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Photo of the cover page: The hyperparasitoid *Alloxysta* sp. (Courtesy *Zine Eddine Labdaoui*)

Guest Editorial

Agriculture and Sustainable Development: Control of Pests with Selective Products

The losses caused by various pests to crops generally vary from 30-50% depending on the region and the crop. In order to satisfy the alimentary needs of an ever increasing population, it is imperative to reduce these losses caused by various pests to crops and stored products. Several methods were used for controlling pests, in which the conventional insecticides such as organophosphorous, carbamates and pyrethroids play an important role. Improving pest control means is a global challenge to preserve human and animal health, secure food reserves and reduce losses while respecting the environment and the rules of sustainable development. It is therefore essential to develop new products and / or new approaches that combine different ways of pest and vector control.

The negative effects of conventional neurotoxins on the environment has resulted in the implementation of integrated pest management approaches applying environmentally-friendly and sustainable methods to control major insect pests of crops and veterinary and human importance. Among these approaches the development of novel insecticides with selective properties acting on biochemical sites or on physiological processes. Based on the advances in insect physiology selective agents such as the insect growth disruptors (IGDs) have been developed. These IGDs affect the hormonal regulation of molting and developmental processes. Since their discovery, these new insecticides have been the subject of several detailed reviews. The following classes of IGDs have been successively developed; the juvenile hormone analogs, the inhibitors of chitin biosynthesis and the molting hormone agonists.

Larval growth and development in insects are regulated by the polyhydroxylated steroid 20-hydroxyecdysone and the sesquiterpenoid juvenile hormone (JH). In the adult stage, these hormones are also involved in the regulation of reproductive maturation. In 1967, C. Williams suggested that compounds that mimic the concept of JH action can be used as third generation insecticides with an eco-toxicological benign profile, and since then, numerous JH analogs (JHAs) have been discovered. Such compounds are toxic during the embryonic, last larval, and reproductive stages of insects, and some have been commercialized as a new class of insect growth regulators (IGRs). Most of the early analogs are based on the terpenoid structure of JH. The most active ones are methoprene and hydropene. More recently, several highly active compounds, such as fenoxycarb, pyriproxyfen and kinoprene have been synthesized. These products have not the expected success, because of their rapid photo-degradation (methoprene) and high toxicity to aquatic organisms (pyriproxyfen).

The insect cuticle is secreted by the epidermal cells and consists mainly of chitin and protein. Chitin is a

polysaccharide (N-acetyl-D-glucosamine polymer). It is present in fungi, diatoms and certain groups of invertebrates such as arthropods and nematodes. The fact that chitin has only a limited distribution among certain organisms makes it an attractive target for the development of specific active agents. The benzophenylureas (BPUs) prevent typically the molting process. They were found to disturb the cuticle secretion (thickness and fine structure) and to inhibit the incorporation of radio-labeled precursors into chitin confirming their primary mode of action on chitin biosynthesis. In addition, these chemical agents affect reproduction in several insect orders, primarily by causing a reduction in egg hatch. Diflubenzuron (Dimilin 25WP) is the most investigated BPU derivative widely used in the control of insect pest in agriculture and forestry. Since its introduction, a number of other BPU derivatives have been developed such as flucycloxuron, triflumuron, hexaflumuron or novaluron.

Besides these IGDs, the non-steroidal ecdysteroid agonists, also called moulting-accelerating compounds (MACs), have been discovered. They mimic the action of the steroid insect moulting hormone, 20-hydroxyecdysone (20E), and manifest their activity by binding to the ecdysteroid receptor complex in a manner competitive with ecdysteroids, inducing a precocious and incomplete lethal moult in several insect orders. Four products have been commercialized. Halofenozide was found to be more toxic to coleopteran pests while methoxyfenozide was more potent against lepidopteran species. The ecdysteroid agonists affect reproduction in different insect orders, mainly by reducing fecundity and egg viability, or sexual behavior.

Conclusively, the use of pesticides is expected to shrink more and more in coming years due to environmental imperatives. Integrated pest management is necessary with a reasoned chemical control based on the appreciation of a threshold of harmfulness or economic tolerance and using selective insecticides such as the IGDs. These new selective insecticides have potential for use in integrated pest management regarding their specific mode of action on insects and their low eco-toxicological risks. They constitute a good alternative to conventional insecticides. For environmental risk assessment, the potential side-effects of these alternative chemical agents on ecosystems particularly on non-target organism like the auxiliary agents, must be investigated. The valorization of plant extracts is a promising alternative. However, the further research must focus on their mode of action and their potential hazards on ecosystems.

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Review Article

Beyond *Bt*: New Bacterial Resources for Insect Biocontrol

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ABSTRACT

Leclerque, A. 2018. Beyond *Bt*: new bacterial resources for insect biocontrol. Tunisian Journal of Plant Protection 13 (si): 1-9.

The Gram-positive bacterium *Bacillus thuringiensis* (*Bt*) is the economically most important entomopathogen for insect biocontrol, and for excellent reason a large part of the effort invested in the development of microbial insecticides has concentrated on *Bt* strains and their *cry* genes. Hundreds of *cry* gene sequences have been determined from nature, and molecular modeling has been used to create artificial recombinant Cry proteins with potentially new properties. However, alternative insect biocontrol agents are increasingly solicited. More recently, research efforts have concentrated upon bacterial insect-pathogens as potential biocontrol resources as, e.g., non-*cry* toxins of *Bt* and other bacteria of the family *Bacillaceae* as well as the bacterium *Saccharopolyspora spinosa* (*Actinobacteria*). Moreover, several different γ-proteobacterial entomopathogens belonging to the species *Serratia entomophila*, *Yersinia entomophaga* or *Pseudomonas entomophila* as well as to the genera *Providencia* or *Rickettsiella* are currently being evaluated for their biocontrol potential. The present literature review gives a brief update on these entomopathogens and toxins.

Keywords: *Bacillus thuringiensis*, microbial biocontrol, *Providencia*, *Pseudomonas entomophila*, *Rickettsiella*, Spinosad, TcABC toxin

Introduction.

Insects are associated with bacteria in relationships ranging from obligate endosymbiosis to pathogenicity. Among the numerous entomopathogens that infect and kill the respective host by a diversity of mechanisms, the Gram-positive rod-shaped bacterium *Bacillus cereus* subsp. *thuringiensis* that is commonly referred to as "*Bacillus thuringiensis*" or "*Bt*", is by far the most studied, best understood and most widely used for biocontrol (Roh et al. 2007; Sanchis 2011; Schnepf et al. 1998; Vega and Kaya 2012). Insecticidal Cry protein toxins produced by *Bt* during the stationary growth phase are localized in parasporal bodies and as a rule display toxicity for a narrow host spectrum (De Maagd et al. 2003). Hundreds of toxin encoding *cry* gene sequences have been described (http://www.lifesci.sussex.ac.uk /home/Neil_Crickmore/Bt/), the general mode of action has been elucidated in molecular detail (Bravo et al. 2007), and insect resistant transgenic crops carrying *cry* sequences have been generated successfully. However, emerging insect resistance to Cry toxins has triggered both a debate on Cry-based integrated pest management (IPM) practices (Bravo and Soberón 2008; Pardo-López et al. 2013) and increased efforts to explore

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alternative biocontrol agents. The present article reviews some of the principal advances in research on alternative bacterial insect biocontrol resources.

Non-Cry *Bacillus thuringiensis* toxins.

Besides Cry toxins, *B. thuringiensis* strains produce three main types of further insecticidal protein toxins designated as Cyt, Vip, and Sip proteins (Palma et al. 2014).

Predominantly dipteran specific "cytotoxic" Cyt proteins display a general cytolytic activity *in vitro* (Butko 2003). They have been grouped into three families, termed Cyt1 through Cyt3. Importantly, besides being themselves entomotoxins, Cyt proteins synergistically increase the insecticidal effects of Cry or Vip3 proteins and thereby hold potential to counteract insect resistance development against Cry proteins (Federici and Bauer 1998; Yu et al. 2012).

The terms "vegetative insecticidal" (Vip) and "secreted insecticidal" (Sip) proteins denote two different classes of insecticidal proteins that are both secreted into the medium by *B. thuringiensis* during the vegetative growth phase (Palma et al. 2014). Vip1/2 forms a binary toxin with insecticidal activity against beetles (Coleoptera) and aphids (Sattar and Maiti 2011). Vip1 functions as a receptor-binding domain that facilitates entry of the Vip2 toxin into the host cell's cytoplasm where it blocks actin polymerization (Barth et al. 2004). Single-chain Vip3 proteins display insecticidal activity against a wide spectrum of Lepidoptera (Estruch et al. 1996; MacIntosh et al. 1990). As for Cry toxins, formation of pores in midgut epithelial cell membranes appears to be a crucial step in Vip3 protein toxicity (Yu et al. 1997). However, the mechanisms of pore formation are different for both toxin classes (De Maagd et al. 2003), and insect resistances against Cry and Vip3 are largely independent from each other (Bommireddy et al. 2007). Vip3 proteins have therefore been proposed as complements to Cry proteins in resistance management programs (Mike et al. 2006). *B. thuringiensis* Sip protein is toxic to coleopteran insects as, e.g., the Colorado potato beetle, *Leptinotarsa decemlineata*, by a yet not deciphered mechanism (Donovan et al. 2006).

Recently, the *Bt* zinc metalloprotease InhA has been shown to specifically hydrolyze antimicrobial peptides that contribute to the humoral response of Lepidopteran insect; combined application of InhA and Cry proteins leads to a mutual increase in toxicity (Dammak et al. 2015).

Insecticidal toxins from further *Bacillaceae* bacteria.

Insecticidal toxins often, but not always, homologous to the different classes of *Bt* toxins have been described from further bacteria belonging to the taxonomic family *Bacillaceae* as, e.g., to the species *Brevibacillus laterosporus*, *Lysinibacillus sphaericus*, and *Paenibacillus popilliae* (Ruiu 2015). The latter species is best known as the causative agent of "milky disease" of scarabaeid larvae and is considered a potential biocontrol agent for these agricultural pests. *P. popilliae* expresses a parasporal Cry-homologous protein of still uncertain significance for pathogenicity (Zhang et al. 1997).

B. laterosporus is both notable as a versatile insect pathogen, infecting and killing Coleoptera, Lepidoptera, and Diptera, and as a producer of both antibacterial and antifungal secondary metabolites (Ruiu 2013). Mosquiticidal activity of certain *B. laterosporus* strains has been demonstrated to depend on the

presence of parasporal inclusion bodies similar to those found in *Bt (*Zubasheva et al. 2010). However, a strain lacking crystal proteins has been found highly virulent to the house fly, *Musca domestica*, and has been developed into a commercially available biocontrol agent (Ruiu et al. 2007; 2012). Moreover, a different group of *B. laterosporus* strains infecting larvae of coleopteran pests as, e.g., corn rootworms, secretes binary insecticidal proteins termed ISPs that are related by primary amino acid sequence to the Vip1/2 toxins of *Bt* (Warren 1997).

Actinobacterial macrolids as entomotoxins: avermectins and spinosyns.

Among the large variety of secondary metabolites produced by different *Streptomyces* spp., several compounds have been demonstrated to possess insecticidal activity (Craveri and Giolitti 1957; Kido and Spyhalski 1950; Oishi et al. 1970). Probably best studied is the class of "avermectins", i.e. large macrocyclic lactones produced by the *S. avermitilis* that have been demonstrated to impair neurotransmission in the insect peripheral nervous system by gamma-aminobutyric acid (GABA) receptor binding (Turner and Schaeffer 1989).

Binding to neuronal receptors has been proposed as the main mechanism of action of the better studied members, namely spinosyn A and D (Kirst et al. 1991), of a complex class of polyketide compounds, collectively referred to as "spinosyns" (Kirst 2010; Salgado and Sparks 2005), that confer broad-spectrum insecticidal activity to actinobacteria belonging to the species *Saccharopolyspora spinosa* (Mertz and Yao 1990; Waldron et al. 2000) and *S. pogona* (Lewer et al. 2009). Mixtures of - both natural and semisynthetic - spinosyn derivatives, marketed under the collective name "spinosad", display activity against a wide variety of Lepidoptera and Diptera, but are considered safe for non-target organisms, particularly for vertebrates (Sparks et al. 2001).

Insecticidal TcABC toxin complexes.

A heterogeneous group of Gram-negative bacterial entomopathogens carries large "TcABC" toxin complexes: component A displays the entomotoxic activity that is under certain conditions potentiated by the action of auxiliary components B and C (ffrench-Constant and Waterfield 2006). TcABC toxins have been identified in *Serratia entomophila* (termed SepABC), i.e. the causative agent of "amber disease" of *Costelytra zealandica* grubs in New Zealand, in *Yersinia entomophaga* from the same geographic and host origin, in a further isolate of *S. entomophila* found associated with larvae of the scarabaeid *Phyllophaga* spp., an important pest of maize plants in Mexico (Nunez-Valdez et al. 2008), and in the nematode symbiotic bacteria *Photorhabdus* and *Xenorhabdus* (Blackburn et al. 1998; Bowen and Ensign 1998; Bowen et al. 1998). Moreover, PCR-based screening has led to the identification of a homologous *tcABC* gene cluster in *B. thuringiensis* (Blackburn et al. 2011). Toxin encoding *tcABC* gene clusters are localized on large plasmids. Whereas the action of SepABC from *S. entomophila* is highly specific for the natural host, *C. zealandica* (Hurst et al. 2007; Jackson et al. 2001), further Tc toxin complexes have been described as considerably less specific (Hurst et al. 2011a; Nunez-Valdez et al. 2008). *Y. entomophaga*, for instance, has been shown to be highly virulent for further Coleoptera as well as Lepidoptera and Orthoptera (Hurst et al. 2011b).

Proteobacterial fruit fly pathogens: *Pseudomonas entomophila* and *Providencia* spp.

The gamma-Proteobacterium *Pseudomonas entomophila* (Mulet et al. 2012) perorally infects and rapidly kills larvae and adults of *D. melanogaster* (Vodovar et al. 2005) and has been employed to develop an infection model for bacterial fruit fly pathogens (Liehl et al. 2006). *P. entomophila* has been shown to produce both hydrogen cyanide HCN (Ryall et al. 2009) and a novel ß-pore-forming toxin termed "monalysin" (Blemont et al. 2013; Leone et al. 2015; Opota et al. 2011). Whole genome sequencing of the specific type strain L48 (Vodovar et al. 2006) has led to the development of a PCR-based diagnostic approach for *P. entomophila* employed in a broad screening of olive fly populations around the Mediterranean (Papagiannoulis et al. 2009).

Enterobacteria of the genus *Providencia* are mainly of clinical importance as opportunistic pathogens typically causing dysentery (Galac and Lazzaro 2012). However, *Providencia* bacteria have been found associated with wild-caught *D. melanogaster* and the Mexican fruit fly, *Anastrepha ludens* (Kuzina et al. 2001), and several species as, e.g., *Providencia sneebia* and *P. alcalifaciens* display pronounced virulence to the common vinegar fly (Juneja and Lazzaro 2009). The species *P. vermicola* appears to be the insecticidal agent carried by certain entomo-pathogenic nematodes, and isolated *P. vermicola* bacteria cause elevated mortality in Lepidoptera (Park et al. 2011; Somvanshi et al. 2006). Moreover, *Providencia* bacteria have been demonstrated to attract both male and female fruit flies from several *Bactrocera* species potentially facilitating infection in IPM measures (Hadapad et al. 2016).

Insect-associated *Rickettsiella* bacteria.

Bacteria of the taxonomic genus *Rickettsiella* have been described as intra-cellular pathogens of a wide range of arthropods (Fournier and Raoult 2005) including - besides both crustaceans and arachnids - insects of agricultural or medical importance as, e.g., scarabaeid grubs (Leclerque and Kleespies 2008; Leclerque et al. 2012), wireworms (Leclerque et al. 2011; Schuster et al. 2013) or ticks (Kurtti et al. 2002; Leclerque and Kleespies 2012). Moreover, a mutualistic relationship has been described for bacteria of the species `*Candidatus* Rickettsiella vidridis´ and their host, the green pea aphid, *Acyrthosiphon pisum*, with the *Rickettsiella* endosymbiont being involved in host pigmentation (Tsuchida et al. 2010; 2014) and providing protection against aphid-pathogenic fungi (Lukasik et al. 2013). Despite the misleading genus name, *Rickettsiella* are not closely related to bacteria of the taxonomic genus *Rickettsia* (*Alphaproteobacteria*), but instead belong to the gamma-proteobacterial order *Legionellales* (Fournier and Raoult 2005). However, morpho- and cytological development inside the host and histopathology of infection resemble those of both *Rickettsia* and *Chlamydia*. In a generalized picture, *Rickettsiella* bacteria typically multiply in cytoplasmic vesicles within fat body cells or hemocytes, and multiplication is typically accompanied by the formation of crystal proteins of presumably lysogenic properties (Jurat-Fuentes and Jackson 2012 and references therein; Kleespies et al. 2014). Currently, *Rickettsiella* bacteria are under intensive evaluation as a new source of insect biocontrol agents.

Conclusion.

Past research has indicated several promising approaches for the solicited complementation, not substitution of *Bt*-based products by other microbial insecticides for biological control and integrated pest management. Present and future intensified and systematic screening for new bacterial entomopathogens will likely reveal further novel biocontrol resources. However, development of these microbial resources into innovative bio-insecticides in most cases is still well ahead.

RESUME

Leclerque A. 2018. Au-delà de *Bt*: Nouvelles ressources bacteriennes pour la lutte biologique contres les insectes. Tunisian Journal of Plant Protection 13 (si): 1-9.

La bactérie Gram-positive *Bacillus thuringiensis* (*Bt*) est l'agent entomopathogène le plus économiquement important pour la lutte biologique contre les insectes et pour une excellente raison, une large partie de l'effort investi dans le développement d'insecticides microbiens s'est concentrée sur les souches de *Bt* et leurs gènes *cry*. Des centaines de séquences de gènes *cry* ont été déterminées à partir de la nature, et la modélisation moléculaire a été utilisée pour créer des protéines recombinantes Cry artificielles avec des propriétés potentiellement nouvelles. Toutefois, des agents alternatifs pour la lutte biologique contre les insectes sont de plus en plus sollicités. Plus récemment, les efforts de la recherche se sont concentrés sur les agents pathogènes bactériens des insectes comme ressources potentielles d'agents de lutte biologique telles que les toxines non-*cry* de *Bt* et d'autres bactéries de la famille des *Bacillaceae* ainsi que la bactérie *Saccharopolyspora spinosa* (*Actinobacteria*). De plus, divers entomopathogènes γ-protéobactériens appartenant aux espèces *Serratia entomophila, Yersinia entomophaga* ou *Pseudomonas entomophila* ainsi que les genres *Providencia* ou *Rickettsiella* sont actuellement en cours d'évaluation pour leur potentiel de lutte biologique. La présente revue de littérature donne une brève mise à jour sur ces agents entomopathogènes et ses toxines.

Mots clés: *Bacillus thuringiensis*, lutte microbiologique, *Providencia, Pseudomonas entomophila, Rickettsiella,* Spinosad, toxine TcABC

ملخص

لوكليرك، أندرياس. 2018. ما بعد *Bt*: موارد بكتيرية جديدة للمكافحة البيو لوجية ضد الحشرات.

Tunisian Journal of Plant Protection 13 (si): 1-9.

إن البكتيريا غرام (+) من نوع *Bacillus thuringiensis* (*Bt*) هي العامل الممرض للحشرات الأهم اقتصاديا في المكافحة البيولوجية ضد الحشرات ولسبب ممتاز، جزء كبير من الجهد استُثمر في تطوير مبيدات ميكروبيولوجية للحشرات تكثّف حول سلالات *Bt* ومورّثاتها *cry*. حُددت مائات التتابعات للمورثات *cry* واستُعمل التصميم الجزيئي لانتاج البروتينات المؤتلفة Cry اصطناعيا ذات خصوصيات محتملة جديدة. إلا أن عوامل بديلة للمكافحة البيولوجية ضد الحشرات أصبحت مطلوبة أكثر فأكثر. في الآونة الأخيرة، تكثفت جهود البحث على عوامل ممرضة بكتيرية للحشرات كموارد محتملة لعوامل مكافحة بيولوجية مثل توكسينات لا-*cry* للبكتيريا *Bt* وبكتيريات أخرى من فصيلة *Bacillaceae* وكذلك البكتيريا *Saccharopolyspora spinosa* (*Actinobacteria*). أيضا، يتم حاليا تقييم إمكانية المكافحة البيولوجية لمختلف الممرضات الحشرية من طائفة γ-بروتيوبكتيريات التي تنتمي إلى الأنواع *Serratia entomophila* و *Yersinia entomophaga* أو *Pseudomonas entomophila* وكذلك الجنسان *Providencia* و *Rickettsiella*. إن هذه المقالة التوليفية تقدم تحيين مقتضب لهذه العوامل الممرضة للحشرات وتكسيناتها.

كلمات مفتاحية: توكسينات TcABC، سبينوراد، مكافحة ميكروبيولوجية، *Bacillus thuringiensis*، *Providencia*، *Pseudomonas entomophila*، *Rickettsiella*

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Morphological and Physiological Changes Induced in the Date Palm Trees (*Phoenix dactylifera*) Exposed to Atmospheric Fluoride Pollution

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ABSTRACT

Ben Amor, A., Elloumi, N., Chaira, N., and Nagaz, K. 2018. Morphological and physiological changes induced in the date palm trees (*Phoenix dactylifera*) exposed to atmospheric fluoride pollution. Tunisian Journal of Plant Protection 13 (si): 11-22.

Air quality bio-monitoring using plant leaves has been applied to assess the effects of atmospheric pollution. This study was conducted to evaluate the effects of fluoride (F) on date palm (*Phoenix dactylifera*) trees situated around a phosphate fertilizer-producing factory constituting a major source of pollution. Monthly observations on the southwest side of a phosphate fertilizer plant located in the coastal zone of the Gabès region have been assessed. This study was focused on the impact of F accumulation on the photosynthetic pigment content, cell membrane, and selected osmoprotectants (proline and soluble sugars) of the surveyed trees. Leaf samples were collected at various distances from the phosphate fertilizer factory (three sites at 0.5, 2.5, and 3.5 km and a control site at 35 km). Date palm trees accumulated significant amounts of F in leaves, with no visible lesions but showed a marked reduction in the photosynthetic pigment content, and damage to the cell membranes, as indicated by an increased malondialdehyde (MDA) content. The significant increases in the proline and soluble sugars contents in response to fluoride accumulation may be considered as defense mechanisms induced in response to fluoride stress. Based on photosynthetic pigment content, malondialdehyde (MDA), osmoprotectants levels and fluoride content, the date palm would be classified as a tolerant species.

Keywords: Adaptation, biochemical responses, biomonitoring, date palm, fluoride

Atmospheric pollution constitutes one of the major problems in industrial environments. Fluoride is one of the most important phytotoxic air pollutants (Weinstein and Davison 2003). Fluorides are absorbed through leaf stomata and move by transpiration into the principal sites of accumulation at the tip and leaf margins, where they can cause physiological, biochemical, and structural damage, and even cell death, depending on the concentration in the cell sap (Jacobson et al. 1966). Atmospheric fluoride can also reach the soil and contaminate plants via the roots. Thus,

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plants can also uptake fluoride from polluted soils (Ahmad et al. 2012; Gritsan, 1992; Jha et al. 2008; Wang et al. 2012).

The interactions between plant and different types of pollutants and the influence of environmental pollution on physiological and ultrastructural aspects were investigated by various researchers. In fact, Weinstein and Davison (2003) reported the existence of a relationship between the fluoride ion (F) concentration and damage in tree leaves. Mezghani et al. 2005 showed large differences in F concentrations among different plant species and large variations in the degree of plant tolerance to F pollution.

Fluoride concentrations in the range 1-10 µg F/g dry weight (dw) are considered as the normal background values in plant leaves (Davison et al. 2006, Brougham et al. 2013).

However, Sheldrake et al. (1978) signaled that other plants such as elderberry (*Sambucus nigra*) and camellia (*Camellia sasanqua*) did not show any toxicity symptoms at up to 3600 mg F/g dw.

Some plant species have been suggested for active and passive biomonitoring of airborne fluoride effects (Franzaring et al. 2013; Junior et al. 2008). Biomonitoring of air quality using plants has been widely applied to detect the effects of air pollution (Anicic et al. 2011; Markert et al. 2003) where many plant groups have been used in monitoring pollution pointing out their advantages; while there has been little focus on evergreen trees (Sawidis et al. 2012).

The assessment of air pollution impact includes analyses of visible injury (Ghosh et al. 1998; Oksanen and Holopainen 2001), accumulation of toxic substances and evaluation of biochemical and physiological pollutant-induced changes in parameters related to photosynthesis, respiration, enzyme activities, lipid synthesis, proteins and other metabolites (Bamniya et al. 2012, Elloumi et al. 2014, Herbinger et al. 2002). In fact, pollution may cause a decline in the number of seeds per fruit and in the leaf number and area (Gupta and Ghouse 1986; Wali and al. 2007). Air pollution can also induce qualitative and quantitative changes in the secondary metabolite composition (Kanoun et al. 2001).

In industrial areas, where mosses and lichens are absents, higher plants have gained special importance and are used as valuable biomonitors. Recent researches on the effects of F on the growth and physiological characteristics were focused on F-sensitive plants, such as *Prunus dulcis* (Elloumi et al. 2005) and *Punica granatum* (Ben Abdallah et al. 2006).

However, the olive plant, *Olea europaea*, is considered as F-tolerant plant (Zouari et al. 2016).

In Gabès, the Tunisian Chemical Group specialized in transformation and treatment of phosphate, constitutes the main source of fluoride pollution in the atmosphere. The atmospheric fluorides and sulfur emitted by the factory primarily occur in gaseous forms, such as sulfur dioxide and hydrogen fluoride, and to a lesser extent in inorganic particulate including sulfur trioxide, calcium fluoride, lead fluoride and calcium phosphate fluoride (Azri et al. 2002; Ben Abdallah et Boukhris 1990). Also, Ben Abdallah et al. (2006) and Mezghani et al. (2005) reported that fluoride is the most important phytotoxic air pollutant in the vicinity of the phosphate fertilizer factory.

In Gabès, the date palm (*Phoenix dactylifera*) trees are typical local trees growing over large areas. Even where

high levels of pollution exist, they can be seen almost everywhere in the industrial and agricultural areas. Therefore, the aims of this present work were (1) to survey the leaves of local date palm trees growing in Gabès industrialized areas for fluoride accumulation and (2) to study some morphological and physiological parameters of some trees exposed to industrial emissions.

MATERIALS AND METHODS

Study area.

The present study was carried out in the industrial area of Gabès located 376 km south-east of Tunis on the southern side of the Gulf of Gabès (Mediterranean Sea, Gabès city), which has an arid climate with a low average rainfall (from 167 to 176 mm average annual pluviometry) and an average annual temperature from 18.8 to 19.3ºC.

In this study, we selected three oases located relatively close to the factory complex (site 1 [S1] 0.5 km, site 2 [S2] 2.5 km, and site 3 [S3] 3.5 km from the factory), and, as a control oasis with less fluoride exposure, a more distant one (35 km from the factory).

