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Effect of pruning severity on the vegetative growth, density and population dynamics of the Spirea aphid, *Aphis spiraecola* in Citrus orchard

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Abstract
In this study, a one-year field experiment was conducted to evaluate the effect of winter tree pruning severity on the abundance, population dynamics and shoot infestation as well as vegetative growth of two citrus cultivars Clementine, *C. clementina* Hort. Ex Tan. and orange, *Citrus sinensis* cv Thomson by the Spirea aphid, *Aphis spiraecola* Patch (Hemiptera, Aphididae) and its natural enemies. Data were collected during the spring in 2015 on 6-years old Citrus trees submitted to 3 different intensities pruning (severe, medium or control and light pruning) following a completely randomized design with five replications in which one single tree-plot was considered as a replicate. Results showed that, for both Citrus cultivars, pruning enhanced the sprouting of newly shoots, the shoot elongation and the number of leaves per shoot especially for Clementine cultivar. The average number of newly growing shoots per tree was maximum in heavy pruned trees (5593±315 shoots per tree) and minimum in lightly pruned trees (2656±855shoots per tree). There was a positive relationship between the number of new shoots per experimental tree and *A. spiraecola* infestation. The trees severely pruned were more infested by larvae, wingless and winged aphids even though this difference may be shifted when the population densities increased exponentially. The densities of aphid natural enemies show mixed results for *C. clementina* but were similar for *C. sinensis*.

Keywords: *Aphis spiraecola*, *Citrus* vegetative growth, pruning severity, natural enemies, Tunisia

1. Introduction

Citrus is the most important fruit tree crop in the world, with a production of more than 1211 million tons in 2014) [1]. In Tunisia, the yearly citrus production exceeds 393000 tons in 2014 [2].
Aphids, the largest group of plant phloem feeders, are plant sucking insects which occur throughout the world [3]. The greatest numbers of species are located in the temperate regions [4]. These insects are known to cause direct removal of plant nutrients and the transmission of viruses reducing plant growth and cause extensive loss of crop productivity [5,6];Aphids also damage new growth by inducing top-roll symptoms and chlorosis. It has been suggested that photosynthesis is inhibited in leaves showing such symptoms [7].
In Tunisia, the inventory of Citrus aphid species as well natural enemies were undertaken by Ben Halima *et al*. [8] Boukhris-Bouhachem [9],Tena and Garcia-Mari [10], in which four main species were considered as primary citrus pests because of their direct or indirect damage; the Spirea Aphid, *Aphis spiraecola* Patch, the Cotton Aphid, *Aphis gossypii* Glover, the Black Citrus Aphid, *Toxoptera aurantii* Boyer de Fonscolombe and the Peach Aphid, *Myzus persicae* Sulzer.
The spirea aphid, *Aphis spiraecola* Patch (Hemiptera: Aphididae) is a key pest of Clementine, *Citrus clementina* Hort. ex Tan. (Geraniales: Rutaceae), in the Mediterranean basin [10-15]. This polyphagous aphid colonizes young, tender Clementine shoots in spring and causes economic losses because it sucks sap, serves as a vector for Citrus Tristeza virus, excretes large amounts of honeydew and curls developing leaves while the colony population is growing [11]
In Tunisia, the species was reported by Boukhris*et al*. [16]. and Ben Halima *et al*. [8] and considered as a polyphagous insect with numerous host plants of over 20 families, especially, Rutaceae, Caprifoliaceae, Compositae, Rosaceae and Rubiaceae [17], But there is no published data regarding the biology and ecology of the insect.
In commercial *Citrus*, growers rely primarily on the use of synthetic chemicals to control Aphids. Tunisian plant protection service recommends the control of *Citrus* aphids to reduce fruit yield loss. Usually 2-3 insecticide sprays were yearly applied particularly in *C. clementina* and *C. sinensis* cv Thomson orchards. Usually these methods are effective when

correctly applied [18,19], but the large scale usage of the chemical pesticides has resulted in several associated adverse effects such as insecticide residues, environmental pollution [20], insect resistance [21, 22, 23] and emergence of secondary pests [24]. There is a need for more reliable, permanent management tactics against *Citrus* aphids. One tactic which has several advantages is cultural practices which include pruning method.

Winter pruning is a training practice used in all fruit orchards to shape fruit trees or to partially adjust crop load. It is generally seen as a mean of controlling tree vegetative growth and vigor [25]. There has been relatively little work in this area; no experimental results have been reported dealing with the effects of pruning intensity applied to the Citrus tree on aphid infestation. Indeed, there are orchard practices that can be used by growers to reduce aphid infestation making the tree to be less attractive for insect growth and reproduction or make the tree less attractive [26]. Pruning practices in *Citrus* orchards are important to support plant health and reduce stress in order to reach an acceptable balance between vegetative and reproductive activities, a key factor in many stages of citrus grove development [27]. The purposes of this article were to compare the Citrus shoot elongation, the population densities of the Spirea aphid, *A. spiraecola* and its natural enemies on young shoots after severe, intermediate (control) and light pruning.

