Preliminary Study on Mealybugs in Two Vineyards of the Cap-Bon Region (Tunisia)

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ABSTRACT

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A study on mealybugs and their parasitoids was conducted from April to December 2007 in two vineyards of the Cap-Bon region (North-East of Tunisia). According to their microscopic characteristics, two mealybug species, *Planococcus ficus* and *P. citri* were recorded on vines. The pheromone-based monitoring system showed that the male flight activity of the two mealybug species extended from April to November with predominance of *P. ficus* males in Takelsa vineyard (6 flight peaks) and of *P. citri* males in Mraïssa vineyard (7 flight peaks). Only one parasitoid, *Anagyrus* sp. near *pseudococci* was identified from the mealybugs.

Keywords: *Anagyrus* sp. near *pseudococci*, male flight activity, *Planococcus citri*, *Planococcus ficus*, Tunisia, Vine

The vine (*Vitis vinifera*), worldwide cultivated, is often attacked by several insects, such as mealybugs (Hemiptera, Pseudococcidae). Among these insects, various species can coexist in the same vineyard and can cause serious damages.

Currently, mealybugs are considered key pests in several countries (8, 23, 32, 33) because of the direct damage caused by their feeding activity and of the indirect damage linked to honeydew production, that supports the growth of sooty mold fungi (5, 12), and to their role as vectors of numerous vine viral diseases (1, 8, 21).

For these reasons, several control strategies were undertaken in order to limit further spread of mealybugs, and therefore to try to contain the serious problem of viral diseases in vineyards.

In this context, synthetic sex pheromones of mealybugs were developed and tested in pheromone-baited traps as a monitoring tool for *Planococcus ficus* in vineyards (5, 33) and for *Planococcus citri* in citrus orchards (15, 27) and it was shown that the practical use of these pheromones could effectively help to highlight new infestations and to follow the mealybug

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male flight activity in order to specify the appropriate control period against these pests (5, 33). In addition, synthetic sex pheromones, used in a mating disruption system, can effectively contribute to suppress mealybug populations in vineyards (7).

Chemical control may be ineffective due to the cryptic lifestyle of mealybugs which often reside in protected locations, such as the bark of trunk (12) and may have also a negative impact on the natural enemies of theses insects (30).

In several countries, biological control has successfully contributed to an important decrease in mealybug populations (6, 7, 13, 16, 31).

In Tunisia, very few studies were carried out on mealybugs, and the two species, *P. citri* and *P. ficus* were recently found on vines in Tunisia (20). However, until recently, the biology of these insects is almost unknown in this country.

In order to have a better and complete knowledge of the Pseudococcid fauna and of the most common parasitoids, the present study was conducted in two vineyards located in the Cap-Bon region (North-East of Tunisia), with the aim of establishing an effective IPM program against mealybugs in vineyards.

MATERIALS AND METHODS

Study sites. The study was conducted from April to December 2007 in two table-grape vineyards located in the Cap-Bon region (Mraïssa and Takelsa) that had a history of *P. citri* infestations (19).

Takelsa vineyard covers an area of 1.5 ha, planted with the Muscat d'Italie variety, led in pergola and furrow irrigated. The vines are 21 years old with a distance of plantation of 4 m x 2 m. Mraïssa vineyard covers a surface of 1 ha, cultivated with the Superior Seedless variety, led in pergola and drip irrigated. The vines are 14 years old with a distance of plantation of 4 m x 2 m.

Climatic data. In Takelsa vineyard, the mean amount of the rain ranged from 0 mm (July) to 214.5 mm (December), while in Mraïssa vineyard, it ranged from 0 mm (July) to 187 mm (October). However, the temperature averages of Beni-Khalled (near studied sites) varied from 10.9 (December) to 26.7°C (July) (Table 1).

