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Aphids and *Citrus* responses to excessive nitrogen fertilization

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Abstract
The aim of this study was to assess the effect of an excessive nitrogen mineral fertilizer on the vegetative growth of *Citrus* as well as on the densities and population dynamics of the main aphid species *Aphis spiraecola* Patch (Hemiptera, Aphididae). Two trials were conducted on clementine trees, *Citrus clementine* Hort. ex Tan. in 2016 and 2017. The quantities of nitrogen delivered varied from no nitrogen (control) to the triple recommended dose of nitrogen (3ARD) in a complete randomized block design. Results showed that all vegetative growth parameters evaluated for the clementine trees responded positively to the amount of nitrogen supplied. The highest values were obtained with the highest N doses in both years. A positive response also was observed for aphids for all doses of N even the lowest, with densities increasing proportionally to N inputs. The highest dose (3ARD) increased aphid populations by 3 times compared to the control. The aphid dynamics differed between the two years, probably under the influence of other factors such as rainfall. Thus, too much nitrogen should be avoided in *Citrus* orchards. Aphid management cannot be separated from the management of fertilization, as this agronomic practice would maintain aphid densities at acceptable levels without the need for repeated insecticide applications.

Keywords: *Citrus* aphids, *Citrus* vegetative growth, nitrogen fertilization, chlorophyll content, Aphid densities, Tunisia

1. Introduction

Citrus is one of the most important fruit crops in the world, with a total production of 130 947 thousand tons in 2015 (F.A.O. 2016) [23]. In Tunisia, *Citrus* is considered a major crop with an annual production ranges from 250,000 to 400,000 tons (Onagri 2019) [49].

Among *Citrus* pests, aphids are economically important, because of their direct and indirect damage that depends on the density of their populations (Blackman and Eastop 2006) [10]. In Tunisia, aphids are considered major *Citrus* pests ranked just after the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Garcia-Mari *et al*. 2018) [25]. The damage on *Citrus* includes leaf deformation, reduced growth of shoots, dropping of flowers and small fruits. Also, aphids are known for the large amounts of honeydew excreted and for sooty mold fungi that in hibit photosynthesis. However, the most serious damage is the transmission of *Citrus* tristezavirus, even if all species do not have the same efficacy as vectors (Dreistadt 2012; Hermoso de Mendoza *et al*. 2006; Veen 1985) [19, 31, 60]. The main aphid species in Tunisia attacking*Citrus* are *Aphis spiraecola* Patch, *Aphis gossypii* Glover, *Aphis* (*Toxoptera*) *aurantii* Boyer de Fonscolombe, and *Myzus persicae* (Sulzer), the first two being the most abundant(Behi *et al*. 2019; BenHalima-Kamel *et al*. 1994*;* Boukhris-Bouhachem 2011) [4, 64, 12].

In conventional *Citrus* orchards, growers rely primarily on the use of synthetic chemicals to control aphids in spring and autumn (Jerraya 2003) [65]. The large-scale use of chemical pesticides has resulted in several adverse effects such as insecticide residues, environmental pollution (Konstantinoua *et al*. 2006) [39], insect resistance (Gubran *et al*. 1993; Li and Han 2004; Nabeshima *et al*., 2003) [27, 42, 47], and emergence of secondary pests (Belaam-Kort and Boulahia-Kheder 2019; Fenemore and Norton 1985) [5, 24]. Therefore, there is a need for reliable and more environmentally friendly tactics against *Citrus*aphids. Cultural practices that slow aphid population growth rate, while preserving and enhancing natural enemies are recommended (Boukhris-Bouhachem 2011) [12]. Use of good farming practices with respect to nitrogen (N) supplementation can save money, prevent pollution and also reduce aphid pressure (Schumann *et al*. 2010) [53], since aphids respond positively to N fertilization (Jansson and Ekbom 2002) [36]. Nitrogen has a central role in all agricultural systems. It is required in adequate amounts by plants since it is an integral constituent of proteins, nucleic acids, and chlorophyll (Alfonso Sandra *et al*. 2018) [2]. Nitrogen should be supplied annually (or as needed depending on horticultural conditions)as fertilizer (Embleton and Jones 1978) [67]. In *Citrus* groves, nitrogen fertilization is essential in spring because new spring flush utilizes a large part of the previously stored nitrogen (Legaz *et al*. 1982) [41].

Commonly, *Citrus* growers supply *Citrus* trees with large amounts of nitrogen in early spring to boost new shoots and to increase yields. However, too much nitrogen does not induceun-limited shoot production, and it can decrease the fruit quality (Bru *et al*. 2003) [14]. It alsofavors insects, particularly sap-sucking ones (Hogendorp and Cloyd 2005; Schoonhoven *et al*. 2005; Singh and Sood, 2017) [63, 52, 54]. Besides, the excess of nitrogen contaminates groundwater (Bru *et al*. 2003; Cui *et al*. 2020) [14, 18], with negative effects on ecological balance and human health. Excess nitrogen pollutes the atmosphere and is incriminated as one factor in a warming climate (Tian *et al*., 2012) [59]. Hence, one of the important aspects of good farming practice is to balance nitrogen fertilization to meet but not exceed the requirements of the plant. An added benefit is potential reduction in aphid pest problems.

