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THE STATUS OF *PEZOTHRIPS KELLYANUS* BAGNALL (THYSANOPTERA: THRIPIDAE) IN CITRUS ORCHARDS OF TUNISIA

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ABSTRACT

Since *Pezothrips kellyanus* first report in Tunisia in 2008 in citrus orchards, this thrips species has been considered as a secondary pest because related damage was rare or absent. However, these last years, fruit scars have been recorded of variable intensity depending on locations and citrus crops. The goal of this work is to study *P. kellyanus* status in Tunisia: abundance, damage caused on different citrus crops, pest dynamics and alternative host-plants. A monitoring during the years 2015 and 2016 showed that *P. kellyanus*, which causes a partial or complete silvery ring at the base of the fruit peduncles or at the opposite, more or less extended, is little abundant in citrus orchards with an average of 0.2 individuals / flower. *P. kellyanus* develops on various citrus crops with a preference for Bergamot and Lemon causing 59 and 45 % of damaged fruits respectively on these crops. *Jasminum officinale* and *Bunium pachypodium* were found as two alternative host plants for *P. kellyanus* in Tunisia. *P. kellyanus*, is currently common in citrus orchards of Tunisia and damages begin to develop however it stays less abundant than in those countries where it causes crop losses.

Keywords: *Pezothrips kellyanus*, citrus , varieties, damage, population.

RÉSUMÉ

STATUT DE *PEZOTHRIPS KELLYANUS* BAGNALL (THYSANOPTERA: THRIPIDAE) EN VERGERS D'AGRUMES EN TUNISIE

Depuis que *Pezothrips kellyanus* a été signalée pour la première fois en 2018 dans les vergers d'agrumes en Tunisie, cette espèce de thrips a été considérée comme un ravageur secondaire puisque les dégâts liés à cette espèce étaient rares ou absents. Cependant, ces dernières années, des cicatrices sur fruits ont été enregistrées avec une intensité variable selon les localités et les espèces d'agrumes. L'objectif de ce travail est d'étudier le statut de *P. kellyanus* en Tunisie : l'abondance, les dégâts causés à différentes variétés d'agrumes, sa dynamique et sa gamme de plantes hôtes. Un suivi durant les années 2015 et 2016 a montré que l'espèce *P. kellyanus* dont les dégâts se présentent sous forme d'un anneau argenté plus ou moins étendu sur fruit, est peu abondante dans les vergers d'agrumes avec en moyenne 0.2 individus / fleur. *Pezothrips kellyanus* se développe sur divers agrumes avec une préférence pour le bergamotier et le citron causant 59 et 45% de fruits endommagés sur ces cultures respectivement. En plus des Citrus, les espèces Jasminum officinale et Bunium pachypodium ont été identifiées comme deux plantes hôtes alternatives de *P. kellyanus* en Tunisie. *P. kellyanus*, est actuellement commun dans les vergers d'agrumes de la Tunisie et ses dégâts commencent à se développer mais il reste toujours moins abondant que dans les pays où il provoque des pertes de récoltes.

Mots clés : *Pezothrips kellyanus*, citrus, variété, dégâts, abondance.

INTRODUCTION

The species *Pezothrips kellyanus* is an Australian thrips that has become an invasive pest of citrus in several other regions of the world (Nguyen et al., 2015). Until the first half of the 20th century, *P.kellyanus* was found exclusively in Australia. In 1950, it was captured for the first time outside of Australia, in New Zealand (Mound and Walker, 1982). It was detected in Greece in 1981 (zur Strassen 1986), and then reported as an important pest of citrus orchards in Cyprus in 1996 (Vassiliou 2010). Italy was the first locality in Mediterranean basin where it was reported as a pest of citrus in 1998 (Conti et al. 2001), and then reported in Portugal in 2002 (Costa et al. 2006), France in 2004 (Moritz et al. 2004), Turkey in 2006 (Teksam and Tunç 2009) and Spain in 2007 (Navarro-Campos et al. 2011). Its presence was also noted in Hawaii (Vassiliou, 2011).

