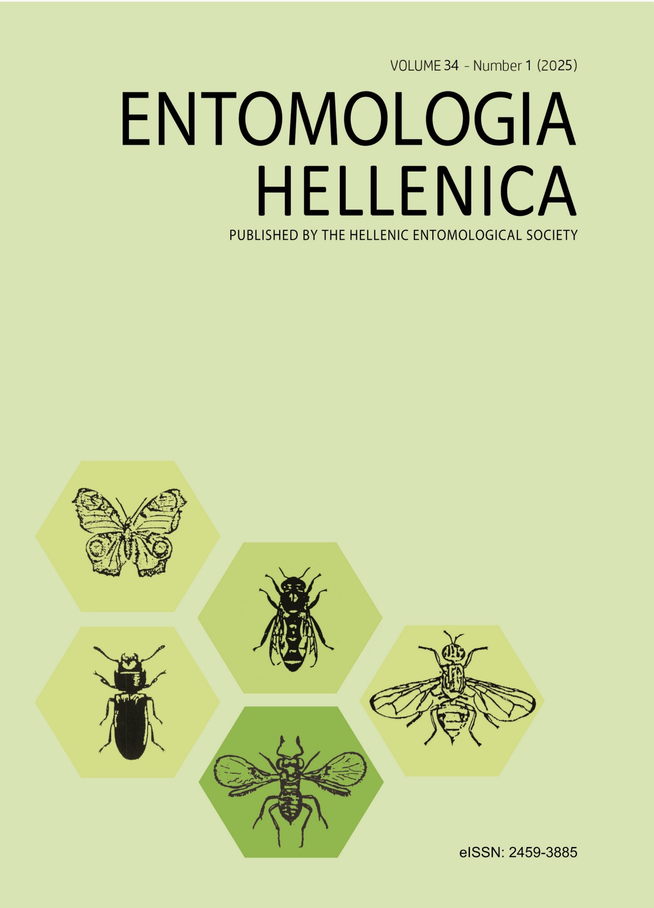

Mineral nutrition as a lever for the control of Parlatoria ziziphi (Hemiptera: Coccomoprha: Diaspididae) in Algeria (Rouiba region)

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Mineral nutrition as a lever for the control of *Parlatoria ziziphi* (Hemiptera: Coccomoprha: Diaspididae) in Algeria (Rouiba region)

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ABSTRACT

This work focuses on the comparative study of *Parlatoria ziziphi* (Hemiptera: Coccomorpha: Diaspididae) on citrus trees and the influence of mineral nutrition on its variation. The results demonstrate that infestations of *P. ziziphi* on lemon trees are more significant than on clementine trees (10078/6842 individuals), especially on older leaves (6850/5250 individuals). The periods of strong infestations occur in January and March. The differences in potassium, sodium, calcium, magnesium, and iron levels are highly significant ($p=0.001$) and vary depending on the variety, leaf age, and time of year. Potassium plays a limiting role in the proliferation of *P. ziziphi* on clementine and lemon trees. It is notably higher on clementine trees, especially in young leaves (2.11% in May), where the infestation has declined. The same applies to sodium, with higher concentrations on clementine trees (1.28% in March) and lemon trees (1.02% in May). The highest levels correspond to a low proliferation of this scale insect, regardless of leaf age in both varieties. However, there is a synchronization between calcium levels and the abundance of *P. ziziphi* populations on both young and old leaves of both varieties. Magnesium, with higher concentrations (0.79%) in lemon trees and in older leaves (0.77%), is inversely correlated with the abundance of the scale insect. Copper and iron have no marked influence on the scale insect, but lemon trees have a higher copper content (0.40 ppm) compared to clementine trees (0.39 ppm), suggesting that these factors promote infestation.

KEY WORDS: *Citrus, lemon, clementine, infestation, mineral nutrition*.

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Introduction

Citrus fruits form a fundamental pillar of agriculture due to their significant economic and nutritional value. Perennial plants, such as citrus trees, offer substantial benefits for conservation and protection within agroecosystems. In Algeria, for instance, citrus cultivation has expanded considerably from approximately 44,820 hectares in 1997 to 71,477 hectares in 2018, according to M.A.D.R (2019). However, the Mediterranean region, including Algeria, faces numerous challenges for citrus cultivation, with homopteran pests being a significant threat.

Among these pests, the scale insect *Parlatoria ziziphi* (Lucas, 1853) is particularly damaging to citrus trees, especially lemon, orange, and clementine, and is classified as a quarantine organism in many countries (Quilici, 2003). The nutritional composition of a host plant plays a pivotal role in the development and behaviour of scale insects, such as diaspines. Saighi (1998) suggested that host plants can modulate the life cycle and behavior of these insects based on their nutritional composition.

Scientific interest in *P.ziziphi* dates back to the work of Balachowsky and Mesnil (1935). In Algeria, research on this species has been intensified since the 1980s, with studies by Ouzzani (1984), Zellat (1984), Lasnami (1993), and Zekri (1993). More recently, focus has shifted to the interactions between the host plant and the insect, particularly regarding nutrition. Mostefa and Boukhors (2004) laid the groundwork for this research, followed by Belguendouz (2014), who delved deeper into the chemical and physicochemical relationships between *P. ziziphi* and citrus trees.

This study aims to analyze the mineral nutritional relationship between the scale insect and its two host plants, lemon and clementine. By doing so, we seek to better understand the factors contributing to the proliferation of this pest and to propose tailored solutions for the protection of citrus crops.

Materials and Methods

The study focused on two varieties of citrus species: 'Four Seasons' lemon (*Citrus limon* var. Eureka) and Clementine (*C. clementina*). The selection of these varieties was based on their economic importance in Algeria and the concerning health status of the orchards, degraded by the proliferation of the scale insect *P. ziziphi*, favoured by the region's climatic conditions (temperature, humidity, wind).

The goal of this study was to evaluate the impact of several factors, such as host variety, leaf age, time of year, and foliar mineral content, on the variability of infestations caused by this pest. By doing so, we aim to better understand the underlying mechanisms of these infestations and identify factors that may exacerbate or mitigate the problem.