This study evaluates the effect of excessive versus recommended ammonium nitrate supplementation on (i) *Citrus* vegetation (nutritional status and spring twig growth), and (ii) aphid response (abundance and population dynamic). Aphid infestation was assessed to recommend the correct nitrogen fertilization to be applied to *Citrus* in spring as a cultural component of Integrated Pest Management against aphids, ensuring an optimal yield without inducing outbreaks.

2. Materials and Methods

2.1. Study site and experimental protocol

The study was conducted during the spring leaf-flushing period (from February to May) for two consecutive years (2016 and 2017) in an experimental *Citrus* grove belonging to 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 orchard of about 2500 $m^2$was planted in 2009 and had 83 clementine, *Citrus clementina* Hort. ex Tan, (CV Kassar) trees arranged in four rows: three of 22 trees each and one of 17 trees surrounded by cypress hedgerows in three sides and 2 rows of C. *sinensis (*L.) Obs. cv Thomson trees on the other side (Fig. 1). The description of the orchard and its characteristics were given by Braham and Amor (2018). The orchard was managed under sustainable production and did not receive pesticide sprays or mineral fertilization from the time of establishment. However, each year, in late December or early January, 15 to 20 kg of cattle manure were incorporated into the soil beneath each tree.

Trees were arranged in a randomized complete block design with three replicates (blocks) and four treatments. Each experimental plot was represented by 5 trees (Fig.1). All trees were pruned similarly on January 20th and 21st 2016, and on January18th 2017. The doses of ammonium nitratein 2016 were the Agronomic Recommended Dose (ARD), half the Agronomic Recommended Dose (ARD/2), double the Agronomic Recommended Dose (2ARD), which was considered as an excessive supply, and no nitrogen fertilization (control) (Table 1). The same amounts of nitrogen were used in 2017 except the 2ARD was replaced by triple the Agronomic Recommended Dose (3ARD) to further investigate the effect excess nitrogen (Table 1). The recommended dose was equal to the nitrogen need of the trees, which was calculated based on the variety, and the nitrogen uptake during vegetative growth and by fruit production in the previous years (CTA, 2009) [16]. These requirements were estimated at200 nitrogen unit per tree (CTA 2009) [16]. The commercial mineral fertilizer used was Ammonitrates 33.5% (Puteaux, France) rating 16.7 units of nitric Nitrogen (NO_3^-) and 16.8 units of ammonia cal Nitrogen (NH_4^+). The fertilizers were dissolved in 60 liters of water and supplied to each experimental tree as flood irrigation within the canopy drip zone. This practice coincides approximately with standard mineral fertilization in commercial *Citrus* in Tunisia (Table 1). Control trees received the same amount of water as fertilized trees (Beniken *et al*. 2013) [9].

2.2. Effect of nitrogen supplementation on *Citrus* twig growth and aphid infestation

The effect of nitrogen supplementation was assessed based on the following parameters: (i) the leaf chlorophyll content in *Citrus* measured by a portable chlorophyll meter (the Soil Plant Analysis Development, SPAD, USA). The SPAD-502 Konica-Minolta meter measures the transmittance of red (650 nm) and infrared (940 nm) radiation through the leaf and calculates a relative SPAD meter value that should ‘‘correspond to the amount of chlorophyll present in the sample leaf’’ that indicates the “in situ” nitrogen status (Evans 1989). Measurements were made on four young fully developed leaves per tree, one leaf per quadrant on 2 trees per treatment totaling 24 trees onMay30th, 2016, and May 22nd 2017, (ii) Twig elongation was evaluated on two labeled trees per block per N dose. In total, 24 trees (2trees x3blocks x4doses) were labeled. On each tree, 4 branches (one per quadrant) were tagged, and the numbers of newly produced twigs were counted. Among these twigs, two per branch were marked (in total, 8 per tree) and their lengths were measured on February 26th, and May 17th in 2016 and March 21st and May 22nd in 2017. The rate of increase in twig length (%) or in number of newly produced twigs (%) was calculated as: (Second date value-First date value/First date value) x100) (iii) Aphid identification and infestation in the study orchard were measured based on samples of10 and 50 infested twigs taken respectively on March 10th 2016 and March 13th 2017. Leaves were examined in the laboratory under a stereo-microscope (Leica MZ). Samples of adult aphids collected on the different observation dates were determined with slide-mounted specimens using a microscope (Leica), and the key provided by Blackman and Eastop (2006) [10]. Voucher specimens were deposited in the Laboratory of Entomology and Insect Ecology at the Regional Research Centre on Horticulture at Chott-Mariem (Tunisia)

Aphid infestation was assessed by counting the nymphs and adults on twig 10 cm long. For each dose 2 trees per blockper compass point were tagged, and these were checked once a week, from 3 February to 12 May in 2016 (except from 3 February to 22 March) and from 8 March to 10 May in 2017. Thus, two twigs per tree per compass point per block per treatment were utilized totalizing 48 twigs per treatment.

2.3. Statistical analysis

The number of newly produced shoots, the *Citrus* twig length and the densities of aphids 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 (IBM, 2015). Before analysis, a Kolmogorov-Smirnov test was applied to assess data normality. When necessary, before analysis, data were transformed to log(x+1) to meet the assumption of ANOVA.