2. Materials and methods

2.1. General

The study was carried out during the Citrus spring flushing period in 2015 at the experimental *Citrus* Orchard of the Research Center of Horticulture and Organic Agriculture located in Sousse region in the Center East of Tunisia (35°51'32"N-10°35'38"E). The climate is Mediterranean with mild winter and hot dry summer. The yearly precipitations vary from 270 to 320 mm and are mainly occurring in January- April and September-December. The annual average temperature ranges from 16 to 19 °C, with a maximum recorded in July and a minimum recorded in January (Fig. 2). The soil texture is silty –clay. The study orchard of about 0.35 ha was planted in 2009 and composed of 83Clementine trees (*Citrus clementina* Hort. ex Tan, cv Kassar), 36 trees of orange, *Citrus sinensis* (L.) Osbeck cv Thomson and 29 trees of sweet orange, *Citrus sinensis* (L.) Osbeck. All were grafted on sour orange, *Citrus aurantium* rootstock. The orchard was faced east- west with 7 rows of 22 trees each and surrounded by cypress hedgerow (Fig. 1). The planting distances were 6 m between rows and 4 m between trees. The height of Clementine tree varied between 2.5 and 3 m and perimeter from 4 to 5 meters whereas the height of Thomson varied from 2 to 2.5 meters and perimeter from 5 to 6 meters.

Trees were irrigated with two micro spray jets per tree giving 2 liters per hour. The irrigation period occurred seven to eight months per year usually between March and October and lasted from one to two hours every three days except in rainfall days. The herbaceous plants were mechanically mowed 2-3 times per year between rows and 2-3 times manually per year beneath trees to reduce water loss. The orchard had received no pesticide sprays or mineral fertilization since its establishment. However, each year, usually on late-December early-January, 15 to 20 kg of cattle manure was incorporated into the soil beneath each tree.

2.2. Pruning severity

Experiments were carried out by following a Completely Randomized Design with five replications in which one single tree was considered as a replicate. The trees were randomly assigned along the plot (Fig. 1) and were manually pruned on 21 January 2015 using wood saw and garden shears. In light pruning practice, thinning out only twigs aged for up to one-year old wood (about 10% wood, 2.68±0.68 kg). Under medium pruning, twigs of one-year old were thinned plus some undesirable branches (from 10 to 20% of tree volume; 2.68±0.68 kg). Heavy pruning consisted of thinning out one-year old wood and 2 to 3 main lateral branches (from 20 to 35% of tree volume; 6.5±0.84 kg of wood was cut). Pruning treatment was randomly assigned in 5 different trees of each cultivar (15 of Thomson variety and 15 of Clementine variety, Fig. 1). Medium pruning was considered as control as growers do each year in the region.

2.3. Sampling and monitoring

To evaluate the population dynamic of *A. spiraecola*, it was sampled weekly 10 shoots at random from trees beginning in February until no more aphid were observed on the trees (around mi-May).

The effect of pruning intensity on shoot elongation and aphid infestation were evaluated as follow:

- The number of newly formed shoots was counted once at the end of flushing period (from April 20th to April 24th) in one quarter of each experimental tree taking a different quarter of the tree at each time. A newly produced shoot was defined as any shoot with a variable number of immature and expanded leaves to fully elongated shoots with expanded, tender leaves [28].
- Five newly produced shoots (one from each cardinal point and 1 from the tree center) were sampled per tree at two –week interval from the beginning of April 2015 until no more aphid were observed on the trees (around mid- May 2015)and transported to the laboratory. The numbers of aphids (larvae, apterous morphs and winged adults) as well as natural enemies were counted.
- -Five newly produced shoots per tree were randomly selected and tagged from all over the crown on 4 March 2015 (at the beginning of the infestation) and observed on 8 April 2015 aiming to measure the shoot elongation and the number of leaves per shoot.
- Fruit production was estimated by weighing and counting all Clementine and Thomson from each experimental tree during the normal harvest in the grove (November 2015). We identify aphid species according to the key provided by Blackman and Eastop [29] and confirmed by Professor Nafria (University of Léon, Spain).Natural enemies were characterized according to ACTA [30] and Navarro-Campos and García-Marí [31].

2.4. Statistical analysis

The number of newly produced shoots, the citrus twig length, the densities of aphids and the yield were analyzed using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test (P<0.05). All statistical analyses were performed using SPSS Statistics version 20 [32]. Before analysis, Kolmogorov-Smirnov test was applied in order to assess data normality. When necessary, before analysis, data were transformed to log(x+1) to meet the assumption of ANOVA.

3. Results

During the study period, the daily temperature averaged was 17.67±5.44 °C (Fig. 2). For both *Citrus* species, two aphid species were identified; the Spirea aphid, *Aphis* *spiraecola* Patch and the cotton aphid, *Aphis gossypii*. The Spirea aphid was the most abundant and occurring in almost all samples (about 85%). *A. gossypii* was found in some flushes but usually in mixed colonies with *A. spiraecola*. Hence, only the Spirea aphid was considered in this study.

