REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE MOHAMED BOUDIAF - M'SILA

FACULTE DES SCIENCES

DEPARTEMENT DES SCIENCES DE LA NATURE ET DE LA VIE

N° :……..

DOMAINE : SCIENCES DE LA NATURE ET DE LA VIE

FILIERE : BIOLOGIE

OPTION : BIODIVERSITE ET PHYSIOLOGIE VEGETALE

Mémoire présenté pour l'obtention
Du diplôme de Master Académique

Par:

HADJI Toka

Intitulé

Physiological and anatomical effects of *Parlatoria blanchardi* Targ. on *Deglet Nour* variety of date palm (*Phoenix dactylifera* L.) in Biskra region

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Année universitaire : 2018 /2019

ACKNOWLEGEMENT

I would like to thank Allah the Almighty who blessed me with living up to this day and giving me the chance to finish Master's dissertation.

I am fortunate that I have been supervised by Mr. BENMEHAIA R.; words would not be enough to deliver my sincerest gratitude to him for his insightful advice and for proposing this topic and guiding me through it.

My acknowledgement goes also to Mrs GUETTOUCHI and Mr BENDIF for accepting to be in the panel of the jury for my dissertation defend.

I have to thank the National of Agronomical Research Institute (INRA) and the Technical Institute of the Saharan Agricultural Development (ITDAS).

I'm also grateful for my sister IKHLAS who supported and encouraged me throughout the year.

Furthermore, I won't forget to thank my family: My mother, my father, MAROUA, SONDOSS, and GHAYA.

Finally, I would like to thank all those who helped me in any way in my project.

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INTRODUCTION

Date palm, *Phoenix dactyifera* L., was cultivated in north Africa, the Arabian Peninsula and Indian subcontinent since ancient times. It is known for its ability to adapt to desertic conditions because of its resistance to high temperatures, drought and salinity. It is characterized by its high production and its fruit's high nutritional value that could go as far as to be one of the main commodities in the national food security (Anonymous, 2002).

Algeria ranks as the fifth most producing country of date palm with 156 thousand tons, and is the first world producer of *Deglet Nour*. Despite this, date palms agriculture today still faces some obstacles, the most dangerous of which are those related to pests such as: Bayoud (*Fusarium oxysporum*) , Khamedj (*Mauginiella scaettae*) , Boufaroua (*Oligonychus afrasiaticus*), Bouguassas (*Apatemonachus*), date moth (*Ectomyelois ceratoniae*), and the white scale insect (Achoura & Belhamra, 2016).

Parlatoria blanchardi Targ. is considered one of the most dangerous pests that infest the Arecaceae family, including date palm in all its cultivation regions. It is one of the most feared pests after Bayoud and has lately become a serious defect, especially in the newly planted regions (Saharaoui & *al.*, 2011 in Gassou, 2015). Young palm trees are more vulnerable to white cochineals than their older counterparts. The insect spreads when appropriate conditions avail on the roofs of all vegetative parts and fruit. The heavy infestation may cause death of offshoots and weak old trees. It can reduce the palm's production to 50-60 % (Munier, 1973).

This study aims to evaluate the effect of high and low infestation of *Parlatoria blanchardi* on the physiology (moist content, chlorophyll pigments) and anatomy (stomatal density, conducting vessels) of date palms of *Deglet Nour* in Biskra region. Our study is contended on leaves because they are the first and the most part infested by the insect.

I. Biological description of date palm

Date palm is considered a crucial component of the world's permanent vegetation cover over large spaces of the earth including tropical, sub-tropical, arid and warm regions.

According to De Candolle (1883) in (Ibrahim & Khalif, 2004), date palm is a native plant of the hot arid regions extending from West Africa to Iran, where it spreads to other parts of the world.

It was named *phoenix dactylifera* by Linne in 1734. The word phoenix originates from the Greek *phoinix*, which means date in Phoenician; *dactylifera* the second part of the name comes from the Greek *dactylos* which means finger, denoting the finger shape of the fruit. (Munier, 1973)

I.1. Systematic description

Date palm is a diploid, dioecious, and monocot plant that belongs to the botanical family Arecaceae which is the only family that belongs to the Arecal order with two hundred and fifty two genus (Ibrahim & Khalif, 2004). Phoenix genus includes twelve species (Munier, 1973).

According to the angiosperm Phylogeny Group III, date palm is classified as follows:

Kingdom	Planta
Branch	Angiosperm
Class	Monocotos
Order	Arecal
Family	Arecaceae
Sub-Family	Coryphyoideae
Genus	Phoenix
Species	*Phoenix dactylifera* L.

I.2. Morphological description

I.2.1. Trunk

The trunk, also called stipe of date palm, is a vertical cylinder that grows on the ground and which is about ten to thirty meters high (Ibrahim & Khalif, 2004).

Munier (1979) and Zaid & de Wet (2002) indicate that the trunk do not branch, but the growth of the basal offshoot gives it a look of a pseudo-branching. Its vertical elongation is insured by a terminal bud called phyllophor. The lateral growth of a trunk is ensured by an extra-fascicular cambium which disappears after giving the trunk its final width.

The trunk is covered with leaf bases that are enclosed in fiber to protect it from herbivorous insects and animals, as well as an insulation to reduce water loss. Through time the appearance of the trunk changes and it becomes smoother with visible remaining cicatrices of the leaves' bases.

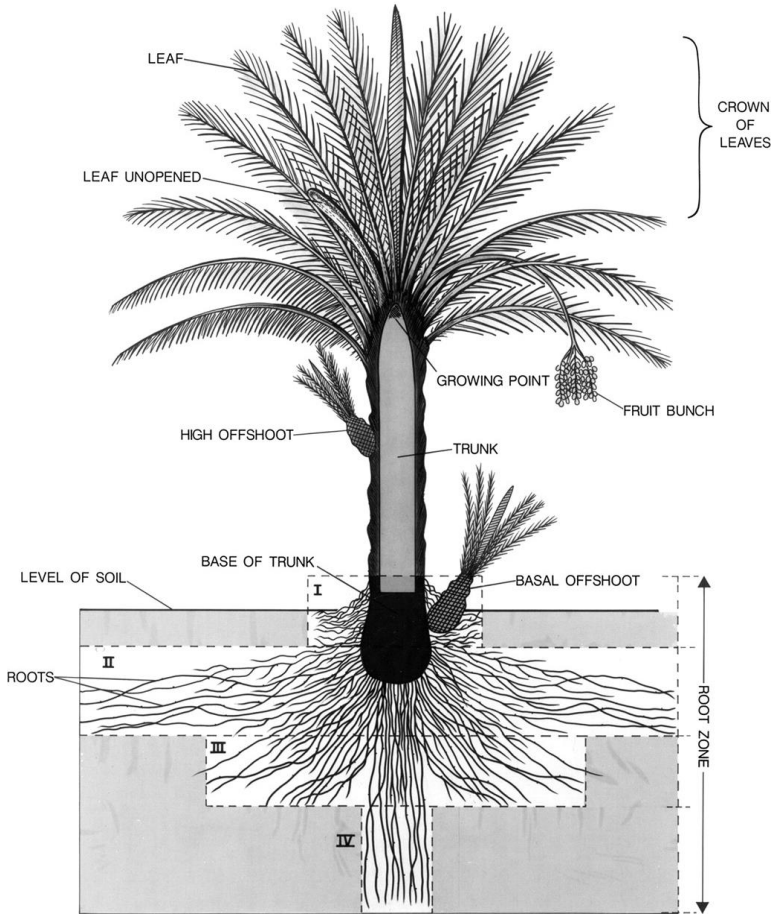

Figure 01: Diagrammatic representation of date palm structure (ChihCheng & Robert, 2007)

I.2.2. Roots

Date palm has dense fasciculate fibrous roots which sprout of an enormous bulb attached to the base of the trunk; furthermore, a part of it emerges from the soil (Munier, 1973). The roots of date palm are distinct according to Zaid and de Wet (2002), the primary roots that develop directly from the seed at the beginning have an average of four to ten meters. They produce similar but shorter secondary roots which also grow thinner tertiary roots from it.

Munier (1973) divided the roots' zone into four sections based on their function (Figure01):

- Zone I: is called the respiratory zone; it starts from the debut of the stipe base and reaches twenty five centimeters into the soil with a lateral distribution of a maximum of half a meter away from the stipe, and it has many aerial adventitious roots growing over the ground. The roots of this zone have a negative geotropism and play a respiratory role.

- Zone II: is called nutritional zone; it extends from the first zone and contains a high proportion of the roots.

- Zone III: is the absorption zone; its importance depends on the mode of culture and the phreatic surface.

- Zone VI: is the largest portion of this zone and is dependent on underground water. At a shallower depth, it becomes difficult to distinguish between this zone and the third zone. The roots in this zone are assembled in beams with positive geotropism.

The depth and the length of the roots depend on several conditions such as the quality of the soil (heavy or light soil), culture mode, water level in the soil, and cultivars among other conditions (Munier, 1973; Hocine, 1978 in Ibrahim & Khalifa, 2004).

I.2.3. Leaves

Date palm leaves, called frond, are compound, pinnate leaves growing on top of trunk and forming a crown (canopy) (Munier, 1973; Ibrahim & Khalifa, 2004).

The crown of a healthy adult date palm holds from a hundred to a hundred and twenty five green leaves with annual formation of ten to thirty new leaves. Each leaf remains active

for a period between four and seven years, then it starts to wither and become yellowish and finally droop from the crown only to be removed later (Munier, 1973).

The leaves develop from a terminal bud that generates close spirals of leaves along the trunk (Toutain, 1967).