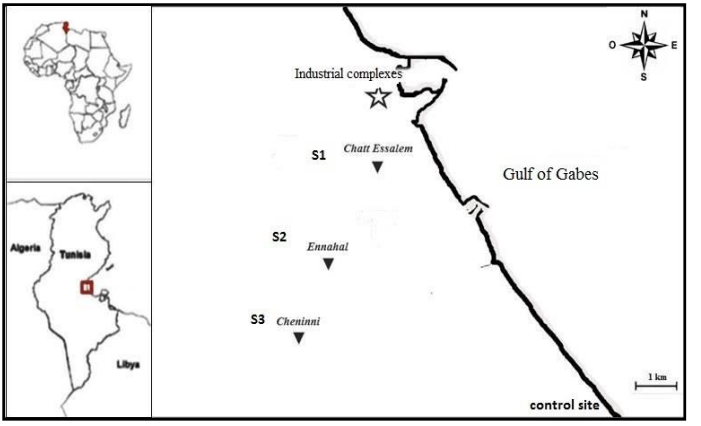

Fig. 1. Map of the study area in south east Tunisia, selected sites are named (S1, S2, S3, and control).

Sample collection.

The sampling of date palm trees,variety 'Bouhattem', was done during April, May and June 2014 to avoid rain washing fluoride.

Date palm leaves were collected with a maximum of 5 m trunk height. Three leaf samples were taken from several branches in different parts of the tree side exposed to the factory fume.

Only leaves occupying the middle of the shoots were taken.

Determination of fluoride content.

Unwashed leaf samples from all trees were dried for 24 h at 105 °C. For each repetition a 2 g powdered sample was heated in an oven at 550 ºC until white ashes were formed.

After extraction of the ash sample into NaOH solution, F concentrations were determined potentiometrically using a fluoride-specific ion electrode and a reference electrode. For measurement of total F (complex and free) in the solution, the NaOH extract was acidified with acetic acid glacial to pH 5.3 and mixed 1:1 with Total Ionic Strength Adjustment tampon buffer (TISAB). The TISAB buffer contained 57 ml acetic acid, 58 g NaCl and 4 g CDTA per litre, adjusted to pH 5.2 with 6 M NaOH and diluted to 1 liter with distilled water (Ben abdallah et al. 2006).

Determination of pigment content.

Chlorophyll a (chl a), chlorophyll b (chl b), and total chlorophyll (chl a+b) determinations were taken from fully expanded leaves of plants. A sample of 0.1 g of leaves was weighed and ground in 5 ml of 80% acetone. After filtration, the extraction was adjusted to 10 ml with 80% acetone, and the content of photosynthetics pigments was determined spectrophotometrically according to Arnon (1949).

Malondialdehyde (MDA) content.

The level of lipid peroxidation in the leaf tissues was measured in terms of malondialdehyde content (MDA a product of lipid peroxidation), determined according to Heath and Packer (1968) with minor modifications as described by Zhang and Kirham (1944).

A 0.25 g leaf sample was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 5 min. Then, 4 ml of 20% TCA containing 0.5% TBA were added to a 1 ml aliquot of the supernatant. The mixture was heated at 95ºC for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was read at 532 nm and the value of the nonspecific absorption at 600 nm was subtracted.

Proline and soluble sugar content.

Proline content was analyzed according to Bates et al. (1973). Soluble sugars were analyzed according to Robyt and White (1987).

Statical analyses.

All statistical analyses were performed with SPSS version 17 software. Duncan's Multiple Range test was used to determine the significance of differences between treatments, at $P \leq 0.05$.

RESULTS

Visual symptoms.

Examined date palm trees did not show any morphological abnormalities such as chlorosis, leaf curling, or necrosis. Leaves did not show any visible lesions. The under surface of the plant leaves tended to accumulate dust and appeared white. Leaves from plants removed from control area did not show any morphological abnormalities.

Fig. 2. Leaves of date palmcollected at site 1, located at 0.5 km from the Gabès phosphate fertilizer factory.

Compared to leaves removed from control site, leaves from polluted sites showed a considerable reduction of the foliar surfaces even though they have the same age.

Fluoride concentration in leaves.

F concentrations of date palm leaves collected at different distances from the phosphate fertilizers are presented in Fig. 3. The maximal contents of fluorine (95.5 µg/g dw) were recorded in leaves removed from site 1 which is the closest site to the source of pollution. At 35 km from the factory, the F concentration decreased (61.7 µg/g dw).

Regarding the temporal evolution of fluorine concentrations, the highest fluorine contents were recorded in May. This increase coincided with the decrease in precipitation. In June, recorded fluorine contents were lower than those of May.

The F concentrations in sampled date palm leaves, noted in April, May, and June were significantly higher than those of control (Fig. 3).

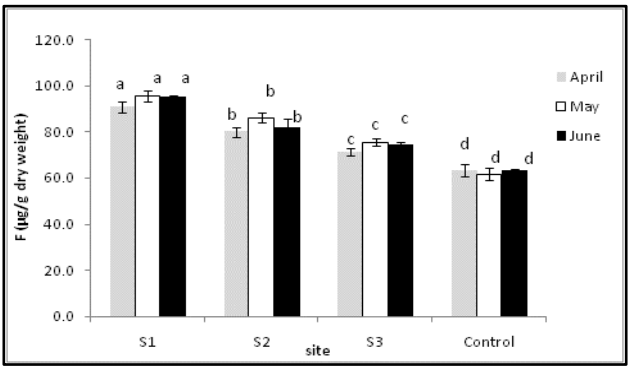

Fig. 3. Temporal variation of fluoride (µg/g dry weight) in date palm leaves at the polluted sites at increasing distances from the phosphate fertilizer factory. S1 at 0.5km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Pigment contents.

The pigment contents of date palm trees are presented in Fig.4. There were no significant differences in leaf chlorophyll content between the sites and months.

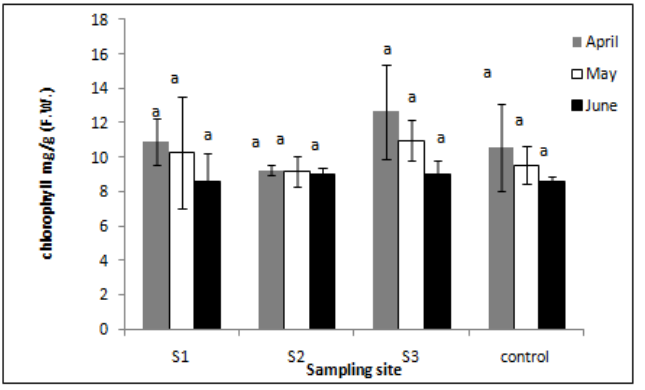

Fig. 4. Total chlorophyll contents (mg/g fresh weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Chlorophyll a (Chl a), Chlorophyll b (Chl b) and total chlorophyll (Chl (a+b) contents were measured at S1, the highest polluted site. Total chlorophylls showed no significant changes in response to fluoride accumulation. However, Chl b showed increase in June. Chl a decrease significantly; it was more sensitive to F stress. Chl *a* content decreased about 65% after three months of exposure to fluoride (Fig.5).

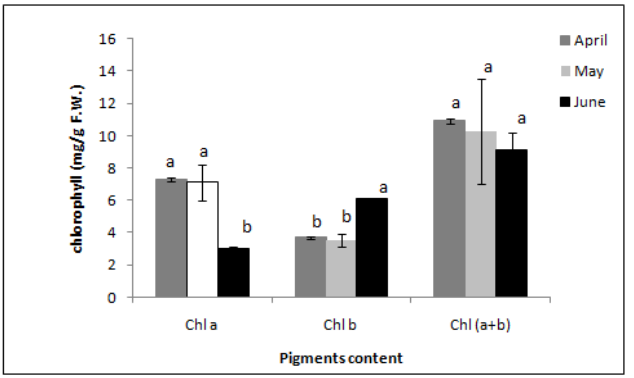

Fig. 5. Chlorophyll a (chl a), Chlorophyll b (chl b), and total chlorophyll (chl a+b) contents (mg/g fresh weight in the leaves of date palm at S1 (at 0.5 km the factory). For each pigment, means with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Malondialdehyde (MDA) content.

As an indicator of oxidative stress due to fluoride toxicity, an enhanced formation of malondialdehyde (MDA) was observed (Fig.6).

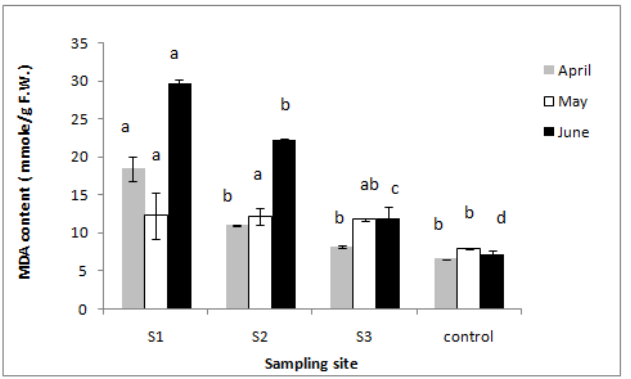

Fig. 6. MDA content (mmol/g fresh weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$

The MDA content rose markedly at the polluted sites indicating enhanced lipid peroxidation. The MDA content in leaves of date palm increased with fluoride contamination. The highest MDA levels were detected in the S1 (29.5 mmole/g fresh weight fw) where they were 4 times higher than those recorded in leaves removed from the control site (7.1 mmole/g fw) .

Large increases in the foliar MDA accumulation during the exposure periods were detected each month at all the sites. MDA content was highest at S1 in June (29.5 mmole/g fw), decreasing at the S2 (22.1 mmole/g fw) and S3 (11.9 mmole/g fw) sites.

Proline and soluble sugar content.

The impact of fluoride accumulation on some selected osmoprotectants (proline and soluble sugars) was determined. Compared to the control site, the proline content at S1 was increased in June (Fig.7). Proline levels increased from 20.8 µmol/g fw in April to 29.8 µmol/g fw in June.

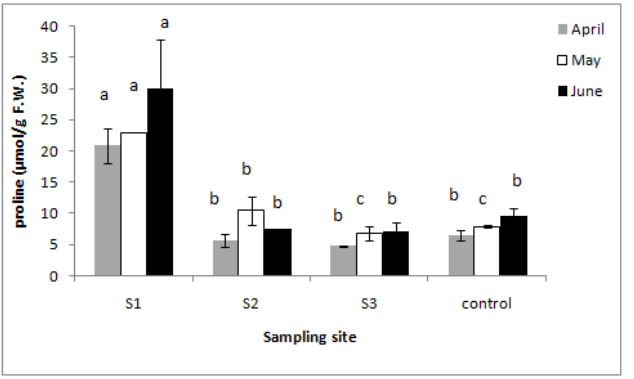

Fig. 7. Proline content (µmol/g fresh weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5 km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

The fluoride content also had a great influence on the leaf soluble sugars concentration (Fig. 8). In fact, at all sites, soluble sugar increased from April to June. As compared to the control site, the soluble sugar content was significantly increased in June in all sites and more importantly in S1 (226.33 mg/g dw).

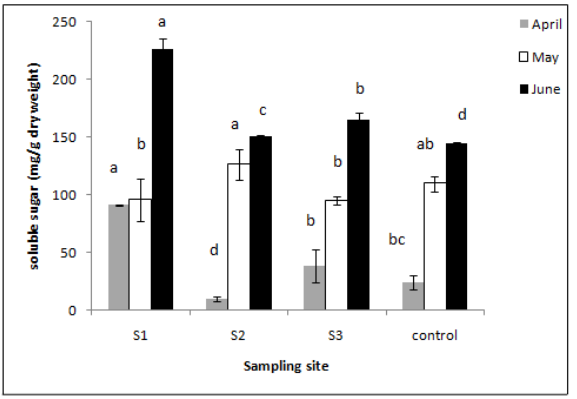

Fig. 8. Soluble sugars content (mg/g dry weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5 km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

DISCUSSION

Leaves of date palm were monitored for the F contamination. The F accumulation in the study area was high in polluted sites. Date palm possesses an important accumulative capacity of fluoride. This fluoride accumulation in leaves differed significantly depending on sampling sites and times. The high fluoride values were noted in sites close to the phosphate fertilizer-producing factory suggesting that this factory constitutes an important source of pollution. The highest concentrations of F were found in leaves sampled in May.

Thus, the dry period is an important factor that strongly influences air pollutant concentrations. In fact, in polluted areas, the dry season accelerates pollutant deposition. Therefore, pollutant availability for plant absorption is greater during drier periods. In arid regions, although high levels of solar radiation, evapotranspiration is high due to the low humidity in these areas (Allen et al. 2006). The decrease in the F contents in June, compared to the values in April and May, coincided with a marked precipitation registered for June (117 mm) (Elloumi et al. 2016). Date palm was found able to accumulate fluoride without exhibiting any symptoms of F toxicity with F concentrations up to 95 µg/g dry weight. As date palm is an evergreen species, air pollution biomonitoring can be done all the yearlong.

The chlorophyll content decreased with the increase of F concentrations. This reduction in the chlorophyll content in sampled leaves, as compared to controls, may be attributed to the high emission and deposition of dust on leaves, which adversely affects the metabolic activity of the plant (Ben abdallah et al. 2006). The reduction in chlorophyll content in the fluoride-stressed date palm trees could be due to structural alterations in the chloroplasts such as disorganization of the thylakoid system and damage to the stroma chloroplasts (Li et al. 2001; Singh et al. 2010). Moreover, according to Elloumi et al. (2015), the decrease of photosynthesis, stomatal conductance, and transpiration rates observed in almond plants grown in a fluoride-polluted zones could be attributed to

abnormalities of the stomata, such as less stomatal density and stomatal closure. The reduction of the photosynthetic performance in the F pollution of date palm trees could be considered as an adaptive mechanism.

In the present study, high MDA levels were detected. MDA is an end product of membrane lipid peroxidation and high MDA levels in plants are used as an indicator of oxidative stress (Niedworok and Bielaszka 2007). Some organic solutes in plants (such as proline and soluble sugars) act osmoprotectants in adaptation to environmental stress such as drought, heavy metals and increased salinity. Sugar metabolism is adversely affected in plants growing under stressful conditions. In many plant species, the accumulation of soluble sugars has been observed in response to various environmental stresses. The accumulation of proline and soluble sugars in stressed date palm plant tissues can be used as endpoints to assess fluoride tolerance (Chakrabarti et al. 2015; Maitra et al. 2013; Ram et al. 2014). Proline accumulation can serve as a selection criterion for the tolerance of most species to stressed conditions (Ashraf and Foolad 2007). The capacity for osmotic adjustment observed in the fluoride stressed date palm trees via the accumulation of proline and soluble sugars could be considered as an adaptive mechanism.

The results of the present work showed that exposure of date palm to fluoride air pollution increased proline and sugar contents. The increase in proline contents is an important factor for providing higher tolerance to fluoride.

The increased proline content is referred to as a protective mechanism due to the generation of reactive oxygen species by fluoride. The accumulation of proline under the effect of stress provides energy for the growth and survival of the plant and helps it to tolerate stress. Thus, date palm could be considered a tolerant species; it is very well adapted to the atmospheric fluoride pollution in Gabès area, with absence of visible damages.

RESUME

Ben Amor A., Elloumi N., Chaira N. et Nagaz K. 2018. Les réponses morphologiques et physiologiques induites chez le palmier dattier (*Phoenix dactylifera*) exposé à la pollution fluorée atmosphérique. Tunisian Journal of Plant Protection 13 (si): 11-22.

La bio-surveillance végétale est un bio-indicateur de l'évaluation de la qualité de l'air et de l'environnement. Cette étude consiste à évaluer les effets des fluorures (F) sur le palmier dattier (*Phoenix dactylifera*) au voisinage des unités de traitement et de production des engrais phosphatés, constituant la principale source de la pollution atmosphérique dans la région de Gabès. Des observations mensuelles sur les teneurs en chlorophylle, la membrane cellulaire et quelques osmoprotecteurs tels que la proline et les sucres solubles ont été effectuées. Trois sites ont été choisis en fonction de leur distance par rapport à la source polluante (à 0,5, 2,5, et 3,5 km, le site témoin 35 km). Les feuilles du palmier dattier ont accumulé les valeurs significatives du fluor, avec l'absence des lésions visibles. Une réduction remarquable des teneurs de pigment photosynthétique a été enregistrée. L'augmentation significative de proline et de sucres solubles en réponse à l'accumulation de fluorure peut être considérée comme une stratégie de défense. En se basant sur les teneurs de pigment photosynthétique, le malondialdehyde (MDA), le contenu de fluorure, les teneurs de sucres solubles et de proline, le palmier dattier pourrait être classé comme une espèce tolérante.

Mots clés: Adaptation, bio-surveillance, palmier dattier, réponses biochimiques

ملخص

بن عمر، عفاف وندى اللومي ونزار شعيرة وكمال الدين نقاز. 2018. الاستجابات المورفولوجية والفيزيولوجية المستحثة لدى نخيل التمر المعرض للتلوث الفلوري للغلاف الجوي.

Tunisian Journal of Plant Protection 13 (si): 11-22.

تعتبر المراقبة البيولوجية استنادا إلى النباتات مؤشرا بيولوجيا فعالا لتقييم نوعية الهواء والبيئة. تهدف هذه الدراسة إلى تقييم تأثير التلوث بالفلوريدات الناتج عن وحدات صنع الأسمدة الفوسفاتية بمنطقة قابس على نخيل التمر المجاور. تم اختيار 3 مسافات مختلفة انطلاقا من المصنع 0.5 و 2.5 و 3.5 كم، أما منطقة الشاهد فتم اختيارها على بعد 35 كم. سجلت أوراق النخيل ارتفاعا نسبيا للفلور دون ظهور أعراض بينما سجل مستوى اليخضور انخفاضا واضحا. ويعتبر ارتفاع مستوى البرولين والسكريات القابلة للذوبان استراتيجيات دفاع عند أشجار نخيل التمر. باعتماد بعض المؤشرات البيوكيميائية (مستوى اليخضور والمالوندیلدهید والفلوريدات والسكريات القابلة للذوبان والبرولين)، يمكن تصنيف نخيل التمر كنوع نباتي متحمل للتلوث الهوائي.

كلمات مفتاحية: استجابة بيوكيميائية، تأقلم، مراقبة بيولوجية، نخيل التمر

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Hepatotoxicity Induced by Chlorpyrifos in 'Wistar' Rats

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ABSTRACT

Chenikhar, H., Djabri, B., Salmi, A., Taib, C., and Rouabhi, R. 2018. Hepatotoxicity induced by chlorpyrifos in 'Wistar' rats. Tunisian Journal of Plant Protection 13 (si): 23-30.

Chlorpyrifos-ethyl is one of the most widely used organophosphorus insecticides for industrial, agricultural and public health purposes. The present work aimed to evaluate the chlorpyrifos-ethyl induced hepatic toxicity on some parameters of oxidative stress in 'Wistar' rats. After daily gavage for 60 days, this insecticide induced cellular damage in exposed rats through the increase in alanine aminotransferease (ALT) and aspartate aminotransferase (AST) activities. In addition, liver parameters showed an increase in hepatic lipid peroxidation as indicated by the malondialdehyde (MDA) contents, as well as a decrease in the catalase (CAT) activity. A reduction in glutathione (GSH) and glutathione-S-transferase (GST) and an increase in glutathione peroxidase (GPx) activities were also noted. These alterations indicated that chlorpyrifos affected the antioxidant system in hepatic tissue, induced hepatic damage and consequently triggered the oxidative stress.

Keywords: Antioxidant system, chlorpyrifos-ethyl, hepatic toxicity, lipid peroxidation, oxidative stress

The widespread use of pesticides in public health and agricultural programs has caused severe environmental pollution and potential health hazards, including acute and chronic cases of human poisoning. Organophosphate insecticides (OPI) constitute one of the most widely used classes of pesticides being employed for both agricultural and landscape pest control (El-Bini Dhouib et al. 2015; Kamath and Rajini 2007). Moreover, residual amounts of pesticides have been detected in the soil, water bodies, vegetables, grains, and other food products (Poet et al. 2004).

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Chlorpyrifos (CPF) is one of the most widely used for domestic, agricultural, and industrial purposes and for public health applications OPI (Akande 2016; Richardson and Chambers 2005). It is able to cause oxidative stress and histopathologic changes in humans and animals, and it can cause embryotoxicity, teratogenicity, immune-logical abnormalities, neurobehavioral changes, developmental and reproductive toxicity, neurotoxicity (Breslin et al. 1996; Ki et al. 2013), hematologic changes (Uzun and Kalender 2013), and testicular toxicity (Joshi et al. 2007). These findings on the various toxicities caused by CPF are of great concern to the general public.

The principle mode of action of CPF is the phosphorylation and subsequent irreversible inhibition of

acetylcholinesterase (Eaton 2008; Wang et al. 2013). This enzyme hydrolyses acetylcholine at cholinergic synapses and neuro-muscular junctions and the persistent inhibition of its activity causes neurotoxic effects (Luis et al. 2015) disrupting cholinergic function in the nervous system (Lassiter and Brimijoin 2008). CPF has also been shown to elicit oxidative stress in biological systems (Ahmed et al. 2010; Alvarez et al. 2008; Vermaet al. 2007).

Oxidative stress caused by reactive oxygen species (ROS) has been reported in membrane lipid peroxidation, DNA damage, mutagenesis and has been associated with the various stages of tumor formation process (Sidhuet al. 2014). Oxidative stress can occur when there is an imbalance between ROS production and antioxidant defenses (Preedy 2013). Acute and chronic exposure to CPF has resulted in considerable liver damage, as evidenced by changes in several liver enzymes (Goel et al. 2000). Liver is the first organ exposed to ingested toxins including pesticides, metals, etc., for biotransformation. Therefore, toxic responses have been reported to occur more in the liver as compared to other organs (Raina et al. 2015) and it is the organ where activation and detoxification of CPF take place. It is demonstrated that OP pesticides like CPF causes hepatotoxicity by changing the profile of liver marker enzymes such as alanine aminotransferase (ALP), aspartate aminotransferase (AST), lactate déshydrogénase (LDH) and histopathological changes (Mansour and Mossa 2009). Therefore, in toxicological studies histopathological lesions have been widely used as biomarkers (Ncibi et al. 2008). For this reason, the aim of this work was to study the alterations in hepatic functions and oxidative damage in hepatocytes following repeated oral exposure to CPF during 60 days in 'Wistar' rats.

MATERIALS AND METHODS

Pesticide.

DURSBAN 4® (Dow agrosciences) containing 480 g chlorpyrifos per liter was used in the present study.

Animals.

Adult female rats of 'Wistar' strain, weighing 190-210 g were purchased from the Pasteur Institute of Algiers, Algeria. They were kept in metal cages at 23±2ºC, 40-60% relative humidity, and light/dark cycle of 12 h. These spleens received water and standard diet and were subjected to the force-feeding every day, during a period of 60 days.

Experimental protocol.

The body weight of all the animals was checked every day. The rats were randomly divided into two groups composed each of five rats. The protocol of rat treatment is given below:

- Group I (control): Animals were administered corn oil only.
- Group II (CPF-treated): Animals were administered CPF dissolved in corn oil (1/25 LD_{50}) (Tanvir et al. 2015).

After 60 days of treatment, the blood and liver tissue were collected for biochemical examinations as indicated below.

Biochemical marker enzymes in plasma.

After 60 days, rats were fasted overnight. Then, they were weighed and sacrificed. The blood samples were collected from each rat and a 2 ml-sample was injected in a glass tube without EDTA and left for 20 min to coagulate at

room temperature, and then centrifuged at 3000 rpm for 10 min to obtain serum samples used for biochemical assays.

Measurement of malondialdehyde level.

MDA can be detected by a colorimetric reaction with thiobarbituric acid (TBA). It was detected after degradation of polyunsaturated fatty acids 3 or 4 Double peroxidized bonds. This is a highly sensitive method for determining lipid peroxidation in vitro. The assay of MDA was carried out according to the method of Esterbauer et al. (1992). Optical density has been measured at λ = 532 nm.

Antioxidant enzymes assay.

Glutathione (GSH) level was determined according to the method of Ellman et al. (1959). This assay is based on the measurement of 2-nitro-5-mercapturic absorbance at λ= 532 nm. The latter resulted from the reduction of the acid 5,5'-dithiobis-2-nitrobenzoic acid (reagent Elleman) by groups (-SH) of glutathione. Once prepared, it must undergo homogenate deproteinization (by 0.25% sulfosalicylic acid) to protect the SH-groups of glutathione.

The enzymatic activity of GPx was determined according to Flohe and Gunzler (1984) method using H_2O_2 as substrate. The spectrophotometric assay of catalase (CAT) activity was performed according to Aebi (1984). The decrease of absorbance was noted for 3 min by a spectrophotometer at a wavelength of 240 nm and an extinction coefficient $\varepsilon = 0.036$ $mM^{-1}cm^{-1}$.

The activity of glutathione S-transferase (GST) was determined according to the method of Habig et al. (1974). It is based on the conjugation reaction between GST and a substrate, the 1-Chloro2,4-dinitrobenzene (CDNB) as a cofactor of GST, the conjugation results in the formation of a new molecule: 1-S-glutathionyl 2-4-Di nitrobenzene to measure the activity of GST.

Statistical analysis.

Data were expressed as mean ± SD of five rats in the group. Significant differences between the control and the treated groups were determined by the Student's *t*-test. Statistical calculations were carried out using Minitab 17.1 statistical package and the Excel 16.0 (Microsoft, Inc.). The level of statistical significance was set at $P < 0.05$.

RESULTS

Effect on liver function biomarkers.

There was a significant increase ($P \leq 0.001$) in ALT activity levels in chlorpyrifos (CPF treated rats (58.66 ± 2.08 IU/L) compared to control animals (38.00 ± 4.00 IU/L). Similarly, the value of AST was significantly ($P \leq 0.001$) increased in CPF group (62.00 ± 2.64 IU/L) compared to control (32.33 ± 2.51 IU/L) (Table 1).

Effects of CPF on lipid peroxidation.

Treatment with CPF induced significant ($P \leq 0.01$) increases in MDA levels in the treated group (0.632 ± 0.140 nmol/mg prot) as compared to control group (0.557 ± 0.071 nmol/mg prot) (Table 2).

Effects of CPF intoxication on antioxidant defense system in the liver tissue.

The administration of CPF caused significant ($P \leq 0.05$) decreases in CAT (0.099 ± 0.015 µmol/min/mg prot), GSH (1.158×10^5 ± 1.093×10^7 µmol/min/mg prot) and GST (0.003 ± 0.048×10^2 µmol/min/mg prot) activities in liver compared to the control group (0.190 ± 0.005 µmol/min/mg prot; 2.469×10^5

±2.376×10^6 µmol/min/mg prot; 0.006 ± 0.001 µmol/min/mg prot, respectively). A significant ($P \leq 0.05$) increase in GPx activity was also noted following oral administration of CPF to rats (0.574 ± 0.056 µmol/min/mg prot) relative to the control ones (0.422 ± 0.076 µmol/min/mg prot) (Table 2).

Table 1. Effect of treatment with chlorpyrifos (CPF) on hepatic injury markers in the plasma of 'Wistar' rats

Treatment	Liver function biomarker	
	ALT (IU/L)	AST (IU/L)
Control	38.00 ± 4.00	32.33 ± 2.51
Chlorpyrifos (CPF)	58.66 ± 2.08***	62.00 ± 2.64***

Values given are means ± SD of the results obtained from 5 rats. Means with at least one common superscript do not differ significantly at $P \leq 0.05$. ALT: Alanine aminotransferease, AST: Aspartate aminotransferase
*** Very highly significant at $P \leq 0.001$

Table 2. Effect of chlorpyrifos (CPF) on lipidperoxidation, reduced glutathione and activities of enzymatic antioxidants in the liver of experimental animals.

Treatment	Control	Chlorpyrifos (CPF)
MDA	0.557 ± 0.071	0.632 ± 0.140**
GSGST	0.006 ± 0.001	0.003 ± 0.048×10^{2*}
GSH	2.469×10^5 ±2.376×10^6	1.158×10^5 ± 1.093×10^{7*}
CAT	0.190 ± 0.005	0.099 ± 0.015***
GPx	0.422 ± 0.076	0.574 ± 0.056

MDA (nmol/mg prot), GST (µmol/min/mg prot), GSH (µmol/min/mg prot), CAT (µmol/min/mg prot), GPx (µmol/min/mg prot). Values given are mean ± SD of the results obtained from 5 rats. Means with at least one common superscript do not differ significantly at $P \leq 0.05$.
* Significant at $P \leq 0.05$; ** Highly significant at $P \leq 0.01$; *** Very highly significant at $P \leq 0.001$.