3.1. Number of newly produced shoots per tree

3.1.1. *C. clementina* cultivar

There was a significant difference between pruning intensities regarding the total number of newly produced shoots per experimental tree ($F_{2,\ 12}$=10.66, P = 0.002). The maximum number was found in heavily pruned trees (5593±315shoots per tree) and the minimum in light pruning (2656±855 per tree). On the other hand, medium pruned trees (control) produced on average 3889±1492 newly shoots (Fig.3a).

3.1.2. *C. sinensis* Thomson cultivar

There was no significant difference between the pruning techniques regarding the total number of newly produced shoots per experimental tree ($F_{2,\ 12}$=1.76; P=0.21). However, heavy pruned trees produced more flushes(Fig. 3b).

There was a positive relationship between the number of new shoots per experimental tree and aphid infestation for both cultivars (*P*<0.00001 for *C. clementina* and P=0.04 for *C. sinensis*, Fig. 4a and 4b). Overall, *C. clementina* trees produced more new shoots than *C. sinensis* cv Thomson trees (on average 4046±1557 versus 1777±844; T Test=9.45 df=29; P=0.000).

3.2. Measurement of terminal shoots length (twig length elongation)

3.2.1. *C. clementina*

On the date of 4th March, 6 weeks following the pruning, the average twig length varied from 1.2 cm to 2.4 cm according to pruning intensity with a significant difference between treatments (ANOVA one factor :$F_{2,352}$ = 26.3; P= 0.001). This difference remains on 8 April 2015 ($F_{2,352}$ = 44.6 ; P= 0.001). So, on average, heavy pruned trees have more elongated twigs. The average number of leaves per shoot varied among pruning intensities (on4 March 2015 ($F_{2,352}$ = 20.1; P= 0.001), on 8 April 2015 ($F_{2,352}$ = 25.2 P= 0.001) with a maximum on Heavy pruned trees (Table 1). Concerning the aphid densities per leaf, there were significant differences between pruning types (on4 March 2015; $F_{2,352}$ = 2.9; P= 0.04 and on 8 April 2015 ($F_{2,352}$ = 5.4 P= 0.005) (Table 1).

3.2.2. *C. sinensis*

The twig length in *C. sinensis* Thomson varies significantly only on 4 March 2015; ($F_{2,352}$ = 8.8; P= 0.001). The number of leaves per shoot did not vary for the two sampling dates On4 March 2015 ($F_{2,352}$ = 0.4; P= 0.6), on 8 April 2015 ($F_{2,352}$ = 0.2 P= 0.7). However the average number of aphid per leaf show mixed results with significant differences among treatments; on 4 March 2015 ($F_{2,352}$ = 0.2; P= 0.7), on 8 April 2015 ($F_{2,352}$ = 3 P= 0.02) (Table 1).

3.3. Aphid infestation

3.3.1. *C. clementina*

Regarding the sampling of shoots, the number of aphid (larvae and apterous adults) per twig varies only for the second date of sampling (on 23 April 2017) with maximum densities for heavy pruned trees indicating the maximum aphid infestation (First sample ($F_{2,338}$= 1.48, P= 0.22), second sampling $F_{2,283}$= 17.58, P= 0.009; third sampling ($F_{2,317}$= 2.23, P=0.10) (Table 2). The number of winged adults is significantly more important in severe pruned trees than light or medium pruned trees (On 9 April 2015 ($F_{2,338}$= 5.4, P= 0.005) on 23 April 2015 : $F_{2,283}$=7.6, P=0.001), However in May (third sampling), there is no significant difference between pruned types (Table 2).

3.3.2.*C. sinensis*

The number of larvae and apterous adults per twig varied significantly among pruning types on 23 April and 7 May 2015 where severely pruned trees were more infested ($F_{2,338}$= 5.4, P=0.005, Table 3). However, the number of winged adults per twig was no significantly different among pruning intensities ($F_{2,338}$= 1.48, P= 0.22, Table 3)

3.4. Natural enemies

For Clementine, the number of natural enemies per shoot according to pruning severities shows mixed results (e.g., there was a significant difference for aphid mummies and empty mummies (on 9 April and 23 April ($F_{2,317}$=9.11, P= 0.001) and no significant difference for predators for all sampling dates (Table 4). Regarding Thomson, there were no significant differences between pruning severities ($F_{2,317}$=1.11, P= 0.32, Table 5). The low densities of natural enemies per shoots may hide such difference.

3.5. Yield

There was no significant difference regarding the fruit weight and number per tree for the two Citrus cultivars (Clementine fruit weight $F_{2,12}$= 0.43; P= 0.66 number of fruits per tree $F_{2,12}$= 0.42; P= 0.6) and Thomson (fruit weight $F_{2,12}$ = 0.40: P= 0.67 ; Number of fruits $F_{2,12}$= 0.9 P= 0.42, Table 6).

