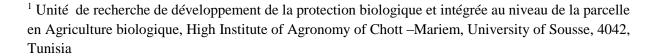


Apple aphid's species and their natural enemies in Tunisian orchards

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Abstract - Aphid apple species and its natural enemies were monitoring in 2013 and 2015 in two different orchards in order to study the species composition and seasonal abundance of aphids as well as to record the populations of their natural enemies. The aphid species *Aphis pomi* De Geer (Hemiptera: Aphididae), *Dysaphis plantaginea* (Passerini) (Hemiptera: Aphididae) and *Eriosoma lanigerum* (Hausmann) (Hemiptera: Eriosomatidae) were recorded. The most abundant aphid specie recorded was *A. pomi* and it was observed in both orchards at the beginning of March. *D. plantaginea* and *E. Lanigerum* were present at the end of March and April. A significant difference of level of population of the three aphids species in the two orchards was demonstrated (F=7.36; df=2; β =0.006; F=16.14, df=2, β =0.001). The natural enemies found were predators belonging to the families of Syrphidae, Coccinellidae, Cecidomyiidae, Anthocoridae, Miridae, Chrysopidae, Cantharidae and Forficulidae. Generally, their numbers were low.

Keywords: Apple, *Aphis pomi, Dysaphis plantaginea, Eriosoma lanigerum*, natural enemies.

1. Introduction

Apple trees can be infested with more than 15 aphid species (Blackman and Eastop 1984). Among them, the green apple aphid (Aphis pomi (de Geer)) (Hemiptera, Aphididae), the rosy apple aphid (Dysaphis plantaginea (Passerini)) (Hemiptera: Aphididae) and the wooly apple aphid (Eriosoma lanigerum (Hausmann)) (Hemiptera: Eriosomatidae) are considered to be serious pests of apple orchards worldwide (Niemczyk 1988, Minarro and Dapena 2001; Jerraya 2003; Ben Halima and Ben Hamouda 2005). A. pomi is a holocyclic and monoecious aphid species that is widespread in the northern hemisphere (Footti et al. 2006; Milenkovich et al. 2013). It colonizes the young shoots causing leaf curling and producing honeydew which results in fruits discoloration making them unmarketable (Blommers 1994). It causes significant losses in yield if not suppressed (Hagley 1989). The species is especially harmful in nurseries and young orchards and it characteristically re-infests apple trees over the May-June period (van Emden and Harrington 2007). D. plantaginea is the major pest of apple in North America and Europe (Hemptinne et al. 1994). It spends the winter as eggs on apple, the primary host. Egg hatch coincides with apple bud break in early spring (Hull and Starner 1983). The first generation or two are exposed on apple buds and expanding leaves, but by the time trees bloom, the leaves begin to curl, thus providing protection to the remaining generations. There are five to seven generations on apple in the spring and early summer (Brown and Mathews 2007), with migration to the secondary host, Plantago spp., especially P. lanceolata L. (Blommers 1999), occurring from mid-May through June. The aphids return to apple in late summer to early fall, where there is a sexual generation that produces oviparous female. It causes malformation of fruits even at low populations (Blommers 1994), longitudinal leaf rolling and formation of red leaf-galls (Forret and Dixon 1975). Finally, E. lanigerum is an important insect that infests apple orchards worldwide (Ateyyat and Al-Antary 2009), and is considered to be critical to the economics of the apple industry (Bus et al. 2007). It infests both the shoot and root parts of the apple tree (Lloyd 1961). Its infestation



reduces vegetative growth and hence production capacity (Brown and Schmitt 1990; Brown et al. 1995). The protection of the orchards is currently only assured by one preventive and intensive chemical fight. However, the misuse of the pesticides by the farmers in particular with the choice of the medicinal agro products, the active matters, the amounts applied, the frequency of the treatments, the equipment of treatment etc, caused diseases on this culture, the destruction of auxiliary fauna as well as the pollution of the environment. Thus, alternative strategies are needed. The growing of resistant cultivars probably provides the best long term solution. Biological control by natural enemies, either by weed strips to enhance the number of aphidophagous predators (Wyss 1995; Wyss et al. 1995), or augmentative releases of indigenous natural enemies (Wyss et al. 1999), may be other powerful tools. However, despite the traditional growing of local apple trees, the community of apple aphids and their natural's enemies has not been still studied in Tunisia. Studies on species composition and abundance of aphids and their natural enemies are required for the development of an integrated management program in apple orchards. Under this concept the aim of the present work was to study the species composition and seasonal abundance of aphids as well as to record the populations of their natural enemies.