Study site

The study was conducted at two experimental stations located in Rouiba [36° 44′ 11″N, 3° 16′ 58″ E] (altitude 20 m) and Boufarik [36° 34′ 00″ N, 2° 55′ 00″ E] (altitude 63 m). Rouiba is situated 25 km east of Algiers and only 7 km from the Mediterranean Sea, in a coastal zone. Boufarik, on the other hand, is at the north of the Blida province, approximately 35 km southwest of Algiers.

Both sites, belonging to private farms, cover an area of 10 hectares each. They are in the vast sub-littoral plain of Mitidja, a region characterized by a sub-humid climate with temperate winters, favourable to agricultural activities.

Sampling and counting

In the field, two plots of 100 trees were delineated, numbered, and divided into 10 blocks. The experimental approach was inspired by the work of Vasseur and Schvester (1957). Sampling was conducted every 10 days, from December 2015 to June 2016, for a total of 7 months, 21 samplings. The method consisted of randomly selecting 2 trees per block. On each tree, two young and two old leaves were collected from the four cardinal points and the center, at about 10:00 am. Each sampling session yielded 200 young and 200 old leaves.

The samples were placed in paper kraft bags, on which sampling information (date, exposure, direction, and site) was recorded. These samples were then transported to the laboratory for observation and counting of individuals under a binocular microscope.

After counting, the leaves were cleaned, dried, and ground into powder using a mixer. The resulting powder was sieved through a 0.02µm ø sieve and subsequently stored in glass vials for further chemical analyses.

Mineral fraction analysis of the leaves

The protocol used in the present study is described in the document "Reference methods for the determination of mineral elements in plants: determination of CA, MG, FE, MN, ZN, and CU by atomic absorption (Pinta, 1969)" based on simple calcination by increasing the temperature to 450°C. The mineral fraction obtained was analyzed to determine Sodium (Na), Potassium (K) by flame emission spectrophotometry, and Calcium (Ca), Magnesium (Mg), and trace elements: Iron (Fe), and Copper (Cu) by atomic absorption spectrophotometry using calibration curves for each element studied (standard solution of K, calcium Ca, sodium Na, Iron, and Mg).

Data exploitation and statistical analysis

In this study, a multi-factor analysis of variance (ANOVA) was used to evaluate the effect of different mineral elements (potassium, calcium, magnesium, iron, and sodium, copper) on the fluctuation of *P. ziziphi* infestations on citrus, based on two factors: leaf age and time.

Factorial ANOVA allows for comparing the mean infestation rates according to the different mineral elements and identifying if significant effects are observed for these factors. Differences were considered statistically significant when $p < 0.05$. In case of significant differences, Duncan's Multiple Range Test (DMRT) was used to separate the means. The analyses were performed using SPSS software (version 23).

Results

Temporal global abundance of *P. ziziphi* on citrus fruits

Our analysis of the overall temporal abundance of *P. ziziphi* on citrus demonstrated that leaf age significantly influenced the susceptibility of citrus to infestation. Older leaves exhibited higher infestation rates than younger leaves. These findings suggest that variations in leaf chemistry or structure associated with leaf age may underlie these differences. Additionally, our results revealed a marked preference of the scale insect for lemon as a host plant (Fig.1).

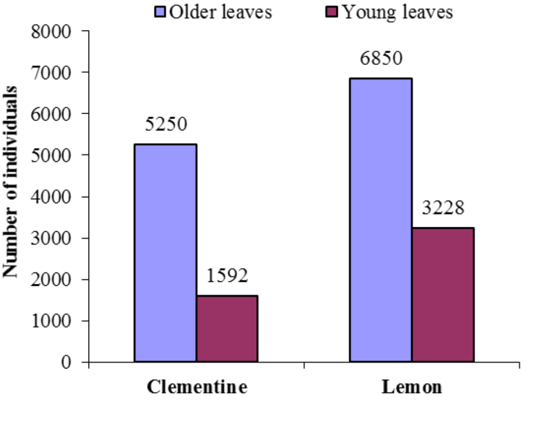

FIG.1: Comparative Evaluation of P. *ziziphi* infestations on clementine and Lemon Tree.

The results demonstrate a marked seasonality in *P. ziziphi* infestation on citrus, with peak incidences occurring in January, March, and mid-April for lemons, and extending until May for clementines. This seasonal pattern suggests a strong correlation between pest activity and both environmental conditions and host phenology. The timing of these peak infestations likely corresponds to critical life cycle stages of the insect, such as oviposition or hatching, that coincide with favorable climatic conditions (Fig. 2).

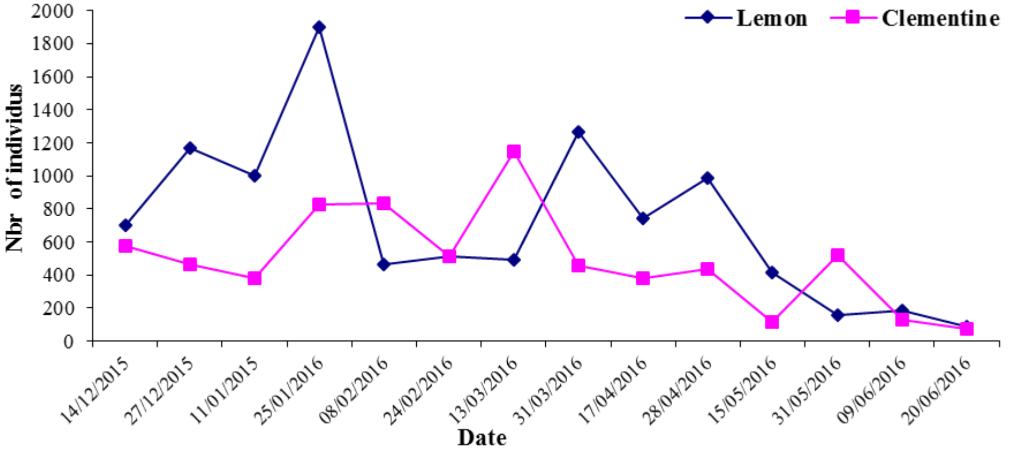

FIG.2: Fluctuation of temporal infestations of *P. ziziphi* on clementine and lemon trees.