4. Discussion

The present study showed that severe winter pruning enhanced the abundance of *A. spiraecola* in Citrus orchards. Few studies have examined the influence of tree pruning intensities on aphid infestation and activities [33, 34, 35, 36]. However, as far as we know, there are no references in the literature to the response of *Aphis spiraecola* to Citrus pruning severity and to the use of winter pruning as a possible alternative technique of aphid management. In this study, full spring period flushing extended from March to April 2015. In Tunisia, *Citrus* trees except lemon, *Citrus limon* produce three main flushes of new growth each year (1) spring flushes period which lasted from late February to April-May. During this period, we observe leaf development, flowering, fruit set and the beginning of fruit growth (2) summer flush occurred in June –July with high temperatures and precipitation nearly absent. For Clementine and Thomson trees, this period is characterized by a fast growth of fruits, which reach their optimal size and (3) autumn flush from August to November. There may be little production of new leaves between these major flushing periods especially after significant heavy rainfall period. The spring flush was also the most abundant flush of the year [3, 38].

Cooper *et al*. [39], studied Citrus flushing pattern in 7 locations in the USA and indicated that Buds began to expand and elongate at Tempe, Riverside, and Santa Paula on February 14, March 1, and February 24, respectively, as compared with February 14 at Orlando and Weslaco. However, the duration of extension growth and of flowering was greatly accelerated or slowed by climatic conditions.

The number of newly produced twigs per tree is relatively high (especially for heavy pruned trees) compared with analog studies conducted mainly in Spain. Indeed, working with 10- to 20-year-old Navelina sweet oranges and Clemenules Clementine mandarins, Garcia-Marı *et al.* [40] reported from 1728 to 3822 new shoots per tree per year in Spain during March through November with 51% to 96% of the flush shoots generated during the spring. This difference may be due to the rootstock, the age of trees as suggested by Knapp *et al.* [41] who indicates that seasonal flushing patterns may differ among regions, varieties, plant age and from year to year. Total leaf area per flush shoot varies from flush to flush in Florida *Citrus* with more flush shoots occurring in trees during the spring than summer but with larger leaves, greater numbers of leaves per shoot, and more leaf area produced during the summer [41]. Indeed, Cooper *et al.* [39] reported data indicating that 76% to 87% of the annual new flush shoots were produced during February and March by mature Valencia orange trees in Florida.

It was found positive correlations between the number of new produced flushes and aphid infestation for both Citrus cultivars (Fig. 4). On the contrary, Muñoz *et al*. [42] found no significant correlation between the spring flushing patterns of Clementine and *A. spiraecola* infestation. Indeed in their study, the authors evaluated the effects of six rootstocks on the Incidence of the Citrus leafminer, *Phyllocnistis citrella* and two aphid species *A. spiraecola* and *A. gossypii*. It is possible that the interaction between the three insects for the same host may influence population dynamics and resource allocation. In addition, the difference in rootstocks used may influence the flushing patterns and hence the aphid infestation; the rootstock used in our study was *C. aurantium*. In fact, it was sampled newly produced twigs where found aphid larvae, adults on the 3 to 5 apical leaves at the most. There is no aphid on basal leaves.

It is well known that Aphids prefer young leaves and stems for nutrition and reproduction for several reasons among them total water content. Slosser *et al*. [43] reported that total leaf water content of cotton was positively correlated with higher *Aphis gossypii* numbers. However, it is unclear if water content is the cause for greater aphid numbers.

It was found that the populations of *A. spiraecola* recorded on Clementine and Thomson tree leaves were abundant during spring season. Indeed, each developmental stage (larvae, wingless and winged adults) occurred at the same time. The most important peak was observed during April early-May (Tables 2and 3), just before the end of flushing period and the occurrence of dry season. The period of maximum aphid infestation was comparable for Clementine trees infested by *Aphis spiraecola* and *A. gossypii* in Algeria [14].

The densities of *A. spiraecola* larvae, apterous adults and to a lesser degree winged morphs show significant difference between pruning intensities for both cultivars especially during maximum infestation (samplings of 23 April and 7 May 2017, Fig 5) where heavy pruned trees were more colonized (Table 2and 3). Aphids realize their great reproductive potential through parthenogenesis and viviparity, synchronize their life cycle to the growth and phenology of host plant and optimize the exploitation of plants food resources which change in both time and space [4]. It is likely that the omnipresence of newly produced shoots which is largely available in heavy pruned trees favors aphid infestation.

The study shows that heavy pruning applied to *C. clementina* and *C. sinensis* Thomson trees increased the abundance of the aphid *A. spiraecola*. It is likely that the enhancement of vegetative growth of shoots and leaves help foraging female to locate suitable host. Growing shoots and leaves are a rich source of food for aphids, which exploit the nitrogen content in these structures. For both Citrus cultivars, the period of growth is relatively short (from1 to 2 months), it is therefore critical for *A. spiraecola* to synchronize its development with its host.

Pruning has been studied primarily in relation to its effect on fruit quality and yield [44]. Severe winter pruning in orchards may be a tool for aphid management by reducing the amount of hibernating eggs [45]. On the contrary, our study demonstrates that the degree of aphid infestation increased with the severity of pruning. Indeed, Grechi *et al*. [35], found similar results for the Peach aphid, *Myzus persicae* infesting peach tree.

