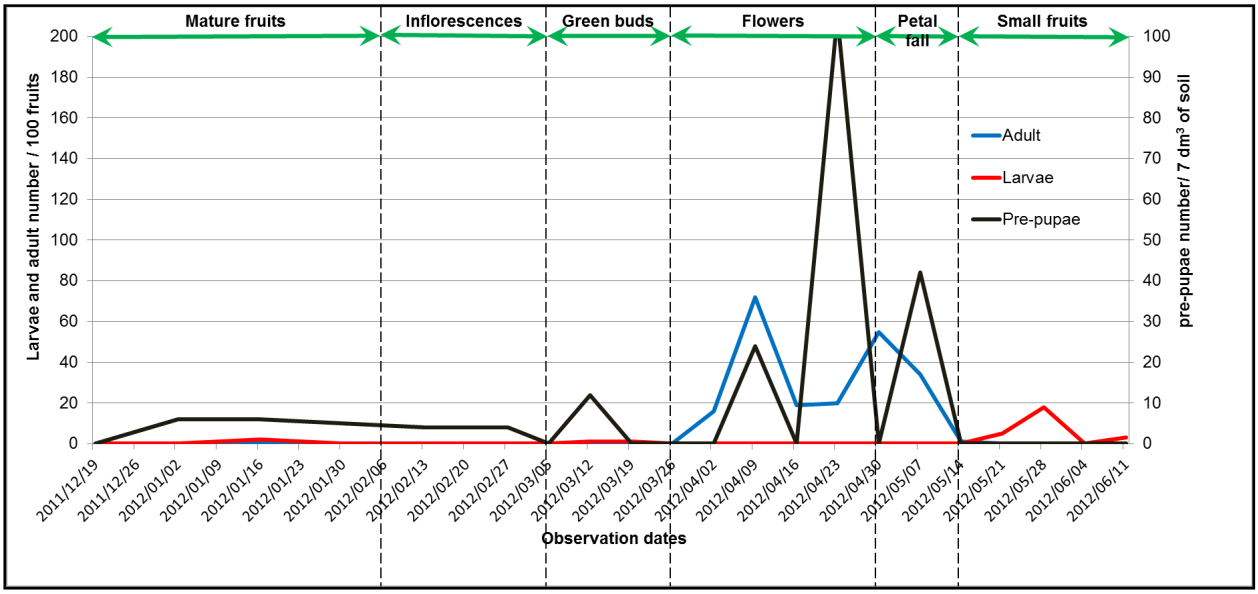

Figure 7: Dynamics of thrips stages – larva, pre-pupae and adult – in the citrus orchard at Bizerte (B_1).

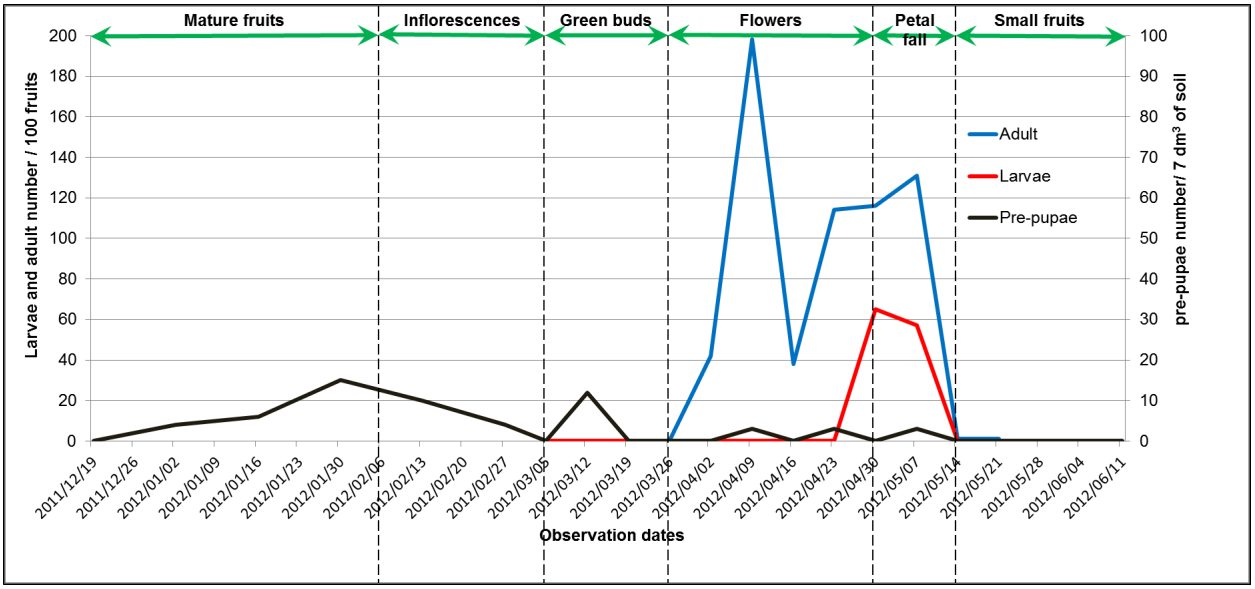

Figure 8: Dynamics of thrips stages – larva, pre-pupae and adult – in the citrus orhards in Bizerte (B_2).