3. Results

3.1. Identification of aphid species

For the two years of study, the dominant aphid species in the orchard was *A. spiraecola* with an abundance of 84 to 94.55%. *Aphis spiraecola* was followed by *A. gossypii and Aphisfabae* Scopoli (Table 2). Only a very few *M. persicae* were observed on March 13, 2017 (0.45%).Hence, only *A. spiraecola* was considered in this study.

3.2. Effect of nitrogen inputs on *Citrus*

3.2.1. Leaf chlorophyll concentration

In 2016 and in 2017, chlorophyll content in the leaves increased significantly proportionally to nitrogen dosage for all treatments compared to the control, with the exception that half the recommended N dose did not differ significantly from the control in 2016 ($F_{3,92}$ = 38.2; P=0.015; $F_{3,92}$ = 46.49; P=0.001 respectively for 2016 and 2017).

The trees that received the triple agronomic recommended dose (3ARD) showed the highest SPAD values (66.6±4.62 SPAD units), and the control trees showed the lowest SPAD values: 45.46 and 30.72 respectively in 2016 and 2017 (Fig. 2a; Fig.2b).

3.2.2. Twig elongation

In both years, for the most part, twig lengths increased significantly proportionally with the levels of nitrogen compared to the control (Table 3). For the first date (i.e. 3-4 weeks after the first N input for both years), all nitrogen doses except ARD/2significantly increased twig lengths compared with the control. Lengths were at least 1.4-1.8 times the length of twigs in control for the ARD and 2.9-3.3 times for the 2ARD and 3ARD respectively in 2016 and 2017 (Table 3).On the second sample date (i.e. 3-7 weeks after the last N input; 22 and 51 days after the third inputs respectively in 2016 and 2017), 2ARD and 3ARD boosted twig elongation to about twice the length of the shoots in the control. ARD produced less than 1.5 times the twig length in the control in both years. Hence, for both years, 2ARD and 3ARD produced the highest twig elongation. The half nitrogen dose mostly had no effect on twig elongation (Table 3 b).

3.2.3. Number of newly produced twigs per branch

In 2016 and 2017, the number of new twigs produced in spring between February and May increased proportionally according to the different levels of nitrogen (Table 4).

The maximum number of twigs in May, which varied between 6.18 and 18.62 respectively in 2016 and 2017, was induced by the highest doses of N: 2ARD and 3ARD. This latter level enhanced more the growth of new shoots with 2.46 times more than control compared to 1.58 more than control with 2ARD (Table 4).

In both years, the lowest number of new shoots was observed in the control (Table 4). However, in 2016, ARD/2 was not significantly different from control for the number of newly produced twigs (Table 4 a), whereas in 2017 ARD/2 was significantly different from the control but not significantly different from ARD (Table 4 b).

The percentage of increase in the number of newly produced twigs was different between years. In fact, in 2016, it was relatively low and varied from 17 to 27% in treated trees compared to 23% in control. Conversely, in 2017, it was high and ranged from 63 to 180% compared to 92% in control (Table 4). Also, unexpectedly the highest doses of N did not allow the highest percentages of increase in the number of new twigs between February and May.

3.3. Effect of nitrogen inputs on aphids

3.3.1. Dynamic of aphid populations

In 2016, in the control and in the lowest nitrogen level, aphid populations showed two peaks (on 29 March and 19 April), and the second peak was higher than the first. On trees supplied by nitrogen (ARD and 2ARD), aphids also showed 2 population peaks but the first was higher than the second (Fig. 3a). Hence, nitrogen inputs might have affected the shape of dynamic curve of aphids.

In 2017, all nitrogen levels and the control produced the same population dynamics for *A. spiraecola* (Fig. 3b). Only one peak was observed in 2017 on April 19th, 3 weeks later than in 2016. The aphids did not respond in the same way to nitrogen inputs in 2016 and 2017.

3.3.2. Density of aphids

In 2016, the number of aphids per top 10 cm of the twig increased with the amount of nitrogen fertilizer applied (one way ANOVA; $F_{3,1724}$ = 65.76; $P<$ 0.0001; N = 384, on seven of eight sampling dates; the first scoring on February 22nd 2016 was excluded in the analysis because of the low aphid densities, values near zero). The averages were, respectively: 18.33±25.25; 9.03±14.68; 30.45±38.98 and 7.87±13.18 for ARD, ARD/2, 2ARD and Control. However, it is noteworthy that for almost all the dates in 2016, the density of aphids was not significantly different between ARD/2 and control. Also, ARD produced a significant difference in aphid densities only during April. Only the highest level of nitrogen, 2ARD, induced a significant increase in aphid populations compared to the control from the beginning until the end of the observation period (March and April) (Table 5a).

In 2017,aphid densities were 3-4 fold higher than in 2016, even for the control (Table 5b).The number of aphids per top 10 cm of twig varied significantly with the amount of nitrogen fertilizer applied (one way ANOVA; $F_{3,956}$ = 36.1; $p<0.0001$; N = 240, for six of eight sampling dates. The samplings of March 8thand May 10th, 2017 were excluded from the analysis because of no aphid infestation). The trees that received 3ARD had the highest aphid densities (Table 5b).The lowest dose of nitrogen (ARD/2) produced aphid densities significantly higher than control on the first date, and this trend remained during all dates.