It was investigated the densities of aphid natural enemies (parasitoids and predators) in which no evidence was detected regarding the pruning intensities (Tables 4and 5). On the contrary Weisser [46] found that plant architecture influences the time-budget of parasitoids which use leaves adjacent to aphid colonies for attacking aphids.

Despite the damage of *A. spiraecola* on leaves for the two cultivars and independently of the pruning severity, fruits weight and fruit numbers per experimental tree were not reduced. Indeed, we do not evaluate the loss of fruits due to aphid infestation, but expected that heavy pruned trees produce less yields due to the stimulation of excessive vegetative growth at the expense of fruit production. This may be due because of naturally flower drop occurred usually in May.

5. Conclusion

This study highlighted the importance of pruning on the population of the key aphid species of Citrus, *A. spiraecola* and its natural enemies. Severe winter pruning enhanced the vegetative growth of new shoots and increased the densities of larvae, apterous adults and winged aphids. However, it is likely that the activity of natural enemies and the weight and number of fruits per tree were not influenced by pruning intensities.

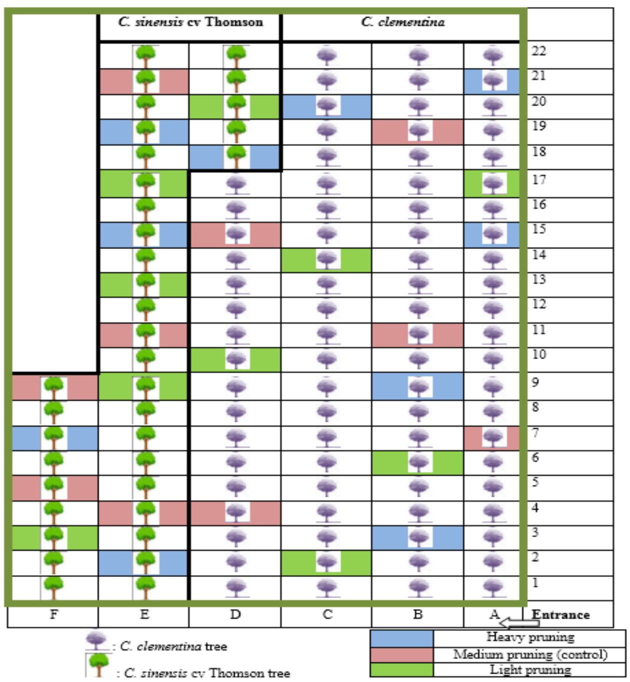


Fig 1: Lay out of pruning experiment in *Citrus* orchard in 2015

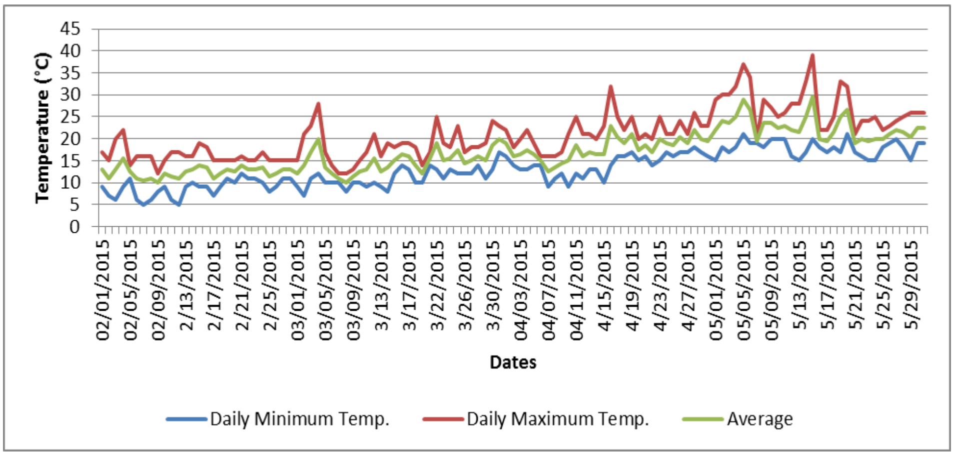


Fig 2: Maximum, minimum and average daily temperatures during the study period (January- June) for the year 2015 starting from the 1st of January.