DISCUSSION

In the present study, we demonstrated that CPF administrated to rats provoked a marked elevation in serum AST and ALT activities. These findings are in accordance with various previous studies (Ambaliet al. 2011; Geolet al. 2005; Heikal 2013; Mansour and Mossa 2011; Ncibi et al. 2008). These increases could potentially be attributed to the release of these enzymes from the cytoplasm into the blood

circulation indicating a necrosis and inflammatory reactions (Acker et al. 2012; Tanvir et al. 2015), and reflect the alteration of the membrane permeability of the hepatocytes (Raina et al. 2015).

Various studies indicate the production of reactive oxygen species (ROS) as secondary means of toxicity (Sidhu et al. 2014). We showed that rats exposure for 60 days to CPF caused an increase in malondialdehyde (MDA) level, which is the major end products of lipid peroxidation, in liver of rats. This effect may be ascribed to an excessive production of ROS by a pesticide-induced oxidative cell (Akande et al. 2014). This result is in agreement with those of other studies showing an increase in lipid peroxidation after treatment with CPF (Gultekin et al. 2000; Kalender et al. 2012; Ojha et al. 2011; Saulsbury et al. 2009; Uzun and Kalender 2013). We have demonstrated that CPF-induced injury is caused by the induction of oxidative stress which was suggested to be an additional factor inducing apoptosis. Tuzmen et al. (2008) demonstrated previously that rats developed lipid peroxidation and liver damage subsequent to CPF exposure.

In the present study, the liver rats treated with CPF showed significant decreases in GSH and GST levels and in CAT activity but there was an increase in the GPx activity. The perturbation of these enzymes may be due to oxidative stress of pesticide intoxication. This is in accordance with previous works (Aggarwal et al. 2014; Ajay et al. 2005; Mansour and Mossa 2011; Raina et al. 2015). Therefore, Adedara et al. (2015) revealed a decrease of catalase and GST activities in treated *Drosophila melanogaster* following CPF treatments. Other researchers also recorded an elevation in CAT activity (Ozkan et al. 2012; Tuzmen et al. 2008; Uzun and Kalender 2013) and in GST level (Cacciatore et al. 2015). However, Aly et al. (2010) recorded an increase in GSH level and GPX activity was unchanged. Thus, CPF increased rate of hepatic lipid peroxidation (MDA), with the depletion in the defense system, suggesting that this alteration induced by CPF involved an oxidative stress.

GSH plays a key role in the detoxification of free radicals. It directly interacts with high affinity with thiol groups (-SH) of GSH. The GPx is a key antioxidant enzyme, which regulates the level of ROS (GPx is capable not only to reduce the hydrogen peroxide in water, but also the resulting hydroperoxides of oxidation of unsaturated fatty acids). Concerning the glutathione S-transferase (GST), this enzyme plays an important role in detoxification of xenobiotics. Catalase (CAT) is the second step in the enzymatic defense system (Preedy 2013).

To conclude, the results from the current study indicate that oral exposure of CPF produces hepatic damage. Furthermore, the oxidative stress and the altered antioxidant system in liver are due to imbalance between oxidant and antioxidant levels in hepatic tissues.

RESUME

Chenikhar H., Djabri B., Salmi A., Taib C. et Rouabhi R. 2018. Hépatotoxicité induite par le chlorpyrifos chez les rats 'Wistar'. Tunisian Journal of Plant Protection 13 (si): 23-30.

Le chlorpyriphos-éthyl est l'un des insecticides organophosphorés les plus largement utilisés à des fins industrielles, agricoles et des applications de la santé publique. Le présent travail consiste à évaluer la toxicité hépatique induite par le chlorpyriphos-éthyl sur quelques paramètres du stress oxydatif. Après

un gavage quotidien pendant 60 jours, cet insecticide a provoqué des dommages cellulaires chez les rats exposés par l'élévation des activités de l'alanine aminotransférase (ALT) et l'aspartate aminotransferase (AST). En outre, les paramètres hépatiques ont montré une augmentation de la peroxydation lipidique hépatique qui a été évaluée par les teneurs en malondialdéhyde (MDA), ainsi qu'une diminution de l'activité de la catalase (CAT). Une réduction de la glutathion (GSH) et de la glutathion-S-transférase (GST) et une augmentation des activités de glutathion peroxydase (GPx) ont également été notées. Ces altérations ont indiqué que le chlorpyrifos a affecté le système antioxydant dans le tissu hépatique, a induit des lésions hépatiques et a déclenché, par conséquent, le stress oxydatif.

Mots clés: Chlorpyriphos-éthyl, peroxidation lipidique, stress oxydatif, système antioxydant, toxicité hépatique

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ملخص

شنيخر،هاجر وجابري بلقاسم وسالمي آية وتايب شهيناز وروابحي رشيد. التسمم الكبدي الناجم عن كلوربيريفوس (chlorpyrifos) عند الفئران 'ويستار'. Tunisian Journal of Plant Protection 13 (si): 23-30.

كلوربيريفوس-إثيل (Chlorpyriphos-éthyl) هو أكثر المبيدات الفوسفورية استعمالا للأغراض الصناعية والزراعية والصحة العامة. يهدف هذا العمل إلى تقييم التسمم الكبدي الناجم عن استعمال كلوربيريفوس-إثيل على بعض معاملات الإجهاد التأكسدي. بعد تزقيم الفئران يوميا لمدة 60 يوم أدى هذا المبيد الحشري إلى تلف الخلايا بارتفاع أنزيمات Aminotransferase (ALT) و Aspartate aminotransferase (AST). كذلك المعاملات الكبدية بينت زيادة مستوى الاكسدة الدهنة الكبدية وذلك من خلال تقييم تركيز Malondialdehyde (MDA)، إضافة إلى انخفاض في نشاط أنزيم كاتالاز(CAT). سجل أيضا انخفاض في Glutathion (GSH) و Glutathion-S-transferase (GST)، مع ارتفاع في مستوى Glutathion peroxydase (GPx). أكدت هذه الاضطرابات أن كلوربيريفوس يؤثر على النظام المضاد للأكسدة في الكبد مؤديا الى جروح في الكبد وبالتالي يسبب الإجهاد التأكسدي.

كلمات مفتاحية: إجهاد تأكسدي، أكسدة دهنية، تسمم كبدي، كلوربيريفوس-إثيل، مضاد للأكسدة

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Impact of Nitrogen Fertilization on Fusarium Foot and Root Rot and Yield of Durum Wheat

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ABSTRACT

Hemissi, I., Gargouri, S., Hlel, D., Hachana, A., Abdi, N., and Sifi, B. 2018. Impact of nitrogen fertilization on Fusarium foot and root rot and yield of durum wheat. Tunisian Journal of Plant Protection 13 (si): 31-38.

This study investigated the influence of nitrogen fertilization on Fusarium foot and root rot. Disease index, percentage of white heads, grain yield, weight of 1000 grains and nitrogen content were evaluated in durum wheat after artificial inoculation with *Fusarium culmorum* under field conditions. The trial was conducted using Karim wheat cultivar during growing season 2016/17. Five nitrogen rates, 0, 50, 100,150 and 200 kg N/ha were evaluated. Nitrogen supply at higher rates (150 and 200 kg/ha) significantly increased disease index, the percentage of white heads, the grain yield, the weight of 1000 grains and nitrogen content. These results suggest that high amounts of nitrogen fertilization may increase infection of wheat by Fusarium foot and root rot disease by influencing the plant physiology.

Keywords: Durum wheat, *Fusarium culmorum*, nitrogen rates

Fusarium foot and root rot is one of the most important diseases of cereals throughout the world and has been reported since the 1970's in Tunisia (Gargouri et al. 2001; Ghodbane et al. 1974). Different cereals can be infected including durum wheat, representing more than 50% of the total cereal areas cultivated in Tunisia (Anonymous 2013; Slama et al. 2005), which is the most susceptible (Wallwork et al. 2004). Up to 26% yield losses have been recorded on durum wheat in Tunisia which were attributed to Fusarium foot and root rot disease (Chekali et al. 2013). The disease is caused by a complex of *Fusarium* species among which *F. culmorum* is the most important ones (Gargouri et al. 2007). This pathogen produces lesions on the coleoptiles, roots, and sub-crown internodes of host plants, and cause browning of the stem bases at or near the soil surface, from soil- or residue-borne inoculum. Damage to cereals is often unnoticed until white heads appear shortly before the crops mature or until shriveled grain is noted during harvest

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(Burgess et al. 2001). Various studies suggest that the application of integral wheat protection measures such as cultivation of resistant cultivars, crop rotation, tillage, and application of appropriate fertilizers and fungicides can significantly reduce wheat infection by *Fusarium* species (Lemmens et al. 2004; Osborne and Stein 2007). The present study investigated the effect of nitrogen fertilization on *F. culmorum* infections in durum wheat under field condition.

MATERIALS AND METHODS

Inoculum production.

Four isolates of *F. culmorum*, obtained from stems of durum wheat collected at Jendouba (2014) were used in this study. The identification on these isolates was based on typical morphological characteristics of the colony on PDA (carmin red, fast growing colony) and the shape of macroconidia (short and stout) as described by Burguess et al. (1994). *F. culmorum* isolates were maintained on PDA medium at 4°C until used. For inoculum production, oat grains were soaked in water overnight and excess water was drained off and a grain sample of 250 cm^3 was autoclaved twice over 2 days at 120°C for 20 min. Pieces from PDA plates colonized with each of the 4 selected isolates were added to oat grains and incubated for 3 weeks at 25°C. The colonized oat grains were air-dried on filter paper ground in laboratory mill and passed through a 2-mm sieve.

Field trial and nitrogen application.

This study was carried during the growing season 2016/17 in the experimental field of the *Institut National des Grandes Cultures* (INGC) in Tunisia using the Karim durum cultivar. After preparation of the seed bed, seeds were sown at a density of 350 seeds/m^2. Each plot consisted of six rows, 2 m long and 25 cm apart. Fungal inoculum (2.5 g/row) was sprinkled on top of seeds in corresponding plot and closed over. The control treatments consisted of non-inoculated plot. Five nitrogen (N) fertilization levels: 0 kg N/ha (T0), 50 kg N/ha (T1), 100 kg N/ha (T2), 150 kg N/ha (T3) and 200 kg N/ha (T4) were applied manually as follows: 30% at the beginning of tillering, 40% at the end of tillering and 30% at heading (Alan and Gash, 2012). The experiment was carried out according to a randomized complete block design (RCBD) with four replicate blocks.

Measured parameters.

At the end of April (anthesis stage), twenty plants from each plot were pulled out and washed. Disease symptoms for each individual plant were assessed by calculating the proportion of the length of stem discoloration and rated using a 0-5 scale (0 = no discoloration; 1 = trace to 25%; 2 = from 25 to 50%; 3 = from 50 to75%; 4 = more than 75%; and 5 = dead plant) as described by Tinline (1986) with minor modifications.

Disease severity was also estimated by counting the number of white heads in each plot. The yield was estimated by weighing the grains harvested per plot and reported as q/ha. The 1000 grain weight and the protein content were also determined.

Data analysis.

Statistical analysis was performed using SPSS 20. Comparisons of means were conducted using analysis of variance (ANOVA) including Tukey-B test ($P \leq 0.05$). Correlation analysis was carried out by calculation of Pearson correlation coefficients.

RESULTS

Effect of nitrogen fertilization on disease severity.

Artificial inoculation significantly increased disease severity ($P < 0.05$) compared to the control (non-inoculated plots). Under natural pathogen pressure, a significant effect of nitrogen on infection was observed. In addition, the effect of nitrogen rate on disease severity was significant ($P < 0.05$). Nitrogen applied at higher rates (150 and 200 kg/ha) significantly increased disease severity (Fig. 1), and nitrogen rates significantly affected the percentage of white heads in inoculated plants (Fig. 2). Nitrogen supply at higher rate (150 and 200 kg/ha) significantly increased the percentage of white heads. However, under natural pathogen pressure no effect of nitrogen on the percentage of white heads was observed (Fig. 2).

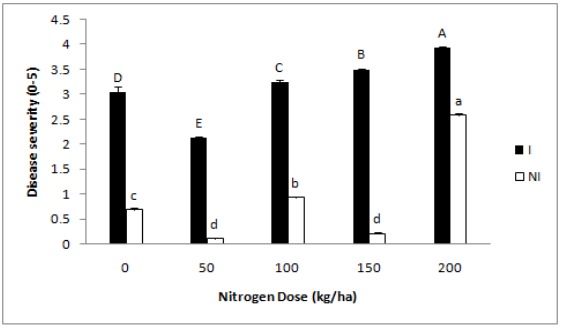

Fig. 1. Effect of nitrogen rates on the severity of Fusarium foot and root rot of wheat under natural infection (NI: non-inoculated) and artificial inoculation (I: inoculated) by *Fusarium culmorum*. Bars with the same letter within the same inoculation treatment are not significantly different according to Tukey test (at $P \leq 0.05$).

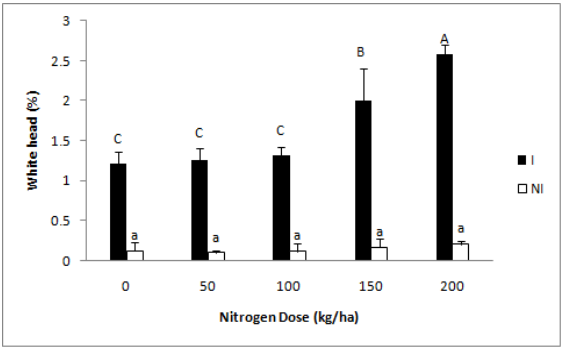

Fig. 2. Effect of nitrogen rates on the percentage of white heads of wheat under natural infection (NI: non-inoculated) and artificial inoculation (I: inoculated) by *Fusarium culmorum*. Bars with the same letter within the same inoculation treatment are not significantly different according to Tukey test (at $P \leq 0.05$).

Effect of nitrogen rates and inoculation by *F. culmorum* on grain yield.

The grain yield of Karim was significantly ($P < 0.05$) affected by the rate of nitrogen (Fig. 3). Grain yield increased significantly with the increase of nitrogen application regardless the inoculation with *F. culmorum*. Moreover, the effect of nitrogen rate on weight of 1000 grains was significant ($P < 0.05$) (Fig. 4). Indeed, nitrogen application resulted in significant increases in 1000 grain weight and the highest weight of 1000 grains was obtained in plants supplied with the highest rate nitrogen rate (200 kg/ha) regardless the pathogen inoculation (Fig. 4).

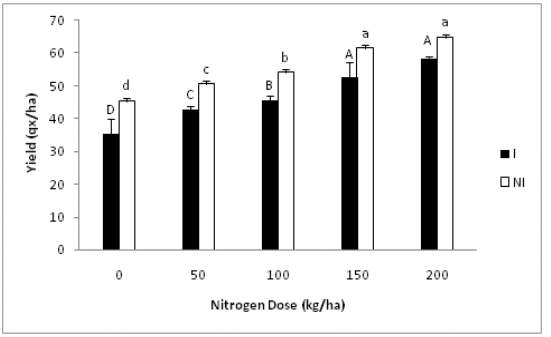

Fig. 3. Effect of nitrogen rates on yield of durum wheat under natural infection (NI: non-inoculated) and artificial inoculation (I: inoculated) by *Fusarium culmorum*. Bars with the same letter within the same inoculation treatment are not significantly different according to Tukey test at ($P \leq 0.05$).

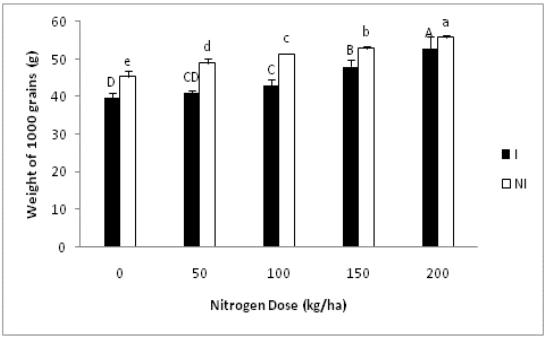

Fig. 4. Effect of nitrogen rates on 1000 grain weight of durum wheat under natural infection (NI: non-inoculated) and artificial inoculation (I: inoculated) by *Fusarium culmorum*. Bars with the same letter within the same inoculation treatment are not significantly different according to Tukey test (at $P \leq 0.05$).

This study revealed a significant difference in grain protein content between the different nitrogen treatments both in inoculated and non-inoculated plots. However, there was no correlation between nitrogen rate and percentage of grain protein content (Fig. 5).

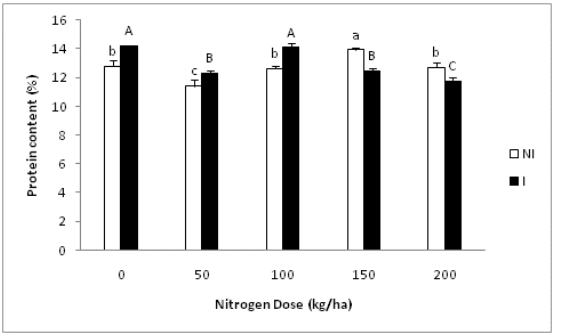

Fig. 5. Effect of nitrogen rates on grain protein content of durum wheat under natural infection (NI: non-inoculated) and artificial inoculation (I: inoculated) by *Fusarium culmorum*. Bars with the same letter within the same inoculation treatment are not significantly different according to Tukey test (at $P \leq 0.05$).

DISCUSSION

F. culmorum is one of the five main *Fusarium* species attacking cereals in the temperate cereal growing areas of the world (Hogg et al. 2007). This pathogen can cause extensive yield losses, especially in durum wheat, with over 50% reductions in grain yield being common observed (Smiley et al. 2005). Various studies suggested weather conditions, plant development, and genetic or morphological cultivar characteristics, nitrogen fertilization as factors influencing the epidemiology of Fusarium-induced diseases (Osborne and Stein 2007). Our results showed that nitrogen supply at higher rates (150 and 200 kg/ha) significantly increased Fusarium foot and root rot disease index, the percentage of white heads, the grain yield, the weight of 1000 grains and the grain protein content. These results are in agreement with field studies of Ma et al. (2004) and Lemmens et al. (2004) who noted an increase in Fusarium head blight in wheat with increasing nitrogen input. Probably the presence of nitrogen leads to the creation of lavish crops with succulent plants sensitive to infection. According to Lemmens et al. (2004), high nitrogen rates (up to 80 kg/ha) significantly affected Fusarium head blight development in wheat where deoxynivalenol (DON) level was also significantly increased. In addition, Martin et al. (1991) showed that an increase in nitrogen rates from 70 to 170 kg/ha significantly increased the occurrence of Fusarium foot and root rot disease in wheat, barley, and triticale.

In summary, field experiments demonstrated a considerable impact of nitrogen fertilization on durum wheat infection with *F. culmorum*. Although nitrogen influence appeared negligible at low inoculum pressure, nitrogen application showed significant effects on Fusarium foot and root rot under conditions with high pathogen pressure. Thus, adjusted nitrogen fertilization may limit this disease. Nevertheless, nitrogen may act differently on infection by other agents belonging to the *Fusarium* species complex or other fungal genera attacking wheat, because of differences in lifestyles, infection strategies, or production of secondary metabolites. These differences should be kept in mind in nitrogen fertilization within an integrated pest management strategy against Fusarium diseases in wheat.

RESUME

Hemissi I., Gargouri S., Hlel D., Hachana A., Abdi N. et Sifi B. 2018. Effet de la fertilisation azotée sur le rendement et la résistance du blé dur à *Fusarium culmorum*. Tunisian Journal of Plant Protection 13 (si): 31-38.

Cette étude a porté sur l'effet de la fertilisation azotée sur la fusariose du collet et des racines du blé dur. La sévérité de la maladie, le pourcentage des épis blancs, le rendement en grains ainsi que la teneur en azote ont été évalués chez le blé dur après inoculation artificielle par *Fusarium culmorum* dans des conditions de champ. L'essai a été réalisé en utilisant le cultivar de blé Karim pendant la campagne agricole 2016/17. Cinq doses d'azote (0, 50, 100, 150 and 200 kg/ha) ont été évaluées. Les doses d'azote les plus élevées (150 et 200 kg/ha) ont augmenté la sévérité de la maladie, le pourcentage d'épis blancs, le rendement en grains, le poids de 1000 grains ainsi que la teneur en azote. Ces résultats suggèrent que la fertilisation azotée excessive peut augmenter l'infection du blé par la fusariose du collet et des racines en influençant la physiologie de la plante.

Mots clés: Blé dur, doses d'azote, *Fusarium culmorum*

ملخص

هميسي، إيمان وسامية قرقوري ودرصاف هلال وأميرة حشانة ونائلة عابدي وبوعزيز صيفي. تأثير التسميد النيتروجيني على محصول القمح الصلب/القاسي ومقاومته للفطر *Fusarium culmorum*.

Tunisian Journal of Plant Protection 13 (si): 31-38.

أجريت هذه الدراسة لتقييم مدى تأثير التسميد النيتروجيني/الأزوتي (N) على المرض الفوزاري للقمح. تم تقييم شدة المرض ونسبة السنابل البيضاء وانتاجية الحب ووزن الألف حبة وكمية النيتروجين في القمح الصلب بعد الإلقاح الاصطناعي بالفطر *Fusarium culmorum* تحت الظروف الحقلية. أجريت التجربة باستخدام صنف كريم خلال موسم الفلاحي 17/2016. تم تقييم خمسة جرعات من النيتروجين (0 و50 و100 و150 و250 كلغ/هك). أدت المعاملات بجرعات نتروجين مرتفعة (150 و 200 كلغ/هك) إلى ارتفاع في شدة المرض ونسبة السنابل البيضاء وانتاجية الحب ووزن الألف حبة وكمية النيتروجين. هذه النتائج تشير إلى أن الكميات المرتفعة من التسميد النيتروجيني قد يزيد من الإصابة بالمرض الفوزاري للقمح من خلال التأثير على فسيولوجية النبتة.

كلمات مفتاحية: جرعات نيتروجين، قمح صلب، *Fusarium culmorum*

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Identification and Occurrence of *Trichoderma harzianum* Associated with Cork Oak in Tunisia

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ABSTRACT

Yangui, I., Zouaoui Boutiti, M., Hlaiem, S., Vettraino, A.M., Vannini, A., Ben Jamaâ, M.L., and Messaoud, C. 2018. Identification and occurrence of *Trichoderma harzianum* associated with cork oak in Tunisia. Tunisian Journal of Plant Protection 13 (si): 39-48.

Trichoderma harzianum is an endophyte fungus of considerable interest because of its effectiveness as a biocontrol agent against various plant pathogenic fungi. In this study, *T. harzianum* was isolated from cork oak trees in three forests in northwest Tunisia. Initially, the fungal characterization was carried out based on macroscopic and microscopic features. Sequencing of the internal transcribed spacers 1 and 2 of the DNAr was carried out to confirm fungus identification at the species level. The aims of this work were to study the occurrence of *T. harzianum*, to understand its relationship with the host plant, and to quantitatively investigate its distribution on the different organs of cork oak trees across three sites (Babouch, Ain snoussi, Ain zana). *T. harzianum* frequency varied significantly ($P < 0.001$) among the surveyed forests. The fungus was more common at Babouch forest and was rarely encountered at Ain zana. Correlation analysis was used to determine the relationship between the dendrometric parameters, the phytosanitary status of the investigated trees and the abundance of *T. harzianum.* The results showed a significant and positive correlation between the fungus frequency and the tree height. A negative and significant correlation was noted between the trees' chlorosis index and fungus abundance. These findings may afford a contribution to the knowledge of *T. harzianum* in Tunisian forests and its relationship with cork oak trees which could help to develop control strategies using *Trichoderma* strains.

Keywords: Cork oak, morphological and molecular identification, occurrence, *Trichoderma harzianum*, Tunisia

Species of the genus *Trichoderma* are filamentous fungi that inhabit diverse environments. They are generally abundant in soils, roots, and woods. It is well known that many *Trichoderma* species are endowed with high ability to establish various heterotrophic interactions with other organisms such as decomposition, parasitism, and opportunistic endophytism (Druzhinina et al. 2006; Zeilinger et al. 2016). Due to their antagonistic properties against a wide range of bacteria, yeasts, and fungi,

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Trichoderma strains are able to survive in a highly competitive environment with other microorganisms (Reino et al. 2008). Dominance of *Trichoderma* species is mainly due to their ability to synthesize a wide array of secondary metabolites, such as terpenoids, steroids and pyrones. Bioactive products of *Trichoderma* species have great potential for a variety of applications in agriculture for the biocontrol of various phytopathogens and for the enhancement of plant growth (Mukherjee et al. 2013; Zeilinger et al. 2016). Currently, they are largely applied as bio-fungicides to prevent several plant diseases (Kumar et al. 2014).

T. harzianum is the most common species of the genus. It is frequently applied on field and greenhouses as a biocontrol agent against plant pathogens (Chaverri et al. 2002). In Tunisia, many studies have been conducted about local *T. harzianum* colonizing soils (Ayed et al. 2006; Sadfi-Zouaoui et al. 2009). However, little data are available about its occurrence on cork oak (*Quercus suber*) trees in Tunisia.

In this paper, we identified a *Trichoderma* species isolated from cork oak and we investigated its distribution in three Tunisian forests and its influence on the phytosanitary status of the trees. A better understanding of the relationship between *Trichoderma* and cork oak trees may lead to further develop new strategies to fight against cork oak diseases.

MATERIALS AND METHODS

Study sites and tree sampling.

In 2016, a survey was carried out in cork oak stands of the northern west of Tunisia in order to evaluate the phytosanitary status of the trees in relation with the occurrence of *Trichoderma* species. Three sites were selected in the main Tunisian cork oak forests at different altitudes: Babouch (36°835'N, 8°709'E - 207m), Ain snoussi (36°86'N, 9°014'E - 520.3m), Ain zana (36°16′ N, 8°24′ E - 924m) (Table 1). At each site, samples were collected from the different organs of ten cork oak trees and dendrometric parameters were noted. A distance of at least 300 m was kept between two trees in order to obtain a representative sampling for each site. Chlorosis index, defoliation index and necrosis index were noted for each tree. A categorical scale ranging from 0 (no symptoms) to 5 (very severe symptoms) was used for each parameter. Four parameters: trunk exudations (1 = presence / 0 = absence), number of cracks, number of cavities and number of cankers were also noted for trunks.

Table 1. Ecological parameters of the investigated cork oak sites

Site	Longitude (E)	Latitude (N)	Altitude (m)	Rainfall (mm)	Temperature (°C)
Babouch	8°709'	36°835'	207	950	20
Ain snoussi	9°014'	36°86'	520,3	1222	16
Ain zana	8°24′	36°16′	924	1780	20

Collection of isolates.

Collected samples were soaked in alcohol (70%). Fragments of 1 mm^2 in size were cut from each sample and placed in Petri dishes poured with potato dextrose agar (PDA) medium. A total of 203 *Trichoderma* isolates was selected based on their morphological characteristics. *Trichoderma* isolates were grouped into morphotypes and the dominant one was proposed to be studied.

Morphological identification.

The dominant morphotype was preliminarily identified according to the morphological characters and the microscopic traits as reported by Samuels et al. (2002). Conidia measurements were taken from colonies after a week of incubation in darkness at 25°C. One representative isolate of the dominant morphotype was selected for molecular identification.