Table 1. Climatic conditions in the studied sites of the Cap-Bon Region (year 2007)

Months		Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
MP (mm)	T	32.9	73.3	201	37.5	16	24.4	0	6.2	62.9	164.7	51	214.5
	M	19.5	40.5	146	39	14.5	13	0	4.5	90	187	32.5	164
MT (°C)	B	11.6	12.2	12 .6	16.2	20.2	23.9	26.7	26.1	22.7	19.3	14.2	10.9

MP: monthly pluviometry; MT: monthly temperature; T: Takelsa; M: Mraïssa; B: Beni-Khalled (near the studied sites)

Pheromone trapping of male mealybugs. The mealybug male flight activity was monitored using pheromone traps with two specific sex pheromone-baited lures of *P. ficus* and *P. citri* (Biosystèmes France s.a.r.l.).

Pheromone lures were made from rubber septa that were loaded with a dose of the racemic synthetic sex pheromone.

Each pheromone lure was placed on a white sticky plate, put inside a white delta-shaped trap (25 x 14 x 12 cm) which was hung in the vine canopy approximately 1.5 m above the ground.

In each vineyard, two pheromone-baited traps (one for *P. ficus* and one for *P. citri*), were installed at a distance of twenty rows.

The white sticky plates were changed weekly from April to November and all adult males of *P. ficus* and *P. citri* found on each sticky plate were counted using a binocular microscope. The pheromone-baited lures were changed every three weeks while delta traps were substituted only when they were dusty.

Identification of mealybug species and their parasitoids. Samples of adult females were collected from different parts of the vineyards in Takelsa and Mraïssa, twice per month during the period from July to December in order to identify the mealybug species.

In each sampling date, at least twelve vines in each vineyard were randomly selected and sampled by collecting mealybugs from the trunk and other parts of the vine. A minimal sample of 3 mealybug females per vine was collected.

The specimens were placed in vials containing a solution of 70% alcohol, recording the number of the sampled vine and its location. Then, microscope slides were prepared following the method described by Williams and Watson (34), in order to identify the mealybug species according to the distribution and presence of multilocular pores and tubular ducts on the adult females (3, 35).

The inventory of parasitoids associated to the mealybug species occurring on vine was made through the intensive sampling method. Thus, twice per month from September to December 2007, several mealybugs were randomly collected from at least 12 vines within different parts of each vineyard. In the laboratory, parasitized mealybugs were individually maintained in tubes until the emergence of the parasitoid. After its emergence, each parasitoid was killed in an ethyl acetate atmosphere and was preserved in a small vial before its specific determination according to the keys to determine the mealybug parasitoids of the Anagyrini (Hymenoptera: Encyrtidae) described by Noyes and Hayat (24).

Statistical analyses. To compare between-mealybug species pheromone trap counts and between-vineyards (localities) pheromone trap counts, a one-way ANOVA was performed and the means were separated by applying the Duncan-Test at a level of significance α = 5%. The statistical analysis was conducted using STATISTICA 6.0 (StatSoft Inc.).

RESULTS

Pheromone trapping of mealybugs. In Takelsa, the male flight activity of *P. ficus* began on the 3rd week of April with 1 male/trap/week and completely declined after mid-November (Fig. 1). During this period, 6 flight peaks were recorded with the two highest trap counts observed in late May and late July with 93 and 57 males/trap/week, respectively.

Males of *P. citri* have been trapped from the first half of April until the 1st week of November with 6 male flight peaks and overall lower catches compared to those of *P. ficus* in the same vineyard (Fig. 1). The highest trap count was recorded on the 4th week of September

with 28 males/trap/week and five other peaks were obtained on the 1st week of May, on the 3rd week of June, on the 3rd and 5th weeks of July and in early November, with 15, 14, 13, 14 and 19 males/trap/week, respectively.

Statistical analyses showed that, in Takelsa, there were no significant differences in the pheromone trap counts of the two mealybug species (*P. ficus* and *P. citri*) ($F_{1; 62}$ = 1.17; P = 0.284).