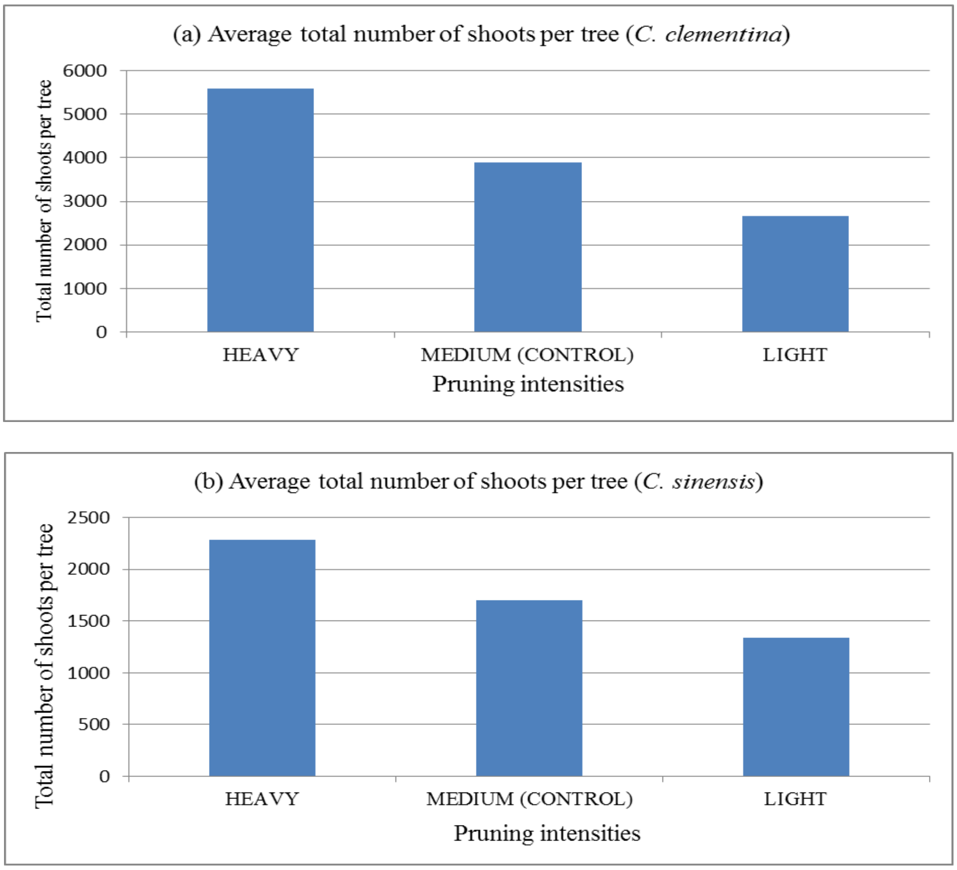
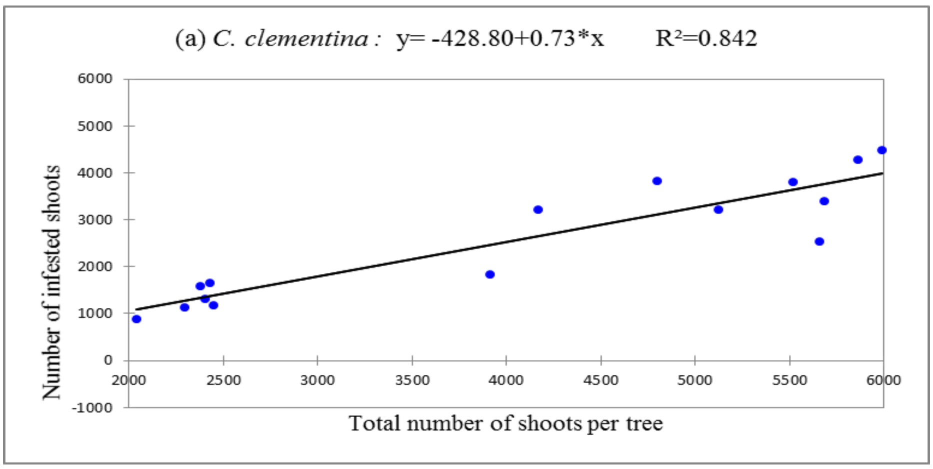


Fig 3: Total number of newly produced shoots per tree according to Pruning intensity in 2015 [(a): *C. clementina;* (b): *C. sinensis*]



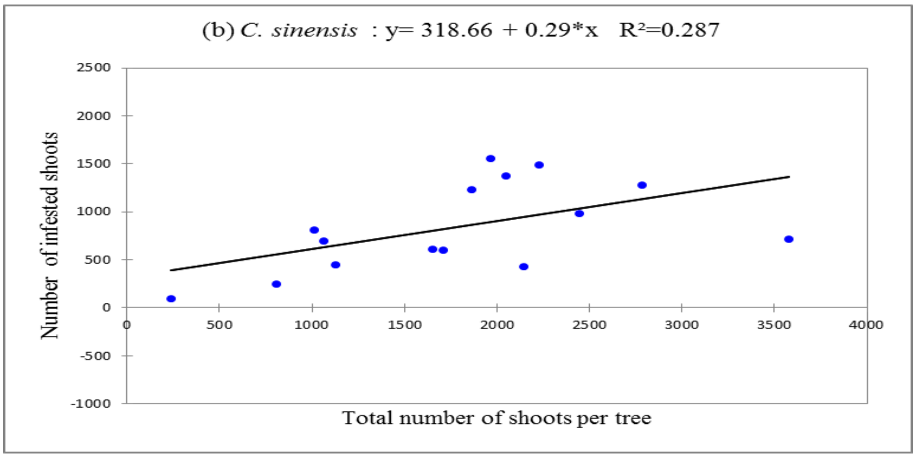


Fig 4: Relationship between the total number of newly produced shoots per tree and aphid infestation (a) *C. clementina* (b) *C. sinensis*