4. Discussion

In Tunisia, *Citrus* growers usually supply their orchards with nitrogen to increase yields and improve the quality of fruits but, without considering the risk of pest build-up. Nitrogen fertilizers mostly are applied by soil incorporation without precise quantification. Such practice could result in inadequate fertilization or excess nitrogen which has at least three types of adverse effects: (i) wasted money, (ii) leaching leading to water pollution, and (iii) enhancement of sap-sucker insects. The aim of this study relates to the third effect. It assessed the effect of excess nitrogen input on the spring vegetative growth of clementine trees and on densities and population dynamics of *A. spiraecola* during two seasons (2016-2017).

4.1. Identification of aphid species

In both years of the study, the aphid *A. spiraecola* was the most abundant species on the clementine trees. Thisresult agrees with data reported recently from Tunisia by Behi *et al*. (2019) [4] and from other Mediterranean regions, e.g. in Spain (Gomez *et al*. 2016; Hermoso de Mendoza *et al*. 2006) [26, 31] andin Algeria (Lebbal and Laamari 2015; Mostefaoui *et al*. 2014) [40, 45].

4.2. Effect of excess nitrogen on clementine vegetation

Our results showed that clementine trees responded positively to the increases in the amount of N received, as measured by chlorophyll content, twig growth, and the number of newly produced twigs. The highest values for these measurements were obtained at the highest N doses in both years. New *Citrus* flush leaves have the greatest benefit from the N applied fertilizer (Martinez *et al*., 2002) [44].

The responses of clementine trees to the lowest N doses differed between 2016 and 2017. In 2016, the minimum dose that produced a response of the tree was the recommended dose, whereas it was half this dose in 2017. One explanation could be that the trees were healthier in 2017 because of water availability in the soil due to a valuable rainfall in autumn of 2016 that would have enhanced vegetative growth in 2017. Consequently, trees could benefit even from small doses of nitrogen. Conversely, in 2016, the trees probably were under water stress, which would have affected their vegetative growth, even with nitrogen supplementing, as noted in other studies (Almenares Garlobo *et al*. 2015; Iglesias *et al*. 2007) [1, 34]. Indeed, rainfall was relatively low from January to April, in 2016 and 2017 (respectively 79 mm and 32 mm) butin autumn 2016 rainfall was more than 3 times higher than in autumn 2015 (respectively 477.8 mm and 125.2 mm from September to December).

Furthermore, the increase in nitrogen rates produced a response in the *Citrus* trees but not without limit. The highest doses (nitrogen in excess) stimulated the number and growth of new twigs, at the beginning of the season; then vegetative growth stabilized. Surprisingly, the lowest rate of increase in twig elongation was found in trees fed by the highest doses of N, whereas trees with the lowest N input (control) showed a significantly greater rate of increase in twig elongation. Thus, even without external nitrogen input, trees seem to have drawn upon their reserves to grow new shoots, suggesting a mobilization of stored resources to renew the foliage in spring. In *Citrus* trees, the accumulation of reserves has been reported for carbohydrates as a survival strategy (Goldshmidt 1997) [28]. Most N accumulated by trees in new growth in spring came from stored N and from N already in the soil (Legaz *et al*. 1982; Martinez *et al*. 2002) [41, 44]. Only one-third of the N fertilizer applied was absorbed by orange trees, and on average 20% was recovered in new growth (Martinez *et al*. 2002) [44]. In fact, N use efficiency by almost fruit trees is lower than 55%, and much nitrogen fertilizer may be lost, resulting in pollution (Carranca *et al*. 2018) [15], or itmay produce excess vigor and vegetative growth rather than flowering (Zekri 2011) [61]. This is in agreement with Liao *et al*. (2019) [43] showing that excess N supply exerted a significant negative effect on N metabolism in the root, leaf, and fruit of the cultivar "Huangguogan" (*Citrus reticulata*× *C. sinensis*)thereby reducing fruit quality and yield.

4.3. Effect of excess nitrogen on the population dynamic and density of aphids

The aphid populations developed in spring mostly between March and April taking advantage of the new succulent shoots. Then aphid populations significantly declined in the first ten days of May, as observed in other studies (BenHalima-Kamel *et al*. 1994; Mostefaoui *et al*. 2014; Braham and Amor 2018) [64, 45, 13]. However, sthe aphid population dynamics in 2016 were different from those in 2016. In 2016, aphid populations developed differently according to N inputs. On the trees fed with the highest doses of nitrogen, aphids increased rapidly with a high peak at the end of March and a second lower peak on April 19th and conversely for trees that received the least nitrogen. Thus, the population growth of aphids was reduced and delayed on trees less supplied with nitrogen. In contrast, in 2017, the doses of nitrogen did not change general aphid population dynamics. For all doses, aphids developed only one peak on April 19th.

In 2016, aphids responded only to the highest N rate, and the lowest rate did not have any effect on densities, whereas in 2017 all doses resulted in a significant and proportional increase in aphid population densities, suggesting that the effect of N was enhanced by the greater water availability in 2017. We noted in 2017that the highest aphid densities in the control were similar to those observed in 2016 with the highest N dose, i.e. 2ARD (64.66 on March 31th 2016 and 71.76 on April 19th 2017 respectively for 2ARD and control N doses). This would mean that in favorable years (as in 2017); aphids can increase their populations even without an additional supply of N, and aphids increase more if their host plant is rich in N. Therefore, depending on the year (with other factors being involved such as rainfall), aphids as well as the host-plant will or will not respond to low inputs of N, but aphids always respond to high N inputs. Consequently, nitrogen inputs have to be optimized and excess must be avoided in *Citrus* orchards because too much nitrogen, will usually favor the increase of aphids, which can lead to significant losses and requires extra insecticide sprays.