2. Material and methods

2.1. Study orchards

Trials were carried out in 2013 and 2015 in two commercial apple orchards. The first orchard of 5 ha at governorate of Ben Arous (36°44′50″ N; 10°20′00″E), consisted of 8-years old apple trees (320 trees and having 4 x 2.5 m row distance) of two different apple cultivars (Aziza and Chahla). The second orchard of 1.6 ha at governorate of Sousse (35°49′33.6″N; 10°38′24″ E), consisted of 260 trees set up in 2009 and having 4 x 2.5 m row distance and two of cultivars (Anna and Lorka). Neither insecticides, acaricides nor fungicides were sprayed in these two orchards in order to allow the presence of both pests and predators.

2.2. Sampling methods

In order to register the presence of aphids, all the trees were assessed weekly during the spring aphid occurrence by visuals controls. Therefore, for aphids' species identification and abundance and in order to minimize the losses of the farmer, 4 rows of 20 trees in each orchard were selected for sampling. The temporal abundance of aphids was studied weekly by taking samples from 7 selected trees using random number. The sampling unit was the upper part of the growing shoot, bearing more than six leaves offers an adequate estimation of the population level of aphids on the tree, since according to Hull and Grimm (1983) 90 or more than 90% of the total aphid population collected from the top on the lower part of the tree, respectively, develops on those leaves. From each tree, 8 shoots (2 from each side: north, south, east, west) were collected. Samples were collected weekly and each shoot was put separately in a polyethylene bug and brought to the laboratory for species identification and for counting. Arthropod predators were also assessed weekly during the spring aphid occurrence by visual controls. Some larvae were reared on aphids' colonies in laboratory in order to determine the species. Mummies founded were also brought to laboratory and put in Petri Dish until parasitoid emergence and determinate species.

2.3. Aphid's and natural enemies' identification

Adult viviparous of aphids collected from apple trees host were cleared and individually mounted on microscope slides using the techniques described by Blakman and Eastop (1984) and were identified with reference to Blackman and Eastop (1984; 2000 and 2006) keys. Hoverflies adults were identified using key of Le Monnier and Livory (2003). Similarly, adults of coccinellids were identified with reference to Chandler (1969) keys.

2.4. Statistical analysis

For the laboratory assays, the experimental results were statistically analyzed using SPSS 17 program, a one way analysis of variance and a S-N-K test, with statistical significance set at β =0.05. The level of population of each aphid in both orchards was compared.



3. Results and discussion

3.1. Aphid's species

Aphid's surveys show the presence of three species on apple trees in the two studied orchards. The most abundant aphid specie recorded was *Aphis pomi* De Geer (Hemiptera: Aphididae) (Figure 1 A and B). It was observed in both orchards at the beginning of March. During period surveys, a high population of *A. pomi* was registered in the second orchard (1585.54±1382.18) in comparison to that in the first orchard (201.17±24.03). *Dysaphis plantaginea* Passerini (Figure 1 C and D), the second abundant aphid was observed at mid March in Sousse (second orchard), therefore, at mid April in Ben Arous (First orchard). A population of *D. plantaginea* in the first orchard is higher than that in the second orchard. The level of population was 857.33 ± 234 and 33.41 ± 24.20 in the first and the second orchard respectively. The third identified aphid specie was *Eriosoma lanigerum* Hausmann (Figure 1 E and F). It was observed at mid May on trunks of apple trees in the first orchard. Therefore, it was seen in shoots and branches at the end of March and disappears in two week in second orchard. A comparison of level of population of the three aphids species in the two orchards demonstrate a significant difference in both orchards (F=7.36; df=2; β =0.006; F=16.14, df=2, β =0.001) (Figure 2).