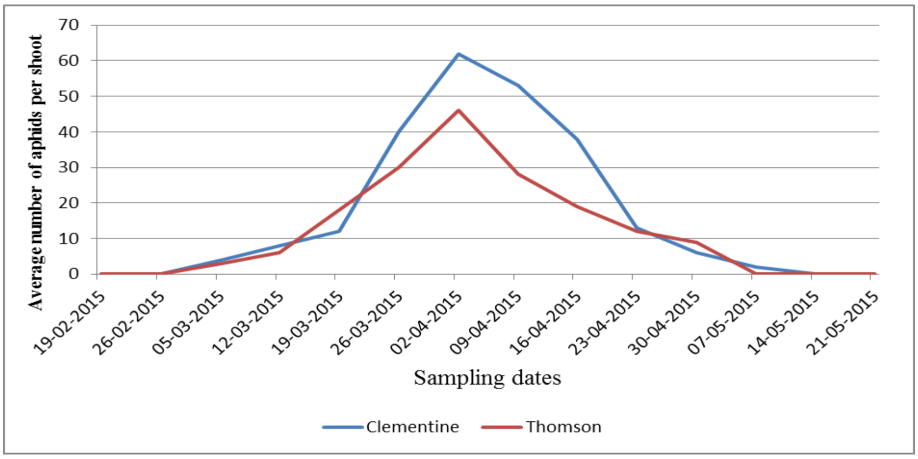


Fig 5: Population dynamic of *A. spiraecola* in Citrus orchard in 2015

Table 1: Measurements of twig length, number of leaves and aphid density on shoots

	C. clementina						
	Twig length$^{(*)}$	**Twig length$^{(\#)}$**	**Perc.$^{(@)}$**	**No.of leaves$^{(*1)}$**	**No. of leaves$^{(\#)}$**	**Aphids$^{(*)}$**	**Aphids$^{(\#)}$**
Light	1.2±1.3a$^{(@)}$	3.5±3.0a	192%	3.7±2.3a	3.9±2.1a	1.1±3.1a	5.4±9.6a
Medium	1.2±1.2a	3.7±2.3a	208%	3.7±1.8a	4±1.1a	0.8±2.1a	2±6.8b
Heavy	2.4±1.6b	7.3±4.5b	204%	5.2±3b	5.7±2.1b	2.3±7.4b	5.6±11.1a
	C. sinensis						
	Twig length$^{(*)}$	Twig length$^{(\#)}$	Perc.$^{(@)}$	No.of leaves$^{(*)}$	No.of leaves $^{(\#)}$	Aphids$^{(*)}$	Aphids$^{(\#)}$
Light	1.6±1.5a	4.9±3.9a	206%	4.3±1.9a	4.6±2a	1±3.3a	5.3±11a
Medium	1.9±1.5a	5±3.2a	163%	4.3±1.8a	4.5±1.9a	0.8±3.1a	3.8±9.1b
Heavy	2.5±1.9b	5.0±3.5a	100%	4.6±2.1a	4.7±2a	1.1±5.4b	6.4±12a

$^{(*)}$ On4 March 2015 (cm)
$^{(\#)}$ On 8 April 2015 (cm)
$^{(@)}$Percentage of shoot growth
Mean ± SD; values within the same row followed by identical letters are not significantly different (p>0.05, one way ANOVA with LSD post hoc test)
No.= number of leaves per twig

Table 2: Average number of aphid per sampled twig (*C. clementina*): larvae, apterous adult and winged adults

Larvae, apterous adult			
Pruning	**9April 2015***	**23 April 2015***	**7 May 2015***
Light	60.5±21.3a(124)**	82.9±25.3a(92) **	16.8±14.9a(109)**
Medium	60.2±21.3a(115)	80.1±33.3a(99)	16.7±14.7a(103)
Heavy	64.1±23.2a(102)	102.7±47.2b(95)	20.4±14.2a(108)
Winged adults			
Pruning	9 April 2015*	23 April 2015*	7 May 2015*
Light	0.4±0.5a (124)**	0.4±0.5a (92)**	0.6±0.4a (109)**
Medium	0.5±0.5a (115)	0.7±0.4b (99)	0.7±0.3a (103)
Heavy	0.7±0.4b (102)	0.6±0.4b (95)	0.6±0.4a (108)

*Values under brackets represent the total number of Citrus leaves (25 twigs, 5 per tree)
**Mean ± SD; values within the same row followed by identical letters are not significantly different (p>0.05, one way ANOVA with LSD post hoc test)