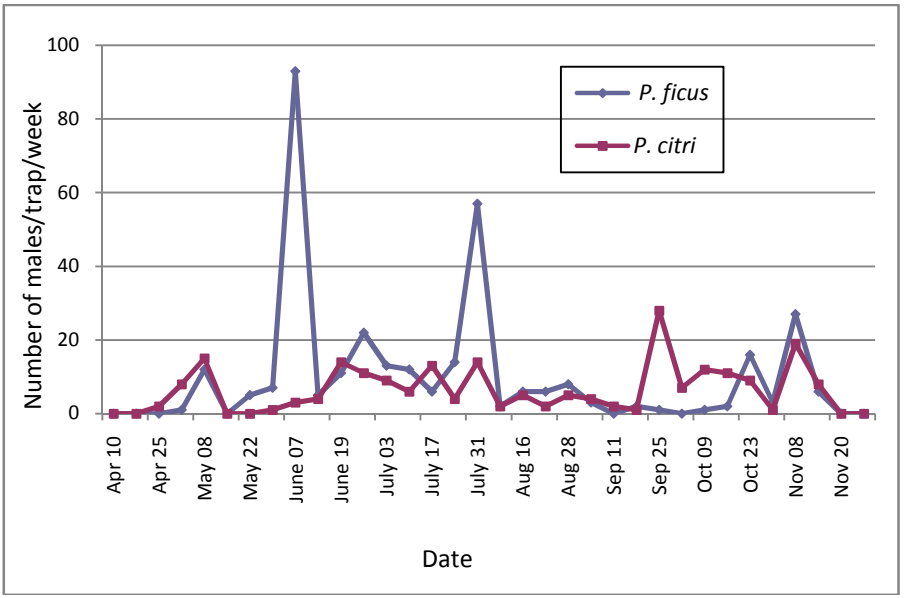

Fig. 1. Male flight activity of *P. ficus* and *P. citri* in Takelsa vineyard (April-November, 2007)

In Mraïssa vineyard, the male flight period of *P. ficus* took place from mid-April to late October with significantly lower trap catches ($F_{1; 64}$ = 6.77; P = 0.011) compared to those obtained in Takelsa vineyard (Fig. 2). Indeed, during this flight period, only one flight peak was recorded in early August with 26 males/trap/week with a severe decline of male flight activity from late May to mid-June and during September.

As regards *P. citri*, the male flight activity was first detected in early April and ended in early November. This species presented seven flight peaks with very high levels of male catches, compared to those observed in Takelsa vineyard ($F_{1; 64}$ = 12.11; P = 0.0009) and to those of *P. ficus* in the same vineyard ($F_{1; 66}$ = 16.45; P = 0.0001). Among these peaks, the highest ones were recorded in late June, in late August, on the 4th week of September and on the 2nd week of October with 123, 228, 128 and 118 males/trap/week, respectively (Fig. 2).

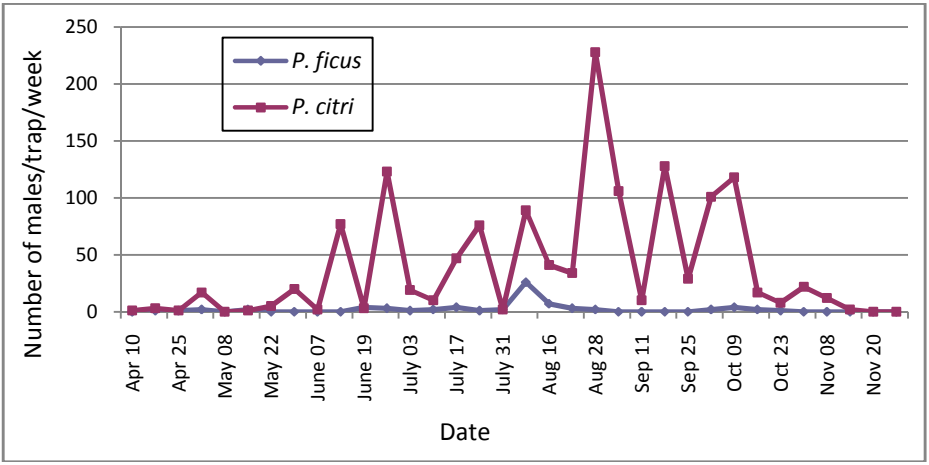

Fig.2. Male flight activity of *P. ficus* and *P. citri* in Mraïssa vineyard (April-November, 2007)

Identification of the mealybugs. Two mealybug species were found in the vineyards, the vine mealybug *P. ficus* and the citrus mealybug *P. citri*, based on their microscopic morphological characters (Table 2).