Role of mineral elements on the fluctuation of *P. ziziphi* infestations on citrus fruits

Potassium. Analysis of variance revealed significant differences ($p<0.05$) in leaf potassium concentrations of lemon and clementine trees, depending on time of year, variety, and leaf age. Potassium levels peaked in spring (2.47% in April and 2.91% in May) and early summer (2.77% in June), indicating increased potassium demand during periods of active growth. Concentrations were significantly higher in lemon compared to clementine trees, suggesting species-specific nutritional requirements. Additionally, young leaves had slightly higher potassium concentrations (2.29%) than older leaves (2.25%) (Fig. 3, 4).

Sodium. Analysis of variance revealed highly significant differences ($p=0.000$) in sodium levels based on factors 1 (time), 2 (variety), and their interactions (1*2, 2*3, 1*3). However, leaf age did not significantly affect concentrations. Sodium levels were higher in spring, being higher in clementine (1.10%) than lemon trees (1.08%). Results suggest that sodium may have an inverse effect on this diaspid population, regardless of leaf age (Fig. 5, 6).

Calcium. A significant variation ($P < 0.05$) in leaf calcium concentrations was observed, influenced by time of year, variety, and leaf age. Maximum concentrations were recorded in winter (December: 7.38%, February: 7.28%), corresponding to a peak in *P. ziziphi* population. Older leaves showed higher (7.03%) calcium levels compared to younger leaves (7.01%). These findings indicate a possible relationship between calcium levels and *P. ziziphi* abundance, suggesting that calcium may play a role in the dynamics of this host-parasite interaction (Fig.7, 8).

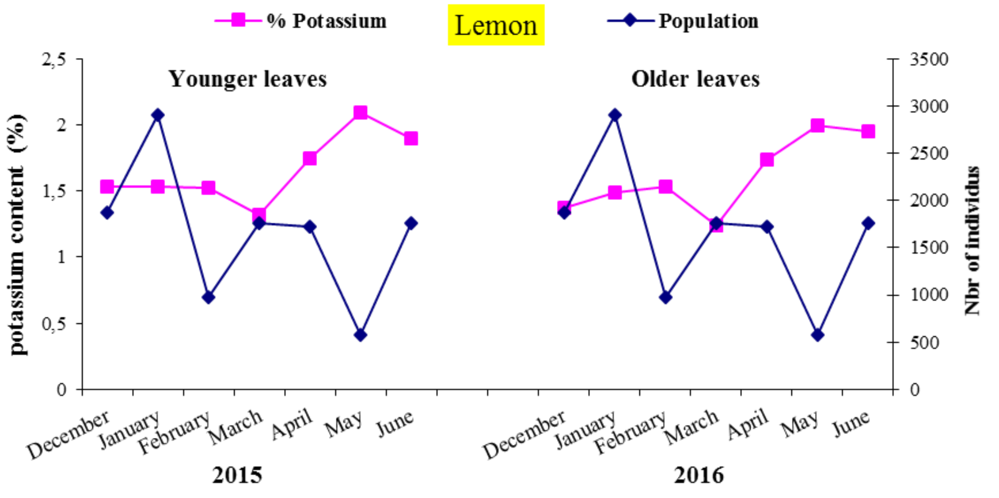

FIG.3: Fluctuations in the *P. ziziphi* populations depending on potassium levels in lemon.

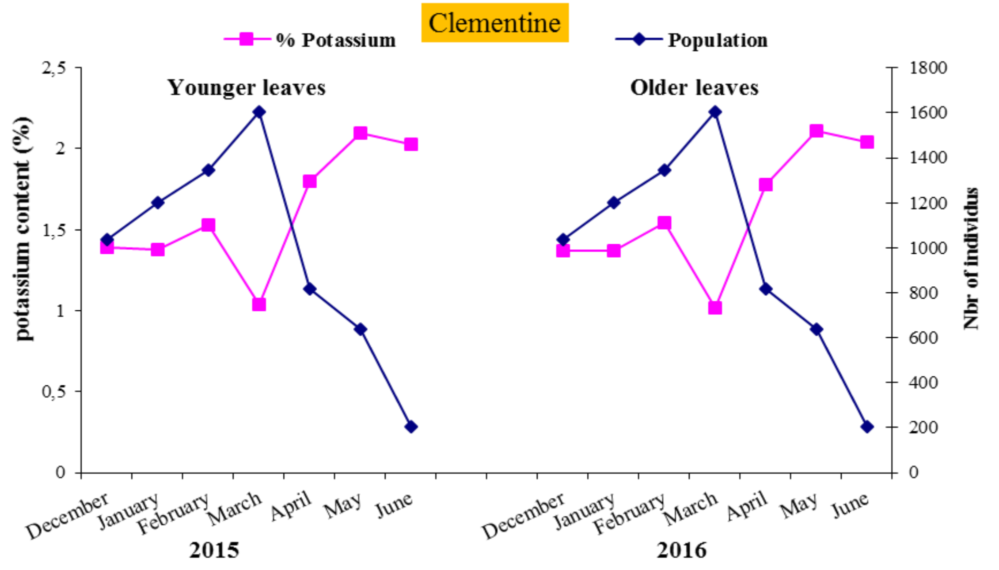

FIG.4: Fluctuations in the *P. ziziphi* population depending on potassium levels in clementine