Table 3: Average number of aphid per sampled twig (*C. sinensis*): larvae, apterous adult and winged adults

larvae, apterous adults			
Light	53.7±20.2a(124)**	20.1±9.1a(92)**	21.8±7.9a(101)**
Medium	49.2±19.2a(115)	33.1±7.3a(90)	35.7±14.7a(98)
Heavy	55.1±18.2a (102)	60.7±19.3b(112)	65±24.2b(108)
winged adults			
Pruning	9 April 2015*	23 April 2015*	7 May 2015*
Light	0.4±0.5a (124)**	1±0.4a (92)**	0.7a±0.4(101)**
Medium	0.5±0.5a(115)	0.7±0.4a(90)	0a(98)
Heavy	0.7±0.4b (102)	0.5±0.3a(112)	0.9±0.5a(108)

*Values under brackets represent the total number of Citrus leaves (25 twigs, 5 per tree)
**Mean ± SD; values within the same row followed by identical letters are not significantly different (p>0.05, one way ANOVA with LSD post hoc test)

Table 4: Number of natural enemies per twig (*C. clementina*)

9 April 2015				
Pruning	**Para.Aphids***	**Empty mummies**	**Predatory larvae(1)**	**Coccinellidae(2)**
Light(3)	0.2±0.5 a	0.3±0.7 a	0.04±0.2a(6)	0
Medium	0.04±0.2b	0.09±0.4b	0a	0
Heavy	0.1±0.4 a	0.3±0.9 a	0.04±0.2a(6)	0
23 April 2015				
Light(3)	1.18±2.0a	2.63±3.68a	0.2±0.4a(7)	0
Medium	0.22±0.74b	0.8±2.5 b	0.12±0.33a(8)	0
Heavy	0.31±0.74b	0.79±1.6b	0.08±0.28a(9)	0
7 May 2015				
Light	0.2±0.5 a	4.9±3.2 a	0.08±0.28a(10)	0a
Medium	0.2±0.5 a	5.1±3.0 a	0.12±0.33a(11)	0.04a(4)
Heavy	0.32±0.5a	4.84±3.4a	0.16±0.38a(12)	0.04a(5)

*parasitized aphids (mummies)
** undetermined species + mummies of *Praon*sp
(1)predatory larvae were represented Chrysopid, syrphid and Cecidomyies
(2)larvae and adults of Coccinellidae
(3)mean ± SD; values within the same row followed by identical letters are not significantly different (p>0.05, one way ANOVA with LSD post hoc test)
(4)1*Scymnus*sp larva
(5)one adult of *C. algerica*
(6) 1 *Aphidoletesaphidimyza* larva
(7) 3 *Chrysopacarnea*larvae +2*Aphidoletesaphidimyza* larvae
(8) 1 C. carnea larvae + 2 *Aphidoletesaphidimyza*larvae
(9) 2 *Aphidoletesaphidimyza*larvae
(10)2*C. carnea*larvae
(11)2*C. carnealarvae* + 1*Aphidoletes aphidimyza* larvae
(12)2*A.aphidimyza*larvae + 2 *Scymnus* sp larvae

Table 5: Number of natural enemies per twig (*C. sinensis* thomson)

9 April 2015				
Pruning	Para.Aphids*	Empty mummies**	Predatory larvae(1)	Coccinellidae(2)
Light(3)	01±0.5a(3)	0.3±0.7 a	0	0
Medium	0.14±0.2a	0.4±0.4a	0a	0
Heavy	0.1±0.4 a	0.3±0.9 a	0.02±0.1a	0
23 April 2015				
Light	2±1.6a(3)	3±3.68a	0.18±0.55a	0.1a
Medium	1.4±0.7a	2.1±2.5a	0.1±0.36a	0a
Heavy	2.7±2.1 a	1.6±1.6a	0.1±0.22a	0a
7 May 2015				
Light	0.2±0.5 a	3.1±3.2 a	0	0.16a(4)
Medium	0.2±0.5 a	2.4±2.6a	0	0.2a(5)
Heavy	0.32±0.5a	3.6±3.4a	0	0.32a(6)

*parasitized aphids (mummies)
** undetermined species + mummies of *Praon*sp
(1)predatory larvae were represented Chrysopid and Cecidomyies
(2)larvae and adults of Coccinellidae
(3)Mean ± SD; values within the same row followed by identical letters are not significantly different (p>0.05, one way ANOVA with LSD post hoc test)
(4)*2 C. algerica*adults + 2 *Scymnus*sp larvae
(5)2*C. algerica* adults + 2Coccinellid larvae + 1*Scymnus*sp larva
(6)5*Hippodamiavariegata* adults + 3 *C. algerica*

Table 6. Average fruit weight and number of fruits per experimental tree

	C. clementina		*C. sinensis* Thomson	
	Fruit weight	**Fruit number**	**Fruit weight**	**Fruit number**
Light	9.2±11a	178.6±208a	7.85±5.4a	40.62±24.2a
Medium	7.7±6.2a	149±120a	7.82±6.3a	40±24.2a
Heavy	4.7±1.7a	92.5±28.3a	5.71±4.5a	25.6±18.8a

Mean ± SD; values within the same row followed by identical letters are not significantly different (p>0.05, one way ANOVA with LSD post hoc test)

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