Table 2. Main distinctive microscopic characters of the mealybug species *P. citri* and *P. ficus* (3, 35).

Planococcus citri	***Planococcus ficus***
Venter of head with 14-35 tubular ducts	Venter of head with 0-4 tubular ducts
Thorax with 7-30 tubular ducts near eight pair of cerarii	Thorax with 0-4 tubular ducts near eight pair of cerarii
Multilocular disc pores situated behind front coxae totalling 0-6	Multilocular disc pores situated behind front coxae totalling 0-17
Multilocular disc pores on posterior edges of abdominal segment VII present in single or double rows	Multilocular disc pores on posterior edges of abdominal segment VII present in single rows
Multilocular disc pores absent near second spiracle, behind femur of metathorax	Multilocular disc pores present near second spiracle, behind femur of metathorax

Identification of mealybug parasitoids. The genus of all collected parasitoids specimens was identified as *Anagyrus* according to the following characteristics as described by Noyes and Hayat (24):

- *Female*: scape almost always broadened and flattened, funicle 6-segmented, funicle segments longer than broad, clava 3-segmented, frontovertex and dorsum of thorax normally matt, mesoscutum without notauli, wings hyaline to distinctly infuscate.

- *Male*: scape slightly broadened and flattened, funicle 6-segmented, scale-like sensilla normally on both sixth funicle segment and clava, frontovertex and dorsum of thorax normally matt, wings hyaline, phallobase variable.

All collected parasitoid specimens (twenty one) were identified as *Anagyrus* sp. near *pseudococci sensu* Triapitsyn *et al.* (28). Indeed, the females of this species have the first antennal funicular segment entirely black while in *A. pseudococci* the same segment is half or 2/3 black and the rest white (Fig. 3).

Fig. 3. Detail of *Anagyrus* sp. near *pseudococci* antenna (x 40).

During the surveys, it was highlighted that *Anagyrus* sp. near *pseudococci* males emerged from second instar nymphs, while females emerged from third instar nymphs and adult females. These two last mealybug development stages were in fact the most frequently parasitized specimens (more than 70%) collected from vines.

Among 21 emerged *Anagyrus* sp. near *pseudococci* specimens, 6 were males and 15 were females; hence, the sex ratio of this endoparasitoid species was female biased (1:0.4) (♀:♂), nevertheless further studies based on the collection of more important numbers of parasitoids are required to confirm this preliminary observation.

Moreover, during the sampling period (from September to December), the encyrtid was constantly present on mealybugs hidden under the trunk bark, but with very low numbers (21 specimens/16 sampling dates).

DISCUSSION

Mealybugs. The results of the survey conducted based on mealybug microscopic characteristics as well as using pheromone traps, clearly suggest that the two species, *P. ficus* and *P. citri*, coexist in both Mraïssa and Takelsa vineyards.

The two species had already been found in other Tunisian vineyards (20), such as in USSR (14), France (26) and Italy (18), unlike California (4) and in South Africa (32), where *P. citri* has not been found together with *P. ficus* in vineyards.

In the present study, the identification of the collected mealybugs was made according to the distribution and presence of multilocular pores and tubular ducts on the adult females. In fact, these microscopic characteristics are, in the majority of cases, difficult to detect, as previously mentioned by Williams and Granara de Willink (35). For that reason, this method appears not obvious for a well-timed and more precise identification of these pests. In this context, it was shown that molecular techniques could provide new interesting horizons for the discrimination of these pests. Indeed, more recently, two methods were successfully used to discriminate the two mealybugs *P. ficus* and *P. citri*, namely the RAPD-PCR (9, 25) and the PCR-RFLP (2). Moreover, according to Cavalieri *et al.* (2), the PCR-RFLP is more reproducible than RAPD-PCR and it provides researchers and technicians with a powerful tool for the rapid, accurate and unequivocal identification of these mealybugs.

