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Infestation and evaluation of damage of the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) on Citrus in Southern Tunisia

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Abstract

The damages caused by the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) were assessed on citrus host fruits namely: Thomson (*Citrus sinensis* (L.) *Osbeck*), clementine (*Citrus clementica Hort. ex Tan.*), mandarin (*Citrus delisiosa* L.), bitter orange (Citrus aurantium L.) and lemon (Citrus limon (L.) Eureka) in two regions in southern Tunisia (Gabes and Tozeur) during 2013-14 - 2015-16. Damage were evaluated by the determination of percentages of fruit infestation (Percentage of punctured fruits) and of fallen fruits. Results showed that *C. capitata* damage varied upon citrus species and sites. The infestation rates were 42. 02, 39.83, 32.5, and 3.44% in Tozeur, against 39.86, 37.18, 30.57, and 3.38% in Gabes, respectively for Thomson, clementine, mandarin, and lemon. Besides, the highest percentages of dropped fruit were obtained for Thomson navel variety (9.02 and 7.84% in Tozeur and Gabes, respectively) against the lowest percentage recorded for the lemon Eureka variety (2.89 and 2.77% in Tozeur and Gabes respectively). In addition, the highest mean number of punctures/fruit was observed for Thomson navel variety in both regions with mean values of 3.17 and 3.38 %, respectively for Gabes and Tozeur.
Results highlighted the severity of *C. capitata* damages in southern Tunisia and the urgency to develop appropriate control strategies in the oasis fragile ecosystem.

1. INTRODUCTION

The Mediterranean fruit fly or Medfly, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) is an economically important insect pest attacking citrus and many other fruits worldwide (Enkerlin et al. 1997). It is one of the most important pests that caused significant damage, especially if no control measures were applied resulting in an early fruit fall reaching till 90% (Jerraya 2003). Damage caused by larvae undergoing their entire developmental cycle occurs inside the fruit's pulp render fruits unmarketable. Females lay their eggs inside the fruits, which usually caused stinging marks. The larvae hatching from the eggs feed on the fruit tissue. The larval tunnels create entrance points to several pathogenic organisms, which live in its rotten masses (Weems 1981). Mature attacked fruits may develop a water soaked appearance, causing a depression on the surface. Medfly puncture also induces premature fruit drop. In Tunisia, *C. capitata* current national control program is mainly based on the applications of organophosphate insecticides (Sadraoui-Ajmi et al. 2022). The problems regarding the destruction of auxiliary fauna, the increase in residue levels in fruits, the resurgence of secondary pests and the development of resistant strains should further restrict the use of this control method against Medfly (Mediouni-Ben Jemâa et al. 2010; Haddaoui et al. 2016). Consequently, alternative control tactics were introduced in Tunisia using mass trapping and attract-and-kill techniques (Boulahia-Kheder et al. 2012; Hafsi et al. 2015; Elimem et al. 2021). Furthermore, a classic biological control program was initiated by the use of the pest's natural enemies (parasitoids, predators and pathogens) and the introduction of available efficient exotic parasitoids (Harbi et al. 2018). Southern Tunisian oases present a succession of various host plants as well as favorable climatic conditions for the development of the Medfly. Thus, particular attention should be given to the damage caused by this pest in southern Tunisia. Indeed, even though *C. capitata* is considered as the major insect pest on citrus and summer fruits in Tunisia, only few researches have been achieved about this pest in southern orchards of the country (Ghabbari 2018). In this respect, Ben Chaabane et al. (2018) and Sadraoui-Ajmi et al. (2022) reported the importance of Medfly damage in south Tunisia particularly in Tozeur and Gafsa regions. At Midès oasis (Tozeur), the percentage of fruit damage of clementines reached 30% at harvest. While for the Thomson variety, the damage was 23%. In Gafsa oasis, the damage attained 25% for Clementine. Additionally, Chermiti et al. (2022) pointed out the importance of *C. capitata* populations in the oasis of Tozeur.

This study aimed to evaluate the susceptibility of some citrus varieties to the damage caused by *C. capitata* in two regions of south Tunisia namely Gabes and Tozeur. Effects of *C. capitata* on: i) infestation percentage at harvest, ii) fallen fruits rate, and iii) mean numbers of punctures per fruit, were investigated for four citrus species.