species. All identified species on citrus are phytophagous except *Aeolothrips intermedius* which is predator of thrips and other small arthropods. Among these species, *Pezothrips kellyanus* is the only one known as pest of economic importance on citrus. These results are quite similar to those found in a previous study in Tunisia in 2010 on citrus showing 10 genera and 13 species in the regions of Cap Bon and Bizerte (Belaam & Boulahia-Kheder, 2012). The species found in Tunisia are similar to those living on citrus in other Mediterranean countries such as Spain, Turkey, Cyprus and Italy (Navarro *et al*., 2008b; Teksam & Tunç, 2009; Vassiliou, 2010; Marullo & De Grazia, 2012). The species *F. occidentalis*, *T. major*, *T. tabaci*, *T. angusticeps* and *P. kellyanus* are among the common species found in the Mediterranean citrus orchards. But only *P. kellyanus* causes feeding damage on citrus fruits in Cyprus (Vassiliou, 2010). However, the

species found in Tunisia differ from those found in Florida, New Zealand and Australia (Childers & Nakahara, 2006; Broughton & De Lima, 2002; Blank & Gill, 1997). Moreover, compared with other countries, the thrips fauna in citrus orchards in Tunisia is not very diversified. Indeed, more than 40 thrips species have been identified in association with citrus in the world (Longo, 1986). In Florida, for example, the inventory of thrips species in 7 Citrus orchards showed 36 species, among which 7 are predatory (Childers & Nakahara, 2006). The low biodiversity may be explained by the sampling of thrips mainly from citrus, while herbaceous plants where can live most species of thrips were not considered in this study.

Regarding the abundance of thrips in the 3 studied regions, thrips were much more numerous in Bizerte groves than in Cap Bon and Mornag, with 95.1 %, 3.8 % and 1.1 % in Bizerte, Cap-Bon and Mornag respectively. Until 2012, Cap-Bon region which includes the major centers of citrus in Tunisia, can be considered as little infested. The difference in the abundance of thrips between regions is probably due to the difference in adjacent crops of citrus and wild plants. The orchards in Mornag and Cap-Bon were located in orchards where only citrus was grown, while in Bizerte the orchards were surrounded by various plantations such as vines and peach trees which are known reservoir hosts for thrips.

The coexistence of mature fruits with the new flowering of others late varieties abundant in orchards of Bizerte, may allow the move of thrips from the mature fruits where they overwintered to the new citrus flowers and cause damage. A study in Valencia showed a significant positive correlation between the percentages of fruits with damages in an area with the percentage of late maturing varieties in the same area; this be explained by the fact that some of plants inside or in the vicinity of citrus orchards show a more extended flowering period than oranges trees so they could act as a breeding site for thrips when the flowering period of citrus has finished (Navarro *et al*., 2013).

Furthermore, among the species found *T. major* was by far the dominant species with 90 % of all thrips collected. Bournier (Bournier, 1963) found similar results in orange groves in North Africa where *T. major* considered polyphagous (Lorens Climent & Lacasa Plasencia, 1996) was the dominant species. It was the only time its harmfulness to citrus, has been reported in North Africa; as in necrosis of the tender fruit around the insertion of the floral peduncle, forming a smaller crown (Bournier, 1963). Another study on the Turkish thrips fauna affecting citrus showed also the predominance of *T. major* species with 84 % of the collected thrips, against an insignificant frequency of *P. kellyanus* (Teksam & Tunç, 2009).