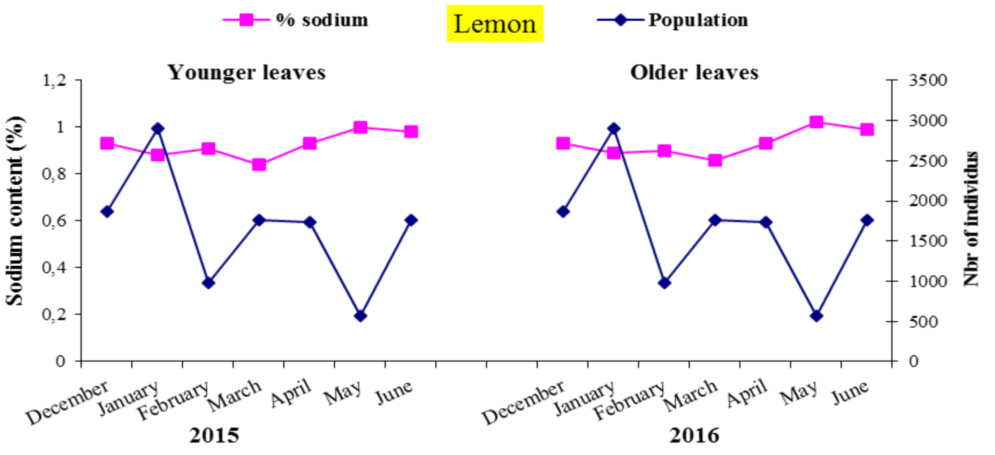

FIG.5: Fluctuations in the *P. ziziphi* population depending on sodium levels in lemon.

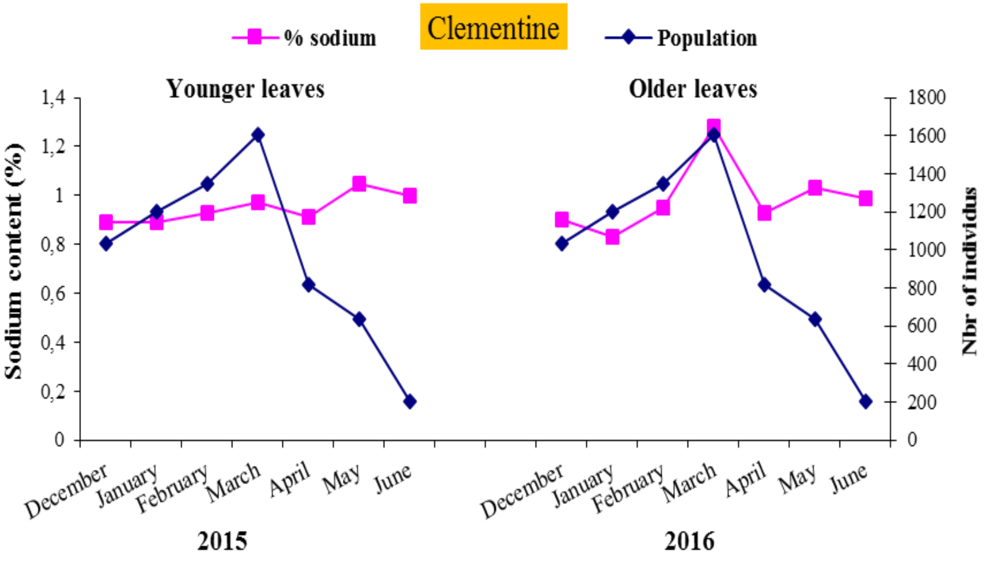

FIG.6: Fluctuations in the *P. ziziphi* population depending on sodium levels in clementine.

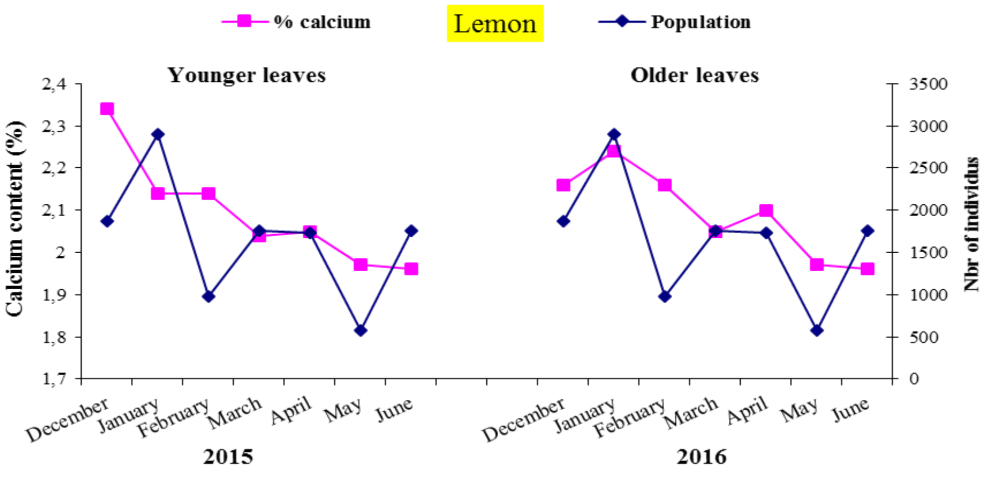

FIG.7: Fluctuations in the *P. ziziphi* population depending on calcium levels in lemon.

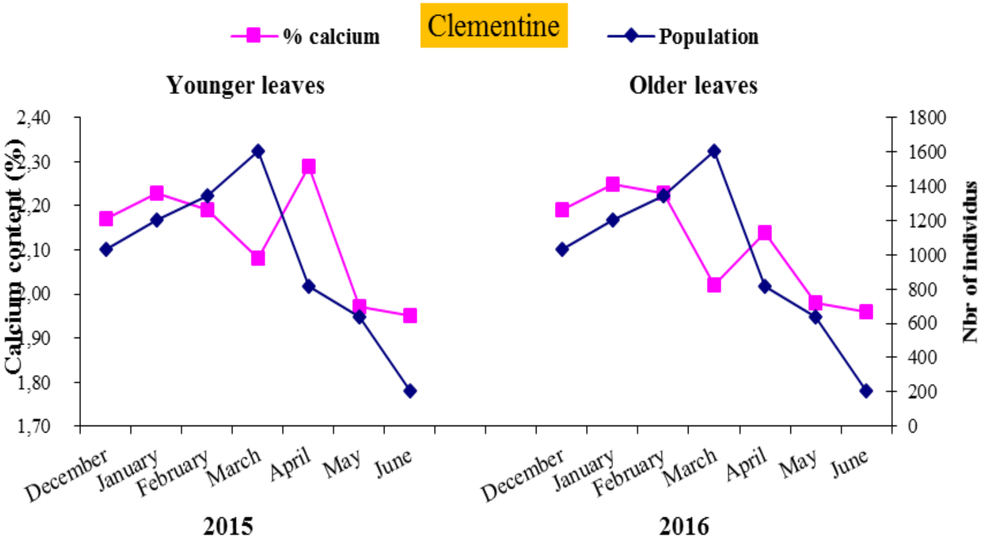

FIG.8: Fluctuations in the *P. ziziphi* population depending on calcium levels in clementine.