P.kellyanus is a polyphagous species living on scented flowers of different plants families that needs pollen for feeding and reproduction (Baker et al., 2002). The female adult lays eggs on the flowers (mainly in the petals) and larvae hatched from the eggs develop through two stages ; at the end of its development the second instar larvae drop from the citrus canopy to the soil to pupate in the upper 20 mm soil-layer (Baker et al., 2002) and this thrips species may develop up to six generations per year (EPPO, 2004).

This species causes a silvery , ring-like thin layer of scarring (halo) or skurfing (marbling) on the fruit surface, mainly around the calyx area of the developing fruitlet (Vassiliou, 2010). This type of damage is caused by larval stages, which were regularly observed feeding on small lemon, orange, and occasionally tangelo fruitlets (Blank and Gill, 1997). In Australia, damages of *P.kellynaus* totalled $ 9 million in 2003 in export value of navel oranges (Baker, 2007).

In Tunisian citrus orchards, *P. kellyanus* was reported for the first time in the north in 2008 (Trabelsi and Boulahia- Kheder, 2009) then it has been found in the center-east in 2010 (Elimem and Chermiti, 2013). This species is relatively a new citrus pest in Tunisia and information about the biology, the ecology and the management of *P. kellyanus* is scarce. Therefore, this research was conducted in citrus orchards in Tunisia aiming (1) to assess the adundance and to study the annual fluctuation of *P. kellyanus* in Tunisia (2) to assess fruit damage caused by *P. kellyanus* on different varieties of citrus (3) and to identify the incidental or breeding alternative host plants of *P. kellyanus*.

MATERIAL ET METHODS

Experimental sites

This work was conducted during 2015 and 2016. An estimation of *P. kellyanus* damages on mature fruits was carried out in 82 orchards in three locations of Tunisia (Cap-Bon, Bizerte and Mornag) with a mixture of varieties. These locations are situated in the north of Tunisia and are the first areas of citrus production in Tunisia with an excellence citrus production in Cap-Bon.

Then, the work was carried out in four citrus orchards of Orange Thomson Navel, two located in Bizerte and two in Mornag. The choice of these orchards was based on the presence of damages of thrips and the presence of contrasted orchard management patterns (1 organic orchard receiving no insecticides and 3 orchards receiving a various number of insecticides per year) .

Dynamics of *P. kellyanus* in the 4 orchard experimental network.

Annual dynamics The population dynamics of *P. kellyanus* was monitored by counting the inviduals collected (larvae and adults) by beating tray according to “Fauvel funnel technique” at the rate of 60 branches by orchard. The thrips were then collected in a vial containing alcohol 70° placed under the funnel. The dynamics was monitored from beginning of April 2015 until December 2016 in the 4 citrus orchards. The *P. kellyanus* samples were collected weekly in spring and bi-monthly or monthly in the rest of the year.

Seasonal dynamics. The seasonal dynamics of *P. kellyanus* was studied by randomly sampling 50 organs (flowers and young fruits) in each of the 4 experimental orchards during 2015 and 2016 from the beginning of April until June, when the diameter of fruits reaches more than 3 cm. In the laboratory, under a binocular magnifier, the number of *P. kellyanus* was counted. It was also studied by installing two white color traps in each orchard from March to the end of Septembre 2016. These traps were serviced once a week for counting *Pezothrips* individuals.

Identification of *P. kellaynus*. The identification of *P. kellyanus* was based on the descriptions of Mound and Walker (1982), Navarro-Campos, and al. (2008) and Marullo (1998, 2003).

Fruit damage caused by *P. kellyanus.* The percentage of fruits damaged by *P.kellyanus* was evaluated by randomly surveying 200 to 1000 mature fruits for each variety, in each of the 82 orchards visited in the 3 regions of Bizerte, Cap-Bon and Mornag in 2015 and 2016. Several citrus varieties have been considered to estimate damages of *P. kellyanus*: Grapefruit, Bergamot, Orange (Thomson, Maltaise, Double-fine, Navel and Meski), Clementine (Hermandina and Kassar) and Lemon. On all of them, we looked for the presence or absence of ring damages that are specific to *P. kellyanus*.

The percentage of damages by *P. kellyanus* was calculated according to the number of fruits with ring damage (mild or severe) * 100 / total of fruits examined .