Several authors found similar results for aphids and other phloem-feeding insects with increasing levels of nitrogen fertilization. For instance, the adult and nymph densities as well as intrinsic rate increase of the melon aphid, *A. gossypii* on cotton were positively correlated with nitrogen fertilization (Nevo and Coll 2001) [48]. Also, the lowest mean generation time and the highest rate of increase was reported for *A. gossypii* fedon chrysanthemum fertilized at 150% of recommended nitrogen fertilizer levels (Rostami *et* *al*. 2012) [50]. In winter wheat, synthetic nitrogen fertilization increased aphid infestations (Hasken and Poehling 1995) [29]. On potatoes, nitrogen applications increased the rate of population growth of the green peach aphid, *M. persicae*, and growth was correlated positively with the concentrations of free amino acids in leaves (Jansson and Smilowitz 1986) [36]. The *Citrus* mealy bug, *Planococcus citri* Risso, showed the highest performance on coleus plants with higher nitrogen concentrations (Hogendorp and Cloyd 2005) [63]. Moreover, the population abundance of Western flower thrips, *Frankliniella occidentalis* Pergande, which is an emerging pest in Tunisian *Citrus* orchards (Belaam-Kort *et al*. 2020a) [6] increased significantly with higher nitrogen levels on chrysanthemum (Chauand Heinz 2006) [17]. These effect are expected on over-fertilized *Citrus* trees.

Unexpectedly on peach, *M. persicae* populations whose host plants were given two different levels of nitrogen increased proportionally with nitrogen content of leaves until the concentrations in the leaves reached intermediate levels. Populations decreased for higher levels of nitrogen (Sauge *et al*. 2010) [51]. At the highest N level, the peach trees continued growing but *M. persicae* populations decreased. The authors suggested that the highest N level has increased concentrations of induced defensive compounds that explain the reduction in aphid numbers.

An increase in insect pest populations in response to high nitrogen levels can result from various mechanisms, depending on the pest species and the host plant. Hermes (2002) [30] reported that nitrogen fertilization decreased plant resistance to spider mites and sap-sucking insects by enhancing the nutritional quality of the plant and/or decreasing secondary metabolite concentrations. According to Hogendorp and Cloyd (2005) [63], a plant with excess nutrients will concentrate its energy on growth, thus producing abundant new succulent leaves. These tissues with high levels of vascular fluids (phloem and xylem) benefit phloem-feeding insects. However, nitrogen use efficiency is finite.

According to Srivastava and Singh (2003) [58] the normally timed soil-applied nitrogen is not the major contribution for growth of spring flush, flowering and vegetative growth. This being the case, excessive N would be lost, resulting in pollution. In addition, excessive nitrogen fertilizer will unnecessarily increase the annual cost of production. Nitrogen fertilization contributes an average of 20-30% of the total production cost (Srivastava 2012) [56]. Consequently, *Citrus* trees must be supplied withan adequate amount of N that matches their nutrient requirements. A combination of foliar and soil analysis is required to calculate N requirements, rather than visible symptoms commonly used by growers (Srivastava 2013) [57]. In addition, the different species of *Citrus* do not have the nitrogen requirements (Boaretto *et al*. 2015) [11]. This specificity has to be considered in the estimation of annual applied nitrogen for the crop.

One of the solutions to improve nitrogen fertilization management is fertigation, which is to apply nitrogen through the irrigation system. Alva *et al*., (2008) [3], showed that fertigation was as beneficial as conventional dry fertilizer application, because it reduced groundwater pollution and development of sap-sucking insects (Martinez *et al*. 2002) [44]. However, in fertigation, the quality of water is critical particularly with respect to salt concentration (Alva *et al*. 2008) [3].