DNA extraction and molecular identification.

Mycelial plugs of the *Trichoderma* isolate were collected from the margin of a PDA culture and transferred into Eppendorf tubes containing 500 µl of potato dextrose broth (PDB) with three replications. PDB cultures were incubated in darkness at 25°C for 7 days. The DNA extraction was performed from the PDB cultures as described by Cenis et al. (1992). The ITS region of the nuclear rDNA gene of the *Trichoderma* isolate was amplified using primers ITS1 (5'-TCGGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). PCR amplification was carried out in a volume of 25 µl containing 1 µl of DNA (100 ng), 1 µl of each primer (1:10) , 12.5 µl of MyTaq HS Mix (2X) (Bioline, UK) and 9.5 µl of water using a PCR program comprised of an initial denaturation at 95°C for 1 min followed by 34 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s and a final extension at 72°C for 7 min. The PCR products were visualized by electrophoresis on a 1.5% agarose gel containing 1% of GelRed, then purified by the Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). The resulting amplicons were sequenced (Eurofins, Italy) and a basic local alignment search tool (BLAST, http://www.ncbi.nlm.nih.gov/) was used for sequence similarity searches.

Isolation frequency.

The isolation frequency of *Trichoderma* isolates (%) from the different organs was calculated using the following formula: IF (%) = 100 × (Ni/Nt), where Ni and Nt are the number of segments colonized by the fungus and the total number of examined segments, respectively (Franceschini et al. 2005).

Data analysis.

The significance of the variation in the isolation frequency of *T. harzianum* among sites or organs was determined using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using SAS version 9. Correlation analysis between the *T. harzianum* frequency, the dendrometric parameters and the phytosanitary variables was carried out using SAS.

RESULTS

Morphological and molecular identification.

According to the phenotypic traits of colonies and their micro-morphology, *Trichoderma* isolates, belonging to the dominant morphotype, probably belonged to *T. harzianum*. In fact, colonies grown on PDA medium were floccose with effuse conidiation covering the entire surface of the plate. Colonies grew very rapidly and reached the edge of the plate after three days of incubation. Conidia were globous and 2.4 to 2.6 µm in diameter. They began yellow and became green to dark green after one week. The conidial production started after less than 48 h. The macroscopic and microscopic characteristics of a representative isolate are illustrated in Fig. 1.

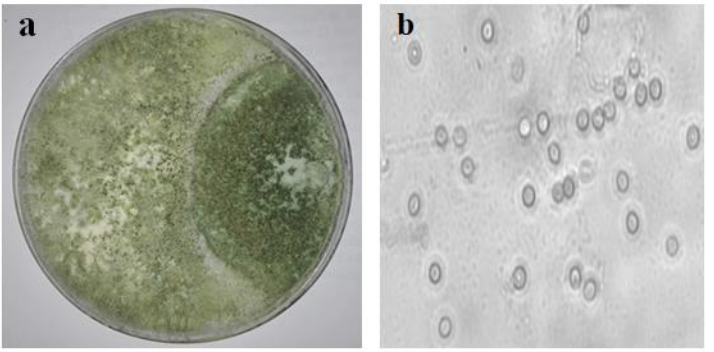

Fig. 1. Morphological characteristics of *Trichoderma harzianum*, a: Colony grown on PDA medium after 7 days of incubation in darkness at 25°C, b: Conidia (40×).

The BLAST searches of the obtained ITS-rDNA sequence resulted in 100% homology with *T. harzianum* isolate 153L (Accession No KF889067) described by Dawidziuk et al. (2014), and nineteen other *T. harzianum* accessions. The obtained sequence was submitted to GenBank and assigned the following accession number: MG675027.

Site differences as measured by dendrometric parameters.

Differences in the total tree height (m) were highly significant (F = 30.51, $P < 0.0001$) among the investigated sites. Babouch was characterized by the tallest trees (12.1 ± 0.87 m). However, the trunk height (m), the trunk circumference (m) and the crown width (m) did not differ significantly between surveyed sites (Table 2).

Table 2. Dendrometric parameters of the investigated cork oak sites

Site	Total height (m)	Trunk height (m)	Trunk circumference (m)	Crown width (m)
Babouch	12.1 ± 0.87 a	2.77 ± 0.28 a	1.35 ± 0.17 a	4.38 ± 0.19 a
Ain snoussi	8.2 ± 0.30 b	2.69 ± 0.21 a	1.29 ± 0.15 ab	4.96 ± 0.46 a
Ain zana	5.99 ± 0.28 c	2.21 ± 0.25 a	1.05 ± 0.12 b	3.97 ± 0.28 a

For each parameter, values followed by the same letters are not significantly different based on Duncan's Multiple Range test at $P < 0.05$.

Among the studied phytosanitary parameters, the chlorosis index showed high significant differences among the investigated sites ($F = 98.28$, $P < 0.0001$) (Table 3).

Table 3. Phytosanitary parameters of the investigated cork oak sites

Phytosanitary parameter	Babouch	Ain snoussi	Ain zana
Chlorosis index	0 ± 0.00 c	1.9 ± 0.1 a	0.8 ± 0.1b
Defoliation index	1.9 ± 0.38 a	1.7 ± 0.3 a	2 ± 0.36 a
Necrosis index	2.8 ± 0.18 a	2.2 ± 0.2 a	2 ± 0.39 a
Trunk exudations	0.7 ± 0.14 a	1 ± 0.00 a	0.3 ± 0.15 a
Number of cracks	0 ± 0.00 b	24.5 ± 5.39 a	0 ± 0.00 b
Number of cavities	0 ± 0.00 b	14 ± 6.18 a	2.7 ± 1.7 b
Number of cankers	1.3 ± 0.5 a	2.1 ± 0.4 a	3 ± 0.15 a

For each parameter, values followed by the same letters are not significantly different based on Duncan's Multiple Range test at $P < 0.05$.

Incidence of *T. harzianum.*

The distribution of *T. harzianum* varied significantly among sites. Babouch exhibited the highest isolation frequency in all the organs (18.4 ± 8.04% in branches, 14±1.63% in leaves and 3 ± 1.52% in trunks). However, Ain zana showed a very low frequency (1.6 ± 1.06%) of *T. harzianum* in branches and a total absence in leaves and trunks (Table 4).

Table 4. Isolation frequency of *Trichoderma harzianum* (%)

Site	Branches	Leaves	Trunk
Babouch	18.4 ± 8.04 a	14 ± 1.63 a	3 ± 1.52 a
Ain snoussi	6.9 ± 2.02 b	9 ± 2.02 b	0 ± 0.00 b
Ain zana	1.6 ± 1.06 c	0 ± 0.00 c	0 ± 0.00 b

Within each column, values followed by the same letters are not significantly different based on Duncan's Multiple Range test at $P < 0.05$.

Correlation analysis showed that the presence of *T. harzianum* in trunks and leaves was found to be significantly correlated to the increase in tree height and circumference. The high frequency of *T. harzianum* appeared to be negatively correlated to the chlorosis index. However, its incidence on cork oak trees was found to be positively correlated to the degree of leaf drying (Table 5).

Table 5. Correlations between *Trichoderma harzianum* infestation and the dendrometric and the phytosanitary parameters

Predictor variable	Correlation coefficient (r)	Significance probability (*P*)
Trunk height (m)	0.715**	<.0001
Trunk circumference (m)	0.409	0.025
Crown width (m)	0.225	0.231
Chlorosis index	-0.361*	0.05
Defoliation index	0.245	0.192
Necrosis index	0.245	0.192
Trunk exudations	-0.236	0.210
Number of cracks	-0.183	0.333
Number of cavities	-0.139	0.462
Number of cankers	-0.306	0.100

** highly significant at $P \leq 0.01$; * significant at $P \leq 0.05$.

The defoliation and the necrosis indexes were not significantly affected by the *T. harzianum* frequency. Furthermore, all parameters indicating the phytosanitary status of the trunks were not significantly influenced by the increase in *T. harzianum* incidence (Table 5).

DISCUSSION

Morphological characterization approach is considered as a potential method to identify *Trichoderma* species (Anees et al. 2010; Gams and Bissett 2002). In our study, the selected morphotype was identified as *T. harzianum* according to colony appearance, growth rate and conidia size. In fact, the small conidia size of *T. harzianum* isolates and their capacity to grow and sporulate at 35°C are the main features that distinguish them from the other *Trichoderma* species (Samuel et al. 2002). However, only colony appearance may not provide enough information to identify the species due to the difficulty to establish an exact description of the colony (Gams and Bissett 2002).

Since the distinguishing morphological features of a fungus are usually too limited to allow its identification, molecular techniques are required. The sequencing of the ITS1-5.8s-ITS2 region of the rDNA remains one of the most reliable methods for the identification of fungi at the species level (Kullnig-Gradinger et al. 2002). In this study, by comparing the obtained sequence of the ITS region to the sequences deposited in GenBank database, the representative isolate was confirmed as *T. harzianum* with homology percentage of 100%.

The second aim of this study was to determine the distribution of *T. harzianum* species on cork oaks in three different sites. Babouch forest, characterized by the tallest trees, revealed

the highest frequencies of *T. harzianum*. Based on correlation analysis, the tree height and the trunk circumference were correlated to *T. harzianum* frequency. Indeed, it was demonstrated in recent studies that *Trichoderma* species may influence the plant development by several mechanisms such as production of growth hormones (Chowdappa et al. 2013). Furthermore, *Trichoderma* species are known to induce large changes in the plant proteome by stimulating unregulated proteins which are involved in carbohydrate metabolism and/or in photosynthesis. These proteins enhance respiratory and photosynthetic rates which may lead to an increase in the plant growth (Shoresh and Harman 2008). Moreover, *Trichoderma* species participate in increasing the availability of essential elements for plants by mineralization of organic matter (Haque et al. 2012, Mukherjee et al. 2012).

Considering the phytosanitary situation of leaves, a significant difference in chlorosis index was noted between the investigated sites. Babouch forest, characterized by the highest frequency of *T. harzianum*, exhibited the lowest chlorosis index. Indeed, chlorosis is a deficiency disease due to an insufficient chlorophyll production, often as a result of a nutrient deficiency or infections by plant pathogens such as viruses, bacteria or fungi (Hale et al. 1946). In this study, the correlation analysis showed a negative correlation between the chlorosis index and *T. harzianum* infestation. Accordingly, we suggested that *T. harzianum* species may avoid chlorosis by increasing the solubility of nutrients like phosphate, zinc and iron and by inhibiting a large diversity of plant pathogens. Thus, our study supports the consideration that *Trichoderma* species are able to act as biocontrol agents by producing defense related compounds which may enhance the plant resistance (Harman et al. 2004, Smolińska et al. 2007, Yedidia et al. 2004). In this context, several previous studies have proved the antifungal potential of *Trichoderma* species against various plant pathogens such as *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp., *Phytophthora* spp., *Pythium* spp., and *Sclerotinia* spp. The antifungal activity of *Trichoderma* was found to be attributed to their ability to produce a wide range of antibiotics and lytic enzymes (Toghueo et al. 2016, You et al. 2016). This may explain the frequent use of *Trichoderma*-based formulations as commercial biocontrol agents for foliar application to treat fungal diseases (Oros and Naár 2017). Nevertheless, correlation analysis did not show any relationship between the phytosanitary situation of the trunks and *Trichoderma* infestation. This suggests that *T. harzianum* species may not be effective in controlling trunk damages which may probably due to infection by wood decay fungi such as *Armillaria* and *Xylaria*. This result supports the idea that most of *Trichoderma* species are more effective for controlling some pathogens, but may be largely inefficient against others (Pratella and Mari 1993). Furthermore, the bioactive agents of *Trichoderma* strains are living organisms and their activities depend heavily on several factors (Kaur et al. 2005; Li et al. 2005). Accordingly, the effective protection exerted by *Trichoderma* strains against pathogens is often unpredictable. As an overall conclusion, our study showed an eventual positive effect of *T. harzianum* species on the development of the tree and its resistance against some plant pathogens. However, further studies are required in order to conclude a causal association between *Trichoderma* species and the phytosanitary status of the host

plant. Other studies on the bioactive compounds of *Trichoderma* species are also necessary to a better understanding of their antagonistic interactions and mechanisms of actions.

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RESUME

Yangui I., Zouaoui Boutiti M., Hlaiem S., Vettraino A.M., Vannini A., Ben Jamaâ M.L. et Messaoud C. 2018. Identification et occurrence de *Trichoderma harzianum* associé au chêne-liège en Tunisie. Tunisian Journal of Plant Protection 13 (si): 39-48.

Trichoderma harzianum est un champignon endophyte d'un intérêt considérable en raison de son efficacité en tant qu'agent de lutte biologique contre divers champignons phytopathogènes. Dans cette étude, *T. harzianum* a été isolé à partir des arbres de chêne-liège du nord-ouest de la Tunisie. Initialement, la caractérisation fongique a été réalisée sur la base des caractéristiques macroscopiques et microscopiques. Le séquençage des espaceurs transcrits internes 1 et 2 de l'ADNr a été effectué pour confirmer l'identification du champignon au niveau de l'espèce. Les objectifs de ce travail étaient d'étudier l'occurrence de *T. harzianum*, de comprendre sa relation avec la plante hôte et d'étudier quantitativement sa distribution dans les différents organes des arbres de chêne-liège dans trois forêts (Babouch, Ain snoussi et Ain zana). La fréquence de *T. harzianum* a varié significativement ($P < 0,001$) selon les forêts étudiées. Le champignon a été plus abondant dans la forêt de Babouch et très rare dans la forêt de Ain zana. L'analyse de la corrélation a été utilisée pour déterminer la relation entre les paramètres dendrométriques et l'état phytosanitaire des arbres étudiés et l'abondance de *T. harzianum*. Les résultats ont montré une corrélation significative et positive entre la fréquence du champignon et la hauteur des arbres. Une corrélation négative et significative a été notée entre l'indice de jaunissement des feuilles et l'abondance de *T. harzianum*. Ces résultats peuvent contribuer à la connaissance de *T. harzianum* dans les forêts tunisiennes et à sa relation avec les arbres de chêne-liège, ce qui pourrait aider à développer des stratégies de lutte en utilisant des souches de *Trichoderma.*

Mots clés: Chêne-liège, identification moléculaire et morphologique, occurrence *Trichoderma harzianum*, Tunisie

ملخص

اليانقي، إسلام ومريم زواوي بوتيتي وسوسن حليم وأنا ماريا فترابنو وأندريا فنيني ومحمد لحبيب بن جامع وشكري مسعود. تشخيص وانتشار الفطر *Trichoderma harzianum* المقترن ببلوط الفلين (الفرنان) في تونس.
Tunisian Journal of Plant Protection 13 (si): 39-48.

الفطر*Trichoderma harzianum* هو فطر داخلي ذو أهمية كبيرة بسبب فعاليته كعامل مكافحة بيولوجية ضد كثير من الفطريات الضارة. في هذه الدراسة، تم عزل الفطر من أشجار بلوط الفلين (الفرنان) المتواجدة في الشمال الغربي للبلاد التونسية. في مرحلة أولى، تم تشخيص الفطر وفقا لخصائصه المضخمة والمجهرية. تم التأكد من النوع الفطري باستعمال تسلسل الفواصل الداخلية 1 و 2 للحمض النووي. تتمثل أهداف هذه الدراسة في التحقيق في تواجد الفطر ودراسة العلاقة بينه وبين شجرة الفرنان و دراسة توزعه الكمي على مختلف أعضاء الشجرة ثلاثة غابات (بيوش وعين السنوسي وعين الزانة). تفاوت توزع الفطر بشكل كبير بين الغابات. كان أكثر شيوعا في غابة بيوش ونادرا جدا في عين الزانة. تم استعمال تحليل الترابط لتحديد العلاقات بين مقاييس الخصائص الفيزيائية للأشجار وحالتهم الصحية ومدى انتشار الفطر. بينت النتائج وجود علاقة ايجابية بين علو الأشجار وانتشار الفطر. كما أثبتت النتائج وجود علاقة عكسية وهامة بين درجة اصفرار الأوراق ووفرة الفطر. يمكن لهذه النتائج أن تساهم في التعرف على هذا الفطر وعلى حقيقة علاقته بشجرة الفرنان مما يساعد على وضع استراتيجيات السيطرة على مسببات الأمراض وذلك باستخدام عزلات من هذا الفطر.

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Identification and Pathogenicity of *Pestalotiopsis chamaeropis*, Causal Agent of White Heather (*Erica arborea*) Dieback, and in vitro Biocontrol with the Antagonist *Trichoderma* sp.

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ABSTRACT

Hlaiem, S., Zouaoui-Boutiti, M., Ben Jemâa, M.L., Della Rocca, G., Barberini, S., and Danti, R. 2018. Identification and pathogenicity of *Pestalotiopsis chamaeropis*, causal agent of white heather (*Erica arborea*) dieback, and in vitro biocontrol with the antagonist *Trichoderma* sp. Tunisian Journal of Plant Protection 13 (si): 49-60.

Plant pathogenic fungi are one of the main causes of forest trees diseases. The symptoms of dieback include a foliage yellowing and fall, a drying and necrosis at branches, cankers, deformations, a blackish fluid and flow of rots at the level of the trunks. Symptoms of wilting were observed on one species of scrub: white heather (*Erica arborea*), located in the forest of "Henchir Kort" northeast of Tunisia. Isolations from the margins of these cankers revealed the fungal genus of *Pestalotiopsis*. Morphological and molecular analysis of the ITS allow to identify the pathogen as *Pestalotiopsis chamaeropis*. The Koch's rules have been verified. The antagonistic effect between *P. chamaeropis* and *Trichoderma* sp. was assessed in vitro. Tests of direct or remote confrontation on PDA medium revealed that *Trichoderma* sp. inhibited mycelial growth of the pathogen compared to the untreated control.

Keyswords: Antagonistic effect, dieback, *Pestalotiopsis chamaeropis*,*Trichoderma* sp., white heather

White heather (*Erica arborea*) is one of a common shrub in the Mediterranean region, North Africa and in Tunisia that can reach 4 m in height (Mezghani et al. 1992). The big woody root of this plant is used in the manufacture of pipes. The honey of this species is very rich in minerals and is an excellent restorative (Mezghani et al. 1992). The plant is known for its antiseptic, urinary, diuretic and astringent properties (Rejeb et al. 2006).There are a very few reports of plant pathogens causing diseases in white heather.

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Pestalotiopsis species are common in tropical and temperate ecosystems (Bate-Smith et al. 1957) and may cause plant disease (Das et al. 2010).They were historically named according to the host on which they were first observed. In spite of this practice, many argued that *Pestalotiopsis* species are generally not host-specific and are found on a wide range of hosts and substrates (Jeewon et al. 2004; Lee et al. 2006). *Pestalotiopsis* species are common phytopathogens causing a variety of symptoms, including

canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots, and various post-harvest diseases(Crous et al. 2011;Maharachchikumbura et al. 2012, 2013a, 2013b;Zhang et al. 2012a, 2013). These pathogensare commonly isolated as endophytes (Kumar et al. 2004; Watanabe et al. 2010; Wei et al. 2004) and some species likely have endophytic and pathogenic phases during their life cycle (Tejesvi et al. 2009; Wei et al. 2007).

The purpose of the present study is to identify the causal agent of white heather dieback, presumed to be a species of *Pestalotiopsis*, and to investigate its possible biological control using a local species of *Trichoderma*. This antagonistic genus has proven efficient in controlling a wide range of phytopathogens (Dominguesa et al. 2000) of different crops through different modes of action (Elad et al. 1982; Papavizas 1985; Ridout et al. 1988; Taylor 1986) in eco-friendly manner.

MATERIALS AND METHODS

The forest of Henchir Kort, located in Northeastern Tunisia (36°.30'.406" N; 10°.38'.780"E) suffered from heavy disease infestations since 2012. In October 2016, twigs diebacks and cankers often associated with gummy exudates were observed in the white heather (Fig. 1). Attacked samples with symptoms of necrosis and dryness were collected from infected white heather plants.

Fig. 1. Disease symptoms observed in white heather (*Erica arborea*).

Isolation and identification of the pathogen.

The isolation of the pathogen was performed by adopting the technique of Franceschini et al. (2005). Samples of white heather were collected in a forest of Henchir Kort. Small woody pieces (2 × 2 mm) were taken from the margin between necrotic and healthy tissues of the twigs following surface sterilization as described by Alves et al. (2013)and incubated in the dark at 25°C for 3 days on Petri plates containing potato dextrose agar (PDA) added with streptomycin sulfate (0.05 g/l) antibiotic. Pure cultures were obtained by plating a small piece of

mycelium from the margin of each colony grown on PDA and incubating them under the same conditions described above.

Pestalotiopsis was identified based on its cultural traits, conidial morphological characteristics and molecular analysis.

Regarding the molecular identification, fungal DNA was directly extracted from mycelia growing on plates, using a commercial Kit Macherey-Nagel- 07/2014, Rev.09.PCR reactions were carried out with ITS1 and ITS4 primers (White et al. 1990) to amplify the ITS region of the ribosomal RNA as described by Alves et al. (2004) using the following conditions: 95°C for 3 min; 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min; final extension at 72°C for 10 min. Products from PCR reactions were electrophoresed on a 1.5%agarose gel, then stained with GelRed, and visualized with UV transilluminator. The size of PCR products was estimated by comparison with a DNA ladder 100 bp plus, TransgenBiotech. Amplified products were sequenced and the sequences obtained were blasted in GenBank.

Pathogenicitytest.

Pathogenicity test was made according to the method of inoculation of Linaldeddu et al. (2014) by inoculating pathogen on five excised green white heather shoots (30 cm in length) from cv. Cannonau. A mycelia plug (3 to 4 mm^2) taken from the margin of an actively growing colony of 7 days on PDA was placed in a shallow wound (3mm) made by a scalpel on the middle of each shoot.

The inoculation point was covered with cotton wool soaked in sterile water and wrapped with Parafilm. The inoculated shoots were placed in a break containing 200 ml of sterile water, whereas the bottom and top end of each cane were sealed with a synthetic grafting resin to prevent drying and contamination and then enclosed in a transparent plastic bag at room temperature (20- 26°C).

Antagonist assay protocol.

The antagonistic agent used in this work is *Trichoderma* sp. which was isolated from asymptomatic tissues of white heather and grown on PDA at 25°C before use. The fungus was identified based on its morphological cultural traits and conidial characteristics.

In vitro antagonist activity of *Trichoderma* sp. against *Pestalotiopsis chamaeropis* was studied according to two methods.

Direct confrontation. This technique consists in placing, in the same plate of Petri dishes containing a PDA medium, two small pieces of mycelium of *Trichoderma* sp. and *Pestalotiopsis chamaeropis* cultures. The two fragments were placed along a diametrical axis 3 cm and equidistant of the center at the same time. Incubation was performed at 25°C for 7 days. Ratings on the inhibition of *P. chamaeropis* growth and invasion of its colony by *Trichoderma*mycelium are noted every day. Control plates were challenged with a transplant of the pathogen only which was placed at the center.

Remote confrontation. This method consists of transplanting the antagonist and pathogen in two separated plates. Later, an assembly was carried out by super-positioningthe two plates (*Trichoderma* downside and *P. chamaeropis* upside). The junction between the two plates was insured by a Parafilm in order to avoid any loss of volatile substances (Daami-Remadi and El Mahjoub 2001). Growing conditions

were identical to those of direct confrontation. The control was formed by stacking plates, the upper one contained a small piece of mycelium of *P. chamaeropis* and the bottom one contained only PDA.

The average diameter of treated colonies was noted when pathogen mycelium in control plates reached the periphery. The percentage of inhibition of pathogen mycelial growth was calculated based on the following formula (Hmouni et al. 1996): I (%) = (1- Cn /Co) × 100, where Cn is the average diameter of the colonies in the presence of the antagonist and Co the average diameter of control colonies.

RESULTS

Pathogen identification.

The culture characteristics were white fluffy aerial mycelium (Fig. 2.a). Black acervular conidiomata on the surface of mycelium were produced after about 10 days (Fig. 2.b). Conidia were 4-septate (5 cells) and were fusoid to ellipsoid, straight to slightly curved (Fig. 2.c), measured 22.5µm (16.9-28.0) × 6.5µm (4.8-8.5). There were three median cells dark brown. Both apical and basal cells were hyaline, the apical cell with 2 or 3 appendages (mostly 3). Morphological and cultural characteristics as well as type of conidia observed were very similar and typical of the genus *Pestalotiopsis.*

The sequence resulting from the PCR amplification of the nuclear rDNA operon using the primers ITS1 and ITS4 revealed a high degree of similarity (100%) with the 16S rRNA sequence of *P. chamaeropis*. The fungus was identified as *Pestalotiopsis chamaeropis* and the representative sequence was deposited in GenBank (ITS: KY996524).

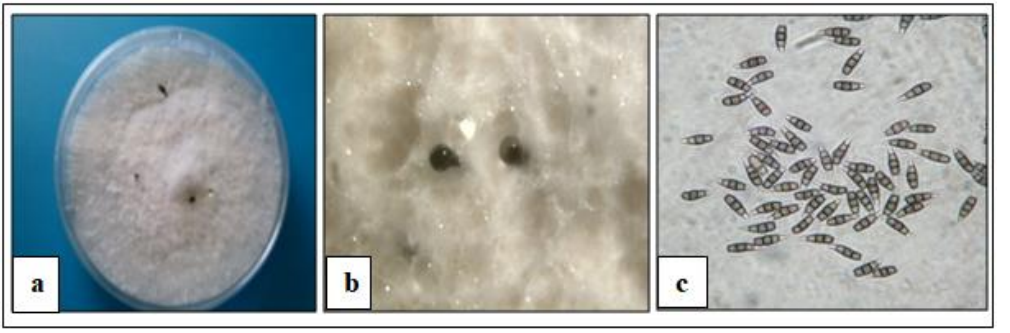

Fig.2.*Pestalotiopsis* colony cultured after 10 days on Potato-Dextrose-Agar at 25°C (a);Acervuli wih exuded conidia(b); Conidia with apical and basal appendages (c).

Morphological and cultural characterization of the antagonist.

On PDA medium, cultures were woolly initially white then green (Fig. 3.a), mycelial growth was rapidreaching more than 65mm after 3 days. Conidia were abundant gray-green, single-celled, smooth, round or ovoid (Fig. 3.b). Cultural characteristics as well as type of conidia observed were typical of the genus *Trichoderma.*

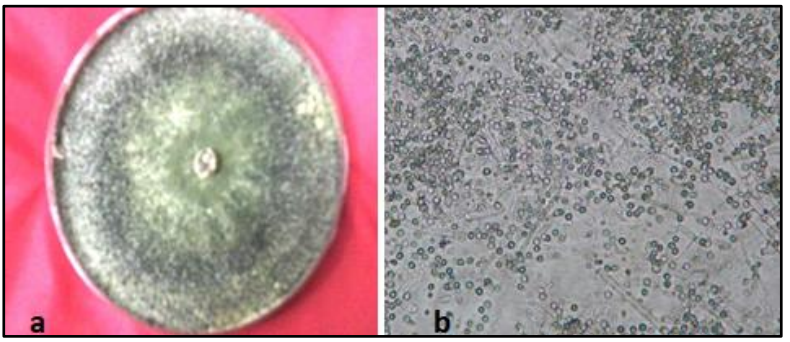

Fig. 3. *Trichoderma* colony growth on PDA medium noted after 7 days of incubation (a); Abundant gray-green conidia (b).

Pathogenicity test.

Five weeks after inoculation, all 5 shoots inoculated by *P. chamaeropis* showed a necrotic lesion around the inoculation point which measured 2.5 ± 0.5 cm. Control shoots, inoculated with sterile PDA plugs only, remained symptomless (Fig. 4).

The fungus was successfully re-isolated from necrotic bark and the margin of symptomatic wood tissues, thus fulfilling Koch's rules.