As stated by Zaid and de Wet (2002), the crown is divided into three main parts or layers of leaves:

a- The outside is photosynthetically-active, green leaves form 50% of the whole crown. According to Tourneur and Vilardebo (1975), the tissue of this part of the crown is aged, in time, it hardens and becomes more coriaceous due to external factor such as the intense infestation by insects and fungi and the corrosion caused by the sand.
b- At the centre, there are fast growing green leaves. The leaves here form an angle of 30° with the principal axe; they are highly active physiologically speaking. (Tourneur & Vilardebo, 1975)
c- Inside, the palm's heart constitutes of white-colored juvenile leaves that are not yet photosynthetic. It is constituted of 8 to 12 leaf attached to each other (Tourneur & Vilardebo, 1975).

The leaf of date palm is a long flexible frond; its length depends on the varieties and the age of the palm. It is between 0.9-1.2 meters in young palms and sometimes reaches 4.8 meters in adult palm. It is composed of a principal axe, rachis or leaf midrib that is narrow on the top and starts to widen as it moves towards the insertion point of the petiole (Ibrahim & Khalif, 2004).

The leaflets are arranged obliquely on the rachis, which can either be isolated or gathered, all folded longitudinally. The underneath leaflets are longer than the ones on top (Munier, 1973); they are usually covered with a thick cuticle and are always coriaceous (Toutain, 1967).

The leaflets are transformed into spines just before the base of the petiole; this latter is attached to a fibrous leaf-sheath which covers the trunk and also helps protect (Munier, 1973; Ibrahim & Khalifa, 2004).

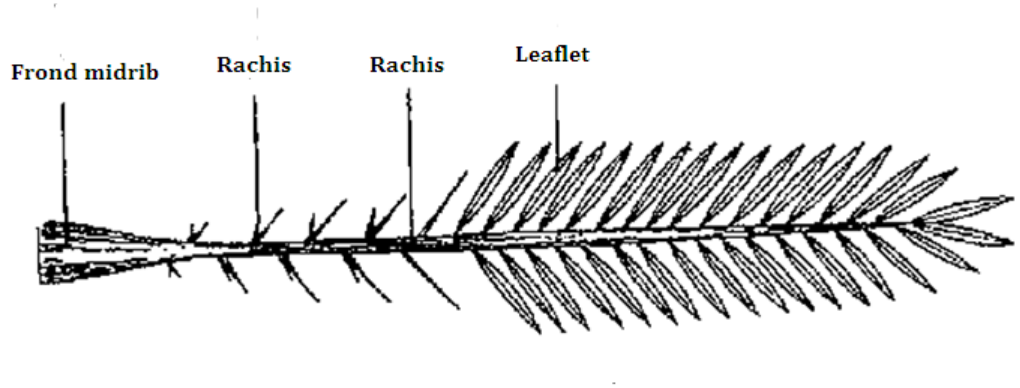

Figure 02 : Diagram of date palm leaf (Zaid & de Wet, 2002)

I.2.4. Reproductive organs

Date palm is a dioecious species with both male and female flowers being produced in clusters (inflorescence) on separate palms and is called spadixes or spikes. The clusters emerge from an auxiliary bud at the base of each leaf of the previous year's growth.

The unisexual flowers (pistillateand staminate) are sessile; they are attached to a fleshy central stem with several spikelets (usually 50 - 150 lateral branches), all of which are enfolded by a membranous sheath that remains entirely closed as long as the flowers are not mature : the spathe. The spathe protects the delicate flowers from shriveling caused by the intense heat until they mature and begin to function. Following maturation, the spathe splits longitudinally exposing the entire inflorescence for pollination purposes.

The male flower with a slightly elongated form is sweet-scented and generally has six stamens. It is surrounded by waxy scale-like petals and sepals; each stamen is composed of two little yellowish pollen sacs.

The female flower, on the other hand, is of a diameter of about 3 to 4 millimeters and has rudimentary stamens and three carpels closely pressed together with the ovary placed above (hypogynous). The three sepals and three petals are connected together so that only the tips diverge.

The color of male and female calyx and corolla are ivory-white, with the female calyx having green color around the edges (Munier, 1973; Zaid & De Wet, 2002).

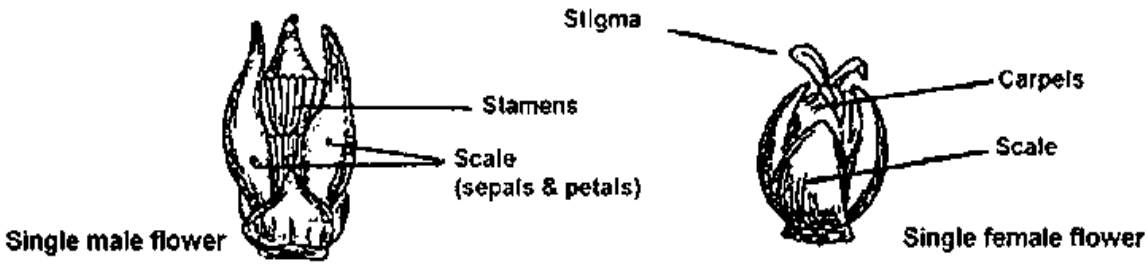

Figure 03: Diagram of male and female flower of date palm (Munier, 1973)

I.2.5. Fruit

The fruit is a one seeded, oblong berry composed from the outside inwards from: an exocarp which consists of an epidermis covered with a cuticle, they both cover a fleshy fibrous mesocarp, which constitutes most of the fruit. This latter is divided into an outer-mesocarp and an inner-mesocarp, with 3-10 layers of tanninferous cells filling the space between them. The final layer is the membranous endocarp which consists of one layer of small cells that separate the mescarp from the seed (Al-Awdat & El-Deajy, 1992 in Sakr & *al*., 2010).

The shape, the size and the color of the fruit depend on the variety, and it measures from 2-11 centimeters in length and from 1-3 centimeters in width. The color of the fruit varies from pale yellow, amber translucent yellow, brown, dark or red (Ibrahim & Khalif, 2004).

Normally, date palm pollination is anemogamous, but to increase the probability of female flowers getting pollinated this needs to be done artificially. Only one ovule per flower is fertilized, leading to the development of a single carpel which in turn produces a fruit, the date, while the other ovules are aborted. The aborted carpels persist as two brown spots in the calyx of ripe fruits. In case of non-pollinated flowers, an immature seedless fruit develops via parthenogenesis (Munier, 1973).

Throughout the year, a date palm generally produces five to ten bunches of fruit; a mature tree produces up to 150 pounds annually (El-Hadrami and Al-Khayri, 2012 in Radwan, 2017).

II. Date palm white scale *Parlatoria blanchardi* Targ.

II.1. History

Date palm white scale, *Parlatoria blanchardii* (Targioni-Tozzetti), is one of the most damaging pests known in most parts of the world, including Algeria. It was discovered for the first time in the Algerian desert in 1868, in the palm groves of Oued Rhig by Blanchard. It was Stickney (1934) and Balachowsky (1953) who gave the final total description of the insect (Smirnoff, 1957).

Date palm white scale have various common names; sometimes it has many names in the same country: cochenille blanche in French, parlatoria date scala in U.S.A., Djreb, sem, Elmen in Algeria, Nakoub, Guelma, Tilichte, Tabkhocht, Tasslacht in Morocco, and Klefiss, Rheifiss in Mauritania (Munier, 1973).

II.2. Classification

Date palm white scale *Parlatoria blanchardi* (Targioni-Tozzetti) is a typical species of the family of diaspididae (Munier, 1973).

Depending on the classification of cochineal that Balachowsky (1954) has proposed, *Parlatoria blanchardi* is classified as follows: (Achour, 2013)

Enbranchement	Arthropoda
Class	Insecta
Division	Exopterygota
Super-order	Hemipteroidea
Order	Homoptera
Sub-order	Sternorrhyncha
Super-family	Coccidae
Family	Diaspididae
Sub-family	Diaspididae
Tribu	Parlatorini
Sub-tribu	Parlatorina
Genus	Parlatoria
Species	*Parlatoria blanchardi* Targioni-Tozzetti

II.3. Geographical distribution

Parlatoria blanchardi is thought to be native to Mesopotamia (Munier, 1973) and Southern Mesopotamia (Toutain, 1967). It can be found in Middle East countries such as Turkey, Iraq, Palestine, Syria, Jordan, Saudi Arabia Iran, in addition to India as well as Turkestan in south Asia where it was detected out for the first time in 1935.

The insect gradually spread over North Africa through the East boarders when date palm offshoots were transported there, in Egypt, Libya, Sudan, Somalia, Sahara, Mauritania, Niger and Chad, and also Algeria (Munier, 1973; Malumphy, 2013). Idder (1991) reported that all phoenicicol regions in Algeria are infested with date palm cochineal.

According to Munier (1973), *Parlatoria blanchardi* spread through the eastern oasis of Algeria startinginTimimoun, 1912; Colomb Bechar, 1920 ; Boussaada, 1925 ;El-Golea, 1926 ; Tidikelt, 1928; Saoura, 1930 All the oasis from Biskra to Ouargla (Balachowsky ,1932); In Marocco, it was observed in Figuig, 1937 ;Tafilalt, 1938 ; Bani et Tata, 1940 ; Goulmina, 1951.

Generally, it exists in areas where date palm is cultivated (Alhadj & Aldaghiri, sd.) except for U.S.A where its extinction was declared in 1936 as a result of an eradication campaign following its transportation there in 1890 via an Algerian plant material (Munier, 1973) where it affected regions of the U.S.A such as California and Arizona (Laudho & Benssy, 1969; Munier, 1973). It has also been transported to Australia in 1894, to Brazil in 1929, and to Argentina through the new plantations of Turkestan in 1935 (Smirnoff, 1954 in Achour, 2013).

Smirnoff (1957) noticed that the initial centers of contamination are located in date palms planted near villages, markets, tracks and rivers infected due to transmission of the pest from regions contaminated by white scale insect.

II.4. Dispersion

The dispersion happens only during the first instar crawler stage which lasts a few hours (36-48 h); thus, it can travel very short distances from twenty to fifty centimeter (Laudeho & Benassy, 1969).