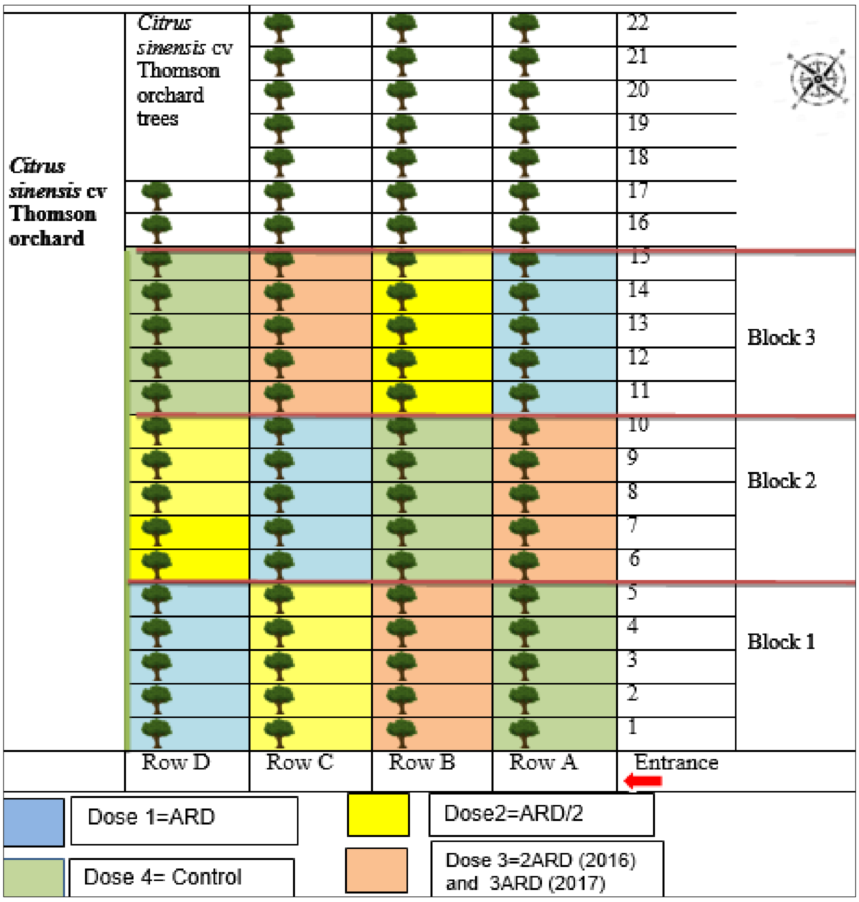
Another alternative to supplement or even substitute soil-applied nitrogen is properly timed foliar spray, which can reduce the risk of nitrogen leaching and avoid excessive vigor of trees (Morton and Woolley 2011; Srivastava and Singh 2003; Zekri 2014) [46, 58, 62]. Among the available sources, urea was the best formulation for foliar applications and was as efficient as soil-ammonium nitrates (El-Otmani *et al*. 2002; Zekri 2014) [20, 62].

Further research is needed to determine the effect of different ways of supplying nitrogen (fertigation, soil, or foliar applications), as well as different sources of N, on aphid development. Thus, growers could modify nitrogen inputs (quality and timing of applications) according to the vegetative growth period with the double goal of maximizing efficiency of fertilizer and keeping aphid levels below the economic threshold. Early nitrogen fertilization was recommended as a component of integrated management against *Citrus* leaf miner (CLM), *Phyllocnistis citrella* Stainton. This cultural practice aimed to maximize spring flush and to minimize the summer and autumn flushes, where CLM can reach high densities (Khedher-Boulahia *et al*. 2002) [66].

The use of natural N sources such as manure, compost, or mulching, and cover crops has been recommended (Carranca *et al*. 2018) [15]. Organic enrichment is known to improve chemical, physical and biological soil properties and to release N more gradually compared to inorganic fertilizers (Srivastava, 2009) [55]. Organic sources of N have, in addition to their nutritional role,a positive effect in decreasing other *Citrus* pests. For example, manure inputs in *Citrus* orchards enhanced soil predators of thrips, thus limiting the thrips population and the damage from thrips pests (Belaam-Kort, *et al*. 2020b) [7].

Srivastava (2009) [55] promoted the concept of integrated nutrient management (INM) combining different sources of nitrogen with emphasis on bio-organics, instead of using only mineral fertilizers. This concept is based on the hypothesis that the economic yield also includes long-term physico-chemical and microbial health of soils.

In conclusion, this study showed that excessive nitrogen stimulated vegetative development of *Citrus* as well as aphid infestation. Pest management cannot be separated from cultural practices, in particular N fertilization. Nitrogen fertilization affects sap-sucking insects such as aphids that rely on new flush for colonization. Farmers should use best management practices (BMPs) that combine IPM and ISM (integrated soil management) to maintain and improve productivity while reducing the use of fertilizers and pesticides. Overuse of nitrogen is detrimental to the environment and a waste of money. Moreover, it also can affect pest management by increasing populations of sap-sucking insects such as aphids. More collaboration between pest management, plant nutrition, and soil science is required for the sustainable management of *Citrus* groves.