Magnesium. A significant variation ($p < 0.05$) in leaf magnesium concentrations was observed, influenced by time of year, variety, leaf age, and their interactions. Maximum concentrations were recorded in spring, reaching 1.26% in May. Lemon trees and older leaves exhibited slightly higher magnesium levels (0.79% and 0.77%, respectively) compared to younger leaves (0.75%). These findings suggest an inverse relationship between magnesium levels and scale insect populations, indicating that magnesium may play a role in plant resistance to this insect (Fig. 9, 10).

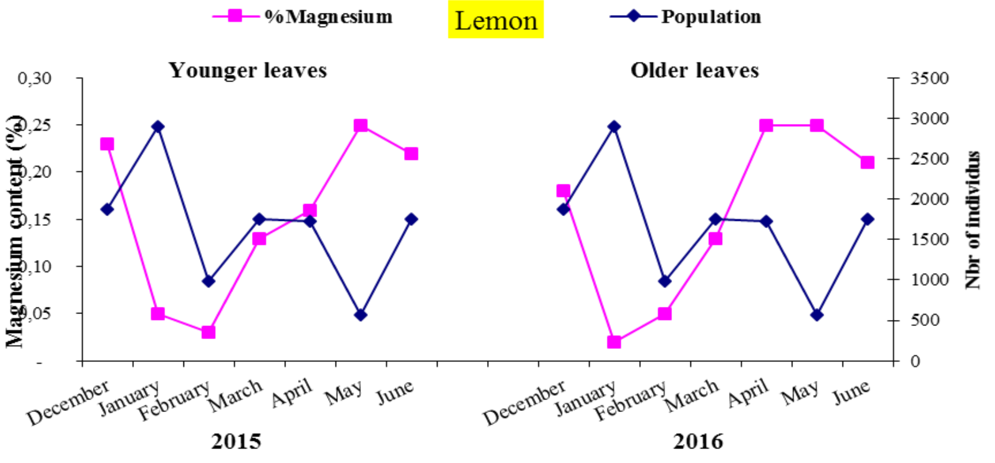

Fig.9: Fluctuations in the *P. ziziphi* population depending on magnesium levels in lemon.

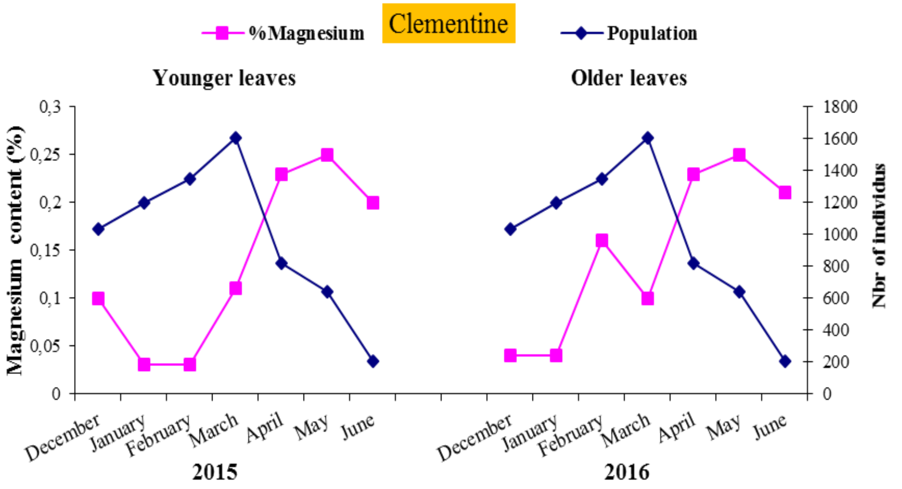

Fig.10: Fluctuations in the *P. ziziphi* population depending on magnesium levels in clementine.

Iron. Analysis of variance showed that both time (season) and citrus variety had a highly significant impact on leaf iron concentrations (P = 1/‰, 5<5%). In contrast, leaf age did not appear to play a significant role. Furthermore, we identified complex interactions among these factors, suggesting that the effects of time and variety on iron content are not simply additive. Results indicate that leaves sampled in spring had the highest iron concentrations (May/0.47 ppm), and that lemon trees accumulated slightly more iron on average (0.40 ppm) compared to clementine trees (0.39 ppm). Despite these variations in iron content, no correlation was found with diaspine populations (Fig. 11, 12).

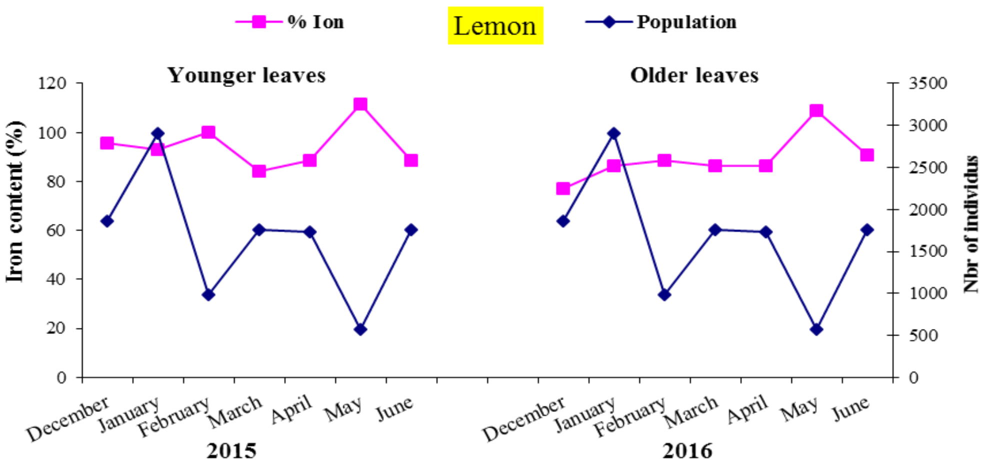

Fig.11: Fluctuations in the *P. ziziphi* population depending on iron levels in lemon.