The infestation is either natural or artificial:

a- Natural infestation

Wind is one of the main natural factors that control the infestation to other oases near the infected one. This type of dispersion only transports larva for short distances. (Hoceini, 1977 in Achour, 2013). Other factors that are considered to be as serious as the wind are water irrigation, density of plantation, in addition to birds that build their nests in the oases such as sparrows (Djoudi, 19992 in Mehaoua, 2006).

b- Artificial infestation

According to Smirnoff (1957), human activity is by and far the most vital reason for the dispersion of date palm cochineal. These insects are carried and transmitted in clothes and animal fur, but they are also transmitted through commercial activities such as selling date palm parts including the trunk, fruit, leaves, offshoots, and also male inflorescence.

II.5. Morphological description

II.5.1. The Egg

Parlatoria blanchardi Targ. eggs are elongated in shape and are about 0.04 millimeters in length, they have a pale pink color, with delicate external envelop. Eggs are laid underneath the scale cover of a female which lays from 6-9 egg, and it can reach up to 59 eggs (El-Haidari, 1980 in meahaoua, 2006) and the incubation period lasts for two days (Tourneur & Lecoustre, 1975)**.**

II.5.2. Nymph

Nymphs are flesh-colored or pale lilac (Smirnoff, 1957), of a length of about0.3 mm with a round white scale (Al-Juboori, 2018).

II.5.3. Female

The young immature female has a pink color which turns into lilac as it grows. The adult female's color gets darker or turns wine red (Madkouri, 1975 in Mehaoua, 2006). According to Dhouibi (1991) in (Mehaoua, 2006), females are largely oval in shape and flattened during all stages, measuring 1.2 to 1.6 mm in length and 0.3 mm in width. Females have a white scale with brown spots on it and measure from 1.3 to 1.8 mm in length and 0.7 mm in width. They live 5-25 days (Watson, 2002).

II.5.4. Male

Males have a white elongated scale measuring 1mm in length and 0.4 mm in width. After hatching, males turn into a yellowish red color, they are winged, with highly developed transparent wings (Mehaoua, 2006).

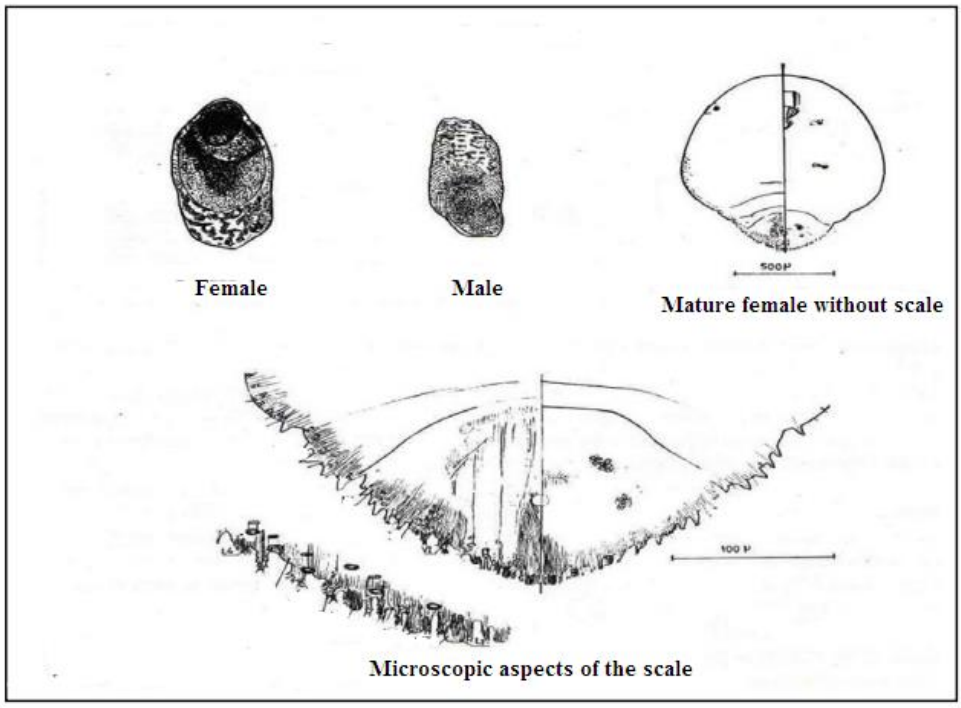

Figure 04: Microscopic characteristic of adult female *Parlatoria blanchardi* Targ. (Smirnoff, 1957)

II.6. Life cycle

Winged males fly during March, May and Juin to fertilize females in young palms, while females fixed on adult ones are often fertilized by micropterous males. The eggs laid under the scale take from 18-20 days to fully mature inside the female insect (Smirnoff, 1954 in Achour, 2013). The spawn lasts for two weeks during early spring, and from two to six days during summer. The eggs that are laid under the scale of the female hatch and the new nymphs stay under the scale for a while before leaving it (Balachowsky, 1950 in Mehaoua, 2006).

According to Laudeho (1969), males and females develop differently starting from the second stage (Annex 07) . The nymph of the first stage walks a short distance before it settles on the leaf and feed on its sap and secretes a white scale. After the first molting, it secrete

another flattened scale with the old one inside it, the result is a nymph of second stage. The second molting gives an adult female in which the scale contains the two previous scales.

The male develops differently, they go through two ecdysis phases forming a protonymph (prenympic) and then a deutonymph (nymph) under the scale before it becomes an adult and leaves it on the leaves.

The dry and hot climate seems to be suitable for cochineal development according to Smirnoff. (Iperti, 1970). The duration of a *P. blanchardi's* life cycle varies from 50 to 60 days during the hot season to 75-85 days in spring and autumn, and can reach 130 to 150 days in winter. In winter, the nymphs of the second and the third stage diapause. The trophic and climatic conditions of the region control the proliferation activity of the insect and also the number of annual generations of Cochineal (Iperti, 1970).

The *Parlatoria blanchardi* is recorded to have three to five overlapping generations annually (Watson, 2002). Nonetheless, it was noted that there is an overlap and rapprochement between generations which make it difficult to define precisely their exact number (Abd El Ahad & Jassim, 1983).

II.7.Insect damage

The infection appears in a waxy oval form, white to gray scales measuring 1-1.5 mm on the leaves of date palm. These scales are the exuviae and waxy secretion left by the insect on the leaves. (Alhadj & Aldaghiri, sd.)

At high population densities, infestation covers the fruit bunches and the fruit stalks. Dense populations may impair development of the palm and cause the fruits to shrink, rendering them unmarketable. In extreme cases it may cause deterioration of the palm. (Blumberg, 2008). Munier (1973) denotes that cochineal feeds on the sap it sucks using its rostrum while, at the same time, it injects a certain amount of a toxin that modifies the chlorophyll resulting into impeding photosynthesis and respiration.

II.8.Insect control

Managing the date palm white scale insect is laborious (Toutain, 1967). Thus, Different methods are applied to control and eliminate it (Idder, 2011).

Iperti (1970) mentioned that certain agricultural, physical, and biological methods can be used jointly or seperately. According to Idder & *al.*(2007) , only three of these methods are used in Algeria.

II.8.1. Physical method

According to Pagliano (1934) in (Idder, 2011), the physical method consists of pruning all the leaves of the crown except those of the heart; pruning can either be partial by trimming and then burning the leaves of the outside of the crown covered with the cochineals, or total in cases when the infestation is more serious and the insect covers all the parts of the crown. The removed leaves are burned at the bottom of the plant trunk (Idder & *al.*, 2007). When burning is done, salty hot water is poured over the remaining leaves of date palm crown (Iperti, 1970).

II.8.2. Agricultural method

This method is the first and less difficult one to process with; the plant is taken care of keeping the vegetative system of the plant healthy. This can be achieved by assuring the plant needs of water by regular irrigation of the grove, enhancing the plant nutrition by fertilization, and pruning and removing surplus offshoots (Iperti, 1970).

II.8.3. Chemical method

The chemical method consists of spraying organophosphoric insecticides on the tree (Idder & *al.*, 2007). Insecticide spray is used on young date palms which facilitates its spread to the infested part of the crown. There are many chemical products used to kill date palm cochineals, and they include: 7% lime sulfur, sulfuric acid, ferrous sulfate (Delassus & Pasquier, 1931in Idder & *al.*, 2007). Cyanhyfric fumigation is also used according to Toutain (1967) and Iperti (1970), although, it requires a specific material which make it expensive.

The chemical method is as efficient as physical method with a mortality percentage of 73.1-80% (Idder & *al.*, 2007).

II.8.4. Biological method

This method consists of using the natural predator of *Parlatorai blanchardi* Targ. When the insect is accompanied with its predator, its extension regresses and the damage is reduced (Toutain, 1967). Introducing natural enemies was used since a long time and it is remarkable both for its frequency and its results (Idder, 2011).

I. Presentation of the study area

Biskra Province is located at the southeast of Algeria, exactly in the eastern part of the northern Sahara at a height of 124m above the sea level; with latitude 34,48°N and longitudes 05,44°E. It is bordered by the wilaya of Batna on the north, Msila to the northeast, El-Oued from the south and from the southwest by Djelfa.

Biskra provides more than 30% of the national date production (5.162.934 quintal) of which 35% is *Deglet Nour* (Messak & *al.*, 2008 in Saighi, 2015).

The station of our study is the palm grove of the Technical Institute of the Saharan Agricultural Development (ITDAS) at Ain Ben Noui, Biskra. The total surface of the grove extends along 20.4 hectares, of which 14 hectares are date palm groves. The total number of date palm trees is1645 tree: 1262 Dglet Nour, 152 Mech degla, 124 Ghars, 107 Dgoul. (ITDAS, 2018).The date palm trees of these groves were planted during the French occupation, but then some of them were removed and replaced by other offshoots between 1991 and1995. In this station, *Parlatoria blanchardi* infested only the younger palm trees of *Deglet Nour*.

