Some Observations on the Predominance of *Aphis spiraecola* on Citrus in Northwestern Algeria

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**ABSTRACT**

*Aphis spiraecola* is the main aphid species found on citrus in Algeria. This study was carried out on *Citrus clementina* in northwestern Algeria, during a two year period (2016-2017) in the first flushing period (spring). The aphid fluctuation of the populations and their natural enemies, especially the parasitoids, were evaluated based on a weekly sampling of 100 leaves taken on 10 trees (10 leaves/tree). *A. spiraecola* colonized citrus trees since the beginning of flushing. The density per young leaf reached a maximum of 78.8 ± 23.4 aphids in 2016 and 44.4 ± 13.0 aphids in 2017 with an average density of 6.0 ± 1.5 aphids/cm² and 4.4 ± 0.6 aphids/cm², respectively, where a significant difference between years (P < 0.05) was observed. The parasitism rate expressed in terms of number of *A. spiraecola* mummies remained very low, varying between 1.6% in 2016 and 3.0% in 2017 with no significant difference (P > 0.05) between years. Also, the emergence number of primary parasitoids was low for both years with 26.6% in 2016 and 10.8% in 2017. The primary parasitoids of *A. spiraecola* in 2016 were *Lysiphlebus testaceipes* and *Binodoxys angelicae* whereas only *L. testaceipes* was found in 2017. The total hyperparasitism rate varying between 16.7% in 2016 and 25.7% in 2017 did not differ significantly between years (P > 0.05). Mummies without adult emergence rate were found to be very high varying between 85 and 100%. This partial parasitic failure observed on *A. spiraecola* underlines many questions related with different factors (climate, ability of aphids to form winged populations to escape to their enemies, impact of hyperparasitoids). The new field of research is concerning the possible presence of endosymbiotic organisms that could give to the aphid a defense reaction against its aggressors.

**Keywords:** *Aphis spiraecola*, citrus, hyperparasitoids, Northwestern Algeria, primary parasitoids

About twenty aphid species were recorded on citrus crops worldwide (Barbagallo and Patti 1985; Blackman and Eastop 2006) but the most harmful species are *Aphis spiraecola*, *A. gossypii*, *Toxoptera aurantii* and *T. citricidus* (Hermoso de Mendoza et al. 2006; Marroquin et al. 2004; Tena and Garcia-Marí 2011). The latest species has not been detected in Algeria yet.

*A. spiraecola* is one of the 14 aphid species of agricultural importance worldwide (Blackman and Eastop 2007). It is known to infest heavily citrus crops in all Mediterranean countries including Algeria (Ali-Arous et al. 2017; Barbagallo et al. 1996; Ben Halima-
Kamel et al. 1994; Boukhris-Bouhachem et al. 1996, 2011; Hermoso de Mendoza et al. 2012; Labdaoui and Guenaou 2015, 2017; Lebbal and Laamari 2016; Mostefaoui et al. 2014; Satar and Uygun 2008). This aphid species is known to be poorly controlled with parasitoids (Hermoso de Mendoza et al. 2006; Satar et al. 2014). In addition to leaf curling, flower falling and ants attracting (Satar and Uygun 2008), A. spiraecola is able to transmit the Citrus tristeza virus (CTV) with 23% of viral RNA detection (Marroquin et al. 2004). In Algeria, this virus is becoming epidemic in Chelif Valley known to be one of the most important citrus-growing region (Ali-Arous et al. 2017).

This aphid species is polyphagous and was recorded on more than 20 plant species (Blackman and Eastop 1984, 2006; Holman 2009). For Algerian private farmers, chemical control is the only issue to limit aphid infestation on citrus. The main objectives of this study were to explore the specific diversity of aphid populations and their parasitoids and to determine the causal relationship between different factors on the predominance of A. spiraecola.

MATERIALS AND METHODS

Study site.

This study was carried out in a Citrus clementina orchard, located at Mazaghran (35°53’ 30.49"N - 0° 5'7.94"E, 1.12 ha) belonging to the university of Mostaganem. Trees were more than 30 years old and grafted on bigarade orange (C. aurantium) rootstock. The orchard was flood irrigated and was free from insecticide-based treatments since 2008.