The most economically important species, *P. kellyanus* which frequency was still low, was detected in the 3 surveyed localities in 3 orchards out of 6 (B_1, B_2 and M). The ring damage specific to this species was detected in 14 of 22 orchards. These results confirm previous studies showing this species in Cap Bon, Bizerte and Mornag (Trabelsi & Boulahia-Kheder, 2009; Belaam & Boulahia-Kheder, 2012). We can therefore conclude that citrus groves of Cap-Bon were currently little infested with thrips. *P. kellyanus*, a key pest in other parts of the world such Italy (Conti *et al*., 2001), Spain (Navarro *et al*., 2011), New Zealand (Blank & Gill, 1996) and Australia (Smith *et al*., 1997), was present in this region but still at very low density. Then, *P. kellyanus* populations should be monitored regularly next years to early detect on the increase multiplication of its density, and deploy control marblings to avoid serious damages. This survey will also allow to understand the factors that maintain *P. kellyanus* densities low. These can be related to climate (Navarro *et al*., 2013) or to citrus variety (Navarro *et al*., 2013; Mound & Jackman, 1998; Varikou *et al*., 2002) or to soil richness in organic matter that affects the biodiversity of mite's predators (Navarro *et al*., 2012b; Navarro *et al*., 2013) or the occurrence of some plants that act as reservoirs for *P. kellyanus* (Fround *et al*., 2001), but also the thrips activity can strongly influenced by the wind direction (Lewis, 1997).

Regarding *F. occidentalis* species, known for its extreme polyphagy, it was found in Bizerte and Cap-Bon on citrus, but it was found also on others plants in Tunisia such as the vine (Besbes, 2011) and pepper greenhouse (Elimem *et al*., 2011). Although quite uncommon on citrus, this species needs a special interest because it can damage this host as has been reported in Japan (Tsuchiya & Furuhashi, 1993). The minor species *T. tabaci*, *T. angusticeps*, *S. graminum* and *M. fuscus* collected

from citrus have a large range of hosts, including the citrus genus, but are not reported as typical citrus pests (Navarro *et al*., 2008b; Tesksam & Tunç, 2009). Therefore, some of these thrips are known to be polyphagous living on weeds or crops but regarding to citrus they seem to be passing species, searching for shelter or food source (Bournier, 1983).
Finally, *A. intermedius*, which was found in Bizerte and Cap Bon, is known for its colonization of herbaceous plants, shrubs and trees in the countries of Europe and North Africa (Llorens Climent & Lacasa Plansencia, 1996); and both the larvae and the adult females are generic predators, even though they present marked dietary preferences (Conti, 2009). *F. vespiformis* which is an important predatory species of thrips was not found in the visited orchards. The study of thrips dynamics showed two adult peaks followed by a larval peak coinciding with flowering and beginning of fruit set. This was similar to *P. kellyanus* in Spain on Valencia late oranges and in Italyon lemon (Navarro *et al*., 2011; Perotta & Conti, 2008) showing that two generations can develop from late March to mid-June. This confirm strong dependence of thrips, larval and adult stages, on flowers, as density decreases with falling petals and with fruit development.

By this study, it seemed that the orchard B_2 was more favorable then B_1 to the thrips development, this was probably due to the fact that B_1 was bordered only by orchards of orange while B_2 included some grapefruit and lemon in addition to orange. On the other hand, B_2 was surrounded by orchards of various fruit crops such as vine and peach, which helps to find reservoir host to the thrips population. In regard to thrips distribution on an orange tree, we found that it was not uniform, and population was more concentrated on North side than in South side. On lemon and grapefruit canopies, the same was shown for KCT (Vassiliou, 2011). This phenomenon can be explained by tendency of thrips to hide in order to avoid sunlight and wind. These results can vary considerably from year to year that's why these investigations have to continue for a longer period considering the seasonal influence and their impact on sampling.

Regarding the estimation of thrips damage on fruits, it reached 70 % in oranges orchards for both types of damage: silver rings caused by *P. kellyanus* and marblings. The latter, caused 11 to 70 % of damage fruit and has been noted in most orchards. These kinds of damages increased with the higher level of the *T. major* population (Bournier, 1963). Nevertheless, these marblings may also be caused by mites such as *Polyphagotarsonemus latus* that has been reported on lemon fruits (Gerson & Vacante, 2012) but also according to several other biotic and abiotic factors (Grafton-Cardwell *et al*., 2003). Such for the species *P. kellyanus,* is not seem a major pest currently because only 0 to 20 % of fruits have rings in 16 orchards in Cap-Bon and in our study orchards it does not exceed 2 %, although its implantation in the country is confirmed. In Turkey, similarly *P. kellyanus* damage are very insignificant (Teksam & Tunç, 2009), while they are severe in Spain reaching 79 % (Navarro *et al*., 2010).
Finally, the findings of this study were an important contribution for a best knowledge of thrips fauna on citrus in Tunisia. Nevertheless, it must be completed by a survey on crops and surrounding vegetation including closed orchards. Moreover, this research has shown that thrips were much more abundant in Bizerte and that Mornag and Cap-bon were very little infested. More investigations and surveys are needed to understand the elucidade factors that keep the thrips densities lower in these latter regions, and those which allowed an increase of population of Bizerte.

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