Figure 05: Geographic situation of ITDAS (Google earth, 2019)

II. Material and methods

II.1. Plant material

The plant material is composed of one variety of date palm, *Deglet Nour*, this variety is rich in sucrose which is preferable by the date palm white scale insect (Khlil, 1989 in Nadji, 2011). Our choice for *Deglet Nour* stems particularly from its being considered one amongst the most infested varieties according to Idder-Ighili & *al.* (2013).

In the station of ITDAS Ain Ben Noui, only the younger palms of *Deglet Nour* are infested, the area where the offshoots are planted is divided into two distinct parts that lay close to each other but with an obvious difference in the infestation level:

- Part 1: in the area where the younger palm trees are planted after removing all the tall trees between 1991-1995, we picked first random sample marked as Sample 1.
- Part 2: some older tall trees were removed and replaced with new offshoots, as the tall trees weren't removed they shadowed the offshoots that are planted between them. We randomly picked our second sample from these offshoots and marked it as Sample 2.

The two samples are the same age and grew under the same ecological conditions.

(A) **(B)**

Figure 06: Experimentation site.
(A) Part1. **(B)** Part2.

II.2. Counting of cochineals to determinate the infestation rate

To determine the infestation rate we chose two fronds from each sample, one from outside of the crown and the other from the inside. We used secateurs to gently remove three sample leaflets from each frond as follows: one leaflet from the top, one from the middle, and one from the base of the frond. The leaflets then were placed in kraft paper bags, the date of sampling and the position of leaflets were indicated on each bag, and were brought to the laboratory.

Under the stereo microscope, we count the cochineals at X40. The count on each sample leaflet is repeated three times, once at the base, once at the middle, and once at the top of the leaflet for the upper and the lower face according to the method of Euverte (1962) in (Laudeho & Benassy, 1969). While counting, we must take into account all the stages of growth of the insect; the living insects as well as dead ones.

For each face of the leaflet we get three values marked as A1, A2, and A3. The density of cochineal's population on each face is given by the following formula:

- For the upper surface: $fs = \frac{A1+A2+A3}{3}$
- For the lower surface: $fi = \frac{A1+A2+A3}{3}$

The total density of cochineals in the leaf is the average of the density on both upper and the lower surfaces:

$$Dt = \frac{fs + fi}{2}$$

II.3.Physiological parameters

II.3.1. Moisture loss

In order to calculate the moister loss in the leaves, we sampled tree leaflets from the fronds of the outside and inside of the crown (top, middle, base) for both Sample 1 and Sample 2. The leaflets were immediately brought to lab and washed gently with water to remove all the insects and dust, the leaflets, then, were dried well using absorbent paper. We measured the fresh weight of the leaflets and recorded it. Thereafter, we placed the leaflets in an electric oven at 103°C for 24 hours and reweighted them; the dry weight of the leaflets was recorded.

The lost moisture percentage was calculated using the formula:

$$\text{Lost moisture}(\%) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \text{ X } 100$$

II.3.2. Chlorophyll concentration

II.3.2.1. Optical concentration

To estimate the optical relative chlorophyll concentration of the leaves we used a chlorophyll-meter *in situ*. Before we pressed the meter's chamber to the leaflets we had removed all the scales of the insect from both surfaces of the leaflets with paper tissue to obtain good optical measurements. The value given by the chlorophyll meter was recorded.

II.3.2.2. Absolute concentration

To study the effect of *Parlatoria blanchardi* on the photosynthetic pigments of the infested leaves, the leaflets were sampled from outside and inside the crown of Sample 1 and Sample 2. The leaflets were wrapped in the aluminum paper and brought to the laboratory where we washed them to remove any residues on the surfaces, and we dried them gently using absorbent paper. We cut the leaflets into very small pieces that weighed 0.5g each.

To extract chlorophyll pigment we used the method of Harborne (1973), whereby we grounded the fresh tissue in a mortar for 5 minutes, then gradually added 40ml of 95% ethanol and continued grounding with adding 0.1g of $CaCO_3$ to prevent the formation of pheophytin. After that, the extract was filtered in the flask using a filter paper. The extract must be protected from the light by wrapping the flask with aluminum paper to prevent any degradation of the pigments.

To measure the absorbance of the extract at 663 and 645nm, we first calibrated the spectrophotometer with ethanol 95%; next, the cell is filled with the extract and placed in the spectrophotometer. The concentration of pigments was calculated using the following formula:

$$\text{Total chlorophyll (mg/l)} = 20.2\ A_{645} + 2.08\ A_{663}$$

$$\textit{chlorophyll a}\ \text{(mg/l)} = 12.7\ A_{663} - 2.69\ A_{645}$$

$$\textit{chlorophyll b}\ \text{(mg/l)} = 22.9\ A_{645} - 4.68\ A_{663}$$

II.4.Anatomical parameters

II.4.1. Stomatal density

As the insect settles on the surface of the leaf, we tested the effect of it on the stomatal density. We used the imprint method whereby the leaf surface was cleaned through applying adhesive tape several times until the last piece of tape appears clean.

Subsequently, we applied a thin layer of transparent nail polish on both surfaces and let them dry; the layer was removed using a dissecting forceps and put on a microscope slide with cover slip on top of it. We counted the stomata under the microscope at X400 and calculated the stomatal density as follows:

$$\text{Stomatal density}(\text{stomata}/\text{mm}^2) = \frac{\text{Ns}}{0.785}$$

In which Ns is the number of counted stomata and 0.785 is the surface of the field of view using X400 (corresponding to OPTIKA microscope)

Our statistical analysis was done using Microsoft Excel

II.4.2. Leaf transverse sections

We prepared transverse sections of the infested leaflets of the two samples under study. The leaflets were first cleaned with water and dried; to facilitate getting a thin section, we placed the leaf into a split that we made in a thick slice of potato and fixed it with rubber bands. We cut the leaflet into section as thin as possible using razor blade. The sections were placed in a watch glass containing diluted bleach (2.6% concentration) for 20 minutes and then rinsed in distillated water to fix the structures. We observed the section under the microscope at 100X and 400X.

III. Results and discussion

III.1. General aspects of the injury

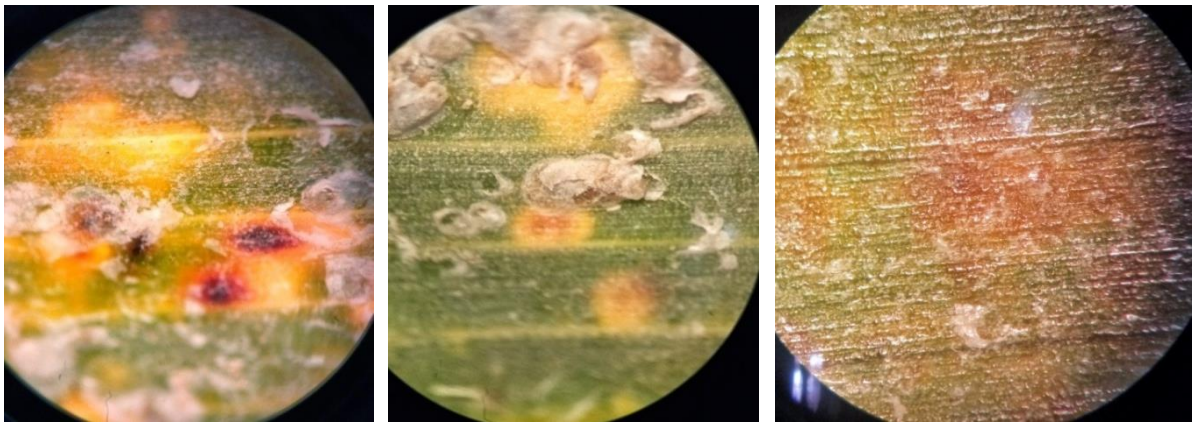

Figure 07: Observable symptoms of the infestation by *Parlatoria blanchardi* Targ**.**

The observation of the infested leaflets under the stereomicroscope showed the presence of yellowish to brown spots in places where the insect settled. Beneath the scales of the insects the centre of the spots has a darker brown color; these spots are a manifestation of chlorosis which is commonly associated with armored scale infestations according to Beardsley (1975).

As Chlorosis is defined as a lack in chlorophyll production due to mineral elements deficiency in the phloem, causing a change in the leaves color (Raven & *al.*, 2007). This transfomration in color is attributted to two principal reasons directly related to the insect of the type pierrcing-sucking : penetrating the plant tissue using its stylet, the insect sucks the sap from all the green parts of the plant which turns the infected area from dark green to pale green or yellow, with some brown spots on (Abd El Ahad & Jassim, 1983). On the other hand, it secretes toxic saliva and plant pathogenes that cause malformed leaf and modify the chlorophyll(Arbab & Nakry; 2016; Munier, 1937; Mansour & *al.*,2017).

III.2. Infestation rate

III.2.1. General infestation rate

The result of the general infastation of sample 1 and sample 2 are presented in Figure 08.

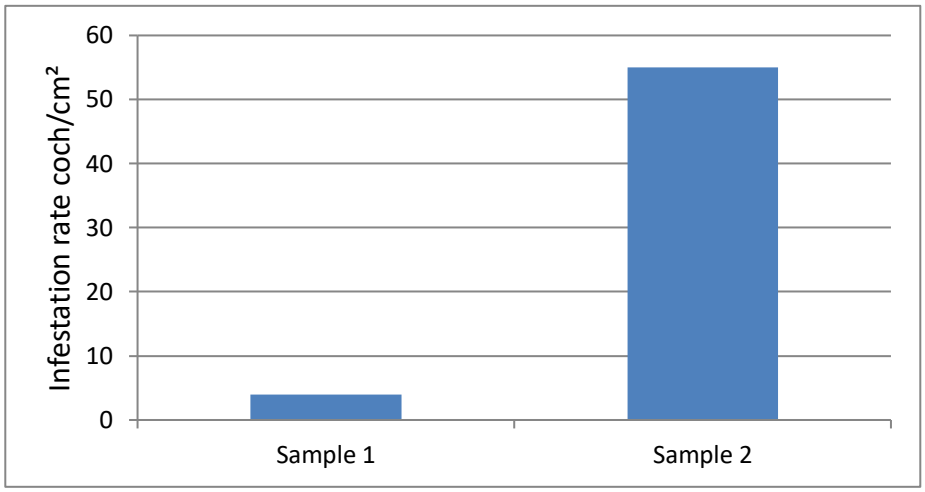

Figure 08 : Infestation rate of sample 1 and sample 2

The histogram of Figure 08 shows that there is a difference in infestation rate of sample 1 with 4 coch/cm² and sample 2 with 55 coch/cm².