Aphid sampling.

This study was conducted in 2016 and 2017 springs. The sampling dates started from the beginning of the flushing (from April, 14 to June, 02, 2016 and from March, 13 to May, 15, 2017). Aphid colonies were marked at the beginning of the spring leaf-flushing period and tracked weekly over the duration of the flush. One hundred young leaves were collected weekly from 10 trees (10 leaves/tree), and placed separately in plastic bags and brought to the laboratory for the monitoring of aphids and their associated parasitoids.

Leaf surface calculation.

The leaf area was calculated according to the formula proposed by Onillon et al. (1973): $s = 0.7707 \times a^{0.9392} \times b^{0.9893}$, where $s$: leaf area (cm²), a: the largest length of the leaf (cm), and b: the largest width of the leaf (cm).

Aphid complex determination.

Samples were observed under a stereo-microscope “Zeiss”. Parasitized aphids were left until mummification. New mummies were added to the total number. Aphids were identified according to several keys (Blackman and Eastop 1984, 2006; Leclant 2000).

Parasitoid complex determination.

Full mummies were smoothly taken and transferred separately in a gelatin capsule until the emerging of parasitoids. Emerged adults were immediately placed in 95% ethanol and stored at 4°C until identification. The percentage of parasitism was estimated by calculating the number of mummified aphids over the total number of aphids. Parasitoids were separated using several morphological keys (Barahoei et al. 2014; Gibson 2001; Hui and Da-wei 2000; Kavallieratos et al. 2001; Rakhshani et al. 2007; Stary et al. 2014; Tomanović et al. 2013). When needed, specialists were
asked for confirmation or correction of misidentification.

**Statistical analysis.**

The software IBM SPSS Statistics version 23 was used to analyze data.

**RESULTS**

**Inventoried aphid species complex.**

The aphid species recorded in the surveyed citrus orchards were *A. spiraecola* which was the most dominant species representing 80 to 100% of the total collected aphids (Fig. 1). *A. gossypii*, *Toxoptera aurantii*, and *Myzus percicae* were found in a very limited numbers. *A. spiraecola* was recorded since the beginning of sampling on 91 and 51% of infested leaves in 2016 and 2017 monitoring, respectively. Its density reached a peak of $78.8 \pm 23.4$ individuals/leaf representing $6.0 \pm 1.5$ aphids/cm² on May 16, 2016 and $44.4 \pm 13.0$ individuals/leaf corresponding to $4.4 \pm 0.6$ aphids/cm² on April 17, 2017. Aphids’ density was found to be lower in 2017 than in 2016 with a highly significant difference between years ($P = 0.0004$). An earlier infestation was observed in 2017 probably related to the climate as expressed by the warmer temperatures occurring in March, 2017 (Fig. 1).

![Fig. 1. Relative density of *Aphis spiraecola* over total aphids collected in 2016 and 2017 and the prevailing temperature data.](image)

**Primary parasitoids complex.**

The parasitism rate on *A. spiraecola* expressed as mummies was very low, reaching a maximum of 1.6% in 2016 (Fig. 10) and 3.0% in 2017 (Fig. 11) with no significant difference noted between the two years ($P = 0.535$).

Only two primary parasitoids emerged from *A. spiraecola* which were *Lysiphlebus testaceipes* (Fig. 2) and *Binodoxys angelicae* (Fig. 3) while
interestingly *B. angelicae* was absent in 2017. The primary parasitoid emergence rate remained very low and did not exceed 26.6% in 2016 and 10.8% in 2017 but this difference was not significant (*P* = 0.937). In 2016, *L. testaceipes* reached a maximum of 10.8% ; however, in 2016, it did not exceed 9.4% of the total parasitism. *B. angelicae* was recorded only in 2016, with a rate of 17.0%.

A high rate of mummies without adult emergence was noted which varied between 70.8 and 87.5% in 2016 (Fig. 10) and between 71.4 and 91.0% in 2017 surveys (Fig. 11).