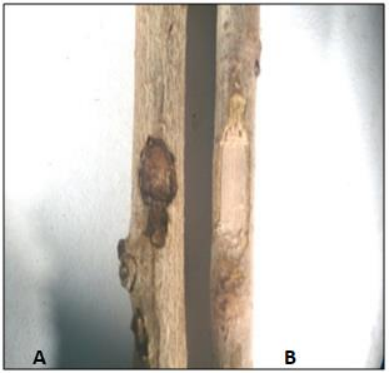

Fig. 4. White heather (*Erica arborea*) shoot showing brown lesion five weeks after inoculation with *Pestalotiopsis chamaeropis* (A). Asymtomatic control shoot (B).

Antagonist activity of *Trichoderma*. sp against *Pestalotiopsis chamaeropis*

Direct inhibition. The simultaneous growing of *Trichoderma* sp. and *P. chamaeropis* in the same plate showed faster growth of *Trichoderma* sp. In fact, after five days of incubation, the Petri plate was invaded by the antagonist, while the pathogen colony measured only 26 mm in diameter as expressed by a growth inhibition of 64% (Fig. 5). The cultivated *P. chamaeropis* control alone covers an area of about 69 mm in diameter. Beyond this period and at the end of seven days, *Trichoderma* invaded *P. chamaeropis* colonies and sporulated on.

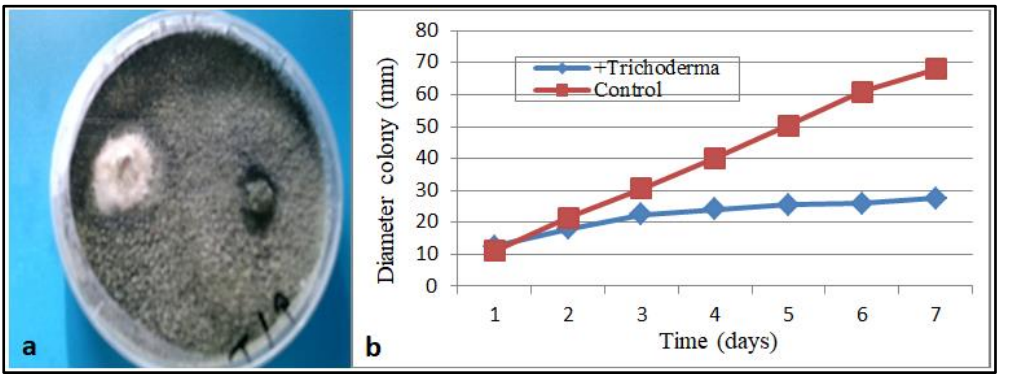

Fig. 5. Inhibition of pathogen mycelial growth in presence of *Trichoderma* sp (a). *Pestalotiopsis chamaeropis* growth in presence and in absence of *Trichoderma* sp. as compared to control (b).

Distant inhibition.This technique enabled us to highlight the inhibiting effect even remotely of the *Trichoderma* sp. on *P. chamaeropis*; this effect was assessed and the obtained results showed a significant reduction in the average diameter of *P. chamaeropis* colonies in presence of *Trichoderma* sp. compared to control. After six days of incubation at 25°C, this reduction reached 58% as compared to control (Fig. 6).

DISCUSSION

Morphological characters of *P. chamaeropis* showed similar characteristics to the ex-type isolate such as pigmentation of the median cells, number of apical appendages (2-3, mostly 3), basal appendages and septa (4-septate) (Maharachchikumbura et al. 2014). The length and width of median, basal and apical cells were shorter. Jeewon et al. (2003) reported that the length of spores, median cells and appendages were not phylogenetically important in identifying the species; but might be informative in identifying species groups. In contrast, pigmentation of the median cells and appendage tip morphology are important factors in phylogenetic identification of the species. *Pestalotiopsis* consists of approximately 205 described species that are easily identified by the presence of relatively fusiform conidia formed within compact acervuli (CABI Bioscience database 2001).

The results of our study indicated that *P. chamaeropis* is a pathogen that could cause canker lesions and dieback symptoms on white heather. To the best of our knowledge, this fungus is a new pathogen for white heather. Dieback of white heather is a serious ecologic problem in Tunisia. There are very few reports of plant pathogens causing diseases in white heather.

Several studies showed that the success of *Pestalotiopsis* infection requires an entry way in the host caused by injury (Espinosa et al. 2008; McQuilken et al. 2004). *Pestalotiopsis* is just one of a complex group of fungi. This fungus is also considered as a weak pathogen (Madar et al. 1991), which penetrates the host through natural openings such as stomata, lenticels and hydathodes (Agrios 2005). Many authors stated that *Pestalotiopsis* species infect only wounded or stressed plants which may be stressed due to insects, pesticides or sun damage. (Hopkins and McQuilken 2000; Wright et al. 1998).

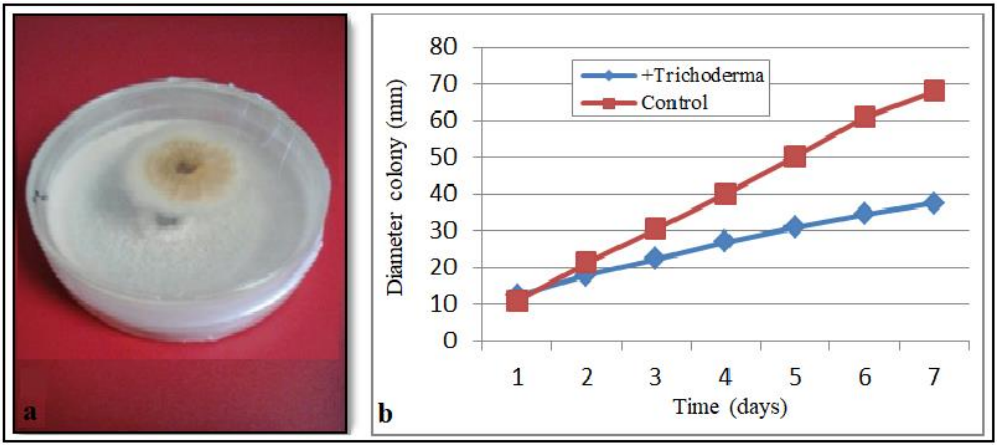

Fig. 6. Distant inhibition of pathogen growth by *Trichoderma* sp. (a); Antagonist effect remotely of *Trichoderma* sp. on *Pestalotiopsis chamaeropis* mycelia growth (b).

Studies undertaken in Australia and America showed that *Pestalotiopsis*-like fungi occurred not only leaves, but also on canes, wood, berries and flowers (Castillo-Pando et al. 2001;Deng et al. 2013; Sergeeva et al. 2001; Urbez-Torres et al. 2009, 2012).

Nowadays biological control is considered as one of the most interesting aspects of the science to find out the mechanisms employed by biocontrol agents for effective disease control (Howell 2003). Vidhya Pallavi et al. (2010) noted that under laboratory conditions, *Trichoderma* spp. exhibited a very good antagonistic potentiality against the grey blight (*Pestalotiopsis* sp.). Similarly, Naglot et al. (2015) tested several isolates of *Trichoderma* spp. and reported their antagonistic potency for the control of this pathogen and these findings are in agreement with our results.

Antagonistic microorganisms, such as *Trichoderma* species reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion (Cook et al. 1983). Several strains of the *Trichoderma* sp. are found to be effective biocontrol agents for the various plant pathogens (Amin et al. 2010) and they are characterized by rapid growth, abundant conidial formation and a high degree of ecological adaptability as reported by several researchers (Bissett 1991; Domsch et al. 1980; Papavizas 1985). *Trichoderma* sp. are capable to induce metabolic changes in plants that increases resistance to a wide range of plant pathogenic microorganisms (Harman et al. 2004).

This study was conducted in order to control the pathogenic plant fungus *P. chamaeropis*. The tests of direct and remote confrontation on culture between the target pathogen and *Trichoderma* sp. revealed the ability of the last to inhibit *P. chamaeropis* mycelial growth. Moreover, beyond this period and after six days, *Trichoderma* sp. invaded pathogen colonies and sporulated abundantly suggesting its highly mycoparasitic potential. Additionally, the results of the remote confrontation showed a significant reduction in the average diameter of pathogen colonies in presence of *Trichoderma* compared to control.

Similar effects of *Trichoderma* were also observed by Benhamou et al. (1997) following its direct confrontation with *Pythium ultimum*. This inhibitory action is due to substances, chemicals released by the strains of *Trichoderma* (antibiosis). Antifungal activity displayed by *Trichoderma* strain may be due to extracellular enzyme production (Davet 1983). These enzymes are responsible for the degradation of cell walls of target pathogenic agents (Lorito et al. 1993).The production of secondary metabolites by different *Trichoderma* species is well documented. It has been reported that *Trichoderma* spp. produce a wide range of volatile and non-volatile substances, antibiotics (Sivasithamparam et al. 1998). *Trichoderma* spp. are known to produce Volatile Organic Compounds(VOCs) that can inhibit fungal growth (Bruce et al. 2000;Wheatley et al. 1997). Specific VOCs, i.e. heptanal, octanal and 2-methyl-1-butanol, have previously been identified as compounds involved in the inhibition of fungal growth(Wheatley et al. 1997).Volatile secondary metabolites produced by *Trichoderma pseudo-koningii*, *T. viride* and *T. aureoviride* affected the growth of the mycelium and the synthesis of proteins in two isolates of *Serpula lacrymans* to various degrees (Humphris et al.2001).

RESUME

Hlaiem S., Zouaoui-Boutiti M., Ben Jemâa M.L., Della Rocca G., Barberini S. et Danti R. 2018. Identification et pathogénie de *Pestalotiopsis chamaeropis*, agent causal du dépérissement de la bruyère blanche (*Erica arborea*), et control biologique *in vitro* avec l'antagoniste *Trichoderma* sp. Tunisian Journal of Plant Protection 13 (si): 49-60.

Les champignons phytopathogènes sont l'une des causes principales des maladies des arbres. Les symptômes du dépérissement consistent à une chute et un jaunissement du feuillage, un dessèchement et des nécroses au niveau des branches, des chancres, des déformations, des écoulements d'un liquide noirâtre et des pourritures au niveau des troncs. Des symptômes de flétrissement ont été observés sur les espèces du maquis: bruyère blanche (*Erica arborea*), situé dans la forêt de Henchir Kort au Nord-est de la Tunisie. L'isolement à partir la bordure de ces chancres a révélé le genre de *Pestalotiopsis*. L'analyse morphologique et moléculaire de l'ITS a permis d'identifier l'agent pathogène comme étant *Pestalotiopsis chamaeropis*. Le postulat de Koch a été vérifié. L'effet antagoniste de *Trichoderma* contre *P. chamaeropis* a été évalué *in vitro*. Les tests de confrontation directe sur PDA ou à distance, ont révélé que *Trichoderma* sp. a inhibé la croissance mycélienne de l'agent pathogène par rapport au témoin non traité.

Mots clés: Bruyère blanche, dépérissement, effet antagoniste, *Pestalotiopsis chamaeropis*, *Trichoderma* sp.

ملخص

حليّم، سوسن ومريم زواوي-بوتيتي ومحمد الحبيب بن جامع وجياني ديلا روكا وسارة بربريني وروبيرتو دانتي. 2018. التشخيص والقدرة الإمراضية لفطر *Pestalotiopsis chamaeropis* العامل المسبب لمرض تدهور الخلنج الأبيض ودراسة المكافحة البيولوجية في الأنابيب باستعمال الفطر المضاد *Trichoderma* sp.

Tunisian Journal of Plant Protection 13 (si): 49-60.

تعتبر الفطريات الممرضة واحد من الأسباب الرئيسية لأمراض الأشجار. تشمل أعراض تدهور الأشجار تساقط واصفرار الأوراق وتجفف ونخر الاغصان والتقرح والتشوه وسيلان سوائل سوداء وتعفن على مستوى الجذع. لوحظت أعراض ذبول على أنواع الاحراش: الخلنج الأبيض (*Erica arborea*) المتواجد في غابة "هنشير الكرت" في شمال شرق تونس. مكن العزل من حواشي القروح من الحصول على فطر من جنس *Pestalotiopsis*. مكنت التحاليل المورفولوجية وكذلك الجزيئية للفواصل الداخلية المسجلة (ITS) من تشخيص الفطر الممرض بأنه *Pestalotiopsis chamaeropis*.

تم التثبت من قواعد "كوخ". تم تقييم التأثير العدائي للفطر المضاد *Trichoderma* على الفطر *P. chamaeropis* في الأنابيب. بينت اختبارات المواجهة المباشرة وعن بعد على المستنبت PDA، أن .sp *Trichoderma* منع النمو الغزلي للفطر الممرض مقارنة بالشاهد غير المعامل.

كلمات مفتاحية: تأثير مضاد، تدهور، خلنج أبيض، *Pestalotiopsis chamaeropis*، *Trichoderma* sp.

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Ophiostomatoid Fungi Associated with the Ambrosia Beetle *Platypus cylindrus* in Cork Oak Forests in Tunisia

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ABSTRACT

Bellahirech, A., Inácio, M.L., Ben Jamâa, M.L., and Nóbrega, F. 2018. Ophiostomatoid fungi associated with the ambrosia beetle *Platypus cylindrus* in cork oak forests in Tunisia. Tunisian Journal of Plant Protection 13 (si): 61-75.

Cork oak (*Quercus suber*) is a unique species of the Western Mediterranean region and over the last decades it has been threatened by several pests and diseases. Amongst the main dangerous pests, the ambrosia beetle *Platypus cylindrus* (the oak pinhole borer) has a key role on the process of cork oak decline namely in Portugal, Morocco, and Algeria. However, in Tunisia, where cork oak forests cover around 90.000 ha of the territory, this insect continues to have a secondary pest status. As all ambrosia insects, *P. cylindrus* is able to establish symbiotic relationships with fungi and it is known as the vector of ophiostomatoid fungi, a group including primary tree pathogens. The aim of this study was to identify these beetle-associated fungi in Tunisian forests and to understand the contribution of this association in cork oak decline by comparing with the results from other countries. The present study was conducted in 2012 in ten cork oak forests in the western-north of Tunisia and focused on ophiostomatoid fungi associated with the cork oak pinhole borer. Twenty four isolates were grouped based on morphological identification, and five representative isolates were included in phylogenetic analyses based on sequence data of ITS and β-tubulin loci. The fungi were assigned to five species namely *Raffaelea montetyi*, *R. canadensis*, *Ophiostoma* sp., *O. tsotsi* and *O. quercus*, some of them were already reported in Portugal and Algeria to be associated with cork oak decline. All these species were identified and reported for the first time in Tunisia to be associated with *P. cylindrus* in cork oak trees and their role in the cork oak loss of vitality needs to be investigated.

Keywords: Ambrosia fungi, oak pinhole borer, Ophiostomatales, *Quercus suber*.

Platypus cylindrus, the cork oak pinhole borer, is an ambrosia beetle infesting cork oaks (*Quercus suber*) that has assumed increasing importance in the Iberian Peninsula, specifically Portugal and in Algeria where it is directly associated with cork oak decline (Belhoucine et al. 2011; Inácio et al. 2011, 2012; Sousa and Debouzie 1999). Nevertheless, in some countries of the North African region, the insect is not a relevant problem, and in Tunisia it is considered as a secondary pest, attacking

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mainly weakened or dead trees (Bellahirech et al. 2015; Ben Jamâa et al. 2010). As an ambrosia beetle, it carries and inoculates fungal inoculum, in specialized organs called mycangia (Beaver1989).Several Ophiostomatales were reported to be associated with *P. cylindrus* in Portugal, Algeria and France, unlike Tunisia where no information has been previously reported until the beginning of new work in 2010 (Bellahirech et al. 2012).

Ambrosia beetles are associated with mutualistic fungi that serve as a nutrition source for larvae and adult beetles (Hofstetter et al. 2015), such as species of the Ophiostomatales (De Beer et al. 2013; Zipfel et al. 2006).This order includesthe genera *Ophiostoma*, *Ceratocystiopsis*, *Leptographium*, *Sporothrix*, *Raffaelea*, and *Graphilbum* (De Beer and Wingfield 2013; De Beer et al. 2014, 2016). More specifically, wood-boring ambrosia beetles are commonly associated with fungi of the genera *Ophiostoma* sensu stricto, and specific fungi, known as "ambrosia fungi", of the genera *Ambrosiella*, *Raffaelea*, and *Phialophoropsis* (Gebhardt et al. 2004; Harrington et al. 2010; Kowalski 1991; Massoumi Alamouti et al. 2009;Mayers et al. 2015).Due to their morphological and ecological similarities, Ascomycota fungi from the orders Ophiostomatales and Microascales are often designated as ophiostomatoid fungi (De Beer et al. 2013; Spatafora and Blackwell 1994). The ophiostomatoid fungi is a convenient term for a group of species that produce either ascospores or conidia, or both spore types, in sticky drops on elevated ascomatal necks or conidiophores (De Beer et al. 2013). The two main ophiostomatoid genera are *Ceratocystis* and *Ophiostoma*, and have been taxonomically confused for many years. Presently, they are placed indifferent orders with Ophiostomatales, including the genus *Ophiostoma*, and Microascales, comprising the genus *Ceratocystis* (De Beer et al. 2013, 2014).

Many of these fungi are serious tree pathogens (Wingfield et al. 2013) and some considered as agents of sapstain(Seifert 1993). For instance, in Japan, *Raffaelea quercivora*is responsible for the mortality of oaks infested by *Platypus quercivorus* since the 1980s (Kubono and Ito 2002; Matsuda et al. 2010; Murata et al. 2005). In addition, *R. lauricola* transported by *Xyleborus glabratus* to the southern United States is the causal agent of the lethal vascular wilt on *Lauraceae* (Fraedrich et al. 2008; Harrington et al. 2010).

Platypus cylindrus carries and inoculates mycelium that develops along the galleries serving both as offspring source of food and to weaken the host tree (Batra 1963). Several ophiostomatoid fungi were reported to be associated with *P. cylindrus* in France (Morelet 1998), Algeria (Belhoucine et al. 2011) and Portugal (Inácio et al. 2012). Based on this relationship developed between insects and associated fungi, the present study was elaborated. The main goals of this work were to (i): identify ambrosia fungi transported by the pinhole borer in Tunisian cork oak stands and; (ii): compare ongoing studies in Tunisia with previous reports in the Mediterranean cork oak region namely in Portugal, where it has been observed that the combined action of *P. cylindrus* massive attacks and extensive boring with the inoculation of ambrosia fungi, leads to an increase of tree mortality. The increase of cork oaks mortality in these past two decades can thus be in part attributed to these new associations between the insect and more aggressive wilt causing fungi (Inácio 2011). Thus, it is of paramount importance to prevent the spread and

establishment of these more aggressive ambrosial fungi in Tunisian cork oak forests where *P. cylindrus* is already infesting trees. The first step is the complete characterization of the insect associated mycoflora and the investigation of the pathogenic potential of each fungal species towards the cork oak trees.

MATERIALS AND METHODS

Study area and sampling.

The present study was conducted between January and July 2012 inselected trees of tencork oak forests (Ain Beya, Ain Drahem, Ain Sarouia, Babouch, Bellif, El Jouza, Mzara, Oued Zen, Sejnène and Tabouba) located in the western-north of Tunisia, the main cork oak region (Fig. 1).

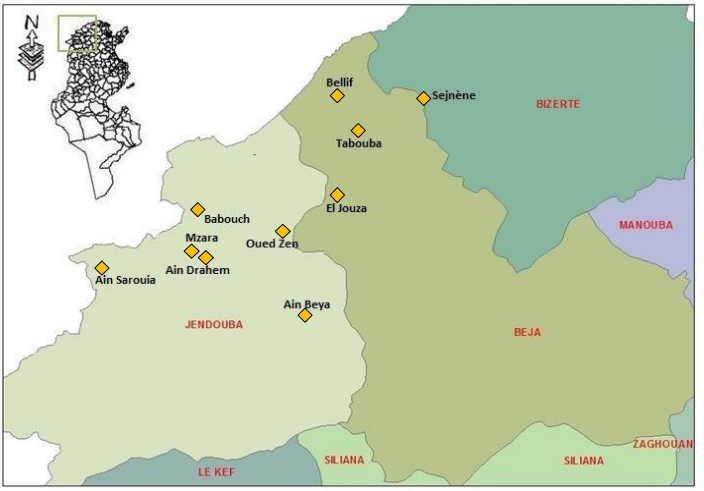

Fig. 1. Localization of studied sites (cork oak forests are indicated with yellow diamond).

One infested cork oak tree from each site was allowed to be cut and sectioned into three logs of 50 cm length. The logs were installed in the laboratory and the insects were captured while emerging in fabric traps attached to the entrance/exit hole (Sousa and Inácio 2005).

Insects were aseptically dissected and their mycangia were delicately detached under a stereo binocular microscope following the procedures of Sousa et al. (1997) and Inácio et al. (2008). After insects' emergence, cross-sections of the logs were sawn and the tunnel system of the beetles in the sapwood was open. Three galleries from each tree were taken and sectioned into small pieces.

Fungi isolation.

Both mycangia and pieces of wood from the trees' galleries were surface sterilized with sodium hypochlorite solution (1%) for one minute and rinsed with sterilized distillated water (Cassieret al. 1996). The material was plated in Malt Extract Agar medium (MEA, Difco) added with streptomycin (500 mg/l) and cycloheximide (500 mg/l). The latter is an antibiotic used to isolate only fungi of the genus *Ophiostoma* (Harrington 1981;

Harrington et al. 2010). Plates were incubated at 25°C in darkness for 15 days.

Morphological identification.

Morphological observations were made on 3 to 10-day-old cultures and after 15 days, slide cultures of each isolate (Riddell 1950) were mounted in lactophenol and examined with light microscopy (Olympus BX-41 with Olympus DP11, Hamburg, Germany).Identification at genus level was based on cultural and morphological features according to Ellis (1971, 1976), Kiffer and Morelet (1997) and Barnett and Hunter (1998).

DNA extraction and PCR amplification.

DNA of the five representative isolates was extracted from fresh myceliumscraped from the surface of the pure cultures growing on agar plates with a sterile scalpel. Mycelium was transferred to a pre-cooled and sterile mortar and pestle, frozen with liquid nitrogen and ground to a fine powder. Total DNA was isolated from approximately 100 mg of the mycelia powder using the DNeasy Plant Mini kit (Qiagen, Germany) following the manufacturer's instructions. DNA concentration and purity were checked using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). The 5,8S nuclear ribosomal RNA gene and the flanking internal transcribed spacers (ITS1 and ITS2) were amplified using primers ITS1F and ITS4 (White et al. 1990) and a fragment of the 5' end of the β-tubulin gene was amplified with primers T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995).

PCR reactions were carried out using the Dream Taq PCR Master Mix (2X) (QIAGEN, Germany) in a Biometra TGradient thermocycler (Biometra, GmbH). Each reaction mixture was performed in a final reaction volume of 25 µl containing 1 µl (50-150 ng) of template DNA, 1 µl of each primer (10 µM stock), 12.5 µl of PCR Master Mix buffer (2X), which included 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP. The thermal cycling conditions were initially denatured at 95°C for 4 min, followed by 35 cycles of 1 min at 94°C, annealing at 50°C for 1.5 min and extension at 72°C for 1 min, followed by a final elongation for 10 min at 72ºC.

The amplified products were loaded into a 1.5 % agarose gel containing 0.5 µg/ml ethidium bromide and 0.5× Tris-borate-EDTA (TBE) running buffer and electrophoresed at 5 V/cm. Amplifications were visualized using the Versa Doc Gel Imaging System (Bio-Rad, USA). PCR products were cleaned using the GeneJET PCR Purification Kit (Fermentas, Germany) according to the manufacturer's protocol.

Sequencing of PCR amplicons and bioinformatics analysis.

Amplicons were sequenced in forward direction at STABVida Sequencing Laboratory (Lisbon, Portugal) on a DNA analyzer ABI PRISM 3730xl (Applied Biosystems). The nucleotide sequences were edited and analyzed using BioEdit v7.2.0 program (Hall 2007). The nucleotide sequences were compared with those of reference available at NCBI (National Center for Biotechnology Information) GenBank database using the BLAST (Basic Local Alignment Search Tool) sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic trees were generated from the aligned sequences in

MEGA version 6 program (Tamura et al. 2013) using a Neighbour-joining analysis method. The evolutionary distances were computed using Maximum Likelihood methodology. Percentage reliability values at each internal node of the trees were obtained by performing 1000 bootstrap analyses.

Outgroup sequences were selected based on their genetic distance to Ophiostomatales used in the phylogenetic analyses Matsuda et al. (2010). Sequence from *Taphrina virginica* was used which is a frequent used outgroup in Ophiostomatales phylogenetic studies. The homologous sequences were retrieved from the GenBank database and their accession numbers were plotted in phylogenetic trees.

RESULTS

Morphological and molecular characterization of isolates.

A total of 200 isolates were obtained. Several of them were cosmopolitan fungi belonging to the genera *Acremonium*, *Aspergillus*, *Fusarium*, *Gliocladium*, *Penicillium*, and *Trichoderma*and are not part of the present work. We could observe five different morphological groups among twenty four ophiotomatales (Fig. 2) and coded respectively as TN11.001, TN12.002, TN12.003, TN12.004 and TN12.005 (Table1). Microscopic observations of conidia and conidiophores are presented in Fig. 3.

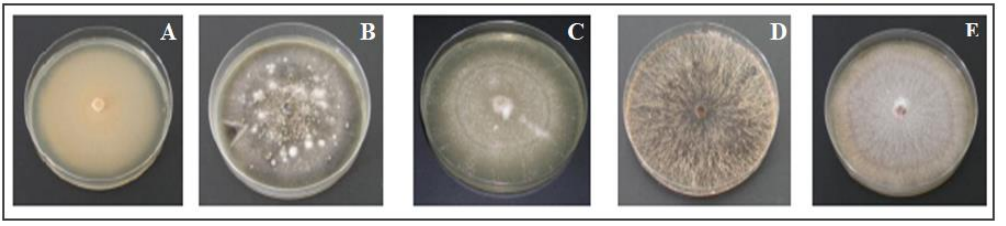

Fig. 2. Ten days old cultures of five representative species of Ophiostomatales:**A)***Ophiostoma* sp1, **B)**. *Ophiostoma* sp2, **C)***Ophiostoma* sp3, **D)***Raffaelea* sp1, **E)***Raffaelea* sp2.

Table 1. Ten days old cultural description of five putative Ophiostomatales species

Isolate	Cultural aspect	Density	Color	Shape
TN11.001	Appearance type yeast to flour with long filaments, vigorous and aerial mycelium	Medium	Fulgurant, light yellowish	Absent
TN12.002	Yeast-like to farinaceous with long filaments, vigorous and aerial mycelium with a central colony	Medium	Fulgurant, olive green	Clear
TN12.003	Yeast-like to farinaceous with long filaments, vigorous and aerial mycelium with a central colony	Not very dense	Light green olive	Clear
TN12.004	Appearance like yeast flour with long filaments, vigorous and aerial mycelium	Not very dense	Light brown	Absent
TN12.005	Yeast-like to farinaceous with long filaments, mycelium and aerial, with a central colony	Medium	Yellowish with a whitish central colony	Absent

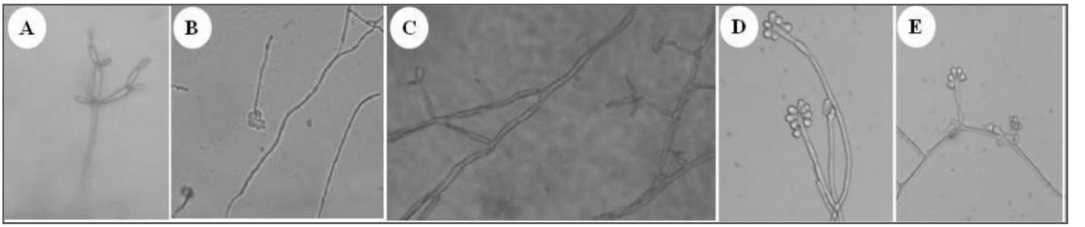

Fig. 3. Conidia and conidiophores of Ophiostomatales isolated from *Playpus cylindrus* and its galleries after 5 days incubation in darkness: **A**) *Ophiostoma* sp.1 (x400), **B)** *Ophiostoma* sp. 2 (x200), **C)** *Ophiostoma* sp. 1 (x400), **D)** *Raffaelea* sp. 2 (x400), **E)** *Raffaelea* sp. 3 (x400).