According to the results of the pheromone-based monitoring system, *P. ficus* appeared to be the dominant species in Takelsa vineyard, as previously found in California (5) and South Africa vineyards (30), while *P. citri* is by far the most abundant mealybug species in Mraïssa vineyard. It should be noted that the dominance of *P. citri*, compared to *P. ficus*, in Mraïssa vineyard could be explained by the fact that this last is surrounded by citrus orchards which are characterized by higher levels of *P. citri* infestations; on the contrary, Takelsa vineyard is localized far from citrus orchards and surrounded by several grape growing areas.

The results concerning *P. ficus* male flight activity in Takelsa vineyard are almost similar to those obtained in California vineyards where the flight period extends from March to November and the highest *P. ficus* male density value occurs in June, with smaller peaks in October and November (5, 22).

However, in South Africa, the male flight period of *P. ficus* extends from mid-September (Spring) to early April (beginning of autumn) with the highest trap count recorded during the period between early December and early February (summer), corresponding to the most important activity of *P. ficus* populations on vine in this country (33). For instance, in the Hex River valley (Western Cape Province), during both seasons, numbers of *P. ficus* males in the pheromone traps increased during December and peaked towards the end of February with more than 60 males/trap/week in each season (10).

On the other hand, it should be noted that the number of peaks reported in the studied vineyards (for instance 6 for *P. ficus* in Takelsa and 7 for *P. citri* in Mraïssa) must correspond normally to the number of generations per year developed by each species. In that sense, a very recent study carried out in Mraïssa vineyard (the same vineyard of this study) showed that *P. citri* developed 7 generations/year with accordingly 7 male

flight peaks of this mealybug species (unpublished data).

These results concerning the male flight activity of both *P. citri* and *P. ficus* obviously provide some baseline information on the biology of mealybugs in the Cap-Bon region and could help in choosing the best control measures and the suitable control moment for these economic pests. In this context, it appears that the pheromone-baited traps could be an excellent tool to find new infestations in vineyards and monitoring them, therefore improving vineyard pest management strategies.

Mealybug parasitoids. In our study, all parasitoid specimens emerged from collected mealybugs were identified as *Anagyrus* sp. near *pseudococci.* This result suggests that this encyrtid species is the most common parasitoid of mealybugs in Mraïssa and Takelsa vineyards.

In a previous study, it was shown that in the North-East of Tunisia, two parasitoids species, *Anagyrus pseudococci* and *Leptomastidea abnormis* (Hymenoptera: Encyrtidae), can coexist in the same vineyard (19).

Our observations corroborate those conducted in California (5, 22), Argentina (29), Israel (11) and Italy (17). Indeed, in California vineyards, two introduced encyrtid parasitoids *L. abnormis* and *A. pseudococci* can effectively attack the vine mealybug *P. ficus* (4, 13, 22), but several studies showed that the introduced encyrtid *A. pseudococci* was the most common (and effective) parasitoid attacking this pest on vine in this state (4, 5, 6, 13, 22). In addition, *A. pseudococci* was the most common parasitoid of mealybugs recovered on vine in Argentina (29) and on citrus in Israel (11). Moreover, Longo *et al.* (17) showed that, in Southern Sicilian vineyards (Italy), two indigenous parasitoids *L. abnormis* and *A. pseudococci* were reported as the most common parasitoid species of the vine mealybug *P. ficus*.

The fact that the majority of collected parasitized mealybugs were females or third instar nymphs clearly indicates that *Anagyrus* sp. near *pseudococci* has a preference for these development stages. This observation corroborates those of Gonzalez (13) who showed that *A. pseudococci* primarily attacks the last nymphal stages and the adult females of *P. ficus* in California vineyards and those of Daane *et al.* (6), obtained also in California, who suggest that the third instar nymphs were the most commonly selected and parasitized instars by several encyrtids.