***P. kellyanus* alternative host plants.** Flowers of plants species present in the 82 citrus orchards visited were collected and examined to detect larvae or adults of *P. kellyanus.* Each plant species was sampled as 1 to 10 plants giving 7 to 20 flowers per sample. These flowers were collected in vials filled with alcohol 70°. In the laboratory under a binocular magnifier, the presence of larvae and adults of *P. kellyanus* was assessed.

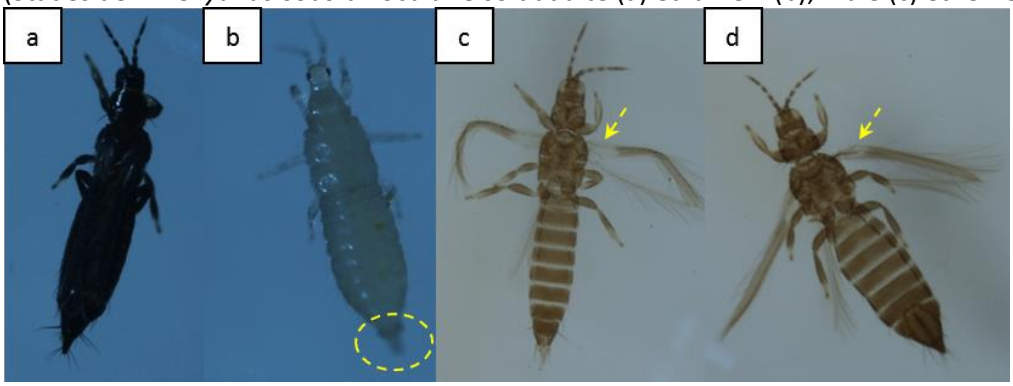
RESULTS

Identification and morphology of *P. kellyanus*

The adults of *P. kellyanus*, females and males, are dark brown colored (Fig. 1a). They have 8 antennal segments with an apical neck in the segments 3 and 4. They have 4 brown wings but slightly paler at the base (Fig. 1c and 1d). The first vein of forewings is with 2 setae on distal half, the second vein with complete row of setae. Sternites are without discal setae and have small circular glandular area in sternites III-VII for the male, which is smaller than female (Fig. 1d).

The larva of the first stage of *P. kellyanus* is whitish or yellowish and become orange in the end of their development. The larva of second stage (Fig. 1b) has sclerotized teeth in the eighth abdominal segment that facilitate their movement between the soil particles when they go to pupate.

Figure 1: *P. kellyanus* stages under binocular i.e adult (a) and larva 2 (b); male (c) and female (d)
(Stades de *P. kellyanus* sous binoculaire soit adulte (a) et larve 2 (b); mâle (c) et femelle (d))



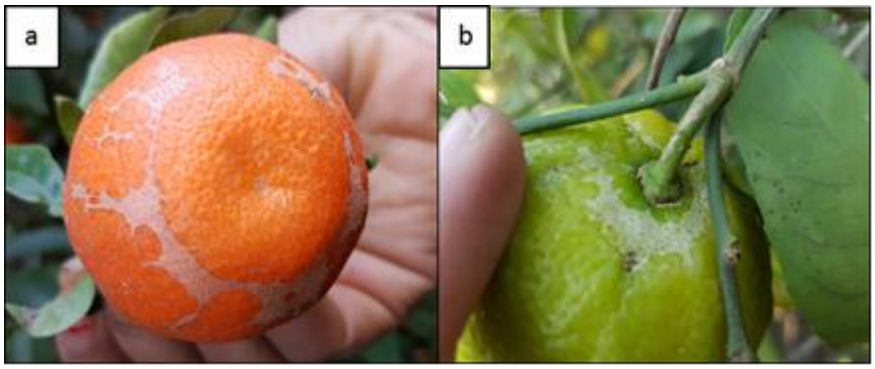
Abundance of *P. kellyanus*. *P. kellyanus* population collected by beating and organ samples in the 4 citrus orchards of study represents only 4.70% in 2015 and 23.83% in 2016 of all others species of thrips collected (Tab. I). Concerning the abundance of *P. kellyanus* on citrus flowers, it was on average of 0.2 thrips/flower, less than one thrips per flower.