2. MATERIAL AND METHODS

2.1. Study sites

Trials were conducted, from 2013 to 2015, in 4 citrus orchards: 2 (Limaoua and Zerkine II) situated in the region of Gabes (33° 52' 53" N; 10° 05' 53" E) and 2 (Tozeur and Tamagheza) in the region of Tozeur (33° 55' 6.722" N; 8° 7' 22.559" E) (Fig.1 & Table 1). These two regions were chosen because they represent extension zones of citrus plantations in southern Tunisia.

Each orchard occupied an area of 5 ha. The experimental plots are cultivated with varietal mixture of various citrus species including Clementine MA3 (*C. clementina Hort. ex Tan.*), orange Thomson Navel (*C. sinensis* (L.) *Osbeck*), mandarin Nova (*C. reticulata* L.) and lemon (*C. limon* (L.) *Eureka*). Trees' spacing was 5 × 5 m. Citrus trees are 18, 10, 21 and 15 years old, respectively in Limaoua, Zerkine II, Tozeur and Tamagheza orchards. For Limaoua and Zerkine II orchards, chemical biweekly treatments using Dimethoate® were carried out during the period September -October. However, no applications of pesticides (insecticides, herbicides) or fertilization were applied at Tozeur and Tamagheza orchards.

2.2. Fruit damage assessment

Fruit damages (Fig.2) were assessed through three parameters: i) infestation percentages, ii) percentage of dropped fruits and iii) mean numbers of punctures/fruit.

To determine the fruit infestation rate at harvesting (September to November), five trees were randomly selected from each species. From each one, the percentage of damaged fruits was calculated as the number of fruits with at least one ovipositional puncture over the total number of 500 sampled fruits of each (100 fruits per tree), according to the following formula: Damage (%) = Number of fruits with at least one oviposition puncture/ Total of Fruits sampled.

The rate of dropped fruits of each Citrus species was calculated. The five trees selected to follow the progress of fruit infestation rate were checked weekly till harvest respectively in the region of Gabes and Tozeur and continued two days before harvest. The total fruit number of each selected tree (five trees of each species) was counted. The fallen fruits with *C. capitata* damages from these trees were collected every week, recorded, and removed from the orchard. The rate of dropped fruits was estimated by dividing the cumulative number of dropped fruit by the total number of fruits counted on the trees.

To assess the mean numbers of punctures per fruit caused by *C. capitata* adults, 100 fruits were collected at harvest from each host plant. Five replications were achieved for each plant (five trees of each species). Each fruit was checked, and the number of Medfly punctures per fruit was determined. The mean number of punctures per fruit was calculated by the following formula:

Mean number per fruit = (NbPF1+NbPF2 + …+NbPFn)/ Total of fruits sampled

where: NbPFn: Number of Punctures counted in the Fruit «n»; n = 100, NbPF2: Number of Punctures counted in the Fruit two, NbPF1: Number of Punctures counted in the Fruit one.

2.3. Statistical analysis

Statistical analyses were performed using the "SPSS statistical software version 16.0. Differences in values of the percentages of infestation, rates of fallen fruits, and the number of puncture per fruit of each year or sites and species were tested by one-way ANOVA, followed by Duncan test. All values given were the means of three replications and were expressed as the mean ± standard deviation. Significant differences were reported as $P < 0.05$.

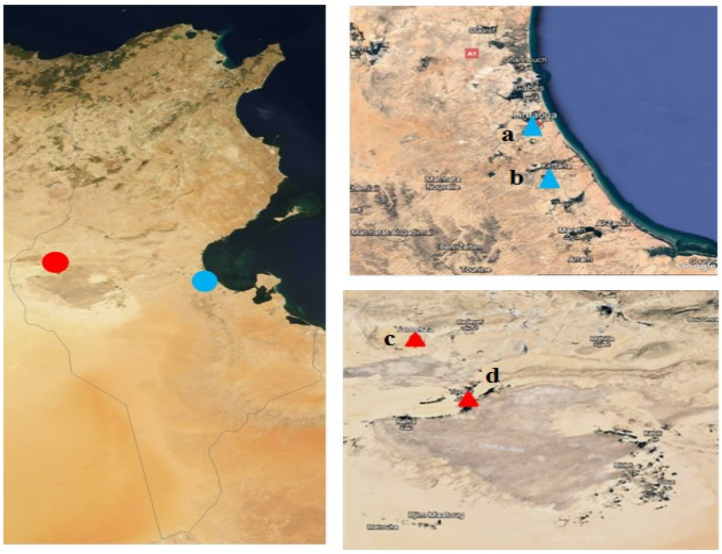

Fig. 1. Experimental Sites in Gabes: Limaoua (a), Zerkine (b); In Tozeur: Tamagheza (c) and Tozeur (d).