In our study, we noted that *Anagyrus* sp. near *pseudococci* males emerged from second instar mealybugs; while females emerged from third instar and mealybug adult females. This observation joins those of Daane *et al.* (6) who showed that *A. pseudococci* sex allocation is influenced by the mealybug size: first and second instar mealybugs yield primarily males (100 and 83.3 ± 1.1%, respectively), whereas third-instar and adult mealybugs yield primarily females (95.4 ± 1.1% and 92.9 ± 2.2%, respectively).

During the period of observation on the parasitoids activity, parasitism rates appeared to be very low. This could be related to the fact that, during the study period, mealybugs resided under the bark of the trunk, where they are protected from parasitoids. Another reason could be related to the activity of ants which feed on honeydew and therefore, effectively protect the mealybugs from their natural enemies, as it was often observed in the two monitored vineyards and as already found in previous studies (4, 5). Finally, insecticide treatments carried out in these

vineyards during the growing season could of course have a negative impact on parasitoid populations.

The presence of *Anagyrus* sp. near *pseudococci* in December shows that this parasitoid overwinters inside mealybugs in vineyards of the Cap-Bon region and therefore it is well adapted to winter temperatures of Tunisia.

All these results provided prerequisite information but many other studies should be performed to evaluate the efficiency of *A. pseudococci* s.l. in regulating mealybug populations in the conditions of Tunisian vineyards, as well as to acquire a better knowledge of the mealybug auxiliary fauna in Tunisia.

RESUME

Mansour R., Grissa-Lebdi K., La Torre I., Zappalà L. et Russo A. 2009. Etude préliminaire sur les cochenilles farineuses dans deux vignobles de la région du Cap-Bon (Tunisie). Tunisian Journal of Plant Protection 4: 185-196.

Une étude sur les cochenilles farineuses et leurs parasitoïdes a été conduite d'Avril à Décembre 2007 dans deux vignobles de la région du Cap-Bon (Nord-Est de la Tunisie). Sur la base de leurs caractéristiques microscopiques, deux espèces de cochenilles farineuses, *Planococcus ficus* et *P. citri* ont été recensées sur vigne. Le système de piégeage sexuel a montré que l'activité de vol des deux espèces de cochenilles farineuses s'étend d'Avril à Novembre avec une prédominance des mâles de *P. ficus* dans le vignoble de Takelsa (6 pics de vol) et des mâles de *P. citri* dans le vignoble de Mraïssa (7 pics de vol). Une seule espèce de parasitoïde, *Anagyrus* sp. near *pseudococci*, a été identifiée à partir des cochenilles farineuses.

Mots clés: Activité de vol, *Anagyrus* sp. near *pseudococci*, *Planococcus citri*, *Planococcus ficus*, Tunisie, Vigne

ملخص

منصور، رمزي وكوثر قريسة-لبدي وإيفان لا توري ولوتشيا زبّالا وأغاتينو روسّو. 2009. دراسة أولية حول حشرات البق الدقيقي بكرمي عنب بجهة الوطن القبلي (تونس). Tunisian Journal of Plant Protection 4: 185-196.

أجريت دراسة حول حشرات البق الدقيقي ومتطفلاتها من أفريل/نيسان إلى ديسمبر/كانون أول 2007 في كرمي عنب بجهة الوطن القبلي (شمال شرق تونس). واعتماداً على الخصائص المجهرية، تم تشخيص نوعين من حشرات البق الدقيقي، هما *Planococcus ficus* و *P. citri*، على كرمات العنب. بينت تقنية المصائد الفيرومونية أن نشاط طيران هذه الحشرات يمتد من أفريل/نيسان إلى نوفمبر/تشرين ثاني مع سيطرة لذكور *P. ficus* بكرم عنب تاكلسة (6 قمم طيران) ولذكور *P. citri* بكرم عنب المريسة (7 قمم طيران). تمّ تشخيص نوع واحد من أشباه الطفيليات داخل حشرات البق الدقيقي وهو *Anagyrus* sp. near *pseudococci*.

كلمات مفتاحية: تونس، عنب، نشاط طيران الذكور، *Planococcus ficus*، *Planococcus citri*، *Anagyrus* sp. near *pseudococci*

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