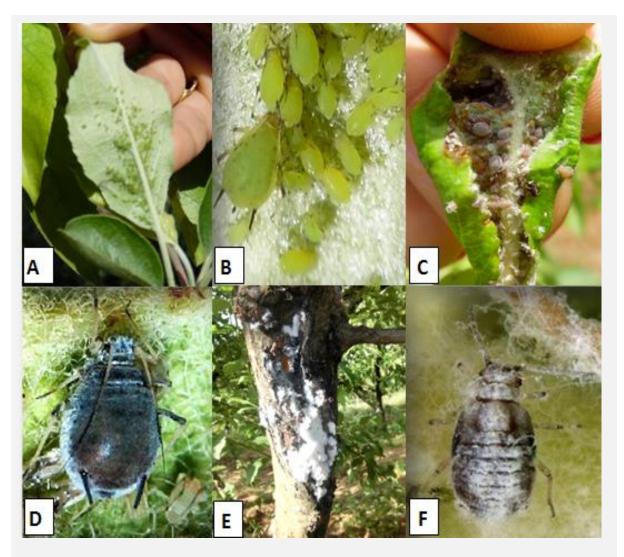


Figure 1. Aphid's species recorded in apple orchards in Tunisia. A: Aphis pomi colony, B: Aphis pomi adult, C: Dysaphis plantaginea colony, D: Dysaphis plantaginea adult, E: Eriosoma lanigerum on apple stem, F: Eriosoma lanigerum adult

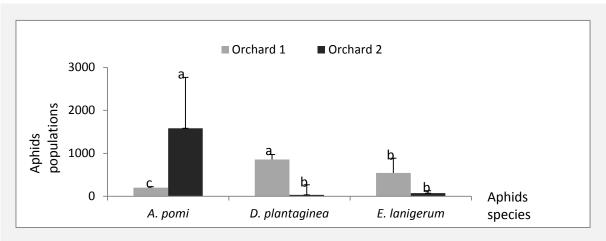


Figure 2. Aphids species populations in two apple orchards (Different letters at the tops of the columns indicate significant differences between treatments based on a S-N-K test and $\beta = 0.05$)

3.2. Natural enemies

The three most abundant predator families in both orchards were Syrphidae, Coccinellidae and Cecidomyiidae (Figure 3) in both apple orchards. Therefore, Anthocoridae and Miridae (Heteroptera), Chrysopidae (Neuroptera), Cantharidae (Coleoptera) and Forficulidae (Dermaptera) were observed preying on aphid's apples colonies only in the first orchard in Ben Arous. The most abundant observed ladybird was Coccinella algerica Kovar (Figure 3 A), although Hippodamia variegata Goeze larvae and adult (Figure 3 B and C) were also recorded. Larvae and adults of these two coccinellids were observed on A. pomi colonies at the beginning of April in the second orchard. Therefore, only C. algerica (larvae and adults) were detected in the first orchard at the end of April. Episyrphus balteatus De Geer and Metasyrphus carollae Fabricius were the two identified syrphids. Eggs and larvae (Figure 3 D, E and F) of these predators were occurred in A. pomi and D. plantaginea larvae colonies at the end of April and in the beginning of May in both orchards. The cecidomyiid fly Aphidoletes aphidimyza was identified from larvae (Figure 3 G) collected from both orchards. It was the second most abundant predator observed for the first time in Mid May in the first orchard in Ben Arous and in Mid April in the second orchard. Anthocorids, Mirids, Chrysopids, Cantharids and Forficulids occurrence was only in the first orchard on D. plantaginea colonies. They were not seen feeding on A. pomi. Concerning parasitoids, Lysiphlebus testaceipes Cresson (Hymenoptera: Aphidiidae)) (Figure 3 I) was identified from the mummies of A. pomi (Figure 3 H). The mummies of A. pomi were detected for the first time at the beginning of May in the second orchard. A mean of 29.24±15.66 of mummies was recorded each surveys from the date of observation.