**Secondary parasitoids complex.**

The rate of both hyperparasitoids reached 16.7% in 2016 and 25.7% in 2017 (Fig. 12) and did not show a significant difference between years (*P* = 0.232).

At least six secondary parasitoids were found namely *Pachyneuron aphidis* (Fig. 4), *Asaphes vulgaris* (Fig. 5), two *Alloxysta* species (Figs. 6 and 8), *Phaenoglyphis* sp. (Fig. 9) and *Phaenoglyphis heterocera* (Fig. 7) which was recorded for the first time in Algeria on 2017 (Ferrer-Suay et al. 2017).
Fig. 6. *Alloxysta* sp. 1

Fig. 7. *Phaenoglyphis heterocera*

Fig. 8. *Alloxysta* sp. 2

Fig. 9. *Phaenoglyphis* sp.

Fig. 10. *Aphis spiraeola* abundance and its parasitism rate in 2016 survey.
Fig. 10. *Aphis spiraecola* abundance and its parasitism rate in 2016 survey.

Fig. 11. *Aphis spiraecola* abundance and its parasitism rate in 2017 survey.
DISCUSSION
In the present study, we have contributed to a better knowledge regarding specific variation in aphids, primary and hyperparasitoids in a representative citrus growing area of the Mostaganem region (Algeria). This study confirmed the predominance of *A. spiraecola* on citrus in this site. Only few colonies of *A. gossypii, T. aurantii* and *M. persicae* were observed which is in accordance with Hermoso de Mendoza et al. (2006) findings where the effect of these aphid species was also negligible. The relatively high density of *A. spiraecola* may be probably attributed to the prevailing favorable temperatures (Fig. 1). A climate conditions corresponding to the optimal range of temperature for *A. spiraecola* population growth reported in Wang and Tsai (2000) study. The density of infested leaves always exceeded the intervention threshold which was established between 5 and 10% according to Barbagallo et al. (2007).

The impact of parasitoids on *A. spiraecola* populations was very limited (below 3%) without significant difference between the two years. This in agreement with Gómez-Marco (2016) finding reporting that in Spain this parameter did not exceed 5%.

In this study, *L. testaceipes* was detected in 2016 and 2017 surveys. As for its historical introduction to the Mediterranean basin, this parasitoid originated from Cuba was introduced in the south of France in 1973 for controlling citrus aphids (Starý et al. 1988). However, it was rarely able to achieve its development on *A. spiraecola* (Boukhris-Bouhachem 2011; Costa and Starý 1988; Michelena and Sanchis 1997; Tremblay and Barbagallo 1983) which could explain the high rate of mummies without adult emergence noted in the current study (Figs. 10 and 11). This failure may be due to a physiological parasitic inadequacy (Starý et al. 1988). Endocrine effects could arrest the development of many parasitized insects.
and prevent them reaching the adult stage (Beckage 1985). This low rate of *A. spiraecola* parasitism could probably be attributed to defensive symbiosis in aphid. In fact, as firstly demonstrated by Oliver et al. (2003), the pea aphid *Acyrthosiphon pisum* was found to harbor heritable infections with bacterial endosymbionts that are involved in their increased resistance to the parasitoid *Aphidius ervi* commonly employed as a biocontrol agent (Vorburger 2017). More recently, a study on intraspecific variation in facultative symbiont infection among native and exotic aphid populations showed the presence of the bacterium *Arsenophonus* sp. in native *A. spiraecola* infesting citrus and interestingly the main endosymbionts bacteria found in most aphid species were absent from *A. spiraecola* (Desneux et al. 2017).

As demonstrated in the current study, *B. angelicae* was not found in 2017. It was also absent in Tunisia (Sellami et al. 2013). Interestingly, in Spain, it was the only primary parasitoid of *A. spiraecola* (Gómez-Maro 2015). The variation of specific richness of primary parasitoid is not clear because of the complexity of the relationship between primary and secondary parasitoids. Other factors could be implicated. The hyperparasitism rate (25%) including 6 species is one of factors making ineffective the control by primary parasitoids. It remains also a problem in Spain with a hyperparasitism of 40% (Gómez-Maro 2015).