Tableau I: Abundance of *P. kellyanus* (larvae and adults) in the experimental citrus orchards (Abondance de *P. kellyanus* (larves et adultes) dans les vergers d'agrumes d'étude)

Year	2015	2016
Number of *P. kellyanus* thrips collected	12	97
% of *P. kellyanus* thrips collected	4.70 %	23.83%
Total of thrips from all collected species	255	407

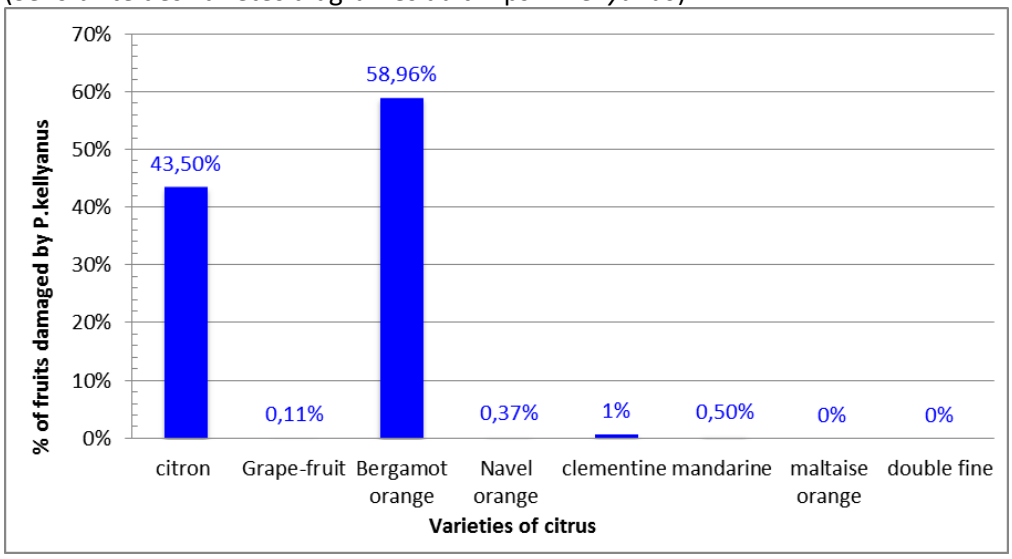
Fruit damages caused by *P. kellyanus.* In the 82 citrus orchards visited, we have observed 2 types of ring: a ring at the base of fruits (Fig. 2a) or/and a ring partial or complete at the base of the peduncles of fruits (Fig. 2b) sometimes accompanied by other lesions.

Figure 2: *P. kellyanus* ring damage at the base of fruit (a) and at the base of fruit peduncle (b) (Dégâts de *P. kellyanus* sous forme d'anneau à la base du fruit (a) et à la base du pédoncule du fruit (b))



Regarding the varietal sensitivity to *P. kellyanus,* the Bergamote appears to be the most damaged one with 59% of fruits showing severe or fine scars and this in 14 orchards out of the 82 monitored orchards. The lemon is the second variety susceptible to *P. kellyanus* with 45% of damages assessed across 35 orchards out of the 82 monitored orchards. Navel orange is less sensitive to *P. kellyanus* with 0.37% of damaged fruits assessed across 72 orchards out of the 82 monitored orchards (Fig. 3). Grape-fruit, clementine, mandarine, Maltaise and double fine orange are also less sensitive to *P. kellyanus* with 0.11%, 1%,0.5% and 0% respectively of damaged fruits assessed across 25, 22,37,57 and 7 orchards respectively out of the 82 monitored orchards (Fig. 3).