ARD = Agronomic recommended dose

Fig 1: Schematic representation of the study orchard and experimental design

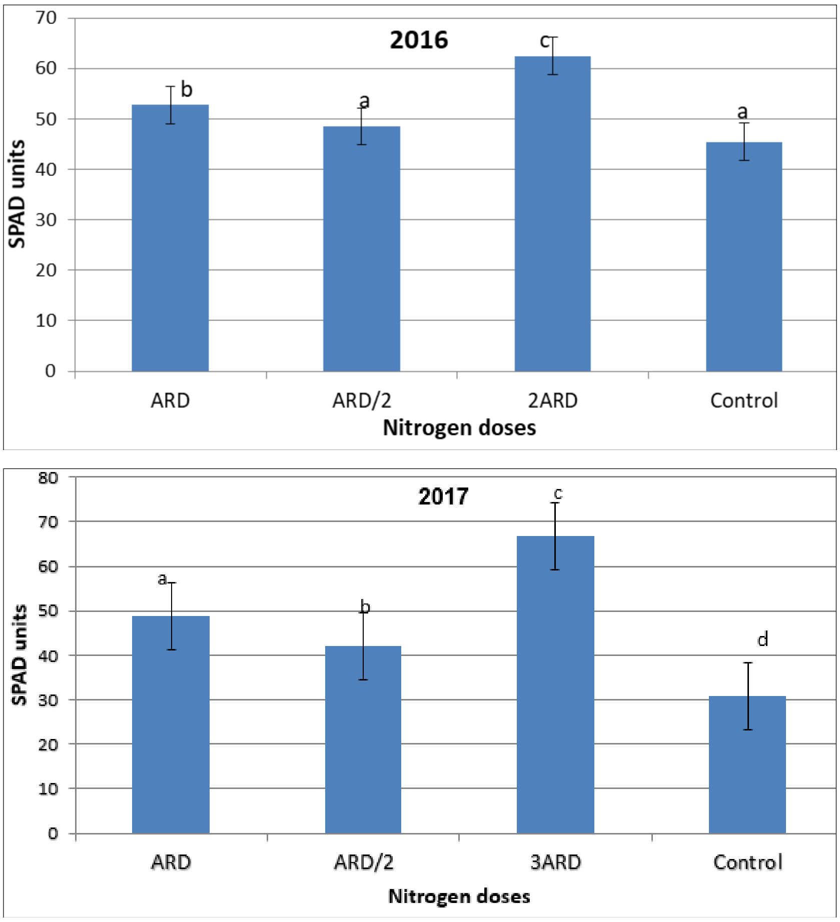


Fig 2: SPAD units (± SE) in clementine leaves in 2016 (a) and 2017 (b). Values with different letters are significantly different at P= 0.05

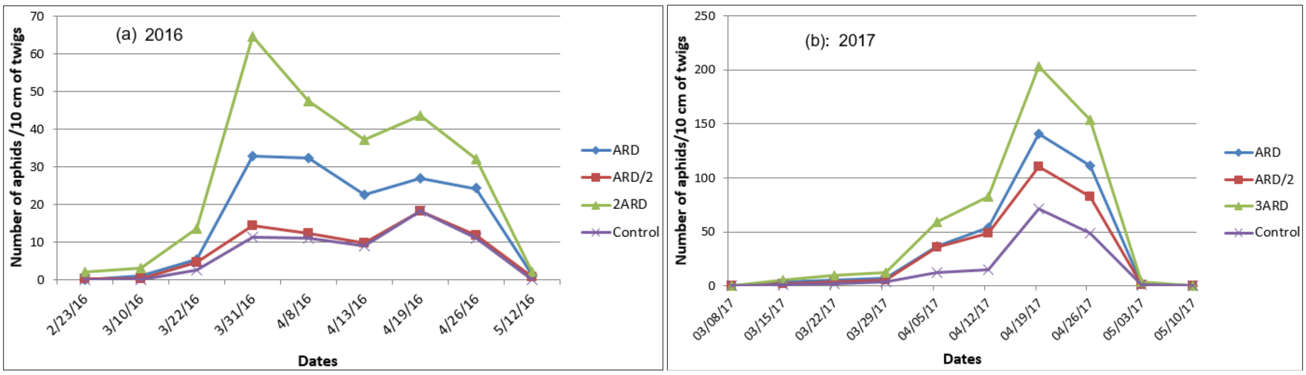


Fig 3: Aphid abundance (average number of nymphs and wingless females) per 10 cm of terminal twig in 2016 (a) and 2017 (b) (the scales in ordinate axis are different, for details see table 5)

Table 1: Amounts and dates of Ammonitrate inputs*

Years	Dates of fertilization	Doses of Ammonitrate at each input	Total amounts of Ammonitrate
2016	4 Feb; 29 Feb and 25 March	- 0.2 kg/tree	- Dose 1 (0.6kg)
		-0.1kg/tree	- Dose 2 (0.3 kg)
		-0.4 kg /tree	- Dose 3 (1.2kg)
		- 0 kg	Dose 4 (control, 0 kg)
2017	23 Feb; 14 March and 31 March	- 0.2 kg/tree	- - Dose1 (0.6kg)
		- 0.1 kg/tree	- Dose2 (0.3 kg)
		- 0.6 kg/tree	- Dose3 (1.8kg)
		- 0 kg	- Dose4 (control, 0 kg)

*Commercial name: Ammonitrate 33.5%

Table 2: Relative abundance of aphid species on *Citrus* twigs in 2016 and 2017

Aphid species	March 10th 2016		March 13th 2017	
	Number(*)	Percentage	Number (*)	Percentage
A. spiraecola	105	84	1040	94.55
A. gossypii	15	12	55	5
A. fabae	5	4	0	0
M. persicae	0	0	5	0.45
Total	125	100	1100	100

(*)Number of all stages taken together (larvae and wingless aphids)

Table 3 a & b: Twig elongation in 2016 (a) and 2017(b)

	Twig length (cm)± SD*	Min -Max	Twig length (cm)± SD**	Min- Max	Percentage of twig elongation
Control	0.84±0.39a	0.2-1.9	5.79±1.63a	2.9-9.2	589.28
ARD/2	1.20±0.59ab	0.4-3	6.4±1.78a	3.1-10.5	433.33
ARD	1.51±0.71b	0.5-3.6	8.5±2.5b	4.2-13	462.91
2ARD	2.46±1.6c	0.8-7.6	10.29±2.62c	5.2-20	318.29
One way ANOVA	$F_{3,188}$=22.58 p< 0.0001		$F_{3,188}$=43.58 P< 0.0001		

* On 26 Feb. 2016 (22 days after the first N input)
**on 17 May 2016 (22 days after the third N input)