Our data are yet insufficient to reach any definitive conclusion concerning the dominance of *A. spiraecola* populations occurring in citrus crops; this study strongly suggested that multiple factors and processes are involved over the season, location and years. Citrus producers should have a good knowledge on IPM-based protection because their basic education is not sufficient to achieve higher efficiency in citrus production.

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RESUME

*Aphis spiraecola* est le puceron le plus dominant dans les vergers d’agrumes en Algérie. La présente étude a été réalisée en verger de clémentinier *Citrus clementina* dans la région nord-ouest d’Algérie, durant deux années (2016-2017), pendant la première poussée de sève. Les fluctuations des populations des pucerons et de leurs ennemis naturels en particulier, les parasitoïdes, ont été évaluées sur la base d’un échantillonnage hebdomadaire, effectué sur 100 feuilles prélevées sur 10 arbres à raison de 10 feuilles/arbre. *A. spiraecola* colonisé les arbres du clémentinier dès l’apparition des premières pousses. La densité moyenne maximale a atteint 78,8 ± 23,4 pucerons par feuille en 2016 et de 44,4 ± 13,0 pucerons par feuille en 2017, ce qui représente respectivement une densité d’environ 6,0 ± 1,5 pucerons et 4,4 ± 0,6 pucerons/cm² montrant une différence significative entre les deux années (*P* <0,05). Le taux de parasitisme exprimé en termes de nombre de momies d’*A. spiraecola* a été très faible, variant entre 1,6% en 2016 et 3,0% en 2017 mais aucune différence significative entre les deux années n’a été enregistrée (*P* >0,05). L’émergence des parasitoïdes primaires a été faible pour les deux années avec un pourcentage de 26,6% en 2016 et de 10,8% en 2017 ; cette différence n’est pas
تعطي للمن مقاومة ضد عدَّة أسئلة. كان معدل الموميَّات بدون بزوغ. تراوحت نسبة أشباه الطفيليات الثانوية بين 85 و 100%. لِللقاح الأولي ضعيفاً في كلتا السنتين بمعدل 4,3 ± 0.4% في 2016 و 4,4 ± 0.4% في 2017 والذي يمثل على التوالي 1,5 ± 0.4% و 1,6 ± 0.4% (p < 0.05) كما يظهر فرقاً معنويًّا بين السنين (p > 0.05).

تم العثور على أوراق الكليمتين من بداية خروج البراعم الصغرى. سجلنا معدل حوالي 78.8 ± 4.4% في 2016 و 44.4 ± 13,0% من ورقة في 2017 والذي كان معدل التطلَّف الذي عبر عنه من حيث عدد الموميَّات على نوعين من أشباه الطفيليَّات الأوليَّة A. spiraecola في 2017، ولا يوجد فرق بين السنين (p > 0.05). كان بزوغ أشباه الطفيليَّات الأوليَّة ضعيفاً في كلتا السنتين بمعدل 16.7% في 2016 و 25.7% في 2017، ولم يبين التحليل الاحصائي اختلاف بين السنين (p > 0.05). تم العثور في 2016 على Lysiphlebus testaceipes و L. spiraecola على نوعين من أشباه الطفيليَّات الثانويَّة L. testaceipes العيني الذي تم العثور عليه في 2017. تراوحت نسبة أشباه الطفيليَّات الثانوية بين A. spiraecola في 2016 و 25.7% في 2017، ولم يوجد اختلاف بين السنين (p > 0.05). كان معدل الموميَّات بدون بزوغ أشباه الطفيليَّات مرتفعاً جداً وتراوحت بين 85-100%. يطرح الفضل الجزيئي للتلف على A. spiraecola متعلقاً بالمناخ وتاريخ المن على تشكيل مجموعات مجنحة وربما وجود كائنات تكافل داخلي تعطي للمن مقاومة ضد مهاجميه.

كلمات مفتاحية: أشباه الطفيليَّات الأوليَّة، أشباه الطفيليَّات الثانويَّة، حماية متكاملة من الآفات، شمال غرب الجزائر، A. spiraecola، قوارض/حمضيات

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