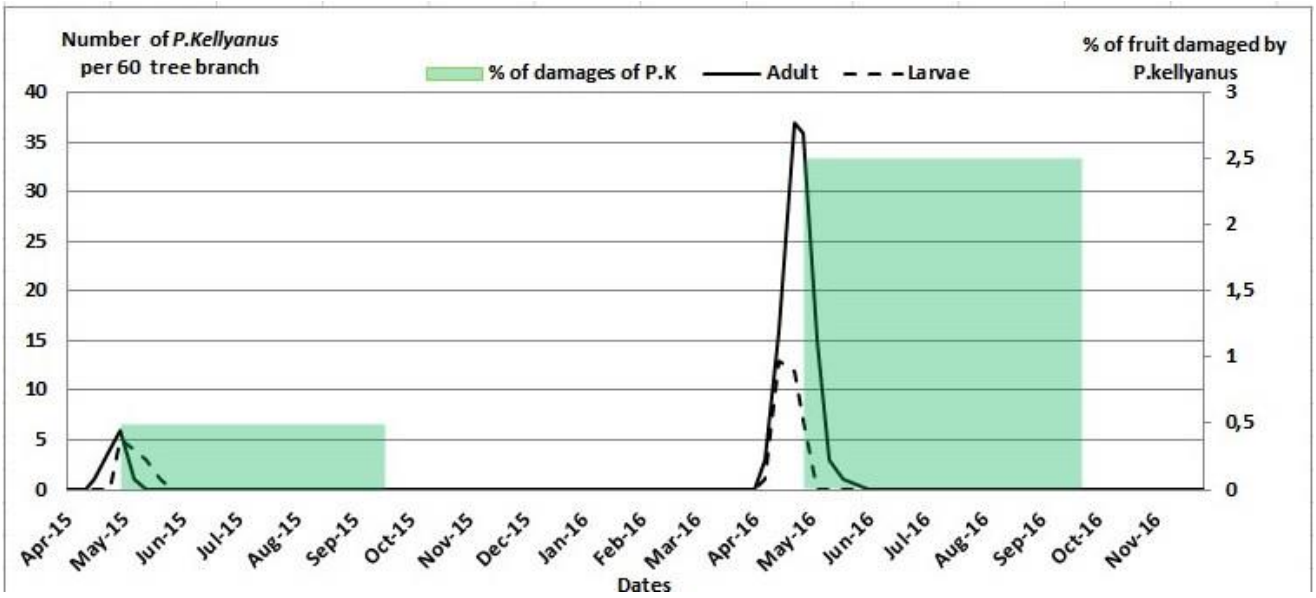
Figure 3: Citrus variety susceptibility to *P. kellyanus* thrips
(Sensibilité des variétés d'agrumes au thrips *P. kellyanus*)



Dynamics of *P. kellyanus* in citrus

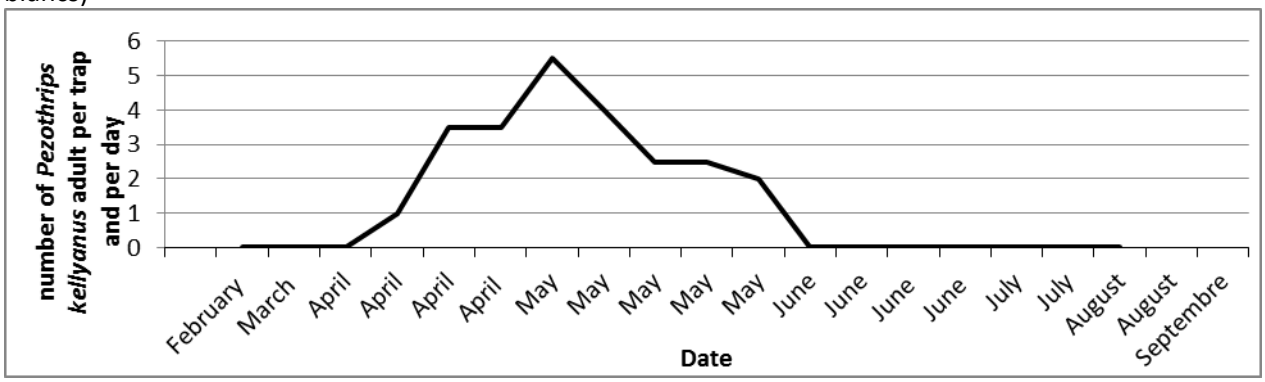
In the 4 orange Navel orchards, the abundance of *P. kellyanus* increases with flower availability. The Figure 4 shows that *P. kellyanus* develops mainly during spring flowering from April to May then decreases progressively to zero in absence of flowers. This result shows that *P. kellyanus* has a single generation per year in citrus and this can probably explain why damage did not increase. On the other hand, this figure shows that *P. kellyanus* population densities were higher in 2016 than in 2015, and that damage on fruit was more important in 2016 than in 2015 without exceeding 2.5%. This damage was irrespective of the way in which orchards are managed and was caused after fruit set when the fruit is still young.