Phylogenetic analysis.

PCR analysis using ITS1F/ITS4 and T1/BT2b primer pairs yielded specific products of approximately 650 bp (ITS-PCR products) and 1000 bp (β-tubulin), respectively. For each pair of primers used in the PCR amplifications, no secondary band was obtained except for the specific products. In addition, no PCR products were obtained for the controls.

Comparison of the sequences with GenBank nucleotide sequences showed that the similarity of some sequences is not very high, but some of them can be assigned to a particular species.

Comparison of the sequences obtained with the *Raffaelea* nucleotide sequences in the GenBank sequence database for both ITS and β-tubulin regions, confirmed high degree of sequence identity of two *Raffaelea* species with *R. canadensis* and *R. montetyi*. The position of the two *Raffaelea* species was consistent, as they were placed in the Ophiostomatales clade (Harrington et al. 2010; Massoumi Alamouti et al. 2009; Matsuda et al. 2010). On the other hand, a similarity of 100% of the TN12.002 isolate with *Ophiostoma tsotsi* and 96% of the TN12.003 isolate with *O. quercus* was noted for the partial β-tubulin gene.

The phylogenetic analysis was carried out separately for both regions (ITS and β-tubulin) and the sequences of *Taphrina pruni* and *T. wiesneri* were used as outgroup, according to Inácio (2011).This phylogenetic tree underlined a separation of isolates into two different groups. A phylogenetic group including isolates TN12.002 and TN12.003, closely related to each other and to *Ophiostoma* strains, whereas isolates TN11.001, TN12.004 and TN12.005 were more similar to strains close to *Raffaelea* (Fig. 4).

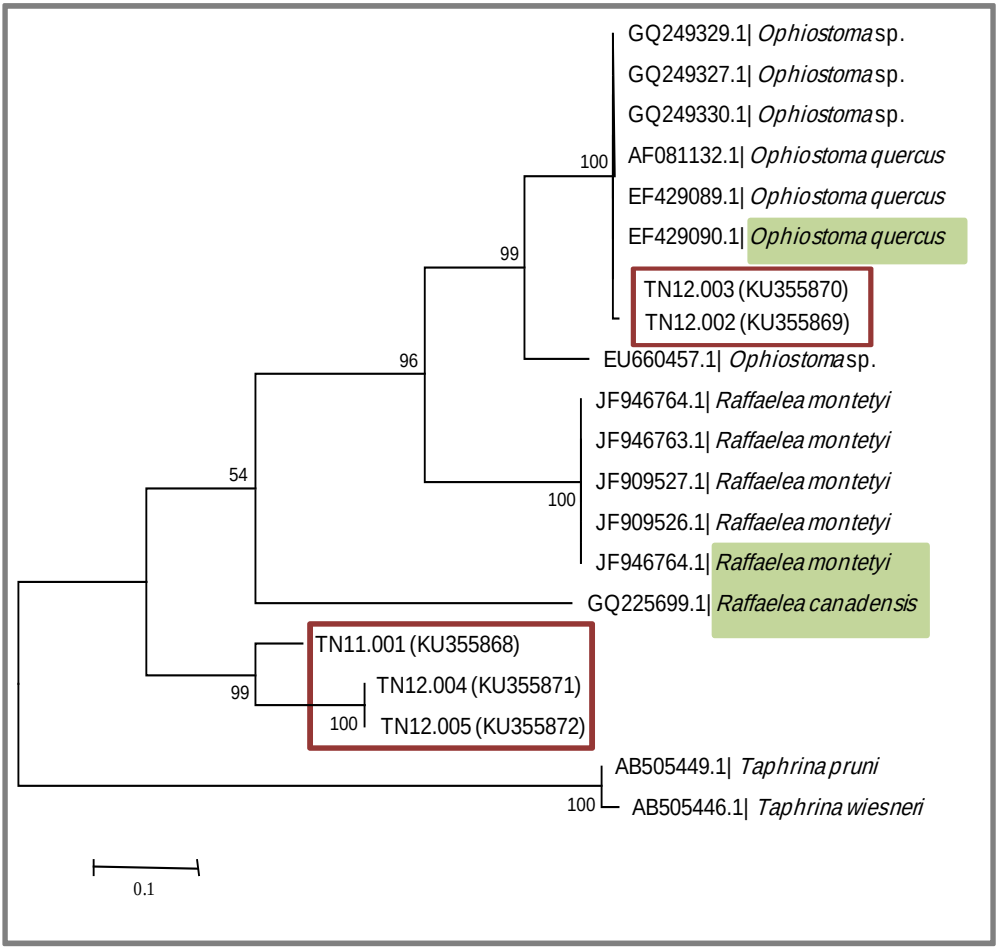

Fig. 4. Molecular phylogenetic analysis of 20 nucleotide sequences of rDNA (5.8S-ITS2-28S) of Ophiostomatales isolated from *Platypus cylindrus* on cork oak stands in Tunisia by Maximum Likelihood (ML) method based on Tamura 3-parameter model (Tamura and Nei1993) and supported by 1000 replicates of bootstraps. Evolutionary analyses were conducted with the MEGA 6 software (Tamura et al. 2013).

The comparison of the partial β-tubulin sequences with sequences already present in the database using the BLAST search of the *Ophiostoma* sp., *Raffaeleamontetyi* and *R. canadensis* sequences yielded the phylogenetic tree below (Fig. 5). A sequence from *Taphrina virginica* was included as outgroup. Homologous sequences were retrieved from GenBank and accession numbers included in the phylogenetic tree.

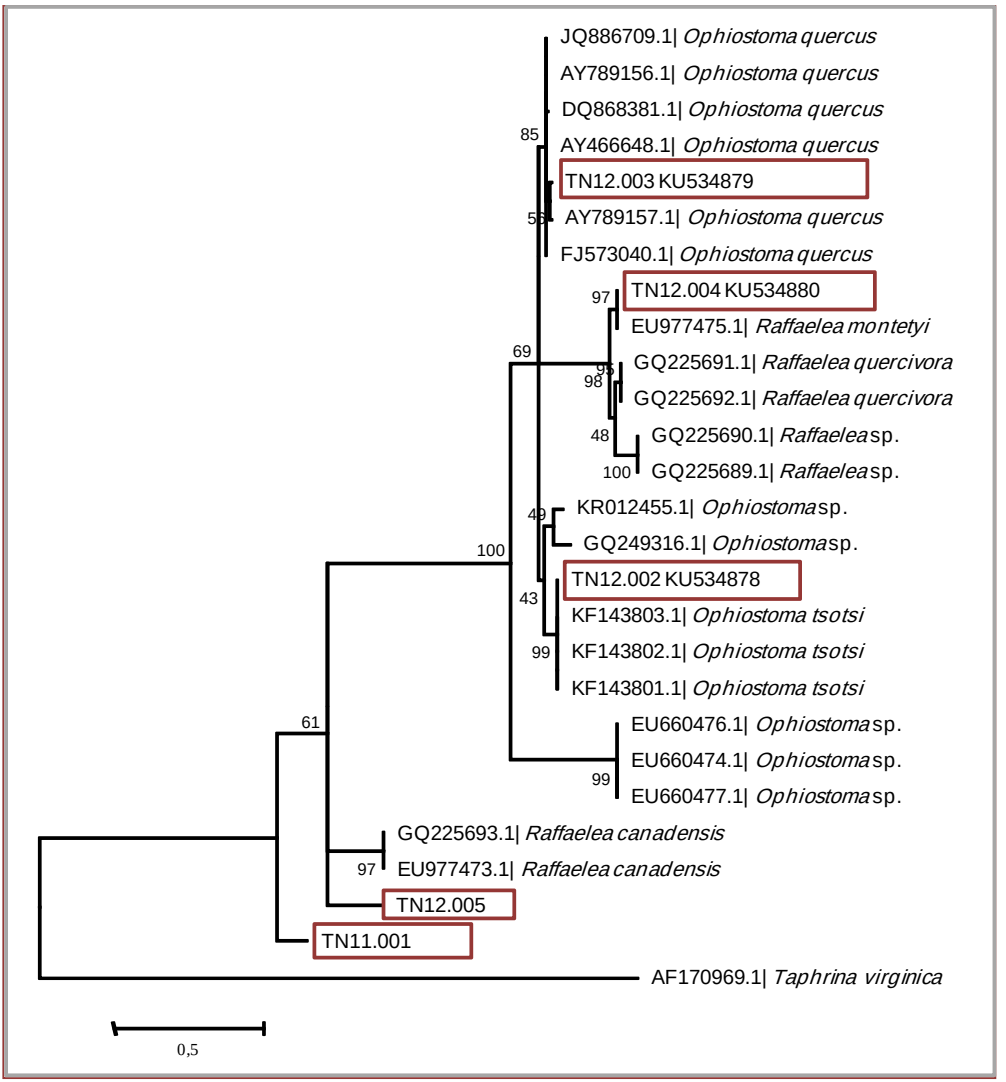

Fig. 5. Molecular phylogenetic analysis of 27 nucleotide sequences encoding the β-tubulin protein gene of Ophiostomatales isolated from *Platypus cylindrus* on cork oak stands using the Maximum Likelihood method (ML) based on the Tamura 3-parameter model. Evolutionary analyses were conducted using the MEGA v.6.0 software (Tamura et al. 2013).

The phylogenetic tree showed that the TN12.005 isolate is directly related to *R. canadensis* whereas TN12.004 is closer to *R. montetyi*. The other *Ophiostoma* isolates belonged to three different species, two of which could be identified, and a third one preliminary called *Ophiostoma* sp. The isolate

TN12.003 has more similarity with *O. quercus* while TN12.002 has more homology with *O. tsotsi*. TN11.001 isolate is *Ophiostoma* sp. since its position on the phylogenetic tree did not allow to assign it to any known species.

The sequences of the isolates obtained by each pair of primers were deposited to GenBank database (NCBI) (http://www.ncbi.nlm.nih.gov/genbank) under the accession numbers from KU534878 to KU534880 for β-tubulin and from KU355868 to KU355872 for the ITS region (Fig. 4 and Fig. 5).

Based on the sequences for both regions of the obtained ophiostomatales associated with *P. cylindrus*, five different species were identified: *Ophiostoma* sp. (KU355868), *O. tsotsi* (KU534878; KU355869), *O. quercus* (KU534879; KU355870), *Raffaelea montetyi* (KU534880; KU355871), and *R. canadensis* (KU355872).

The distribution of the fungal species in the sampled forests revealed some differences between sites (Table 2).

Table 2. Presence of the fungal species in the Ophiostomatales order associated with *Platypus cylindrus* in Tunisian cork oak forests (in grey)

Fungi	Wed Zen	Ain Sarouia	Ain Beya	Babouch	Mzara	Ain Drahem	El Jouza	Tabouba	Bellif	Sejnène
Raffaelea montetyi										
R. canadensis										
Ophiostoma quercus										
O. tsotsi										
***Ophiostoma* sp.**										

R. montetyi is present in seven forests and absent in Babouch and Mzara while *R. canadensis* occurs only in three forests namely Ain Beya, Bellif and Sejnène. The genus *Ophiostoma* represented by three species is less present. *O. quercus* was isolated from trees of the forests of Babouch and Mzara, which in addition to Ain Sarouia forest reveals the presence of *O. tsotsi*. As for the unidentified *Ophiostoma* species, it has been isolated from the forests of Wed Zen and Ain Beya.

DISCUSSION

Morphological and molecular identification of Ophiostomatales associated with *P. cylindrus* and its galleries revealed the presence of the fungi *O. quercus*, *O. tsotsi*, *R. montetyi* and *R. canadensis* and a third species of *Ophiostoma* not yet fully characterized, *Ophiostoma* sp.

Taking into account previous studies on *P. cylindrus* in Portugal (Henriques 2007; Inácio et al. 2011,2012) and in Algeria (Belhoucine et al. 2011), the presence of *R. montetyi* in Tunisia confirms this symbiotic relationship with the insect that appears to be an ecological adaptation aiming at colonizing the host in the fastest and most efficient way.

R. montetyi was detected in the forests of Ain Beya, Ain Drahem, Ain Sarouia, El Jouza, Tabouba and Wed Zen which are characterized by a humid bioclimate with mild and temperate winters and by the presence of dense and abundant shrubs (Bellahirech 2016). These conditions seem to enhance the presence of this fungal species. Among *Raffaelea* genus, several species were related to the massive mortality of oaks in Japan (Kubono and Ito 2002; Matsuda et al. 2010) and of laurel wilt (Fraedrich et al. 2008) and avocado (*Persea americana*) in the United States (Eskalen and McDonald 2011). In Portugal, *R. montetyi* was shown to be pathogenic towards cork oak (Inácio et al. 2012), therefore playing an important role in the cork oak decline. In Portugal, a *Raffaelea* species closely related to *R. canadensis* was noticed for the first time and in association with the ambrosia beetle (Inácio et al. 2012). In Tunisia, *R. canadensis* is reported for the first time in North Africa, and it has been detected only in Ain Beya forest where insect infestation did not exceed 10% (Bellahirech 2016).

Regarding molecular studies, the ITS region was widely used for fungi diagnosis and proposed as a universal marker for fungal DNA barcodes (Schoch et al. 2012). Unfortunately, the locus is particularly difficult to use for *Raffaelea* fungi (Harrington et al. 2011; Jeyaprakash et al. 2014). Thus, the differentiation between *Raffaelea* species was achieved only through the analysis of both ß-tubulin and ITS regions.In the present work, we identified two species of the genus *Ophiostoma* namely *O. quercus* and *O. tsotsi*. *O. quercus* was isolated for the first time in Yugoslavia from *Q. pedunculata* (Georgevitch 1927). This fungus was associated with the decline of various oaks in central Europe (Cech et al. 1990). In Spain, Luque et al. (2000) isolated *O. quercus* from cork oak, and was considered to be the agent of blue stain and xylem discoloration of many other trees (De Beer et al. 2003; Geldenhuis et al. 2004; Nkuekam et al. 2008). *O. quercus* is associated with many insects, particularly bark beetles (Kirisits 2007; Zhou et al. 2006). In Norway, *O. quercus*was associated with *Scolytusrafzeburgi* in *Betula* sp. (Linnakoski et al. 2009). It was also found in Finland and Russia (Linnakoski et al. 2008).

O. quercus was reported in Algeria on cork oak associated with *P. cylindrus*, (Behoucine et al. 2011) and in Tunisia,as shown in the present work, in Babouch and Mzara forests with presence average of 16.6 and 8.3%, respectively. These forests are characterized by similar climatic, edaphic and phytosanitary conditions and dense vegetation cover. Phylogenetic β-tubulin analyzes confirmed that the TN12.002 isolate associated with *P. cylindrus* and its galleries was 100% similar to the *O. tsotsi* species. This group of fungi is closely related to *O. quercus* and to the Dutch disease pathogens, *O. ulmi* and *O. novo-ulmi*, in the clade of the O. *piceae* complex (Grobbelaar et al. 2010). This is coherent with our results which show, through the phylogenetic tree of the ITS region, an assembly of these two species under a monophylogenic line (*O. quercus*). The differentiation between these species was only possible through

the β-tubulin analyzes alone, due to a better resolution.

It is noteworthy that *O. tsotsi* is frequently found in association with *O. quercus* and it is not surprising that it remained hidden from recognition as a discrete entity. The available knowledge of this fungus is limited, but it appears that its distribution and host range are confounded with those of *O. quercus* (De Beer et al. 2003; Grobbelaar et al. 2010; Harrington et al. 2001).*O. tsotsi* was found on hardwood trees in South Africa (Grobbelaar et al. 2010) and it is considered a native species in the northern hemisphere (Brasier 1990). Its presence in Tunisia suggests that it was introduced from this region which also represents all the industrialized countries (Europe, Asia, and North America). This could easily occur with the commercial trade of timber and its products from the northern hemisphere to Africa (Grobbelaar et al. 2010) including Tunisia. On the other hand, this fungus was identified on *Eucalyptus* in China (Grobbelaar et al. 2011) and Australia (Nkuekam et al. 2011). It is important to verify that *O. tsotsi* was always isolated in association with *O. quercus* (Grobbelaar et al. 2010, 2011; Nkuekam et al. 2011). This observation corroborates our study where the two species were isolated in the same forest (Mzara) where *P. cylindrus* infestation is about13% and the global phytosanitary situation of trees is good (Bellahirech 2016).

In Tunisia, these species are newly reported in symbiotic relationship with the cork oak pinhole borer. *Ophiostoma* and *Raffaelea* fungi were obtained from both insect mycangia and galleries. Without *P. cylindrus* as vector, it would not be possible for these fungal species to infect new hosts because Ophiostomatales require pre-existing wounds to infect their hosts (Nkuekam et al. 2012).On the other hand, without these fungal associates, the insects would have enormous difficulty in colonizing new trees and to continue their life cycle. Hence, there is thus an obligatory relationship of symbiosis as defended by Lieutier (2007). In addition, several studies showed that only one or a few species of fungi are associated with a particular ambrosia insect (Batra 1963; Funk 1970). However, more recent studies have pointed out that symbiotic relationships are more diverse,more promiscuousand more competitive than assumed and that *Raffaelea* species compete with each other for growth into mycangia (Harrington et al. 2011; Inácio et al. 2012).

Understanding the ecology and population dynamics of *P. cylindrus*-associated fungi is important for the surveillance and management of the beetle-fungal complex, and could improve prediction and modeling. Biological control of the pathogens may prove possible through manipulation of the mycangial mycoflora (Inácio 2011).

Finally, it is important to point out that the research described herein contributed to clarify aspects of putative reasons to trigger the decline of trees in Tunisian cork oak forests. This investigation should continue, namely through the pathogenicity assessment of the identified Ophiostomatales, considering however that it is rather difficult to perform such studies in adult trees. The clarification of these fungal symbionts role and the prevention of their spread will help to preserve the natural patrimony in Tunisia and cultural heritage of the unique cork oak stands in Mediterranean Basin.

RESUME

Bellahirech A., Inácio M.L., Ben Jamâa M.L. et Nóbrega F. 2018. Champignons ophiostomatoïdes associés à l'insecte ambrosia *Platypus cylindrus* dans les forêts de chênes-lièges en Tunisie. Tunisian Journal of Plant Protection 13 (si): 61-75.

Le chêne-liège (Quercus suber) est une espèce unique de la région méditerranéenne occidentale et, au cours des dernières décennies, il a été menacé par plusieurs ravageurs et maladies. Parmi les principaux ravageurs dangereux, l'insecte ambroisia *Platypus cylindrus* (le platype du chêne) qui joue un rôle clé dans le processus de déclin du chêne-liège, notamment au Portugal, au Maroc et en Algérie. Cependant, en Tunisie, où les forêts de chênes-lièges couvrent environ 90 000 ha, cet insecte continue d'avoir un statut de ravageur secondaire. Comme tous les insectes ambrosia, *P. cylindrus* est capable d'établir des relations symbiotiques avec les champignons et il est connu comme étant le vecteur des champignons ophiostomatoïdes, un groupe comprenant les pathogènes primaires des arbres. Le but de cette étude était d'identifier ces champignons associés au coléoptère dans les forêts tunisiennes et de comprendre la contribution de cette association dans le déclin du chêne-liège en comparant avec les résultats des autres pays. La présente étude a été réalisée en 2012 dans d'importantes forêts de chênes-lièges de l'ouest-nord de la Tunisie et s'est concentrée sur les champignons ophiostomatoïdes associés au platype de chêne-liège. Vingt-quatre isolats ont été regroupés en fonction de l'identification morphologique et cinq isolats représentatifs ont été inclus dans les analyses phylogénétiques basées sur les données de séquençage des locus ITS et β-tubuline. Les champignons ont été attribués à cinq espèces: *Raffaelea montetyi*, *R. canadensis*, *Ophiostoma* sp. *O. tsotsi* et *O. quercus*, certaines d'entre elles ont déjà été signalées au Portugal et en Algérie en relation avec le déclin du chêne-liège. Toutes ces espèces ont été identifiées et signalées pour la première fois en Tunisie en association avec *P. cylindrus* sur chêne-liège et leur rôle dans la perte de vitalité du chêne-liège devra être étudié.

Mots clés: Champignons ambrosia, Ophiostomatales, platype, *Quercus suber*

ملخص

بلاحيرش، أماني وماريا لوردس أناسيو ومحمد الحبيب بن جامع وفلومينا نبرجا .2015. مساهمة الفطريات ophiostomatoids المرتبطة بالحشرة *Platypus cylindrus* في غابات بلوط الفلين بتونس.

Tunisian Journal of Plant Protection 13 (si): 61-75.

بلوط الفلين/الفرنان (*Quercus suber*) هو جنس فريد من نوعه في منطقة غرب البحر الأبيض المتوسط، وخلال العقود الماضية، يتعرض باستمرار لخطر مختلف الآفات والأمراض. من بين الآفات الخطيرة الرئيسية، هناك الحشرة الأمبروزية *Platypus cylindrus* (ناخرة الخشب) التي تتواجد في دول أخرى ولها دور رئيسي في عملية تدهور بلوط الفلين، خاصة في البرتغال والمغرب والجزائر. رغم ذلك، في تونس حيث تغطي أشجار بلوط الفلين حوالي 90.000 هكتار، تظل هذه الحشرة آفة ثانوية. مثل جميع الحشرات الأمبروزية، الحشرة *P. cylindrus* قادرة على إقامة علاقات تكافلية مع الفطريات، وتعمل كناقلة للفطريات ophiostomatoids، وهي مجموعة من الفطريات التي تضم مسببات الأمراض الأولية للشجرة. كان الغرض من هذه الدراسة هو تشخيص هذه الفطريات ذات الصلة بالحشرات لفهم مساهمة هذه العلاقة في تدهور اشجار بلوط الفلين. أجريت هذه الدراسة في عام 2012 في عشر غابات في شمال غرب تونس وتركزت على رتبة الفطريات Ophiostomatales المرتبطة بالحشرات الناخرة في غابات بلوط الفلين التونسية. تم توصيف أربع وعشرون عزلة بناءً على التشخيص المورفولوجي، وتمت دراسة خمس عزلات في تحليل جيني استنادا إلى تتابع منطقتيْ ITS وβ-tubulin. تم ربط الفطريات إلى خمسة أنواع هي *Raffaelea montetyi* و *R. canadensis* و sp. *Ophiostoma*. و *O. quercus* و *O. tsotsi*. تمت الإشارة إلى البعض منها في البرتغال والجزائر لارتباطها بتدهور بلوط الفلين. وقد تم تشخيص جميع هذه الأنواع وإرادها لأول مرة في تونس مع ارتباطها بـ *P. cylindrus* في أشجار بلوط الفلين ويبقى دورها في فقدان الفرنان لحيويته بحاجة إلى بحث.

كلمات مفتاحية: بلوط الفلين، ناخرة الخشب، فطريات أمبروزية، Ophiostomatales

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Impact of Captopril on *Ephestia kuehniella*: Ovarian Nucleic Acid Amounts and Protein Analysis

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ABSTRACT

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Ephestia kuehniella is a serious stored product pest, especially in whole and milled grains. Knowing the mechanisms that control the reproduction and development of these pests is therefore of fundamental and economic interest. Captopril, an inhibitor of angiotensin converting enzyme, was tested in vivo by topical application on reproduction of *E. kuehniella*. The drug was dissolved in acetone and topically applied (10 µg/pupa) on newly molted pupae. In follow-up experiment, the adults that survived from treated pupae were investigated for different reproductive event parameters. Captopril significantly reduced the ovarian contents of proteins and nucleic acids. The electrophoretic separation of proteins on sodium dodecyl sulfate polyacrylamide slab gels showed differences in the number of protein fractions between control and treated series. We noted the absence of three protein fractions in treated series.

Keywords: Biochemistry, captopril, electrophoresis, *Ephestia kuehniella,* ovaries, reproduction

Little is known about the role of the invertebrate angiotensin-converting enzyme (ECA). The vertebrate counterpart (ACE, EC 3.4.15.1) plays an important role in the rennin angiotensin system. It regulates blood pressure and water by converting angiotensin I into the water by converting angiotensin I into the vasoconstrictive peptide angiotensin II and deactivating the vasodilator, bradykinin I (Corval et al. 2004). ACE is therefore crucially involved on the homeostatic regulation of blood pressure and electrolyte balance and is strongly linked with a number of cardiovascular and renal diseases (Bernstein et al. 2005; Shen et al. 2008b). More recently, insect cell extracts were found to exhibit in vivo antihypertensive activity without any extra digestion requirement (Staljanssens et al. 2011). Since the discovery of an ACE in *Musca domestica*, ACE-like activity has been detected in several insect species from different orders (Fernandez et al. 2001; Lemeire et al. 2007; Wijffels et al. 1996; Williams et al. 1996). Therefore, inhibiting ACE activity is expected to interfere with the peptidergic endocrine system and to have detrimental effects on growth,

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development and reproduction of insects (Issac et al. 2007).

The Mediterranean flour moth, *Ephestia kuehniella*, is a serious worldwide pest of flourmills (Caspari and Gottlieb 1975). Recently, *E. kuehniella* has been used as a lepidopteron model to investigate normal development (El Ouar et al. 2010; Yezli-Touiker and Soltani-Mazouni 2011). Therefore, these data provide a strong basis to investigate the effect of various xenobiotics on development and reproduction of pest insects, particularly Lepidoptera. In *Tenebrio molitor*, we have shown that captopril affected the morphometric measurements of ovaries and no significant effect was observed on both thickness and fine structure of chorion (Soltani-Mazouni et al. 2007). Kirane-Amrani and Soltani-Mazouni (2012) tested three ACE inhibitors (namely captopril, enalapril, and lisinopril) in *E. kuehniella* pupae and showed that only lisinopril significantly reduced the ecdysteroid contents in whole body extracts at day 3. When captopril and fosinopril, two ACE inhibitors, were added to the rearing water of mosquitoes, high levels of larval mortality were observed of *Aedes aegypti* and *Anopheles gambiae* (Abu Hasan et al. 2017). More recently, we found that captopril affects the fecundity and the egg viability in *E. kuehniella*. This compound also caused a decrease in ovarian protein, lipid and carbohydrate amounts, suggesting interference with the vitellogenesis process (Yezli-Touiker et al. 2016). In order to complete these findings, experimentations on the effects of captopril on reproduction were performed with special attention to the mechanism by which this decrease of fecundity occurs. Thus, the separation of ovarian proteins by polyacrylamide gel electrophoresis (PAGE) was examined. Since the decrease in fecundity following treatment with some xenobiotics, like insect growth disruptors, may be due to the inhibition of ovarian DNA synthesis (Mitlin et al.1977; Soltani-Mazouni and Soltani 1994), the DNA and RNA amounts were also determined in ovaries of surviving adults from treated pupae.

MATERIALS AND METHODS

Insects.

Last instars larvae of *E. kuehniella* were collected from flour infested, separated according to their sex and placed in plastic boxes. These last were then put in a drying oven at a temperature of 27°C and a relative humidityof 80% as reported before (Soltani-Mazouni et al. 2012). Newly molted female pupae (< 8 h old) were collected every day and treated immediately. The surviving adults from treated pupae were collected. Paired ovaries from the newly emerged adult females (< 8 h old) were dissected from control and treated series.