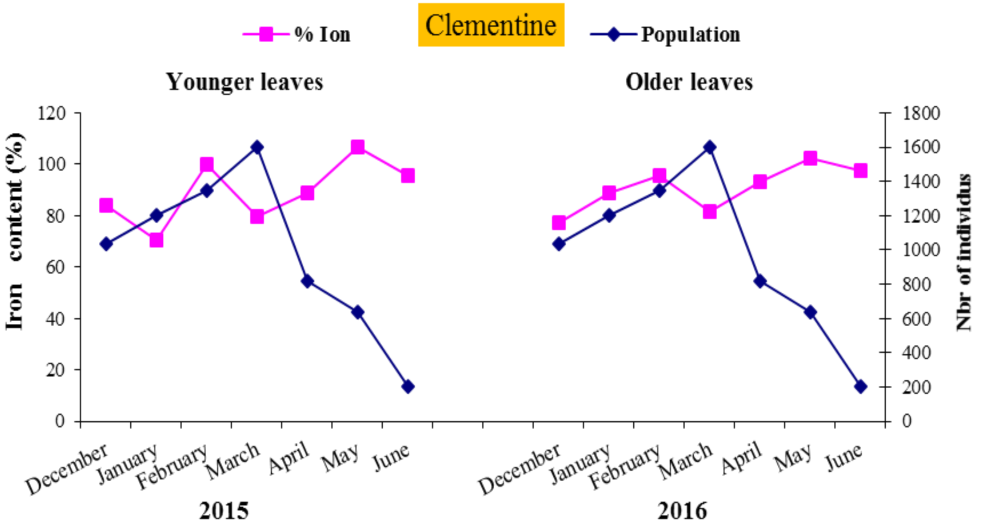

Fig.12: Fluctuations in the *P. ziziphi* population depending on iron levels in clementine.

Copper. The results of the analysis of variance indicate that there were no significant differences in copper levels among samples based on time, variety, or leaf age (P > 0.05). This suggests that copper is not a primary factor influencing the population dynamics of scale insects (Fig. 13, 14).

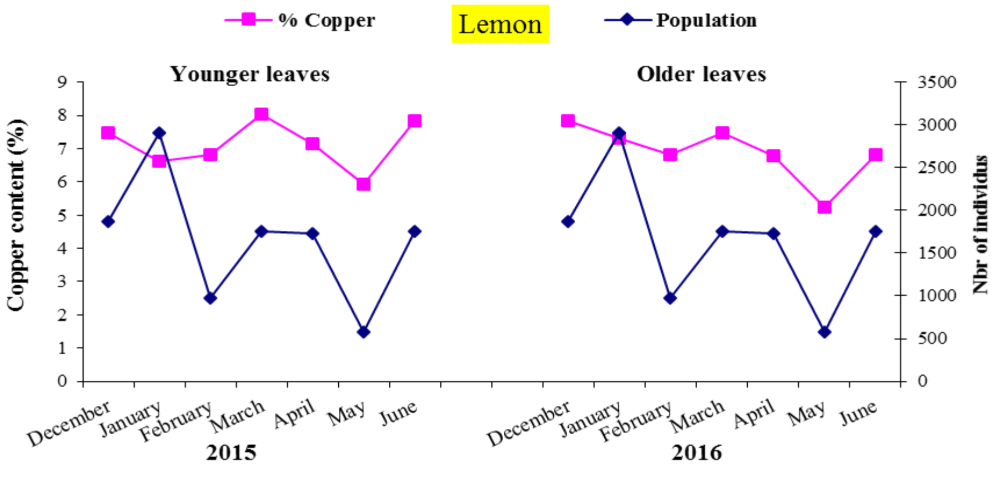

Fig.13: Fluctuations in the *P. ziziphi* population depending on copper levels in lemon.

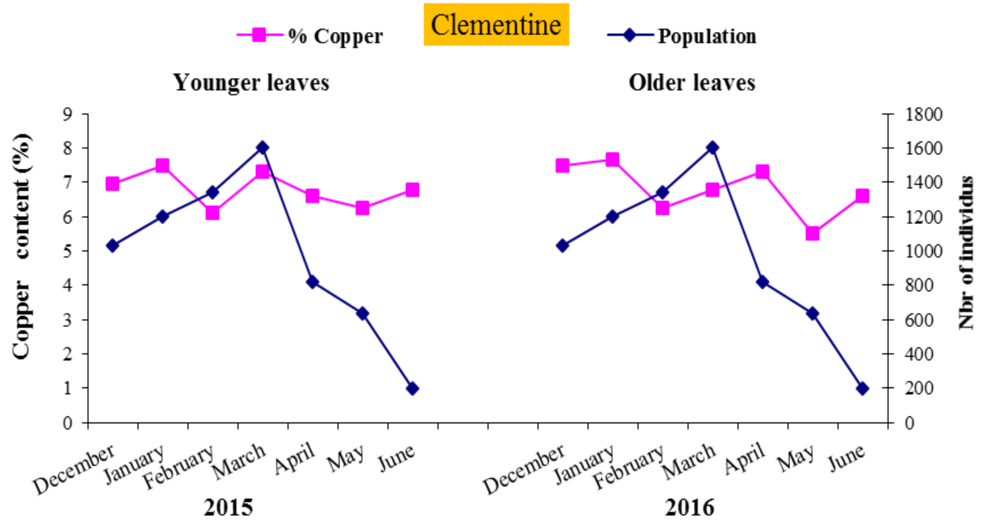

Fig.14: Fluctuations in the *P. ziziphi* population depending on copper levels in clementine.