The analysis of variance of the infestation rate of sample 1 and sample 2 (Annex 1.1) showed a significant difference between Sample 1 and Sample 2, $F(3.97) = 27.55$, $P<0.05$.

III.2.2. Infestation rate in terms of foliar crown

The results of the infestation rate of the lower and upper crown are given in figure 09.

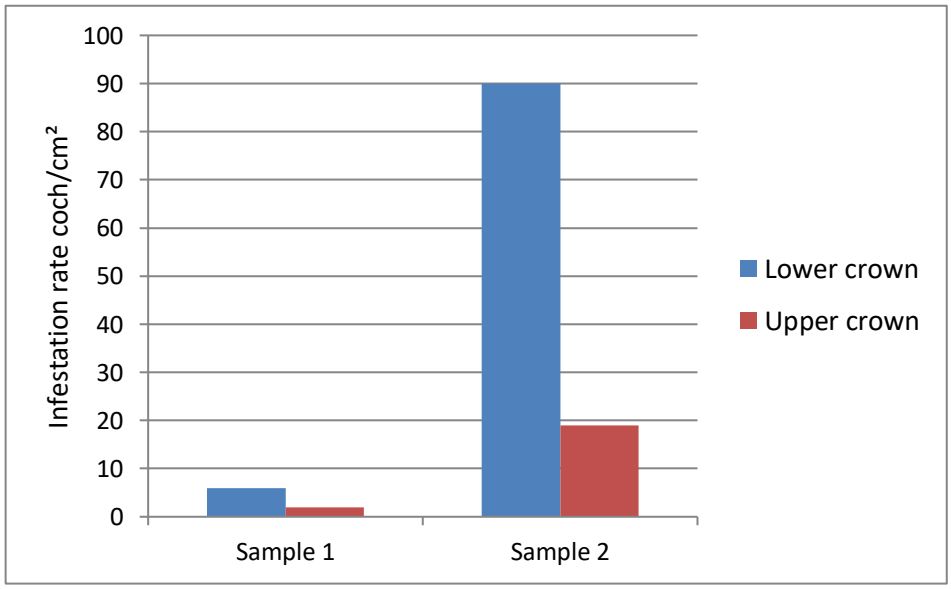

Figure 09: Relationship between the infestation rate and the crown level

The results obtained in Figure 09 indicate that the infestation rate of the lower crown is higher in sample 2 with 90 coch/cm² than in sample 1 with 6 coh/cm², in the same way it is higher in the upper crown of sample 2 with 19 coch/cm² than in sample1 with 2 coch/cm². White cochineals infested the lower crown more extensively than the upper in the two samples. These results match those of Allam (2008) in Algeria.

To test if there is a significant effect of the crown level on the infestation rate we used the two-way ANOVA (Annex 1.2). The analysis of the variance shows a significant difference between the two levels of the crown, $F(3.98) = 22.8$, $P<0.05$. The interaction effect between the crown level and infestation rate is also significant, $F(3.98) = 18.87$, $P<0.05$.

III.2.3. Infestation rate in term of leaflet surface

The results of the infestation rate of both sides of the leaves are shown in figure 10.

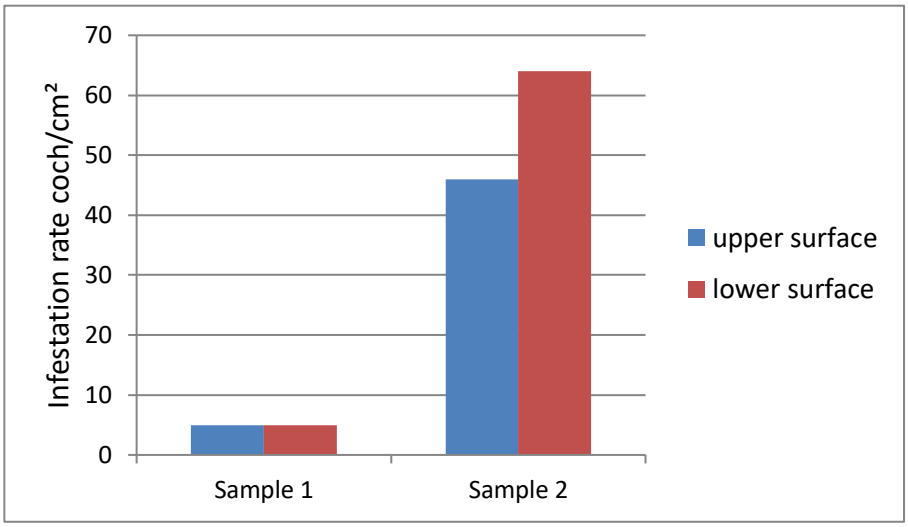

Figure 10: Relationship between the infestation rate and leaflet surface

The results obtained in figure 10 indicate that the infestation rate of the lower surface of the leaves is higher in sample 2 with 64 coch/cm² than in sample 1 with 5 coch/cm, it is also higher in the upper surface in the sample 2 with 46 coch/cm² than in sample 1 with 5 coch/cm². Sample 1 is equally infested on both sides of the leaves while sample 2 is more infested in the lower surface.

To test the effect of the leaflet surface on the infestation rate we have used the two-way ANOVA (Annex 1.3). The analysis of variance shows that the main effect of leaflet

surface is not significant, F (3.98) =0.69, P<0.05. Also, there was no significant interaction between leaflet surface and infestation rate, F (3.98) =1.12, P>0.05.

Date palm white scale prefers generally humid areas distant from the sun (Al-Jaghoub, Al-Laham, & Barhum, 2003), this explains why the infestation is higher in sample 2 sampled from the part of the grove where young date palm trees are planted between tall aged palms that shadow them and provide more humidity.

P. blanchardi is a phloem-feeding insect, and it triggers the lower parts of the crown that seem to be highly photosynthetic than the upper ones (Tourneur & Vilardebo, 1975).

Moreover, the leaves of the lower crown are fully mature which means that they don't use the glucose produced through photosynthesis, but, instead, they transport it through phloem sap to other growing organs or stock it in the form of sucrose, preferred elements by the insect (Khlil, 1989 in Nadji, 2011). On the other hand, the leaves of the upper crown are still developing, thus, half of the photosynthesis products are used to provide energy to accomplish the final growth of the leaves; the other half is transported through phloem, the sucrose is by result not often stocked in immature leaves.

III.3. Physiological parameters

III.3.1. Moisture loss

The results of moisture loss of sample 1 and simple 2 are shown in figure 11.

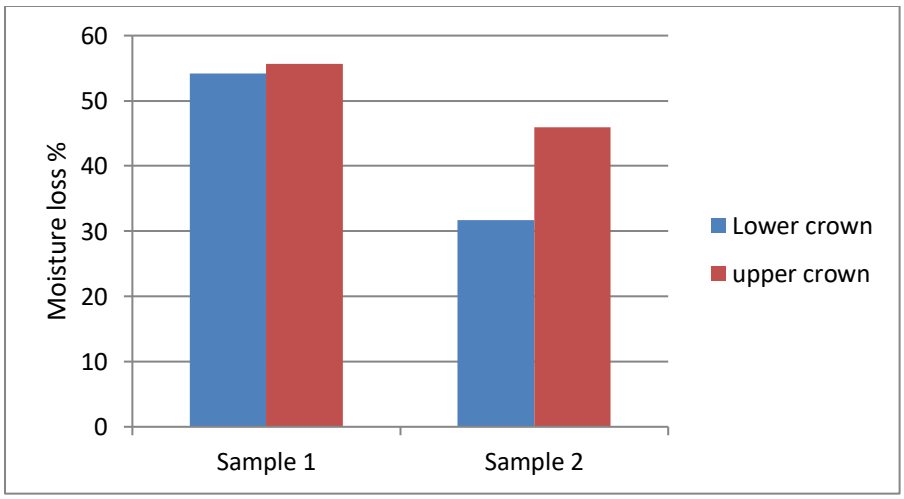

Figure 11: Moisture loss of the upper and lower crown

The percentage of moisture loss is higher in the lower crown of sample 1 with 37.09% than in sample 2 with 31.69%, in the same way it ways higher in upper crown of sample 1 with 55.65% than in in sample 2 with 45.92%, in the two samples the lost moisture perscentage is higher in the upper crown than in the lower.

To test the effect of the infestation rate on moisture loss in leaves we have used the two-way ANOVA (Annex 2). The analysis of variance of the infestation rate on moisture loss shows a significant difference in infestation level, $F(5.31)=18.22$, $P<0.05$, and a significant difference in crown level concerning moisture loss, $F(5.31)=7.139$, $P<0.05$; although there was no significant interaction between the two factors, $F(5.31)=5.29$,$P=0.05$, which means that the infestation rate affects the two levels similarly and on the same direction.

The results of the correlation indicated a negative correlation between the infestation rate and moisture loss , $r= -0.68$. Our result concerning moisture loss matches the first parameter (infestation rates), which means the more the insect infests the plant the more it causes the dryness of its leaves. That explains why the lower crown was more dry than the upper crown; the same goes for sample 1's leaves being more dry than sample 2.