Chemical and treatment.

Captopril (D-3mercapto-2-methyl-propionyl-L-proline) used was purchased from Sigma Co. (98%, Bornem, Belgium). A stock solution of ACE inhibitor captopril was prepared in acetone (5 mg/ml). Newly molted pupae (< 8 h old) were topically treated with a dose of 10 µg/pupae according to previous studies (Soltani-Mazouni et al. 2007; Yezli-Touikeret al. 2016). The drug was easily diluted in acetone, allowing more effective diffusion throughout the cuticle (Soltani et al. 1983; Soltani-Mazouni et al. 2012). In the control groups, pupae were treated with 2 µl of acetone alone.

Extraction and determination of nucleic acid amounts.

Six pairs of ovaries were taken from newly emerged adult females from control and treated series. Nucleic acid extraction from each pair of ovaries was performed according to Shibko et al. (1966). The assay was performed by a colorimetric method using as reagents diphenylamine for DNA (Schneider 1957), and orcinol for RNA (Burton 1956).

Protein quantification.

The surviving adults from treated pupae were collected from control and treated series and ovaries were dissected from newly emerged adult females. Each pair of ovaries was individually extracted following the procedure of Shibko et al. (1966). In brief, each sample consisting one pair of ovaries was homogenized in 1 ml of trichloroacetic acid (20%), and then centrifuged (5.000 g for 10 min). The pellet added with a mixture of ether and chloroform (1/1 v/v) was subjected to a second centrifugation (5.000 g for 10 min). The second resulting pellet added with NaOH (0.1N) served for total protein quantification in accordance with (Bradford 1976) assay using Coomassie blue brilliant (G 250, Merck) as a reagent and bovine serum albumin (Sigma) as standard. Absorbance was read at 595 nm wavelength. Protein contents were expressed as µg/mg of wet weight of tissues.

Electrophoresis.

For electrophoretic analysis of ovarian proteins, 12.5% sodium dodecyl sulfate-polyacrylamide slab gels (SDS-PAGE) were prepared following the procedure of Laemmli (1970) under native conditions. Gonads were dissected at appropriate times (< 8 h old) from females. Each pooled sample (each containing 12 ovaries per series) was placed in phenyl methyl sulfonylfuoride (PMSF, 45 mg/ml ethanol) at 0.1% (500 µl) and stored at -20°C until analysis. Proteins were extracted from samples as previously described by Soltani et al. (2002).

Statistical analyses.

Results were expressed as mean ± standard deviation (SD). Comparison of mean values were made by Student's *t*-test. All statistical analyses were performed using the MINITAB Software (Version 16, PA State College, USA) and $P < 0.05$ was considered to be a statistically significant difference.

RESULTS

Effect on ovarian nucleic acid amounts.

The effect of captopril applied topically on newly molted pupae was examined on the nucleic acid amounts in each ovary collected from newly emerged adult females. The compound caused a significant decrease in both amounts of DNA ($P = 0.02$) and RNA ($P \leq 0.05$) (Table 1).

Effect on ovarian protein amounts.

Results presented in Table 2 showed that captopril significantly ($P < 0.001$) reduced the ovarian amounts of proteins when compared to control series.

SDS-PAGE separation of ovarian proteins shown that there were 11 bands in control females (Fig.1, Table 3). Table 3 presents the different protein bands with their respective molecular weights. As compared to the protein pattern from controls, captopril caused the absence of three bands (the fourth, the fifth and the tenth ones).

Table 1. Effect of captopril (10 µg/pupa) applied topically on newly molted pupae of *Ephestia kuehniella* on nucleic acid amounts in ovaries from newly emerged adults

Treatment	DNA (µg/mg tissue)	RNA(µg/mg tissue)
Control	4.85 ± 0.30 a	12.76 ± 0.38 a
Captopril	3.69 ± 0.19 b	8.91 ± 0.27 b

Values are presented as means ± SD; n = 6 females. Within each column, values followed by the different letter are significantly different based on Student's t-test at $P < 0.05$).

Table 2. Effect of captopril (10 µg/pupa) applied topically on newly molted pupae of *Ephestia kuehniella* on amounts of proteins in ovaries from newly emerged adults

Treatment	Protein amount (µg/mg tissue)
Control	28.72 ± 1.06 a
Captopril	10.02 ± 0.66 b

Values are presented as means ± SD; n = 6 females. Values followed by the different letter are significantly different based on Student's t-test at $P < 0.05$).

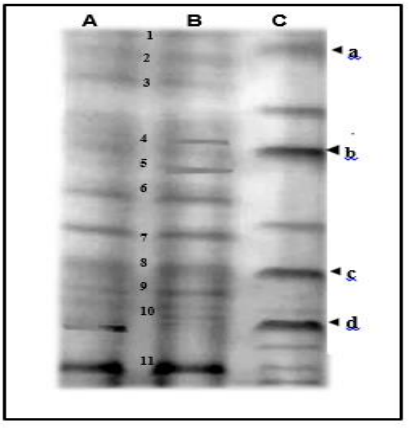

Fig. 1. SDS-PAGE patterns of ovarian proteins from newly emerged adults of *Ephestia kuehniella*in control (**A**) and captopril-treated (**B**) series as compared to protein markers (**C**). **a**: myosin 200 kDa, **b**: phosphorylase 100 kDa, **c**: albumin 75 kDa, **d**: ovalbumin 50 kDa; Numbers**1 to 11**: correspond to the numbers of protein fractions.

Table 3. Electrophoretic separation of ovarian proteins: number and molecular weight (MW) of fractions in control and treated series of *Ephestia kuehniella*

Fraction		Control	Captopril
N°	MW (kDa)		
1	206.06	+	+
2	181.55	+	+
3	150.31	+	+
4	124.45	+	-
5	96.60	+	-
6	83.52	+	+
7	80.50	+	+
8	76.03	+	+
9	63.57	+	+
10	53.96	+	-
11	29.04	+	+

Presence +; -: Absence.

DISCUSSION

In this study, we evaluated the effect of captopril an ACE inhibitor applied topically on reproduction of *E. kuehniella*. Results revealed that this drug reduced significantly ($P < 0.05$) the ovarian amounts of both RNA, DNA and proteins recorded at the end of vitellogenesis process (i.e. at adult emergence). Previous data obtained in a coleopteran species *T. molitor* reported that captopril affects the morphometric measurements of ovary but no significant effect was observed on both thickness and fine structure chorion (Soltani-Mazouni et al. 2007). Bensalem et al. (2013) showed that enalapril and lisinopril applied on newly molted *E. kuehniella* pupae exerted an inhibitory effect on both the amount of testicular proteins and a significant increase on testicular contents of DNA and RNA.

In lepidopteron species, such as *E. kuehniella*, the process of vitellogenesis takes place during the pupal stage and the oocyte accumulates and organizes yolk from precursors imported from hemolymph, while the cells of follicular epithelium synthesize additional components of the yolk as well as the vitellin envelope and chorion (Telfer 2009). The intermediary metabolism is directly involved in several physiological processes (growth, immunity, molt, reproduction and sexual maturation) which require a quantitative and qualitative contribution of various metabolites namely proteins, carbohydrates, lipids, and glycogen (Telfer 2009). In addition, proteins play an important role in the formation of gametes (Borsa and Millet 1992).

Several studies suggest a physiological role for the enzyme in insect reproduction. When *Anopheles*

stephensi females were fed with a blood meal containing either captopril or lisinopril, a reduction in fecundity in a dose-dependent manner was observed (Ekbote et al. 2003). Captopril was found to decrease the duration of the oviposition period and the morphometric measurements of ovaries, the fecundity and the egg viability in *E. kuehniella*. Moreover, biochemical analyzes revealed that treatment reduced total protein, lipid and carbohydrate amounts of ovaries, respectively. Lastly, enzyme immunoassay measurements of ovarian ecdysteroids indicated that captopril increased the amounts of both total and free ecdysteroids, and decreased that of conjugated (Yezli-Touiker et al. 2016). The reduction of reproductive capacity was probably due to an interference of captopril with the vitellogenesis process and/or ecdysteroid biosynthesis. This is supported by the reduction of vitellogenin titers in *Neobellieria bullata* (Vandingenen et al. 2001) and altered oviposition in *Spodoptera littoralis* (Vercruysse et al. 2004). In the current experiments, we noted that captopril caused significant reduction of protein amount in the ovaries. Moreover, PAGE of ovarian proteins revealed that three protein bands of 124.45, 96.60 and 53.96 kDa, respectively, were missing. Our results agree with data obtained after topical treatment of captopril on *T. molitor* adults (Soltani-Mazouni et al. 2007). Indeed, captopril caused a significant reduction in both the weight of ovaries, the number of oocytes per paired ovaries, and the size of basal oocytes as compared to controls. Captopril reduced the number of eggs per female in *E. kuehniella* (Yezli-Touiker et al. 2016) probably via the protein accumulation process and consequently the formation of eggs. The lack of three bands in the ovaries from treated series supports this hypothesis. ACE should be considered as a potential target for the development of new insect growth regulators (Issac et al. 2007).

Captopril was found to affect the ovarian contents recorded at the end of vitellogenesis process of both RNA, DNA and proteins of *E. kuehniella*. Mitlin et al. (1977) suggested that a decrease in sexual activity among *Anthonamus grandis* results in part from inhibition of DNA synthesis in female adults by diflubenzuron an inhibitor of chitin biosynthesis. This compound also caused a decrease in ovarian protein content in *Cydia pomonella* (Soltani and Soltani-Mazouni 1992), suggesting interference with the vitellogenesis process. The decrease in fecundity observed in several insect species may be due to the inhibition of ovarian DNA synthesis (Soltani-Mazouni and Soltani 1994).

Although, the exact mode of action of captopril is still unknown, our findings suggest an interference with the reproductive events. Further experiments are needed to determine the action mechanisms of captopril more in detail, and the functions of ACE in insects.

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RESUME

Yezli-Touiker S., Taffar A., Meskache R. et Soltani N. 2018. Impact du captopril sur *Ephestia kuehniella*: Quantités des acides nucléiques ovariens et analyse des protéines. Tunisian Journal of Plant Protection 13 (si): 77-85.

Ephestia kuehniella est un lépidoptère ravageur des denrées stockées qui provoque des dégâts principalement sur la farine. Le captopril est un inhibiteur de l'enzyme de conversion de l'angiotensine. Il a été testé *in vivo* par application topique sur la reproduction d'*E. kuehniella*. Ce médicament a été dilué dans l'acétone et administré par application topique chez les chrysalides femelles nouvellement exuviées à la dose de 10 µg/chrysalide. Les adultes qui ont survécu au traitement des pupes ont été étudiés pour différents paramètres de reproduction. Le captopril a réduit significativement le taux des acides nucléiques et protéines ovariens. La séparation électro-phorétique des protéines sur des gels polyacrylamide additionnés de dodécylsulfate de sodium a montré des différences dans le nombre de fractions protéiques entre les témoins et les traitées. Le profil électro-phorétique a révélé l'absence de trois fractions chez les séries traitées.

Mots clés: Biochimie, captopril, électrophorèse, *Ephestia kuehniella*, ovaires, reproduction

ملخص

يزلي تويقر، سميرة وأسماء طفار ورانية مسقاش ونور الدين سلطاني. 2018. تأثير كابتوبريل على حشرة *Ephestia kuehniella* : مقادير الأحماض النووية المبيضية وتحليل البروتينات.

Tunisian Journal of Plant Protection 13 (si): 77-85.

تعتبر الحشرة *Ephestia kuehniella* إحدى الآفات المضرة بالمواد المخزنة. كبتوبريل هو مانع أنزيم تحويل الأنجيوتنسين. أجريت التجارب المخبرية عن طريق المعاملة السطحية. تم تخفيف هذا الدواء في الأسيتون واستعمل عن طريق الدمج الموضعي لعذارى الإناث بجرعة (10 مكم/عذراء) . تمت دراسة البالغين الذين نجوا من المعاملة السطحية للعذارى اعتمادا على معايير التكاثر. خفض كبتوبريل من كمية البروتينات والأحماض الأمينية في الرحم. بين الرحلان الكهربائي SDS-Page على خارطة الكسور البروتينية المتأثرة بكبتوبريل اختفاء ثلاثة كسور عند الإناث المعاملة.

كلمات مفتاحية: بيوكيمياء، تكاثر، رحلان كهربائي، كبتوبريل، مبيض، *Ephestia kuhniella*.

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Effects of Latex from *Pergularia tomentosa* and the Aggregation Pheromone, Phenylacetonitrile, on *Locusta migratoria* Larvae

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ABSTRACT

Miladi, M., Abdellaoui, K., Regaieg, H., Omri, G., Acheuk, F., Ben Halima-Kamel, M. **2018**. **Effects of latex from *Pergularia tomentosa* and the aggregation pheromone, phenylacetonitrile, on *Locusta migratoria* larvae. Tunisian Journal of Plant Protection 13 (si): 87-98.**

Despite being a serious risk to human health and environment, chemical insecticides remain the most used for locust control. Searching for alternative control methods, effective and compatible with the environment, has become of increasing interest. Plant latex is an endogenous fluid secreted from highly specialized laticifer cells and has been suggested to act as a plant defense system. The aim of the present investigation was to study the insecticidal potentialities of *Pergularia tomentosa* latex at different concentrations, alone or in combination with the penylacetonitrile (PAN), on the 4th instar larvae of *Locusta migratoria*. The obtained results showed that the latex revealed an interesting insecticidal activity against *L. migratoria* larvae, resulting in a mortality reaching 96.49 %, 6 days after treatment. Toxicity bioassays revealed that PAN, associated with the latex, is able to accelerate and to increase the mortality rate. Pheromone-based treatment affected the health of treated insects by significantly reducing their respiratory rhythms. PAN was shown able to alter, quantitatively and qualitatively, the larval blood cells as expressed by the significant decrease in the number of the differential haemocyte counts (prohemocyte, plasmatocytes and granulocytes) and the important cell lysis.

Keywords: Latex, *Locusta migratoria*, *Pergularia tomentosa*, phenylacetonitrile, toxicity

Locust plague is one of the world's most critical plagues and remains a major constraint to food security and social stability for many rural populations living from agriculture with climatic elevated risk. The migratory locust *Locusta migratoria* is the most widespread locust species (Lomer et al. 2001). Nymphs in the gregarious phase show an aggregating behavior and move in bands to seek food, whereas adults swarm and migrate over long distances

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causing significant crop and pastures damage (Magor et al. 2008). To limit damage to crops and pastures, treatment is required with large amounts of broad-spectrum chemical pesticides. Although effective in reducing the locust population and their incidences, pesticides had some potentially negative environmental effects. In addition, frequent applications of insecticides have led to the occurrence of resistance in some field locust populations (He et al. 2004; Yang et al. 2009). In order to reduce both locust damage and insecticide input, it is important to develop alternative control methods as part of an integrated pest management (IPM) program. Research in recent years has been turning more towards selective bio-rational pesticides. Among these, botanical insecticides have attracted the greatest attention and have been reviewed extensively (Abdellaoui et al. 2013; Ma et al. 2014; Rattan 2010). Botanical insecticides, known as plant secondary metabolites, are expected to be possible alternatives to the traditional synthetic insecticides and have proved to be suitable candidates that fit reasonably well in locust management programs (Acheuk et al. 2012).

Among the natural compounds produced by plants, the latex appears to directly or indirectly influence the patterns of growth and reproduction of associated phytophagous (Chavan Bhagyashri et al. 2015). Latex is the milky sap of plants secreted by the specialized plant cells called laticifers, upon tissue injury and do not have any role in the primary metabolism of plants (Agrawal and Konno 2009). It has been reported in 12500 plant species representing 22 families (Evert 2006). The latex contains a variety of chemicals and proteins, such as various terpenoids, alkaloids, rubber, and cardenolides as well as various proteins and enzymes such as proteases, chitinases, and glucosidases (Konno 2011). It may include also various toxic compounds: for example, the neurotransmitter dopamine in the Persian poppy (*Papaver bracteatum*), narcotic alkaloid morphine in the opium poppy (*Papaver somniferum*), and insecticidal compounds such as the glycosidase inhibitors 1,4-dideoxy-1,4-imino-d-arabinitol (d-AB1) and 1-deoxynojirimycin (DNJ) in mulberry (Hagel et al. 2008). In addition, cysteine protease in latex of papaya (*Carica papaya*) and wild fig (*Ficus virgatalatex*) is toxic to caterpillars of herbivorous insects (Konno et al. 2004). Asclepiadaceae (milkweeds) is a large family of about 230 genera and almost 2000 species distributed through the tropics and temperate areas of the world (Arribere et al. 1998). The family is renowned for cardenolides-containing plants, such as *Asclepias, Pergularia, Gomphocarpus* and *Calotropis* (Nenaah 2013) which usually develop secretory tissues (laticifers) that produce white corrosive latex (Goyder 2006).

Pergularia tomentosa, is a perennial herb which extends in the Saharian and Sub-Saharian countries of North Africa (Babaamer et al. 2012). The plants are 25 cm tall, usually with many leaves and branches having pale green-white stems that are mostly ascending. Nutritional composition of this plant species and its antifungal, molluscicidal and insecticidal activities were recently reported. According to numerous reports, *P. tomentosa* leaves are a rich source of flavonoids and cardenolides and can be a good source of natural antioxidant, antibacterial, cytotoxic, and allelopathic compounds (Cherif et al. 2016; Hosseini Kahnouj et al. 2017; Lahmar et al. 2017). Despite *P. tomentosa* has several traditional uses and various biological effects, very little information is available

concerning their effects on the arthropod fauna and only few studies refer to the insecticidal activities of this plant (Acheuk and Doumandji-Mitiche 2013; Paul et al. 2011).

In the same way of research, Hassanali et al. (2005) discovered that Phenylacetonitrile (PAN), a pheromone produced by the adult male of the desert locust, significantly affected the behavior of *Schistocerca gregaria* larvae and increased their sensitivity to insecticides. Bal and Sidati (2013) have demonstrated that the addition of small amounts of PAN allows to half the quantities of insecticides used in the control of the locust larvae, while maintaining the same efficiency. The present investigation is an attempt to explore the effects of the latex of *P. tomentosa* and the aggregation pheromone, PAN, against fourth instar larvae of *L. migratoria*. For this purpose, we studied (i) the latex activities against *L. migratoria* larvae at various concentrations, and (ii) the effect of latex and PAN in binary combination on some physiological parameters of treated larvae (respiratory activity, and immune system).

MATERIALS AND METHODS

Insects.

Insects used for testing came from a gregarious stock, which had been reared in breeding cages measuring 50 cm^3 and containing a few hundred specimens as previously described (Abdellaoui et al. 2013). The temperature was kept at 30 ± 1°C and a light/dark cycle of 12/12 h was used. *L. migratoria* were fed on fresh sorghum leaves supplemented with wheat bran. The substratum used for oviposition was composed of 2/3 peat and 1/3 sand.

Plant material and latex extraction.

The latex of *P. tomentosa* was collected during spring seasons (March-April 2017) from the region of Hergla (N: 36.1°, E: 10.3°), Tunisia. The crude latex was collected from the healthy aerial parts of *P. tomentosa* by cutting the petiole of youngest leaves, and left to flow into sterile Eppendorf tubes. The extract was gently agitated during collection to overcome the tendency of the coagulation-like effect of the materials. After being collected, lattice was immediately brought to the laboratory and stored at + 4°C until use in the experiments.

The aggregation pheromone, phenyl-acetonitrile.

Phenylacetonitrile or benzyl cyanide is an aromatic organic compound of formula $C_6H_5CH_2CN$. The PAN (~ 98%, Sigma) is the main component (~ 80%) of the pheromone issued by the gregarious male adults of desert locust (Amwayi et al. 2012).

Treatments.

The bioassay for insecticidal activity of fresh latex (FL) of *P. tomentosa* against *L. migratoria* was conducted on newly ecdysed (< 6 h post molting) 4^{th} instar larvae using four concentrations 1, 5, 50, and 100% (v/v) prepared in distilled water and denoted, respectively as FLC1, FLC2, FLC3, and FLC4. The FL was sprayed directly on *Sorghum vulgare* leaves which were subsequently offered as a mono-specific diet for larvae of the migratory locust. In the control experiment, the insects received the same quantity of distilled water used as solvent for preparing the different concentrations. Control and experimental larvae (n = 60 for each concentration) were placed in separate cages (30 cm^3) under the same conditions described above for the mass rearing. The mortality was assessed daily via direct observation for a period of 6 days

(according to the duration of the 4th instar larvae under the mass rearing condition), and when no antennal movements were observed, the insects were considered dead. Insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott 1925). Probit analysis (Finney 1971) was conducted to estimate the LC_{50} and LC_{90} values with their 95% confidence limits.

Other experiments were conducted to assess the effect of the FL and PAN in binary combination. The larvae were firstly exposed to PAN for 6 h and then treated by the FL at the lowest concentrations used (FLC1 and FLC2) as described previously. PAN has been associated at the concentration of 2% prepared in paraffin oil. PAN solution was applied onto Whatman No.1 filter paper disks (2 cm in diameter) which were then introduced into 2-liter plastic boxes containing the larvae. Controls were exposed with solvent alone and each treatment was replicated three times.

Differential haemocyte counts (DHCs).

This trial was conducted to investigate the effect of the latex and PAN on the immune system of *L. migratoria* larvae. Four treatments were applied: untreated larvae, larvae exposed to PAN (2%) for 6 h, larvae treated topically with the FLC1 and FLC2, and larvae exposed to the PAN for 6 h then treated with the FL. About 72 h after treatment, larvae (n = 5 for each concentration) were sampled and bled with a sterile needle on the first hind leg. A 5 µl-sample of hemolymph from each treated and control larvae were collected with a calibrated microcapillary tube and the haemolymph from two individuals was never mixed. Differential hemocyte counts (DHCs) were evaluated according to Guzo and Stoltz (1987). The obtained hemolymph from each treated or control larva was applied to a microscope slide and smeared to a thin film. The smears were first stained with diluted May-Grunwald solution for 3 min, and washed several times with distilled water, and then dipped in tap water. They were stained for a second time with diluted Giemsa for 10 min then washed again in distilled water. The slides were examined and photographed with a Leica LAS EZ (V 3.1.0) microscope. The analysis concerned the prohaemocytes, plasmatocytes, and granulocytes which are easy to identify.

Effect on the respiratory rhythm.

This experiment was carried out on 3-day-old 4th instar larvae. The insects were treated by ingestion with the latex alone or after exposure to PAN (2%) for 6 h. The effect on the respiratory rhythm is achieved by counting the opening rhythm of the metathoracic spiracle under a binocular microscope for one minute, after immobilizing the insect and releasing its hind legs.

Statistical analyses.

Results are expressed as means ± standard deviation. The significance between control and treated series was estimated using Student Newman Keuls (SNK) test at $P \leq 0.05$. All data were statistically analyzed by SPSS (Version 13.0.).

RESULTS

Effect of fresh latex on larval mortality.

The results reported in Fig. 1 show that the FL of *P. tomentosa* exhibited insecticidal activity against *L. migratoria*. Treatment of newly emerged 4th instar larvae resulted in a significant mortality which reached 96.49 ± 6.07% 6 days after treatment in insects fed directly on sorghum leaves treated with undiluted

fresh latex (FLC4). The influence of FL is widely correlated to the concentration used and the analysis of variance considering FL concentration as classification criteria revealed a significant difference among treatments (F = 12.7, df = 3, P = 0.0021).

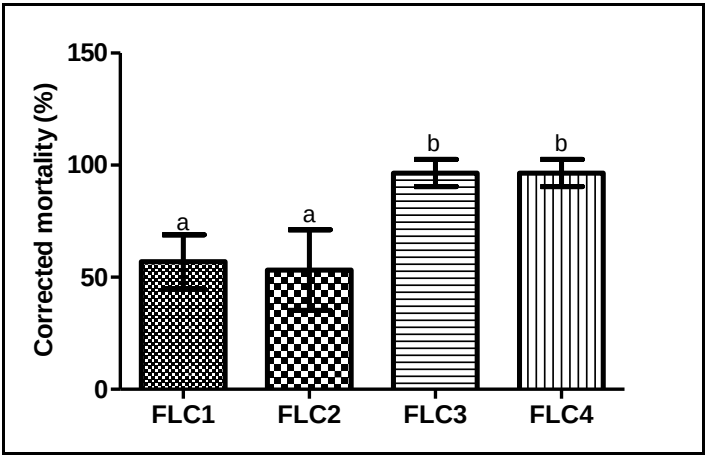

Fig. 1. Corrected mortality (mean ± SD, %) of *Locusta migratoria* larvae noted 6 days after treatment by the fresh latex of *Pergularia tomentosa*. Mean value represents three replicates, each containing 20 insects. Means followed by the same letter were not significantly different based on SNK-test at $P < 0.05$.

The evaluation of Fl toxicity was also estimated by LC_{50} and LC_{90} values, and their 95% confidence limits expressed as percentage after 6 days of treatment (Table 1). The corresponding LC_{50} and LC_{95} values were respectively 8.56% and 52.49%.

Table 1. Toxicity of *Pergularia tomentosa* fresh latex to newly emerged 4th instar larvae of *Locusta migratoria*

LC_{50} [a, b]	LC_{90} [a, b]	df	$\chi 2$
8.56 (5.28-32.7)	52.49 (29.84-206.07)	13	494.6

[a] Units LC_{50} and LC_{95}= %.
[b] 95% lower and upper fiducial limits are shown in parenthesis.

Effect of PAN/FL combination.

Toxicity bioassays also showed that the combination of PAN with FL (PAN/FL) significantly increased the larvae mortality (F = 34.58, df = 4, $P <$ 0.0001). Indeed, the mortality rates observed in the larvae treated with the fresh latex alone at the lowest concentrations used (FLC1 and FLC2) were 56.92 ± 12.1 and 53.21 ± 18.05%, respectively, 6 days after treatment. These same concentrations caused mortality rates of 92.98 ± 6.84 and 96.29 ± 6.41%, respectively, when the larvae were exposed for 6 h to the aggregation pheromone (PAN) at the concentration of 2% before feeding on FL-treated leaves (Fig. 2).

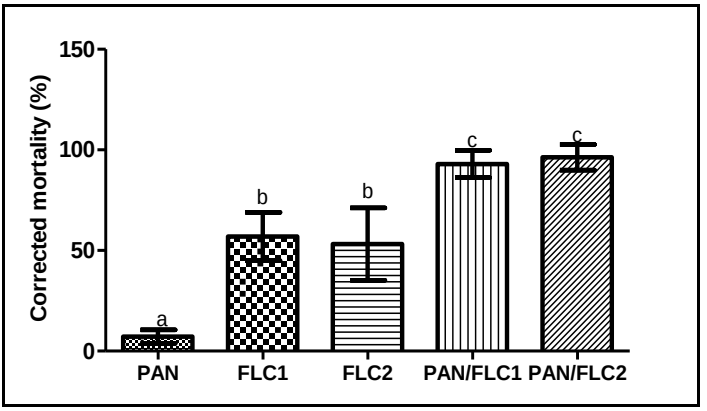

Fig. 2. Corrected mortality (mean ± SD %) of *Locusta migratoria* larvae noted 6 days after treatment by the fresh latex of *Pergularia tomentosa* in combination with the aggregation pheromone, phenylacetonitrile (PAN/FL). Mean value represents three replicates, each containing 20 insects. Means followed by the same letter were not significantly different based on SNK-test at $P < 0.05$.