Proteins. Analysis of variance revealed a significant positive correlation between soluble protein levels and scale insect populations. The highest protein concentrations (0.86-0.89% DM) were observed in February, March, and April, coinciding with increased scale insect populations. These results suggest that soluble proteins may serve as a food resource, promoting the development of scale insects, especially during periods of favorable climatic conditions (Fig. 15, 16).

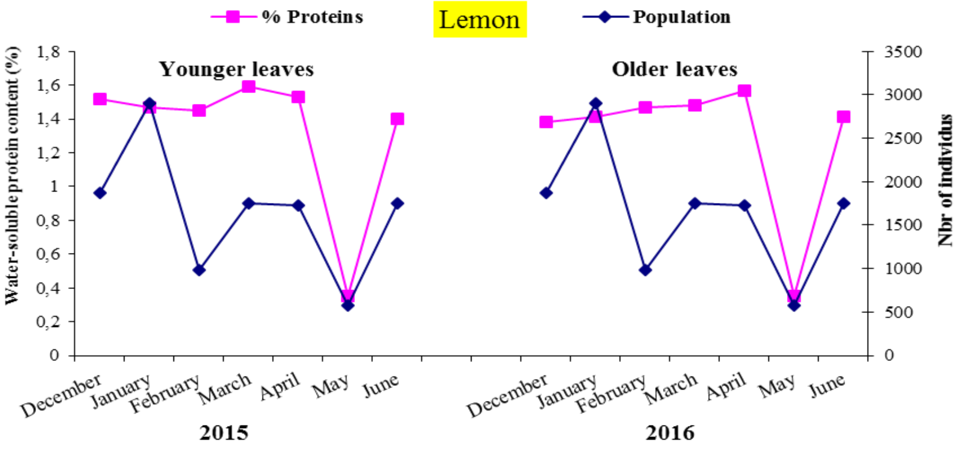

Fig.15: Fluctuations in the *P. ziziphi* population depending on protein levels in lemon.

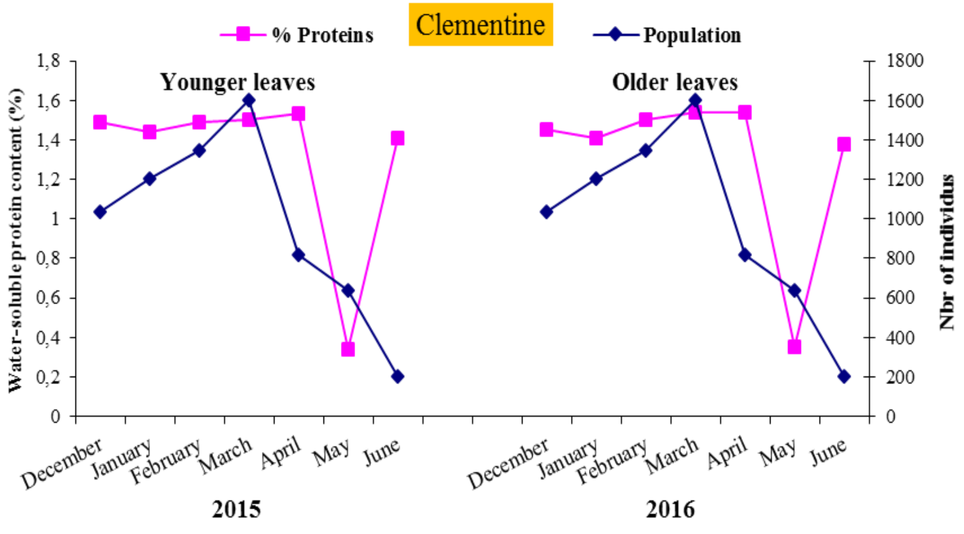

Fig.16: Fluctuations in the *P. ziziphi* population depending on protein levels in clementine.

Discussion

The study of P. *ziziphi* population dynamics on lemon and clementine trees and its interaction with mineral elements has revealed several key findings. The results indicate a clear preference of the scale insect for lemon trees and a marked seasonality of attacks, with infestation peaks coinciding with periods of active tree growth and climatic conditions favorable to insect development. Leaf age also influences susceptibility, with older leaves being more heavily infested. These findings align with previous studies (Biche and al., 2012; Belguendouz, 2014; Takarli et al., 2015; Boukhobza, 2016; Aroua and al., 2020; Boukhobza and al., 2020 (a); Boukhobza and al., 2020(b); Zaabta and al., 2020; Biche and al., 2022; Aroua., 2024) that have shown the determining role of host phenology in scale insect population dynamics.

Analyses revealed significant variations in leaf concentrations of potassium, sodium, calcium, magnesium, iron, and copper, depending on the variety, season, and leaf age. While some elements, such as potassium, calcium, and magnesium, appear linked to plant growth and could indirectly influence resistance to attacks, others, like sodium, do not show a clear correlation with scale insect populations.

Potassium seems to play a limiting role in the proliferation of this scale insect. Chaboussou (1975) demonstrated that potassium fertilizers significantly reduce scale insect attacks on citrus. Moreover, Mostefa and Boukhors (2004) confirmed that foliar potassium content has an inverse effect on the proliferation of *P. ziziphi* on lemon and clementine trees. Some studies suggest that excesses of certain mineral elements can modify the physicochemical properties of plant tissues, making them less appetizing or less nutritious for insects (FAO, IFA, and IMPHOS, 2003). Based on these results, we can conclude that optimizing citrus nutrition with potassium would be doubly beneficial, both by maximizing plant growth and yield and by reducing the multiplication of this scale insect.