The dryness in leaves is caused by the action of feeding on phleom sap of the plant by the insect (Mansour & *al.*, 2017); as the phloem sap is composed from 80% of water and 20% of (Meyer, Reeb, & Bosdeveix, 2013), the insect's feeding on it causes a significant moisture loss in plant tissues. On the other hand, the dryness in leaves may be attributted to other reasons: The studies of Al-Dosary (2009) and Moussa & *al.*(2012) showed a significant declin in epicuticular wax in the infested date palm trees, this may lead to a reduction in the thickness of the cuticular layer which may by consequence enhace transpiration.

The reduction of moisture in tissue may cause guard cells plasmolysis resulting in stomata closure and disturbance in transpiration process. As xylem sap circulation is controlled by stomatal transpiration (Meyer, Reeb, & Bosdeveix, 2013), stomata closure may reduce water absorption and circulation in conductiong vessels, as well as mysophyll CO_2 and net photosynthesis (Neves & *al.*, 2006).

Sharkey (1985) also stated that the interruption of water transport caused by poor stomatal conductance can lead to a decrease in leaf water potentials (Hoback & *al.*, 2015).

III.3.2. Chlorophyll concentration

III.3.2.1. Optical chlorophyll concentration

The results of of optical chlorophyll concentration are given in the following figure.

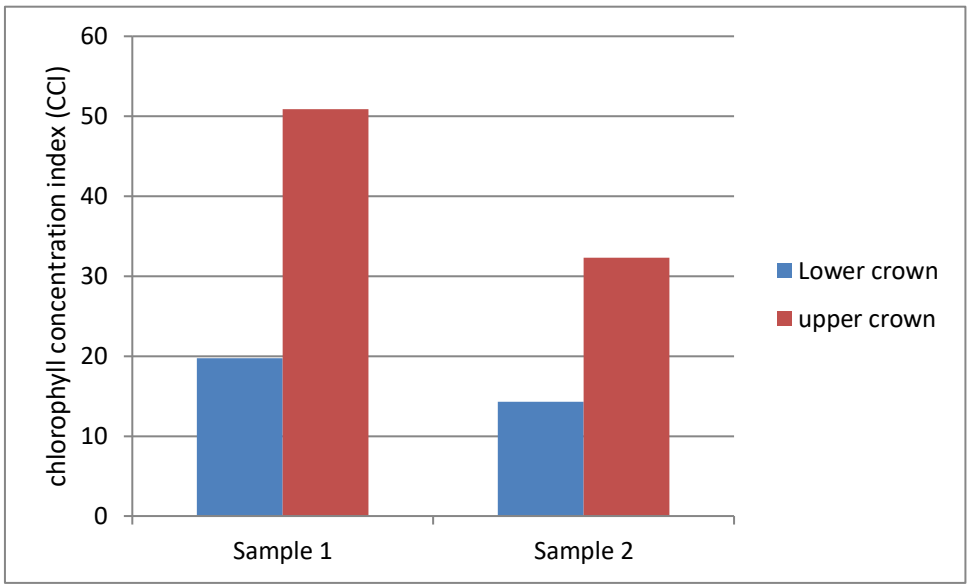

Figure 12: Optical chlorophyll concentration in the upper and lower crown of sample 1 and sample 2

The results of optical chlorophyll concentration are showed in Figure 12. chlorophyll concentration is higher in the upper crown of sample 1 with a value of 50.9 CCI than in sample 2 with 32.31 CCI, it was also higher in lower crown of sample 1 with 19.76 CCI than in in sample 2 with 14.31 CCI, the chlolophyll concentration was higher in upper crown than in the lower in the two samples.

To test the effect of the infestation rate on chlorophyll concentration in the leaves we have ran a two-way ANOVA (Annex 3). The analysis of variance showed that there is a significant difference between sample 1 and sample 2 in chlorophyll concentration, $F(4.15)=10.61$, $P<0.05$, also there was a significant difference between the two levels of the crown, $F(4.15)=44.36$, $P<0.05$, although there was no significant interaction between them, $F(4.15)=3.17$, $P>0.05$, which means that the infestation rate affects the two levels of the crown similarly concerning the chlorophyll concentration.

We have found a weak negative correlation between the infestation rate and chlorophyll concentration index in the leaves, $r= -0.53$. This means that the more the tree is infested the less the chlorphyll concentration in their leaves would be.

III.3.2.2. Absolute chlorophyll concentration

The results of absolute chlorophyll concentration are presented in the Figure13 and Figure 14.

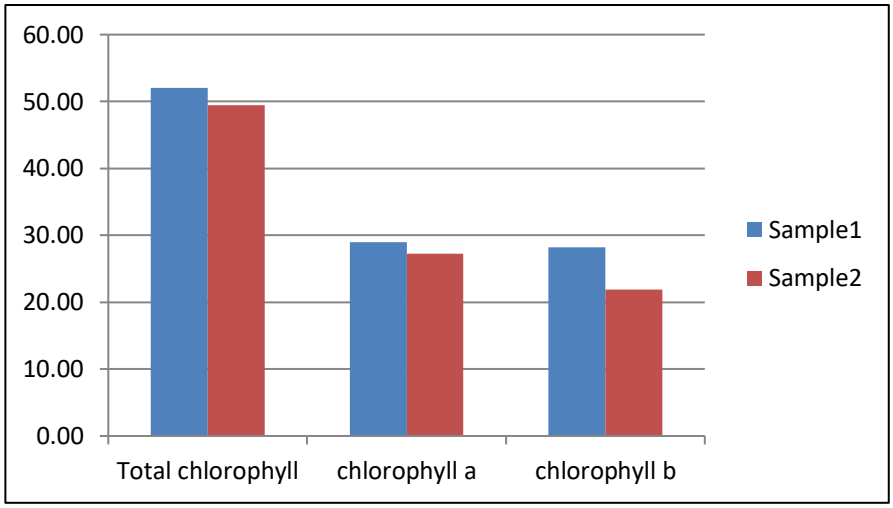

Figure 13: Concentration of total chlorophyll, chlorophyll a, and chlorophyll b in the upper crown

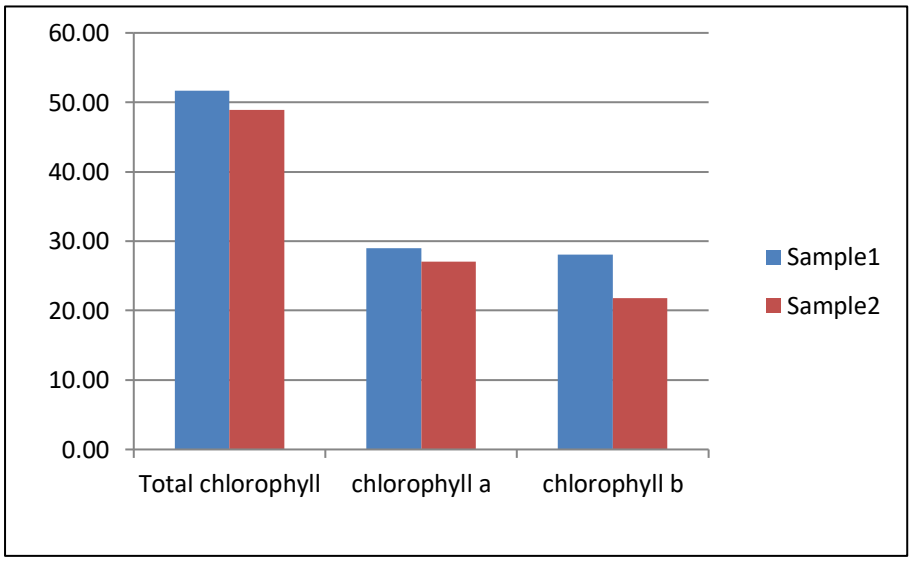

Figure 14 : Concentration of total chlorophyll, chlorophyll a, and chlorophyll b in the Lower crown

Figure13 shows that total chlorophyll of the upper crown is higher in sample 1 with 51.65 mg/l than in sample 2 with 48.90 mg/l, the same way chlorophyll a and b is higher in sample 2 with 28.95 and 28.01, than in simple 2 with 27.08 and 21.83 for chlorophyll a and chlorophyll b respectively.

Figure 14 also shows that total chlorophyll of the lower crownis higher in sample 1 with 52.03 mg/l than in sample 2 with 49.50 mg/l, the same way chlorophyll a and b is higher

in sample 2 with 28.92 and 28.21, than in simple 2 with 27.29 and 21.90 for chlorophyll a and chlorophyll b respectively.

A one-way ANOVA (Annex 05) showed that the difference between the two sample in absolute chlorophyll concentration was significant, $F(4.38)=38.67$, $P<0.05$.

Our optical measurments *in situ* matches the absolute chlorophyll concentration *in vitro*; there was a positive correlation between them, r=0.69. This result was confirmed by Parry & *al.*(2014).

There was also a negative weak correlation between the absolute chlorophyll concentrations and the infestation rate of sample1and sample 2, r= -0.62.

The poor optical measurement indicates a lack in chlorophyll concentration. The reduction in chlorophyll concentration is due to two main reasons, the first is the inhibition of chlorophyll pigments biosynthesis due to the poor passive absorption in the roots which is caused by low transpiration rate (Montanaro & Dichio, 2012); the result is a lack of mineral nutrition which provide essential elements to synthesize chlorophyll pigments such as Mg^{+2} which is the central ion in chlorophyll molecule. On the other hand, the toxic saliva injected by the insect seems to alter the chlorophyll (Munier, 1973; Arbab & Nakry; 2016;; Mansour & *al.*,2017), resulting in green color change and so poor optical and absolute mesurments.

III.4. Anatomical parameters

III.4.1. Stomatal density

The results of stomatal density are shown in the figure below.