Figure 4: Annual dynamics of *P. kellyanus* in the 4 orange navel orchards by beating tray (Dynamique annuelle de *P. kellyanus* dans les 4 vergers d'orange Navel par la technique de battage)



The captures of *P. kellyanus* by aerial traps showed similar result as with the beating capture. The adults of *P. kellyanus* were captured only between April and May. In the rest of the year, the capture of individuals was nul (Fig. 5).

Figure 5: Seasonal dynamics in 2016 of *P. kellyanus* in the 4 orange navel orchards by aerial sticky trap
(Dynamique saisonnière de *P. kellyanus* en 2016 dans les 4 vergers d'orange Navel dans les pièges blancs)



Alternative host plants for *P. kellyanus*. Both larvae and adults of *P. kellyanus* were found in large numbers on *Jasminum officinale L*. (jasmine) in 3 citrus orchards out of the total visited (Table II). Larvae and adults of *P. kellyanus* were also found on *Bunium pachypodium* (Bunium heavy foot). Only one adult of *P. kellyanus* was found on *Diplotaxis erucoides* (White wall-rocket) whilst larvae were absent. On this species, others species of thrips larvae and adults were found. *P. kellyanus* was absent from several other plants present in the citrus orchards (Table II).

The absence of alternative host plants of *P. kellyanus* in citrus orchards in Tunisia could be one of the factors that prevent this species to complete many generations through the year. That could explain the relatively low and non-persistent populations of *P. kellyanus* in citrus orchards.

Tableau II: Weeds present in citrus orchards of Tunisia
(Plantes adventices présentes dans les vergers d'agrumes en Tunisie)

Weeds collected across 82 citrus orchards	Number of samples	Number of *P. kellyanus*	
		Larvae	Adult
Calendula bicolor	20	-	-
Globionis coronarium	7	-	-
Chrysanthemum coronarium	11	-	-
Bunium pachypodum	3	2	6
Otospermum glabrum	5	-	-
Medicago sativa	10	-	-
Sinapis arvensis	2	-	-
Oxalis cernua	15	-	-
Fumaria parviflora	2	-	-
Legousia hybrid	4	-	-
Rapistrum rugosum	1	-	-
Diplotaxis erucoides	15	-	1
Diplotaxis muralis	5	-	-
Urtica dioica	20	-	-
Jasmimum officinalis	5	>17	>25

DISCUSSION

The thrips *Pezothrips kellyanus* has progressively spread throughout the citrus area of the 3 regions of Tunisia (Bizerte, Mornag and Cap-Bon) from 2008 to 2016. In 2010, damages of this species were detected in 8 out of 22 citrus orchards (Belaam, 2011). In 2016, the number of orchards with *P. kellyanus* damages was 51 of 82 orchards. So, the percentage of *P. kellyanus* infested orchards has doubled reaching around 62% in 2016. Despite *P. kellyanus* spreading in citrus orchards, its damages in these orchards were very low these years and this is explained by the very low pest densities.

According to Perrotta *et al* (2004), if 5 to 10 % of 100 fruits are occupied by larvae, a treatment is recommended. Indeed, the abundance of *P. kellyanus* on citrus flowers was less than one thrips per flower in average (0.2 thrips/flower). This value is lower than in other citrus growing areas in the world where this species is a primary pest. In New Zealand for instance, it is up to 50 inviduals /citrus flower (Blank and Gill, 1997). In Spain, it was about 2.21 and 0.35 individual /flower respectively in 2008 and 2010 (Navarro- Campos, 2013). In Italy, this species was more abundant on lemon than in other citrus crops with 10.28 individuals per flower on lemon, 7.0 on bergamot and 5.1 on orange in 2011(De Grazia and Marullo, 2015). The population of *P. kellyanus* appears to depend on the variety, the flower presence and abundance, and the duration of the flowering stage (Vassiliou ,2010).