Fig. 2. Damages caused by *Certatis capitata*

Table 1. Number of orchards and host fruits samples checked in the fields

Host fruit species	Number of sites	Number of sampled fruits
Thomson Navel	4	8450
Clementine	4	8298
Maltese	1	4729
Mandarin	3	6789
Lemon	3	731
Bitter orange	1	4371

3. RESULTS

3.1. Infestation percentage

Results regarding the infestation rates of different fruits species present in Gabes and Tozeur regions during 2013-2015 are shown in Table 2.

In Gabes region, variance analysis revealed that the host fruit had a highly significant effect on the rate of punctured fruits (Duncan test, p = 0.0000). Statistical analyses showed at the same year, significant differences among the levels of hosts' infestations. Results indicated that according to the importance of infestations, the order of ranking of host fruits was as follows: Thomson navel oranges (39.86%)> Clementine (37.18%)> Maltese oranges (32.58%) > Bitter orange (31.01%) ≥ Mandarin (30.57%), The lowest affected fruit was lemon with an infestation rate of 3.38% recorded during the season 2013-14.

On the other hand, the effect of the factor "year" was not manifested in an extreme way (Table 2). Indeed, Thomson navel oranges were always subject to the highest infestations.

Similarly in the region of Tozeur, the infestation rate was significantly different among the host fruit species, during the three seasons of study (Duncan test, F= 5311.296, ddl= 3, P<0.05). Results revealed that Thomson navel oranges were the most affected host by *C. capitata*, followed by clementine, mandarins, and finally lemons (Table 2). Furthermore, comparison of *C. capitata* damage among the three sampling periods (March 2013; March 2014 and March 2015), showed non-significant differences among the infestation percentages of various host fruits (Duncan Test, p>0.05).

3.2. Dropped fruit rate

Table 3 reported results related to the determination of dropped fruits percentages in Gabes and Tozeur regions during the period 2013-2015.

In the region of Gabes, the analysis of variance showed the influence of the factor "host fruit" on the number of dropped fruits following attacks by *C. capitata*, while non-significant differences were detected among years. This influence of the different hosts was highly significant (F= 439.545; ddl= 5; p= 0.000). The host fruit factor had the greatest impact on the number of fallen fruits. Indeed, statistical analyses showed that the rates of dropped fruits recorded for the various host fruits were 8.11; 7.84; 7.14; 7.07; 6.13 and 2.77%, respectively for mandarins, Thomson navel oranges, bitter orange, clementine, maltese oranges, and lemon Eureka variety in March 2013 (Table 3).

Fallen fruits rates caused by *C. capitata* in the region of Tozeur varied depending on the hosts. Indeed, statistical analyses revealed a high significant difference of the percentage of fallen fruits according to the host fruit (Duncan test, p<0.0001). Thus, during 2013, Thomson navel oranges showed the highest rate of fallen fruits with an average of 9.02%. Thereafter, the order was as follows: Clementine (7.05%), Mandarins (7.02%) and finally Lemons (2.88%). An analysis of variance indicated that the rate of fallen fruits varied highly significantly (F= 255.841, ddl= 3, p = 0.0000) depending on hosts (Table 3).

Table 2. Infestation (%) of host fruits in the régions of Gabes and Tozeur during the three seasons (2013-2015)

Site	Fruit species	Infestation (%)		
		2013	2014	2015
Gabes	Thomson navel	39.86f	42.06e	42.67f
	Clementine	37.18e	38.31d	41.20e
	Mandarin	30.57b	30.81b	31.45b
	Lemon	3.38a	3.43a	3.53a
	Maltese	32.58d	32.84c	33.74d
	Bitter orange	31.01c	31.18b	32.03c
	P Duncan (P<0,05)	0.000	0.000	0.000
Tozeur	Thomson navel	42.06d	42.54d	42.63d
	Clementine	39.83c	41.44c	42.03c
	Mandarin	32.24b	33.67b	34.02b
	Lemon	3.44a	3.79a	3.74a
	P Duncan (P<0,05)	0.000	0.000	0.000

At the same study region at the same year, the percentages of infestations were compared among the species (lowercase letter). The means followed by the same letters are not statistically different by Duncan's test, threshold P <0.05. Each total fruit number values present the mean of the total number of fruits of ten trees.