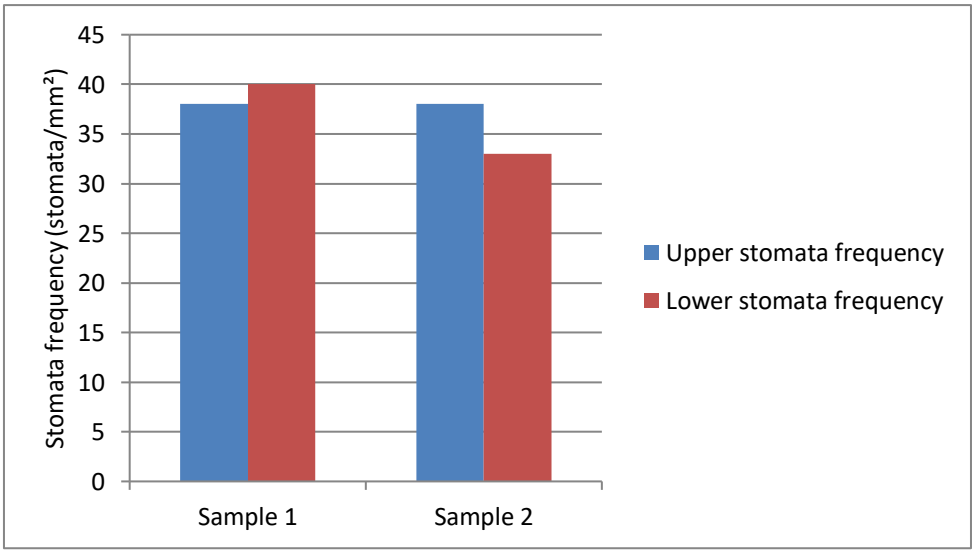

Figure 15: Upper and lower stomata frequency

The results of the stomatal density of the upper and the lower leaflet surfaces Figure14 showed that the two samples had the same upper stomatal density 38 stomata/mm² while there was a small difference in the lower stomatal density in which it was higher in sample 1 with 40 stomata/mm², than in sample 2 with 33 stomata/$mm^{2.}$

The analysis of the variance of the effect of the infestation rate on stomata frequency showed no significant difference in stomtatal frequency in the higly infested sample 2 and the barely infested sample 1, $F(4.15)= 2.29$, $P>0.05$, the main effect of leaflet surface is also not significant, $F(4.15)=0.69$, $P>0.05$, no interaction was detected between the two factors, $F(4.15)=2.29$, $P>0.05$.

In monocots, the stomatal density is often higher in the lower surface of the leaves than in the upper surface, this was observed in sample 1 which is barely infested by the insect. The reduction in the lower surface of sample 2 is considered insignificant and it may be due to manipulation error.

Meister and Bolhàr-Nordenkampf (2001) states that stomatal density in leaves with small area can change due to different factors including water availability, light intensity, temperature and CO_2 concentration. Despite that, the white scale insect doesn't seem to affect stomatal densities of date palm trees. In contrast, a study of the effect of *Melanaspis glomerata* on sugar cane showed an increase in stomatal density of the stem in attacked varieties (Beardsley & Gonzalez, 1975).

III.4.2. Transversal section of the leaves

The histological observation of highly and barely infested transverse sections of leaves shows a normal and similar structure. The mesophyll tissue is composed of palisade cells right below the upper and the lower epidermis, approaching the centre, the cells become spongy without gaps between cells. Under the abaxial and adaxial epidermis, sclerenchyma bundles of different sizes are observed along, these bundles, common to monocots, strengthen the leaves and give them some rigidity. At the centre of the msophyll, the vascular bundles arranged parallel to each other, the large vascular bundles are permeated by small ones.

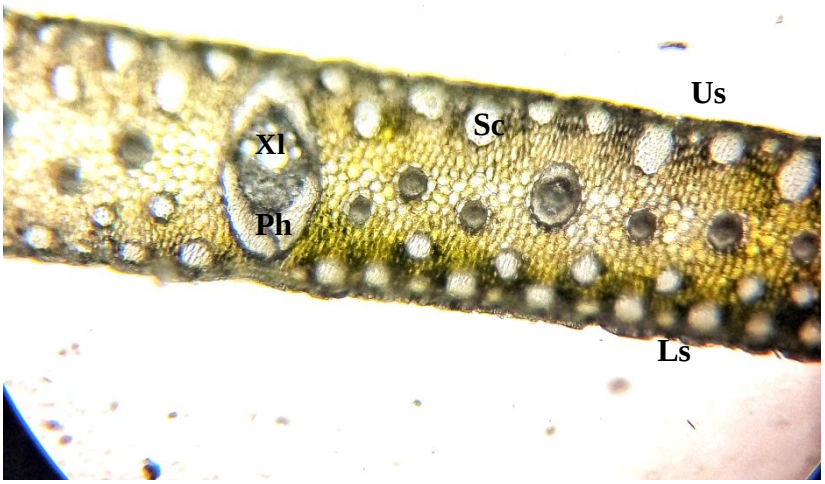

Figure 16 : Transverse sections of leaflet in sample 1 observed at X100.

Us= upper surface, Ls= Lower surface, Xl= Xylem, Ph= Phloem, Sc= Sclerenchyma bundles.

The sections don't show the path of the insect's stylets, nor does the feeding sites. The xylem, phloem and parenchyma cells look normal in the two samples but this should not exclude the existence of a difference in the number and the size of them, These results were obtained by Enser (1941) in (Beardsley, 1975) who found that the number, the size and the arrangement of phloem and xylem cells differs in woody tissue if stem infested by San Jose scale. Although, Leaf vascular bundles penetrating by stylets bundles has been reported in only two species of scales (Washington & Walker, 1990) Juárez-Hernández & *al.* (2014) also reported that The red scale insect avoids vascular tissues especially in leaves, where parenchyma is the main feeding site, and that the penetrating of vascular bundles was more common in stem. Moreover, they didn't detect any cell lysis in the pierced cells or any reduction of their volumes due to loss of cell turgor, he assumed that the sucking was too slow that water could refill the pieced cell again before it collapse. Washington & Walker (1990) had the same observation where the cells pierced by California red scale appeared normal.

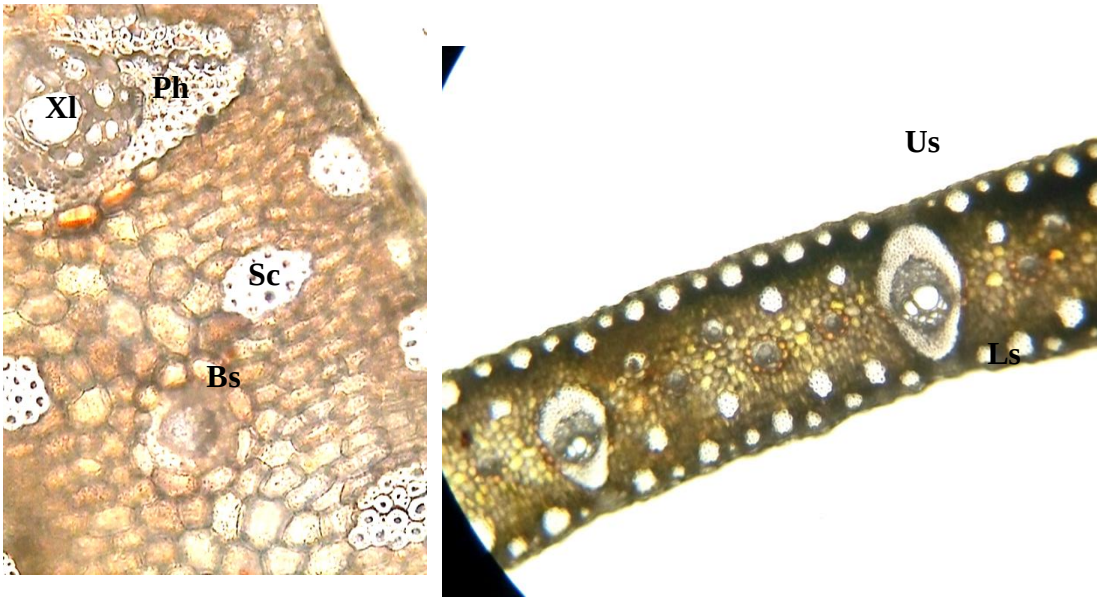

Figure 17 : Transverse sections of leaflet in sample 2 observed at X100 and X400.

Us= upper surface, Ls= Lower surface, Bs= bundle sheath cells, Xl= Xylem, Ph= Phloem, Sc= Sclerenchyma bundles

CONCLUSION

In our study we attempted to verify the negative effect of *Palatoria blanchardi* on the physiology of date palm of *Deglet Nour* and reveal the anatomical aspects of the injury.

The samples from shadowed date palm trees were more infested by the insect although they receive the same maintenance efforts, while samples from trees exposed directly to the sun were barely infested. In the two samples, the infestation rate was higher in the lower crown than in the upper one, and there was no significant difference between the infestation rate of the adaxial and abaxial surface of the leaves.

The infestation by date palm white scale insect has serious effects on the palm physiology. The highly infested samples contained less moisture than the barely infested ones, the upper crown of the two samples was also less infested than the lower, the result was a negative correlation between the infestation rate and moisture loss in leaves. The optical chlorophyll concentration measurement *in situ* matched the absolute chlorophyll concentration *in vitro*, there was also a negative correlation between the infestation rate and chlorophyll concentration, indeed, the chlorophyll concentration was less in the highly infested samples than in the hardly infested ones.

Anatomically, there was no difference between stomatal density of the leaves, whether they are highly or barely infested. The transverse sections showed also no difference in leaves' tissues, and we didn't observe any pierced cells or traces of stylet bundle target through cell's tissues, thus, we suggest using another precise sectioning technique.