Mineral elements do not act in isolation but interact with each other to influence plant physiology and their susceptibility to pests. According to Chaboussou (1975), an optimal cationic balance, characterized by adequate levels of potassium, calcium, and magnesium, is essential to limit the development of scale insects. Our results support this, showing that high calcium concentrations are associated with lower *P. ziziphi* abundance. Chaboussou (1975) observed that a certain predominance of calcium and magnesium over potassium leads to unfavourable repercussions by favouring the multiplication of *A. aurantii* and *L. beckii* on citrus. These observations underline the importance of maintaining a balanced mineral nutrition of citrus to limit damage caused by these insects.

The most striking result of this study is the positive correlation between soluble protein levels and the abundance of *P. ziziphi*. Proteins appear to be an essential food source for insect development, especially during periods of active growth. Particularly, soluble proteins emerge as a key food source for scale insects, partially explaining their rapid development during periods of heavy infestation. It would be interesting to delve deeper into this relationship by identifying the specific types of proteins that attract and promote the development of this insect. A proteomic analysis of leaves could reveal the presence of proteins induced by infestation or proteins that are particularly attractive to scale insects. Additionally, it would be relevant to study the impact of different protein sources (e.g., nitrogen fertilizers, foliar treatments) on scale insect populations.

Conclusion

The main objective of our study was the evidence of the influence of the mineral elements of the plant on the outbreak of the cochineal *Parlatoria ziziphi* on clementine and lemon.

Clementine seems to be more favourable than the lemon tree to the attacks of this scale. This is mainly due to nutritional factors, whose differences in population levels well linked to differences in the chemical composition of plants, and particularly their SAP. The rates of the mineral elements potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) were higher during the spring period, while sodium (Na) had the highest rate in winter. The rates of potassium (K), magnesium (Mg), and iron (Fe) were significantly higher in lemon compared to clementine, on the contrary to calcium (Ca) and sodium (Na), which were higher in clementine. Higher concentrations of potassium (K) and sodium (Na) were recorded for young leaves. While for magnesium (Mg) the rate was significantly higher in the older leaves. In addition, leaf age did not affect the calcium (Ca) and iron (Fe) levels, as well as time, variety, and leaf age which showed no influence on copper (Cu) levels.

Foliar diagnosis shows that an excess of calcium, or more accurately a balance of the cationic elements characterized by a certain predominance of calcium and magnesium in relation to potassium, promotes multiplication of this cochineal. However, this phenomenon can be reconciled by the fact that potassium nutrition is insufficient and there is the phenomenon of outbreak of the cochineal when the proteolysis overtakes the photosynthesis.

It also appears that a high rate of sodium (Na) restricts the number of individuals of *P. ziziphi* and that the rate of copper (Cu) and iron (Fe) have no effect on the outbreak of this cochineal.

These results open up interesting perspectives for the integrated management of *P. ziziphi*. A combined approach involving cultural practices that promote tree vigour (balanced fertilization, judicious pruning) and targeted interventions on scale insect populations during critical periods could help limit the damage caused by this insect. Further research is needed to better understand the underlying molecular and physiological mechanisms of these interactions, in order to develop more sustainable and effective control strategies.

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Τα θρεπτικά στοιχεία ως μοχλός ελέγχου του *Parlatoria ziziphi* (Homoptera: Diaspididae) στην Αλγερία (περιοχή Rouiba)

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ΠΕΡΙΛΗΨΗ

Η παρούσα μελέτη εστιάζει στη συγκριτική ανάλυση της παρουσίας του *Parlatoria ziziphi* (Hemiptera: Coccomorpha: Diaspididae) σε δέντρα εσπεριδοειδών και στην επίδραση των θρεπτικών στοιχείων στη διακύμανση του πληθυσμού του. Τα αποτελέσματα δείχνουν ότι η προσβολή από το *P. ziziphi* είναι σημαντικότερη στις λεμονιές σε σύγκριση με τις κλημεντινές (10078/6842 άτομα), ιδιαίτερα στα παλαιότερα σε σχέση με τα νεότερα φύλλα (6850/5250 άτομα). Οι εντονότερες προσβολές εντοπίζονται του μήνες Ιανουάριο και Μάρτιο. Οι διαφορές στα επίπεδα καλίου, νατρίου, ασβεστίου, μαγνησίου και σιδήρου είναι ιδιαίτερα σημαντικές (p=0,001) και μεταβάλλονται ανάλογα με την ποικιλία, την ηλικία των φύλλων και την εποχή του έτους. Το κάλιο φαίνεται να παίζει περιοριστικό ρόλο στον πολλαπλασιασμό του *P. ziziphi* στις κλημεντινές και τις λεμονιές. Οι συγκεντρώσεις του είναι υψηλότερες στις κλημεντίνες, ιδιαίτερα στα νεαρά φύλλα (2,11% τον Μάιο), όπου η προσβολή μειώνεται. Το ίδιο ισχύει και για το νάτριο, με υψηλότερες συγκεντρώσεις στις κλημεντίνες (1,28% τον Μάρτιο) και στις λεμονιές (1,02% τον Μάιο). Οι υψηλές συγκεντρώσεις καλίου και νατρίου συσχετίζονται με περιορισμένη προσβολή από το έντομο, ανεξαρτήτως ηλικίας των φύλλων και ποικιλίας. Αντίθετα, υπάρχει συγχρονισμός μεταξύ των επιπέδων ασβεστίου και της αφθονίας των πληθυσμών του *P. ziziphi* τόσο στα νεαρά όσο και στα παλιά φύλλα και των δύο ποικιλιών. Το μαγνήσιο, το οποίο παρουσιάζει υψηλότερες συγκεντρώσεις στις λεμονιές (0,79%) και στα παλαιότερα φύλλα (0,77%), έχει αντίστροφη συσχέτιση με τον πληθυσμό του εντόμου. Όσον αφορά στον χαλκό και στον σίδηρο, δεν φαίνεται να επηρεάζουν σημαντικά την παρουσία του *P. ziziphi*, ωστόσο οι λεμονιές παρουσιάζουν ελαφρώς υψηλότερη συγκέντρωση χαλκού (0,40 ppm) σε σχέση με τις κλημεντίνες (0,39 ppm), γεγονός που υποδηλώνει ότι αυτοί οι παράγοντες μπορεί να ευνοούν την προσβολή.