3.3. Punctured fruits rate

The damage of *C. capitata* was estimated by the average number of punctures per fruit. Results of the puncture counts per fruit are recorded in Table (4).

In the region of Gabes, the results showed that there was a high significant difference among three groups of host fruits in terms of mean of number of punctures value (F=41.374; ddl=5; p=0.000). The first group with the highest mean number of punctures was presented by Thomson navel, clementine, mandarin and maltese. The second group is composed by Bitter orange, and finally the third group included lemon with the lowest average number of puncture/fruit.

Similarly, in the region of Tozeur, results showed three groups of host fruits. The first group composed by the oranges Thomson navel, the second group included clementine and mandarin and in the last group come lemons with the lowest average of punctures/fruit. Analysis of variance of the results showed that the year factor did not influence the number of punctures/fruit (P = 0.968) (Fig.3).

Table 3. Dropped fruits (%) according to the host fruits in the two regions of Gabes and Tozeur during three seasons 2013-2015

	Fruit species	2013	2014	2015
Gabes	Thomson navel	7.84 d	8.29 e	9.11 d
	Clementine	7.07 c	7.99 d	8.1 c
	Mandarin	8.11 d	8.44 e	9.1 d
	Lemon	2.77 a	2.84 a	2.92 a
	Maltese	6.13 b	7.14 b	8.35 c
	Bitter orange	7.14 c	7.41 c	7.77 b
	P Duncan (<0,05)	0.000	0.000	0.000
Tozeur	Thomson navel	9.02 c	9.03 c	9.8 d
	Clementine	7.05 b	8.52 c	9.05 c
	Mandarin	7.02 b	7.7 b	7,99 b
	Lemon	2.88 a	3.19 a	3.29 a
	P Duncan (<0,05)	0.000	0.000	0.000

For the same study area and year, the average numbers of fallen fruit were compared among varieties (lower case letter). Means followed by the same letters were non- statistically different with Duncan's Test, threshold P <0.05.

Table 4. Mean number of punctures per fruit according to the host fruits in the regions of Gabes and Tozeur during three agricultural seasons

	Fruit species	2013	2014	2015
Gabes	Thomson navel	3.17 c	3.2 b	2.82 bc
	Clementine	2.8 c	2.82 c	2.02 c
	Mandarin	2.62 c	2.63 bc	3.22 c
	Lemon	0.99 a	1.05 a	2.84 a
	Maltese	2.77 c	2.78 c	1.09 c
	Bitter orange	1.98 b	2. b	1.42 b
	P Duncan (<0,05)	0.000	0 ,000	0.000
Tozeur	Thomson navel	3.38 b	3,6 c	3.66 c
	Clementine	2.74 b	2,84 b	2.88 b
	Mandarin	2.76 b	2,76 b	2.82 b
	Lemon	0.94 a	0.99 a	1.01 a
	P Duncan (<0,05)	0.000	0.000	0.000

For the same study area and year, the number of punctures per fruit was compared between varieties (lowercase letter). Means followed by the same letters were not statistically different with Duncan's Test, threshold P <0.05.