The abundance of *P. kellyanus* in citrus orchards increases with flower availability. This species develops mainly during spring flowering from April to May then decreases progressively to zero in absence of flowers. This suggests that *P. kellyanus* needs the pollen for its reproduction and larval growth (Baker *et al*.,2002). *P. kellyanus* was not detected on mature fruits conversely to other countries where larvae and adults were observed during winter in the coldest months (Navarro-campos *et al*., 2011).

The absence of *P. kellyanus* on mature fruits in Tunisian citrus orchards, could be explained by the fact that the breeding sites for this species are not available all year round as in others countries where larvae and adults were observed on a longer period of time. Indeed in Tunisia, only 2 alternative host plants for *P. kellyanus* were found: *Jasminum officinale* L. (in only 3 orchards out of 82) and *Bunium pachypodium* (in only one orchard out of 82). This is unlike in other countries where many breeding hosts of *P. kellyanus* were observed in citrus orchards, where white and sweetly scented flowers are usually found such as *Jasminum* spp., *Lonicera* spp., *Gardenia jasminoides* Ellis, and *Araujia sericifera* (Kirk,1987; Mound and Jackman,1998; Froud et al.,2001; Navarro et al.,2013). In our study only one adult was identified on *Diplotaxis erucoides*, and immature thrips were absent on the flower of this plant; this is similar to results from Navarro *et al*. (2013) so *Diplotaxis erucoides* should be considered as incidental rather than a breeding host for *P. kellyanus*.

In addition to the absence of alternative host plant, the low density of *P. kellyanus* in citrus orchards of Tunisia can be related to the presence of soil-predatory mites that can reduce the population and to the use of insecticides for controlling others citrus pests which are effective against P. *kellyanus* (Navarro *et al*.,2013; Planes et *al*., 2015) .

Various insecticides were evaluated in citrus orchards against larval stage such as the organophosphate chlorpyrifos, the carbamate methomyl, and the neonicotinoid acetamiprid that were the most effective ones (Conti *et al.,* 2001; Vassiliou, 2007). In citrus orchards of Tunisia, chemical control of some citrus pests is made after flowering when the larvae of P. *kellyanus* are present; chlorpyrifos and spinosad known by their high efficacy against nymphs of P. *kellyanus* are insecticides amongst those used in citrus orchards of Tunisia and can significantly reduce the percentage of damaged fruit (Planes et *al*., (2015),

On the other hand, Navarro et *al*., (2012) demonstrated that a high population of the soil-dwelling predatory *Hypoaspis aculeifer* (Mesostigmata, Laelapidae) was associated with a low abundance and fruit damage caused by *P. kellyanus*. So work is needed to have an information about the

relationship between *Pezothrips* populations and soil mite fauna of Tunisia as well as the effect of insecticides on thrips population. That will help to prevent thrips damage by developing an effective biological control strategy against thrips.

Furthermore, high temperatures occurring during the critical period when damage are produced may be the cause of a low density of *P. kellyanus*. According to Varikou *et al.,* (2009 and 2012) in laboratory conditions, the abundance of *P. kellyanus* is impacted by air temperature. In field conditions, Navarro et *al*., (2013) demonstrated that the lowest level of damage was observed when there were more weeks in which mean temperatures were below +10.2C which is the developmental threshold for *P. kellyanus* (Varikou et al., 2009). Once again research is needed in order to make a link between climatic conditions, especially the temperature in spring and summer often characterized by sirocco (hot and dry wind from south), and the small abundance of *P. kellyanus* in citrus orchards of Tunisia.

CONCLUSION

In this research, the species *P. kellyanus* is present in citrus orchards of Tunisia but it does not pose any problems since a percentage of minimal damage appears in the form of ring on the fruits of orange Navel and since its density is low on flowers knowing that the variety Maltaise and Navel occupies the first place in citrus production in Tunisia. However, the damage of this species was noted on other varieties (Bergamot and Lemon tree) wih an important percentage. These sensitive varieties are less present in the north of Tunisia. Researchers are needed to dedicate more time to study *Pezothrips* biology on these varieties, including the relationships between temperature and damages of *P. kellyanus.* Then the role of predatory mites in the soil shall also be studied as it could be another factor limiting the multiplication of this species in our country.

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