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ANNNEX 01

1.1 One-way ANOVA of infestation rate of *Parlatoria blanchardi* of sample 1 and sample 2

Source of variation	Sum of squares	Freedom degree	Mean squares	F	Probability	Critical F
Between groups	1860,5	1	1860,5	27,55	0.0038	3,97
Within groups	4727	70	67,52			
Total	6587,5	71				

1.2 Two-way ANOVA of the effect of the crown level on the infestation rate

Source of variation	Sum squares	Freedom degree	Mean squares	F	Probability	Critical F
Infestation level	1860,50	1	1860,50	43,16	0.001	3,98
Crown level	982,72	1	982,72	22,80	0.024	3,98
Interaction	813,39	1	813,39	18,87	0.032	3,98
Within groups	2930,89	68	43,10			
Total	6587,50	71				

1.2 Two-way ANOVA of the effect of leaflet surface on the infestation rate by

Source of variation	Sum squares	Freedom degree	Mean squares	F	probability	Critical F
Infestation level	1860,50	1	1860,50	27,48	0.0041	3,98
leaflet surface	46,72	1	46,72	0.69	0.40	3,98
Interaction	76,06	1	76,06	1.12	0.29	3,98
Within groups	4604,22	68	67,71			
Total	6587,5	71				

ANNNEX 02

Two-way ANOVA of the effect of infestation rate on moisture loss in the leaves

Variation souce	Sum squares	Freedm degree	Mean squares	F	Probabiliy	Critical F
Infestation level	814,683	1	814,68	18,22	0,00	5,31
Crown level	319,05	1	319,05	7,139	0,02	5,31
Interaction	236,59	1	236,59	5,29	0,05	5,31
Within groups	357,53	8	44,69			
Total	1727,86	11				

ANNNEX 03

Two-way ANOVA of the effect of infestation rate on cohlorophyll concentration

Variation source	Sum squares	Freedom drgree	Mean squares	F	Probability	Critical F
Infestation level	1299,60	1	1299,60	10,61	0,002	4,15
Crown level	5434,15	1	5434,15	44,36	0,00	4,15
Interaction	388,75	1	388,75	3,17	0,08	4,15
A l'intérieur du groupe	3919,76	32	122,49			
Total	11042,26	35				

ANNNEX 04

Two-way anova of the effect of the infestation rate on upper and lower stomatal frequency

Variation source	Sum squares	Freedom degree	Mean squares	F	Probability	Critical F
Infestation level	78,03	1,00	78,03	2,29	0,14	4,15
Leaflet face	23,36	1,00	23,36	0,69	0,41	4,15
Interaction	78,03	1,00	78,03	2,29	0,14	4,15
Within groups	1090,89	32,00	34,09			
Total	1270,31	35,00				

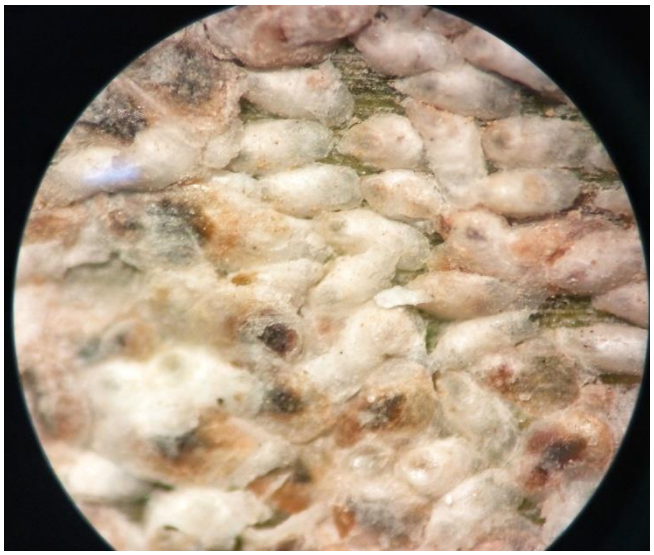
ANNEX 05

One-way ANOVA of the absolute chlorophyll concentration in sample1 and sample2

Variation source	Sum squares	Freedom degree	Mean of square	F	Probability	Critical F
Infestation level	1578,92	5	315,78	38,68	0,0002	4,39
Within groupe	48,99	6	8,16			
Total	1627,91	11				

ANNEX 06

White scale insect under the stereo microscope at X40 (original)

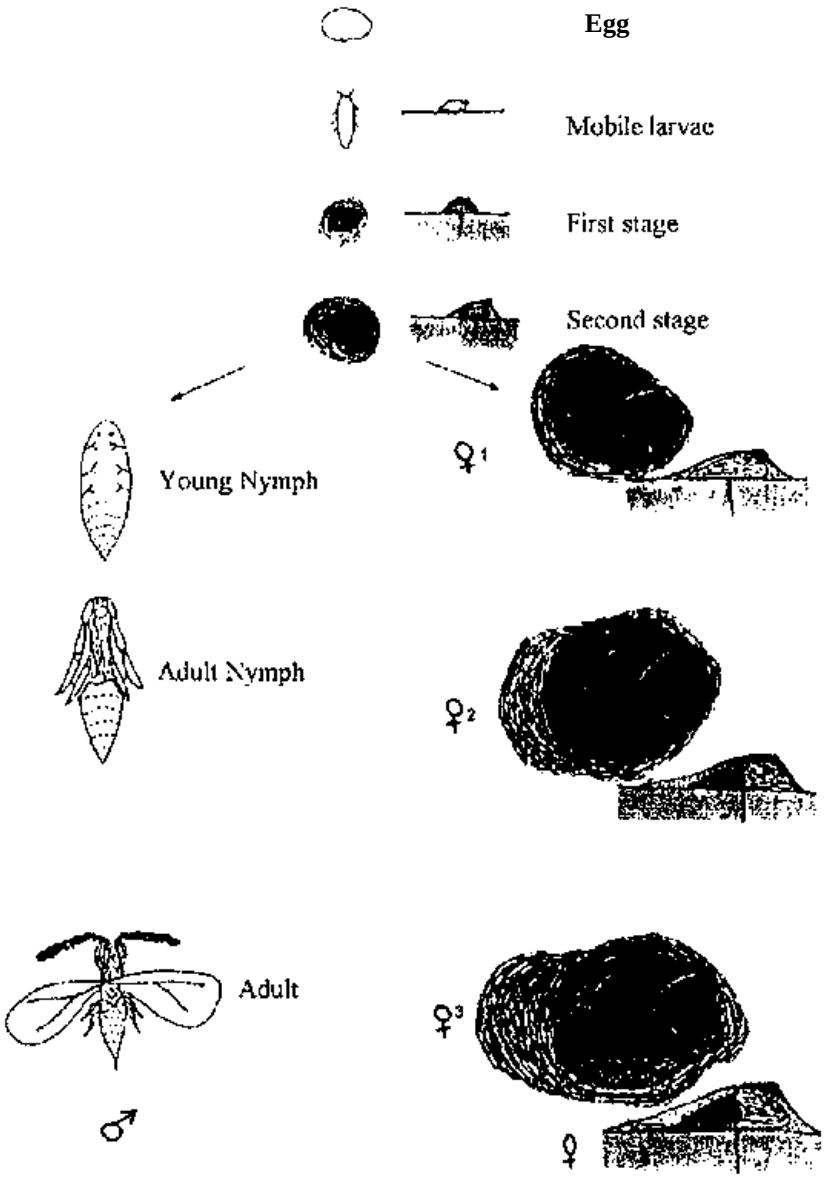


ANNEX 07

Infested leaves

ANNEX 08

Life cycle of *Parlatoria blanchardi*



Abstract

Date palm white scale insect is one of the most dangerous pests that threaten the date palm production in Algeria. It attacks all parts of the try but mostly the leaves. Our study aims to evaluate the physiological and anatomical effects of the in insect on the leaves of date palm of *Deglet Nour* in Biskra. We found that there was a reduction in leaves' moisture; the reduction was more serious when the infestation rate is high. There was also a reduction in the total concentration of the absolute and optical chlorophyll, as well as pigments (chlorophyll a and b). We didn't observe any effect on the stomatal density of the leaves even when the infestation is heavy, the histological sections of the leaves also didn't show any difference or abnormal structure in tissues.

Key words: white scale, date palm, Biskra, moisture, chlorophyll, stomatal density.

Résumé

La cochenille blanche du palmier dattier est l'un des dangereux ravageurs menaçant la production de palmier dattier en Algérie. Elle attaque toute les parties du palmier mais surtout les feuilles. Notre étude vise à évaluer les effets physiologiques et anatomiques de cette insecte sur les feuilles de la variété *Deglet Nour* dans la région de Biskra. Nous avons trouvé qu'il existe une réduction d'humidité des feuilles, la réduction est plus sérieux lorsque le taux d'infestation est élevé. Nous avons constaté une réduction dans la concentration total de chlorophylle absolue et optique, aussi bien dans les pigments (chlorophylle a et b). Nous n'avons pas constaté une différence dans la densité stomatique même quand le taux d'infestation est sérieux. Les coupes histologique des feuilles n'avaient pas révélé aucune différence ou des structure anormale dans la structure des tissues.

Mots clés : cochenille blanche, palmier dattier, Biskra, humidité, chlorophylle, densité stomatique.

ملخص

تعد حشرة النخيل القشرية البيضاء واحدة من أخطر الآفات التي تهدد إنتاج التمور في الجزائر. تهاجم الحشرة جميع أجزاء النخلة و لكن بشكل خاص الأوراق. دراستنا تهدف إلى تقدير الضرر الفيزيولوجي و التشريحي لهذه الحشرة على أوراق نخيل دقلة نور في ولاية بسكرة. وجدنا أن الرطوبة في الأوراق المجتاحة من قبل الحشرة تنقص كلما زادت نسبة الاجتياح. وجدنا أيضا نقصا في تركيز الكلوروفيل الكلي و كذلك في تركيز الصبغات (كلوروفيل أ و ب) .هذا ولم نسجل أي تغير في كثافة الثغور الخاصة بالأوراق حتى مع ارتفاع نسبة اجتياح هذه الأوراق. كما لم تظهر المقاطع النسيجية أيضا أية فروق أو بنيات غير طبيعية للأنسجة النباتية الخاصة بالورقة.

الكلمات المفتاحية: الحشرة القشرية البيضاء, النخيل, بسكرة, رطوبة, كلوروفيل, كثافة الثغور.