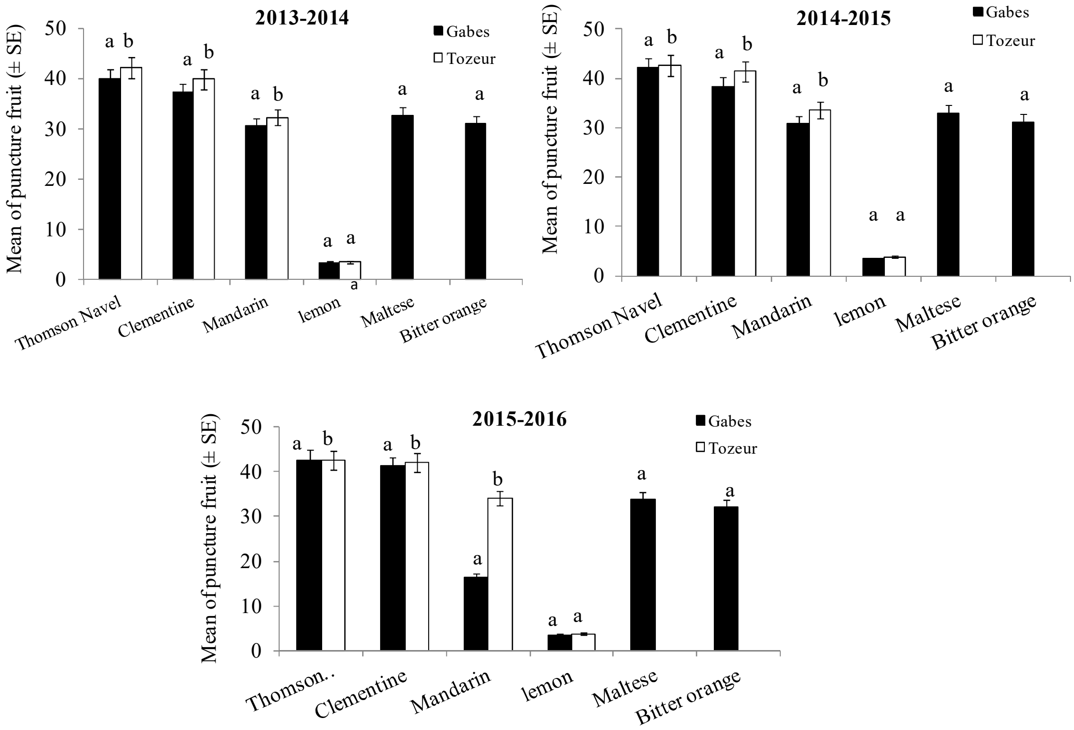

Fig . 3. Variation in the number of puncture per fruit of host fruits in the two regions of Gabes and Tozeur during three agricultural seasons. For the same fruit species (except Maltese and Bitter orange), comparisons were made between the number of puncture per fruit in the two regions of Gabes and Tozeur.

The averages followed by the same letter are not statistically different at the 5% threshold (Duncan test).

4. DISCUSSION

The assessment of *C. capitata* pest status in the two study sites in southern Tunisia was performed as a comparison at harvest between the infestation rates, the rate of dropped fruits and the mean number of punctures/fruit for each Citrus host species. Results indicated significant differences among hosts for the damage levels, rate of fallen fruits and the number of punctures per fruit (Duncan test, $P < 0.05$). On the other hand, results demonstrated that the most important damage was observed for Thomson navel oranges and Clementine. Indeed, the damage of *C. capitata* was noticed from the end of September, especially on figs and prickly pears (Sadoudi et al. 2007). Moreover, the damage resulted from the coincidence of the ripening period of early hosts (Clementine) with the outbreaks of *C. capitata* adults. Indeed, it was during the periods of growth and ripening that citrus trees are more likely to host *C. capitata* larvae. In this context, Martin (1950) reported that larval penetration of the pulp was not observed until October. Similarly, there was a significant increase in the activity of *C. capitata* from September (Lekchiri 1982).