	Twig length (cm)± SD	Min –Max	Twig length (cm)± SD	Min- Max	Percentage of twig elongation
Control	0.91±0.17a	0.5-1.3	6.66±0.76a	5.2-8.4	631.86
ARD/2	1.1±0.21a	0.7-1.5	7.26±0.94b	5.2-9.8	560
ARD	1.3±0.22b	0.9-1.7	8.4±1.04c	6.2-10.8	546.15
3ARD	3.02±0.53c	1.2-3.9	14.66±1.81d	9.7-17.9	385.43
One way ANOVA	$F_{3,188}$=267 p< 0.0001		$F_{3,188}$=417 P< 0.0001		

*On 21 March 2017(at 27 days after the first N input)
**On 22 May 2017(at 51 days after the third N input)

Table 4 a & b: Number of freshly produced twigs per tagged branch in 2016 (a) and 2017(b)

	Number of twigs*	Min –Max	Number of twigs**	Min-Max	Increase of twig number (%)
Control	3.16±0.84a	2-5	3.89±0.94 a	3-7	23.10
ARD/2	3.48±0.86ab	2-5	4.08±0.78a	3-6	17.24
ARD	3.95±1.06b	2-7	5.04±1.08b	3-8	27.59
2ARD	4.93±1.29c	2-8	6.18±1.18c	4-9	25.35
One way ANOVA	$F_{3,188}$=26.27 P< 0.0001		$F_{3,188}$=51.03 P< 0.0001		

* On 26 Feb 2016
** On 17 May 2016

	Number of twigs*	Min –Max	Number of twigs**	Min-Max	Increase of twig number (%)
Control	3.91±1.13a	2-6	7.54±1.74a	5-10	92.83
ARD/2	4.08±1.6ab	2-7	11.45±2.28b	7-15	180.63
ARD	4.41±1.93b	2-7	11.51±2.10b	8-15	160.99
3ARD	11.41±2.04c	8-15	18.62±2.28bc	14-22	63.19
One way ANOVA	$F_{3,92}$= 107.3; *P*< 0.0001		$F_{3,92}$= 114.3; *P*<0.0001		

*On 21 March 2017
** On 22 May 2017

Table 5 a & b: Number of aphids (nymphs, apterous and winged adults) per terminal 10 cm of twigs in 2016 (a) and 2017 (b)

	23Feb2016	10March	22March	31March	8April	13April	19April	26April	12May
Control	0a	0.08±0.5a	2.6±8.8a	11.23±20.22a	10.9±14.20b	8.96±13.44b	18.2±13.6b	11±9.5a	0a
ARD/2	0.33±1.6a	0.19±0.9a	4.6±11.1a	14.46±26.36a	12.3±14.41b	9.67±10.47b	18.3±15.4b	11.9±10.5a	0.85±2.9ab
ARD	0a	1.02±0.5a	5.48±13.9a	32.92±46.8a	32.25±23.9a	22.5±17.6a	27±17.8a	24.21±15.9b	1.19±4.1ab
2ARD	1.96±5.7b	3.17±6.7b	13.46±26.1b	64.67±73.37b	47.48±35.7a	37.25±25.6c	43.6±20.3c	31.9±17.6c	1.98±3.9ab
One way Anova	$F_{3.188}$=4.65 P= 0.004	$F_{3.188}$=5.51 P= 0.001	$F_{3.188}$=4.17 P= 0.007	$F_{3.188}$=13.27 *P*< 0.001	$F_{3.188}$=25.8 *P*< 0.001	$F_{3.188}$=27.10 *P*< 0.001	$F_{3.188}$=23.7 *P*< 0.001	$F_{3.188}$=2.5 *P*< 0.001	$F_{3.188}$=3.1 P= 0.02

	88 March2017	15 March	22 March	29 March	5 April	12 April	19 April	26 April	3 May	10 May
Control	0	1±1.31a	2.4±1.9a	3.93±2.4a	12.1±2.9a	15.3±4.3a	71.7±7.3a	48.7±11.9a	0.9±1.4a	0
ARD/2	0	2.2±1.5b	4.2±2.4b	5.9±2.1b	36.1±5.5b	48.7±7.7b	110.2±13.1b	82.6±18.2b	1.3±1.4a	0
ARD	0	3.4±1.6c	5.5±2.3c	7.6±2.5b	37±5.5b	53.8±7.1b	140.4±11.2c	110.9±26.1c	2.0±1.8a	0
3ARD	0	5.6±2.1d	9.8±3.1d	12.5±3.10c	59.4±6.7c	82.5±7.2d	203.2±13.5d	153.9±32.2d	3.9±2.4b	0
One way ANOVA		$F_{3,116}$=38.7 *P*< 0.001	$F_{3,116}$=48.1 *P*< 0.001	$F_{3,116}$=58.9 *P*< 0.001	$F_{3,116}$=378 *P*< 0.001	$F_{3,116}$=485 *P*< 0.001	$F_{3,116}$=669 *P*< 0.001	$F_{3,116}$=104 *P*< 0.001	$F_{3,116}$=15.1 *P*< 0.001	

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6. Conflicts of interest
The authors declare that they have no conflict of interest.

7. Involving Human Participant sand/or Animals
This research does not involve human participants and /or animals

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