In the present study, the aphid species A. pomi, D. plantaginea and E. lanigerum were found to colonize apple trees in Tunisian orchards. A. pomi was recorded in both orchards but in much higher population densities in the second orchard than D. plantaginea whilst E. lanigerum was present in both orchards in very low number. The presence of these three aphids in Tunisian apple orchards was reported in several works. Indeed, Jerraya (2003) and Ben Halima kamel and Ben Hamouda (2005) show the presence of A. pomi, D. plantaginea and E. lanigerum. Nevertheless, Ben Halima Kamel and Ben Hamouda (2005) show the presence of three other aphid species on apple trees in Tunisia (Aphis citricola Van Der Goot, Allocotaphis quaestionis Börner and Aphis gossypii Glover) but in very low number. In Greece, Perdikis et al. (2008) proved that A. pomi, D. plantaginea and E. lanigerum were recorded colonize apple trees. Similar in the present study, Perdikis et al. (2008) proved that A. pomi was recorded in much higher population densities than D. plantaginea and E. lanigerum. As far as, Laamari et al. (2010) in Algeria reported the presence of these three species in Algerian apple orchards. As for date of appearance, our results revealed that A. pomi appeared early in March while D. plantaginea and E. lanigerum appeared later at the end of March and at the beginning of April. This is in contrast with Perdikis et al. (2008) study. Authors show that D. plantaginea and E. lanigerum appeared earlier in May, while A. pomi appeared later (after Mid-May) and it was present in the samples till the end of September. In another study (Brown 1991) the population of D. plantaginea became very low at the end of June in Nova Scotia. D. plantaginea occurred only in May, in an apple orchard in West Virginia, whilst Aphis spp. population densities were higher in June-July (Kozar et al. 1994). Our results demonstrate also that natural enemies found were predators belonging to a wide range of taxa (Syrphidae, Coccinellidae and Cecidomyiidae, Anthocoridae, Miridae, Chrysopidae, Cantharidae and Forficulidae) and a single species of parasitoids (L. testaceipes). Several species of these predators were reported on apple aphid population in several regions (Carroll and Hoyt 1984; Wyss et al. 1995; Wyss et al. 1999). Indeed, Gontijol et al. (2012) show that, on wooly apple aphid E. lanigerum colony in Washington State, the most common predators encountered were Syrphidae, Chrysopidae and Coccinellidae. In the same context, several works show that the major predators of Aphis spp. on apple have been reported to be Aphidoletes aphidimyza (Rondani) (Diptera: Cecidomyiidae) (Brown and Lightner 1997), chrysopids (Brown and Lightner 1997), and mirids (Hagley and Allen 1990). Bouchard et al. (1986) referred 60 predators and parasitoids active against A. pomi while in California apple orchards, the main predator was the coccinellid Coccinella novemnotata Herbst (Oatman and Legner 1961) and in Quebec the midge Aphidoletes aphidimyza (Rondani) (Stewart and Walde 1997). In central Washington 39 predators and 2 parasitoids were recorded (Carrol and Hoyt 1984). In Ohio apple orchards, syrphids, hemerobiids, coccinellids and in low numbers the anthocorid *Orius insidiosus* (Say) (Holdsworth 1970). Concerning efficiency of these predators against aphids on apple, most of them are generalist and efficiency was in relationship with climatic conditions and aphids abundance (Wyss et al. 1999). Concerning parasitoids, in the course of the current study, L. testaceipes was the sole parasitoids recorded parasitize A. pomi. Cockfield et al. (2012) show that this parasitoid was the most common primary parasitoid of D. plantaginea. Also, Praon unicum Smith and Aphelinus mali Haldeman have been reported on apple aphid colonies (Cockfield et al. 2012; Peusens et al. 2006). Almatni et al. (2002) show that Aphelinus mali Haldeman was observed with a high effectiveness at late summer and at the growing season on colonies of the wooly apple aphid E. lanigerum in Syria.



Figure 3. Natural enemies recorded on aphids colonies in apple orchards. A: *Coccinella algerica*, B: *Hippodamia* variegata larvae, C: *Hippodamia* variegata adult, D: syrphids larvae, E: *Episyrphus balteatus*, F: *Metasyrphus carollae*, G: *Aphidoletes aphidimyza* larvae, H: mummie of *A. pomi*, I: *Lysiphlebus testaceipes*



4. Conclusion

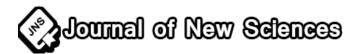
The preliminary observations of aphid species and their natural enemies in apple orchards in Tunisia featured three aphid species and some efficient auxiliaries. However, further studies are needed to clarify their respective roles in the aphid population control. Relationships between some of them and the following weeds occurring in the apple orchard should be investigated to be possibly managed. Besides, mass rearing and release of parasitoids like *L. testaceipes* might be tested.

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