Regarding the impact of *C. capitata* on the infestation rate, the present study showed significant differences depending on the host fruits. The infestation rates recorded were 39.86; 37.18; 30.57; 3.38; 32.58 and 31.01%, respectively for the hosts; Thomson navel oranges, clementine, mandarins, lemons, Maltese oranges, and bitter oranges in the region of Gabes against, 42.06; 39.83; 32.24 and 3.44%, respectively for the hosts Thomson navel oranges, Clementine, mandarins, and lemons in the region of Tozeur for 2013-14. Lemon was the least attacked fruit. According to Spitler et al. (1984), lemon tree is the almost immune to its infestation, despite the presence of sterile stings. This was mostly explained in part to host plant compounds of the peel (i.e. essential oils, 5, 7-dimethoxycoumarin, linalool in the flavedo) (Greany et al.1983; Salvatore et al. 2004), to physical characteristic such as peel thickness of the flavedo and peel resistance to pressure (Papachristos and Papadopoulos 2009). Other researchers like Da Silva Branco et al. (2000) demonstrated that oxygenated monoterpene aldehydes from *Citrus limon* (L.), were responsible for the chemical resistance of lemon be attacked by medfly. Similar studies done by Papadopoulos et al. (2015) showed that citrus resistance/sensitivity depends specifically on the flavedo layer which oil glands contain essential oils can be toxic to newly hatched larvae. The amount and quality of the principal components of citrus oils determined the toxicity of citrus fruits (Papachristos et al. 2009). However, the high infestation of bitter orange observed in the region of Gabes was related to the loosening of the peel tissue (Delanoué and Soria 1962). In addition, it seems clear that Thomson navel cultivar was more susceptible to *C. capitata* stings than those of clementine. Similarly, Thomson navel was reported as the most preferred variety for Medfly (Laamari et al. 2015; Settaoui et al. 2017). Comparable results were found by Smail and Keddouci (2000) and Dekhli (2006) who reported that Thomson navel oranges as a preferential host for *C. capitata*. This is explained by the attractiveness of this host (*Citrus sinensis*, Thomson navel) which would be due to its volatile compounds emitted well before fruit ripening. These compounds are olfactory stimuli for *C. capitata* (Sampaio et al. 1984; Quillici 1993). The role of some chemical parameters such as the essential oil content of the peel and the nature of the volatiles substances have been reported in attracting or repelling of the pest (Dhouibi et al. 1995). In addition, the evolution of fruit coloration and the appearance of carotenoid pigments give the fruit an attractive pale yellow color for *C. capitata* (Lekchiri 2000). Similarly, the highest infestation rate was attributed to the high susceptibility of this cultivar in relation to the structure of the very smooth and relatively fine peel of this fruit species. The high infestation rate of Clementine maybe related to their very thin skin, allowing to female of *C. capitata* to lay their eggs directly in the pulp. Moreover, the high infestation recorded in clementine was explained by the fact that clementine is the early host fruit of citrus whose receptivity is maximum in October.

For the rate of fallen fruits, results showed that the highest number of fallen fruits was observed in the region of Tozeur for Thomson navel oranges (9.02%) while in the region of Gabes, it was the mandarins (8.11%).

The importance of *C. capitata* damage was also estimated by the average number of punctures per fruit of each host. The results revealed that Thomson navel oranges had the highest number of punctures with an average of 3.38 punctures per fruit in the region of Tozeur (and 3.18 in Gabes). The causes of varietal preference of the *C. capitata* in citrus orchards and that Thomson navel seemed to be the most attractive. This difference in susceptibility is important and it is probably related to the evolution of the fruits coloration, which reaches its maximum of receptivity to *C. capitata* at ripening. Indeed, Lekchiri (2000) proved that the appearance of carotenoid pigments of this variety is done in a homogeneous way on the entire surface, which confers to the fruit a pale yellow coloration particularly attractive for the insect. Similar to other fruit flies, Papanicolaou et al. (2016) demonstrate that the spectral sensitivity and visual attraction of medfly revealed a broad range of yellow-green colors (485-500 nm). The visual assessments cover also dimensional host characteristics concerning shape and size (Nakagawa et al. 2011). Previous work has reported that *C. capitata* was attracted more to larger fruits (Katsoyannos et al. 1986; Katsoyannos et al. 1997; Papadopoulos et al. 2001). In addition, Féron (1962) showed that *C. capitata* was attracted to the fruits with a dry surface and yellowish color. Similarly, Delanoue and Soria (1962) demonstrated that *C. capitata* preferentially attacked cultivars with an oily rind texture. Similarly, Ekesi et al. (2007) reported that the evolution of the insect-plant interaction was determined by female preference in part, and by the performance of *C. capitata* larvae, which varies according to the nutritional value of the fruit on the other part. In addition, the abundance of citrus fruits, the spreading of the season of these fruits as well as the favorable climatic conditions during the month of October until mid-December caused the increase of the damage observed in citrus fruits.

5 . CONCLUSION

The variations in the importance of the damage of *C. capitata* according to the regions can be explained by the configuration of the varieties of the sites and the periods of their fructification. Indeed, we note more important damages in the orchards of Tozeur which had an early fructification compared to the region of Gabes. The damage of *C. capitata* varied according to regions, species and varieties of citrus. The results obtained showed that the staggering of the harvest period of citrus fruits as well as summer fruits, especially peaches, also contribute to the increase of the damage of *C. capitata*. This may lead to a phenological coincidence between a peak of fruit production and a high abundance of fertilized females ready to lay eggs.

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