Bioassay was also designed to determine the LT_{50} and LT_{90}, effective times to cause mortality of 50 and 90% of treated insects. The lethal time bioassay results showed that the PAN pheromone seemed able to accelerate the mortality of the larvae that became more sensitive to the latex treatment. The LT_{50} values for the fresh latex, applied alone, were respectively 4.8 and 4.5 days. When the larvae were initially exposed to the pheromone, the time required to cause 50% mortality of the larvae became shorter and reached 1.93 days (Table 2).

Table 2. LT_{50} and LT_{90} values (days) of the fresh latex of *Pergularia tomentosa* alone (FL) or in combination with the aggregation pheromone, phenylacetonitrile (PAN/FL) for *Locusta migratoria* 4th instar larvae

Treatments	LT_{50} (days)*	LT_{90} (days)*	*df*	χ^2
FLC1	4.88 (4.58-5.18)	9.1 (8.33-10.17)	16	6.02
FLC2	4.52 (4.19-4.82)	9.08 (8.28-10.19)	16	7.32
PAN/FLC1	2.87 (2.15-3.47)	5.64 (5.05-6.53)	16	11.86
PAN/FLC2	1.93 (1.6-2.21)	4.63 (4.2-5.24)	16	3.96

*95% lower and upper fiducial limits are shown in parenthesis. FLC1 = 1%, FLC2 = 5%. PAN/FL: Larvae exposed to the PAN for 6 h then treated win the FL. FL: fresh latex, PAN: Phenylacetonitrile.

Differential hemocyte counts.

Data illustrated in table 3 show the effect of *P. tomentosa* fresh latex, alone or in combination with the aggregation pheromone, phenylacetonitrile, on the DHC of the 4thinstar larvae of *L. migratoria* noted 72 h after treatment. In this study, the DHC was expressed in relative numbers of prohemocytes, plasmatocytes, and granular cells. Haemocytes were distinguished on the basis of morphological characteristics and staining affinity. As summarized in Table 3, quantitative determination of larval

blood cells recorded in treated and normal larvae showed that the FL significantly reduced the number of circulating hemocytes (prohemocytes, plasmatocytes, and granulocytes) as compared to control series. The effect became more apparent by exposing the larvae to PAN before treatment with FL. Indeed, the combined treatment (PAN/FLC2) induced the highest reduction for the three cell classes. Results also showed that PAN, applied alone, significantly reduced the DHC (Table 3). The analysis of variance revealed a significant difference among treatments for prohemocytes (F = 48.13, df = 6, P < 0.0001), plasmatocytes (F = 75.42, df = 6, P < 0.0001), and granulocytes (F = 89.16, df = 6, P < 0.0001).

Table 3. Differential haemocyte counts (DHCs) (cell/µl, mean ± SD) of *Locusta migratoria* larvae determined at 72 h post-treatment with *Pergularia tomentosa* fresh latex (FL) alone or in combination with phenylacetonitrile (PAN/FL)

Haemocyte	Control	PAN	FLC1	FLC2	PAN/FLC1	PAN/FLC2
Prs.	446.9 ± 89.8 a	75.1 ± 8.3 c	140.7 ± 10.5 b	160.7 ± 8.5 b	81.6 ± 14.4 c	62.6 ± 14.9 c
Pls.	462.9 ± 69.1 a	98.6 ± 4.6 bc	124.8 ± 1.1 bc	138.5 ± 6.5 b	105.4 ± 37.9 bc	78.1 ± 9.1 c
Grs.	537.4 ± 31.7 a	119.7 ± 22.7 b	129 ± 33.2 b	107.8 ± 33.4 b	97.2 ± 7.1 b	62.1 ± 2.5 c

For each haemocyte category, means followed by the same letter were not significantly different based on SNK-test at P < 0.05. Prs: prohemocytes, Pls: plasmatocytes, Grs: granulocytes, FL, fresh latex, PAN: phenylacetonitrile.

Qualitatively, the application of FL and PAN, in addition to abnormal counts, also caused great abnormalities to the different blood cells. These abnormalities were manifested by distortion of the cytoplasmic and nuclear membrane, rupturing of cell wall, enlargement of cells and abnormal staining of the haemocytes (Fig. 3).

Effects on the respiratory activity.

The obtained results concerning the respiratory rhythm of the 4th instar larvae of *L. migratoria* are shown in Fig. 4. It can be deduced that the fresh latex with the two concentrations FLC1 and FLC2, alone or in a binary combination with PAN (2%), could significantly decrease the respiratory activity of the larvae compared to the untreated control (F = 142.1, df = 5, P < 0.0001). The analysis of the variance followed by the SNK-test gave three significantly different groups, which proved that PAN was able to improve the efficiency of FL by further reducing the respiratory rhythm of *L. migratoria* larvae. Indeed, we counted 39.25 ± 1.47 and 35.37 ± 2.48 opening of the metathoracic spiracle per minute for FLC1 and FLC2, respectively. However, these values decreased significantly after exposure of the larvae to PAN pheromone to reach 23.8 ± 4.16 and 23.2. ± 3.54 for both concentrations, respectively (Fig. 4).

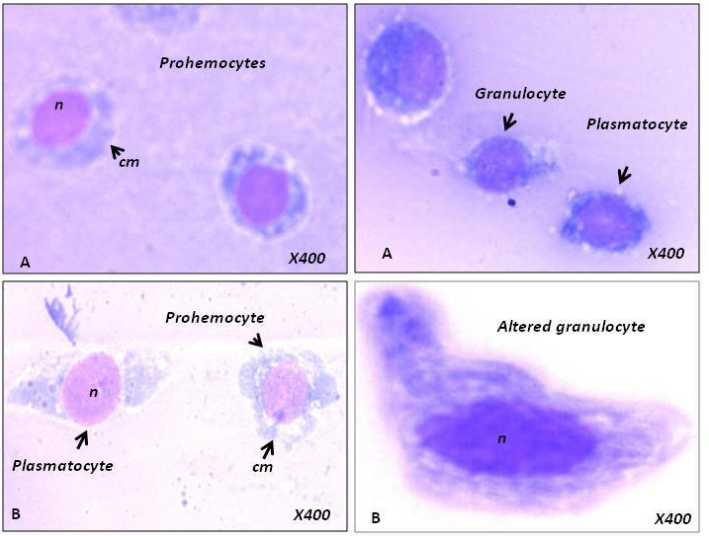

Fig. 3. Light microscope observation of circulating haemocytes of normal *Locusta migratoria* larvae (A) and treated with PAN/FLC2 (B). ×400 original magnifications. Note the disorganization of the cytoplasmic and nuclear membrane and the abnormal staining of the haemocytes. cm: cytoplasmic membrane, n: nucleus.

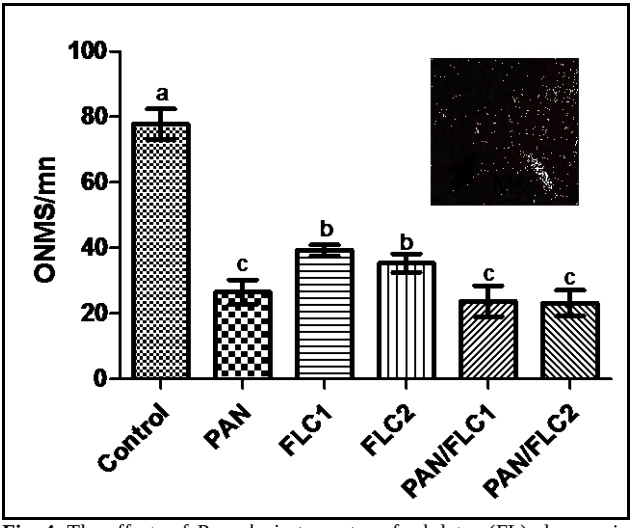

Fig. 4. The effects of *Pergularia tomentosa* fresh latex (FL) alone or in combination with the aggregation pheromone, phenylacetonitrile (PAN/FL) on the respiratory activity (mean ± SD %) of *Locusta migratoria* larvae after 3 days of treatment. Means followed by the same letter were not significantly different based on SNK-test at $P < 0.05$. Ms: metathoracic spiracle, ONME/mn: opening number of metathoracic spiracle per minute.

DISCUSSION

Botanical insecticides may be the future of locust control programs. Plant derivate active compounds, have potential uses as growth and reproduction inhibitors, repellents and as oviposition deterrents (Shahi et al. 2010). In the literature, it is well known that *Asclepia* latex, the protoplasmic content derived from the laticiferous cell which characterizes this genus, contains specialized substances including the poison, asclepione (Hayes 1947).

Nevertheless, only a few researchers have focused on the valorization of Asclepiadaceae plant latex as bio-insecticide. During the present study, the latex of *P. tomentosa* was tested against one of the most redoubtable enemies of culture *L. migratoria* in 4th instar larval stage alone and in combination with the aggregation pheromone PAN. This treatment significantly affected the respiratory rhythm, reduced the number of circulating hemocytes and caused significant mortality.

Some researchers have already discussed the biological activities of *P. tomentosa*. Indeed, Acheuk and Doumandji-Mitiche (2013) showed that alkaloids extracted from the aerial part of this plant exhibited a potent larvicidal effect against the 5th instar larvae of *L. migratoria* with a dose-dependent relationship. The mortality rate reaches 100% with the highest dose 240 µg/larvae 10 days after treatment. Likewise, Hussein et al. (1999) showed that the ethanolic extract of *P. tomentosa* killed test snails within 24 h after treatment. According to these authors, the rapid death of treated snails indicates the presence of highly sensitive target(s) which may be exploited in discovering new specific and effective molluscucide.

We also demonstrated that the exposure to PAN reduced the concentration of fresh latex from 100% to 1% while preserving the same efficiency. Results found in this research are similar to those of other authors who indicated that the addition of small quantities of phenylacetonitrile could make it possible to decrease by half the quantities of insecticides used in the control of desert locust nymphs, while preserving the same effectiveness (Bal and Sidati 2013). Moreover, Ramos et al. (2010) found that the proteins contained in the latex of *Calotropis procera* showed very significant adverse effects on the growth and development of *Callosobruchus maculatus*. Similarly, Nenaah (2013) reported that latex protein of *C. procera* exhibited considerable toxic and feeding deterrent effects against adults of *Sitophilus oryzae* and *Rhyzopertha dominica* in a dose-dependent manner.

Furthermore, our finding showed that the treatment of the 4th instar larvae of *L. migratoria* with *P. tomentosa* latex, alone or in combination with the PAN, significantly disturbed their haemogram by reducing the number of the differential hemocyte counts. This situation has already been mentioned by Guzo and Stoltz (1987) in *Schistocerca gregaria* treated by *Metarhizium anisopliae*. This decrease is probably the result of the depletion of these cells following the phagocytic activity induced against the treatment. Similarly, Duressa et al. (2014) demonstrated that the locusts lost over 60% of the circulating hemocytes within 30 min following β-1,3-glucan injection. Our results are also consistent with those of Aylin et al. (2017) demonstrating that azadirachtin used at 100 ppm is able to reduce total hemocyte counts and to induce significant alterations in differential hemocyte counts. According to these authors, azadirachtin decreased

the counts of circulating hemocytes due to the induction of autophagic or apoptotic pathways resulting in cell death. It was reported in many studies that azadirachtin leads to apoptosis and autophagy in insect cell lines originated from ovarian tissues (Huang et al. 2010). The decrease in the rate of opening rhythm of the methathoracic spiracle reminds the results of Moussa (2003) who showed that the use of neem (*Azadirachta indica*) oil reduces the respiratory rate of *L. migratoria.*

Although the results obtained are very interesting, further studies are still needed to evaluate with more details the possible interferences of PAN with the behavior and physiology of *L. migratoria* larvae and adults. We can also consider in future experiments a chemical study of *P. tomentosa* latex to determine its major active compounds.

RESUME

Miladi M., Abdellaoui K., Regaieg H., Omri G., Acheuk F. et Ben Halima-Kamel M. 2018. Effets du latex de *Pergularia tomentosa* et de la phéromone d'agrégation, phénylacétonitrile, sur les larves de *Locusta migratoria*. Tunisian Journal of Plant Protection 13 (si): 87-98.

Bien qu'ils constituent un risque sérieux pour la santé humaine et l'environnement, les insecticides chimiques restent les plus utilisés dans la lutte antiacridienne. La recherche de méthodes alternatives de contrôle, efficaces et compatibles avec l'environnement, est devenue indispensable. Le latex végétal est un fluide endogène sécrété par des cellules laticifères hautement spécialisées et il a été suggéré qu'il agissait comme un système de défense des plantes. Le but de la présente étude est d'étudier les potentialités insecticides du latex de *Pergularia tomentosa* à différentes concentrations, seul et en combinaison avec le pénylacétonitrile (PAN), sur les larves du quatrième stade de *Locusta migratoria*. Les résultats obtenus ont montré que le latex révélait une activité insecticide intéressante contre les larves de cet insecte, entraînant une mortalité pouvant atteindre 96.49 %, 6 jours après le traitement. Les bio-tests de toxicité ont montré que le PAN, associé au latex, semble accélérer et augmenter le taux de mortalité. Le traitement à base de cette phéromone a affecté la santé des insectes en réduisant considérablement leur rythme respiratoire. Il a été également démontré que le PAN semble altérer, quantitativement et qualitativement, les cellules sanguines des larves qui se manifestent par une diminution significative du nombre des hémocytes (prohémocytes, plasmatocytes et granulocytes) et une importante lyse cellulaire.

Mots clés: *Locusta migratoria*, Latex, *Pergularia tomentosa*, phénylacétonitrile, toxicité

ملخص

الميلادي، مريم وخميس عبداللاوي وهاجر رقيق وغفران عمري وفاطمة عشاق و منية بن حليمة الكامل. 2018. مفعول لبن نبتة *Pergularia tomentosa* وفرمون التجمع penylacetonitrile على يرقات الجراد المهاجر *Locusta migratoria*. Tunisian Journal of Plant Protection 13 (si): 87-98.

على الرغم من أنها تشكل خطرا جسيما على صحة الإنسان والبيئة، لا تزال المبيدات الكيميائية للحشرات الأكثر استخداما في مكافحة الجراد. لذلك أصبح من الضروري البحث عن طرق مكافحة بديلة فعالة ومنسجمة مع المحيط. اللبن النباتي هو سائل تفرزه خلايا متخصصة للغاية ويساهم في حماية النباتات من الأمراض. يهدف هذا العمل إلى اختبار مفعول لبن نبتة *Pergularia tomentosa* وفرمون التجمع penylacetonitrile على فيزيولوجيا الجراد المهاجر *L. migtatoria*. أظهرت النتائج المتحصل عليها أن لهذا المستخلص فاعلية كبيرة على يرقات الطور الرابع للجراد حيث تسبب في نسبة موت عالية تصل الى 96.49% في خلال 6 أيام. كما أثبتت الاختبارات أيضا بأن استخدام الفرمون مع اللبن النباتي يساهم في تسريع وارتفاع نسبة موت اليرقات. وقد أثر هذا الفرمون على صحة الحشرات ويتبين ذلك من خلال

اضطرابات واضحة في التنفس وتغيرا، كميا ونوعيا، في الخلايا الدموية لليرقات حيث سجل انخفاض كبير في عدد الخلايا وبعض التشوهات الخلوية.

كلمات مفتاحية: جراد، سمية، فرمون التجمع، لبن نباتي، مهاجر، *Pergularia tomentosa*

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Evaluation of the Resistance of Different Barley Accessions to the Russian Wheat Aphid *Diuraphis noxia*

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ABSTRACT

Laamari, M., and Benyahia, L. 2018. Evaluation of the resistance of different b**arley accessions to the Russian** w**heat** a**phid *Diuraphis noxia*. Tunisian Journal of Plant Protection 13 (si): 99-106.**

This study aimed to assess the natural resistance of 72 barley accessions to the Russian wheat aphid *Diuraphis noxia.* Three parameters were used to evaluate the response of barley accessions (chlorosis, yield and morphological characteristics). A limited chlorosis rate was recorded for the accession 23 (11 to 20% which was associated with important spike weight (3.02 g) and 1000-grain weights (53.84 g)). In spite of its chlorosis rate situated between 31 and 50%, the accession 68 presented a dry weight (6.49 g) and a number of tillers (3) the most important compared to the rest of accessions. In resistant accessions, the high number of hairs on their leaves has probably limited the action of this aphid on yield, especially the weights of the ears and the 1000 grains. The local accession Saida was the most susceptible to the Russian wheat aphid.

Keywords: Aphid, barley, *Diuraphis noxia*, resistance

The Russian wheat aphid *Diuraphis noxia* was reported for the first time in Algeria in May 1938 on samples of wheat harvested near Algiers (Mimeur 1941). In 1989, the area of wheat infested by this aphid in Setif (northeastern Algeria) was estimated to about 200 ha and in the same year it was reported in Sidi Bel Abbes and Tiaret (northwestern Algeria) (Miller et al. 1993). In 1991, it was found in the province of Constantine on *Hordeum vulgare* and *Triticum aestivum* and in 1992 in the Batna region on *Phalaris brachystachys* (Laamari 2004). In a recent study, Laamari et al. (2016) showed that this aphid is present in all semi-arid regions of Algeria where cereals are mainly grown. This aphid receives the attention of farmers through its devastating effect on cereals, particularly in durum wheat and barley. Its toxic saliva produces longitudinal chlorosis on foliage associated with winding, growth retardation, yield drop, deterioration in grain quality and even total drying of the plants. In South Africa, losses caused by *D. noxia* on wheat reached 92% (Calhoun et al. 1991). In the USA, 850 million dollars was spent to fight against this aphid in 1986 (Kiplagat 2005). Phytosanitary control strategies applied to aphids in general attach more importance to chemical control. Nevertheless, there were failures in this choice, notably due to adverse effects on

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the surrounding environment and the emergence of new strains resistant to aphicides. In addition to cultural, biological and chemical methods, the choice of resistant varieties is currently a major component of integrated protection. For its various advantages, several researchers consider that the selection of resistant plants to insects is one of the new perspectives in the field of plant protection. It is in this context that the International Center for Agricultural Research in Dry Areas (ICARDA), in collaboration with the Technical Institute of Field Crops (ITGC, Algeria) at experimental station of Constantine, introduced 71 accessions of barley to assess their level of resistance to the Russian wheat aphid compared to the local variety "Saida". In this study, foliar chlorosis, dry weight of overhead biomass, weight of ears, weight of 1000 grains, and the number of tillers are considered as criteria for the evaluation of the plant resistance. Moreover, the thickness of the epidermis and the number of hairs on the leaves are taken into consideration in order to highlight their contribution to this resistance.

MATERIALS AND METHODS

This work was carried out on 72 accessions of barley (*H. vulgare*). *D. noxia* individuals used for the artificial infestation of the plants are obtained from a strain kept in continuous breeding in the laboratory of plant protection in the Department of Agronomy at the Institute of Veterinary and Agronomic Sciences, University of Batna 1, Algeria. After planting in glasshouses and in plastic bags (30 cm deep and 17 cm in diameter), 5 plants from each accession were artificially infested by 25 adult aphids at the 3-leaf stage (5 aphids per plant). The measured parameters are developed below.

Chlorosis rate.

To evaluate foliar chlorosis, observations were performed at 3-phenological stages. The first one was made at the tillering stage, the second at the elongation stage of the main stem and finally the last at the stage heading-flowering. Depending on the extent of leaf chlorosis, the accessions were ranked based on a 1-6 rating scale (Calhoun et al. 1991):

- Class 1 (highly resistant): chlorosis covering only 0-5% of the leaf surface.
- Class 2 (resistant): chlorosis covering 6-10% of the leaf surface and limited to a few tillers.
- Class 3 (moderately resistant): foliar chlorosis accounts for 11 to 20% on various tillers.
- Class 4 (moderately susceptible): chlorosis occupying 21 to 30% of the leaf surface on various tillers.
- Class 5 (Susceptible): chlorosis extending at 31-50% on various tillers and the leaf tips were necrotic.
- Class 6 (very susceptible): chlorosis exceeding 51% on various tillers and the extension of necroses reaching the whole foliage.

Components of yield.

At the mature stage, the whole plants were sectioned at the collar level and placed separately in paper bags. Once brought to the laboratory, they were dried in an oven at a temperature of 75°C for 72 h. The parameters measured were the weight of the aboveground biomass, the number of tillers, the weight of the ears and the weight of 1000 grains.

Morphological characteristics of accessions.

In order to evaluate the thickness of the epidermis of the leaves, a scalpel was used to perform 6 very thin transverse sections in the middle of the 3rd

leaf of each accession. After thinning and staining, the samples were placed on a micrometer slide, with an accuracy of 0.01 mm, and measured at the microscope. The hair counting was carried out on a surface of 1 mm^2 removed from the upper face of the third leaf of each plant.

Statistical analyses.

To analyze the action of Russian wheat aphid on some of the yield components of 72 barley accessions, statistical analyses were performed using SPSS version 20.0 statistical software. All values given were the mean of five replicates and were expressed as the mean ± standard deviation. Significant differences between the mean values ($P \leq 0.05$) were determined using the Student's test. Correlation analyses (Pearson correlation coefficient) were established between the yield components of these different accessions and their morphological appearance, especially the thickness of the epidermis and the number of hairs on the leaves.

RESULTS

Ranking of accessions based on leaf chlorosis.

At the heading-flowering stage, chlorosis affected at least 21% of the leaf area of all accessions, with the exception of the accession 23 which was found to be moderately resistant (Class 3). Chlorosis affected more than 50% of the leaf area in the accessions 1, 8, 32, 33, 35, 38, and 56 (Table 1).

Ranking of accessions based on yield components.

In addition to its resistance to chlorosis, the accession 23 produced higher aerial biomass (5.49 g), ears weight (3.02 g) and 1000-grain weight (53.84 g) compared to the other accessions (Table 2). Despite its susceptibility to chlorosis, the accession 68 produced a relatively interesting aerial biomass (6.49 g) and three tillers more than all the other accessions. The local variety Saida (accession 1) was found to be the most susceptible to the Russian wheat aphid as it showed the lowest values of aerial biomass (1.77 g), ear weight (0.29 g), weight of 1000 grains (7.51 g) and number of tillers (0.4 tillers).

Morphological traits of tested accessions.

The thickest epidermis was noted in the accessions 4, 7, 9, and 11 (0.040 to 0.043 mm). In the accessions 2, 5, 16, 17, 19, 20, 23, 24, 29, 31, 32, 37, 41, 45, 49, 52, 54, 55, 56, 59, 60, 64, 69 and 70, the thickness of the epidermis did not exceed 0.037 mm. Accessions 1, 3, 10, 13, 22, 34, 57, 61, 63 and 67 were characterized by a very thin epidermis as the noted thickness did not exceed 0.017 mm. Regarding the hair numbers, the accession 23 presented a density of 5.5 hairs/mm^2. Accession 1 (the local variety Saida) was a poorly grain-producing plant and its leaves were covered by only 2 hairs/mm^2. In accessions 2, 5, 12, 15, 16, 20, 25, 28, 49, 50, 61, 63, and 65, the hair density varied between 4 and 6 hairs/mm^2. Leaves of the accession 34 were entirely glabrous. The results obtained revealed no correlation between the thickness of the epidermis and the yield components in the different accessions of barley (Table 3). Conversely, the high density of hairs on the leaves registered in some accessions has probably contributed to their resistance to *D. noxia*.

Table 1. Classification of tested barley accessions based on chlorosis severity noted at three phenological stages

* Classes	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
Chlorosis (%) Stage	**0-5%**	**6- 10%**	**11- 20%**	**21- 30%**	**31- 50%**	**> 51%**
Tillering	2, 3, 4, 5, 6, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 25, 26, 27, 37, 44, 45, 47, 48, 49, 51, 52, 53, 55, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72.	7, 8, 10, 18, 19, 28, 29, 30, 31, 35, 36, 38, 40, 46, 50, 56.	1*, 9, 11, 12, 33, 34, 39, 41, 42, 43, 54.	32		
Total	**44**	**16**	**11**	**1**	**0**	**0**
Elongation	2, 4, 5, 6, 13, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 44, 45, 47, 48, 50, 51, 52 53, 55, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70	3, 14, 15, 17, 30, 31, 41, 46, 49, 57, 58, 59, 60, 71, 72	7, 8, 9, 10, 11, 12, 19, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 56	1, 54		
Total	**36**	**15**	**19**	**2**	**0**	**0**
Heading-Flowering			23	2, 5, 12, 15, 16, 20, 21, 25, 28, 49, 50, 61, 63, 65	3, 4, 6, 7, 9, 10, 11, 13, 14, 17, 18, 19, 22, 24, 26, 27, 29, 30, 31, 34, 36, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 51, 52, 53, 54, 55, 57, 58, 59, 60, 62, 46, 66, 67, 68, 69, 70, 71, 72	**1**, 8, 32, 33, 35, 38, 56
Total		**0**	**1**	**14**	**50**	**7**

* Class 1: Highly resistant, Class 2: Resistant, Class 3: Moderately resistant, Class 4: Moderately susceptible, Class 5: Susceptible, Class 6: Very susceptible. (**1**: Local accession Saida).

Table 2. The values of certain performance components estimated in tested barley accessions

Acc.	Dry weight (g)	Ear weight (g)	Weight of 1000 gains (g)	Number of tillers	Acc.	Dry weight (g)	Ear weight (g)	Weight 1000 gains (g)	Number of tillers
1*	1.77 ± 0.96 d	0.29 ± 0.58 d	7.51 ± 15.21 f	0.4 ± 0.5 e	**37**	4.64 ± 0.85 bc	1.92 ± 0.57 c	39.71 ± 1.06 c	2.0 ± 0.1 d
2	5.52 ± 1.11 b	2.59 ± 0.44 bc	48.77 ± 4.14 b	2.4 ± 0.9 c	**38**	2.87 ± 0.45 d	0.51 ± 0.16 d	15.92 ± 4.40 e	1.2 ± 1.2 e
3	4.78± 0.74 bc	2.29 ± 0.30 c	49.25 ± 4.21 b	1.2 ± 0.4 e	**39**	3.98 ± 1.34 c	1.65 ± 0.72 cd	45.91 ± 3.57 c	1.6 ± 0.6 e
4	**6.37 ± 0.88 a**	2.58 ± 0.24 bc	**53.42 ± 6.12 a**	2.0 ± 0.1 d	**40**	4.20 ± 0.73 c	1.66 ± 0.63 cd	35.45 ± 2.63 cd	1.4 ± 0.5 e
5	4.74 ± 0.52 bc	2.94 ± 0.45 ab	51.85 ± 3.06 ab	1.4 ± 0.5 e	**41**	4.31 ± 0.86 c	2.04 ± 0.60 c	45.53 ± 16.16 c	1.4 ± 0.5 e
6	5.54 ± 1.27 b	2.34 ± 0.74 c	48.68 ± 2.72 b	1.8 ± 0.5 d	**42**	3.94 ± 1.91 c	2.36 ± 0.33 c	29.21 ± 9.31 d	1.2 ± 0.5 e
7	3.93 ± 0.41 c	1.98 ± 0.48 c	43.03 ± 6.08 c	1.0 ± 0.1 e	**43**	4.03 ± 0.69 c	1.05 ± 0.42 d	26.08 ± 3.81 d	2.0 ± 0.1 d
8	1.43 ± 0.26 d	0.21 ± 0.29 d	6.742 ± 1.29 f	0.4 ± 0.6 e	**44**	4.12 ± 0.85 c	1.59 ± 0.39 cd	29.09 ± 1.05 d	1.2 ± 0.5 e
9	4.72 ± 0.89 bc	2.38 ± 0.50 c	37.67 ± 4.45 cd	2.2 ± 0.4 d	**45**	4.57 ± 1.61 c	2.08 ± 0.71 c	34.12 ± 16.65 cd	1.8 ± 0.6 d
10	4.69 ± 1.10 bc	2.53 ± 0.62 bc	47.28 ± 5.89 b	1.2 ± 0.4 e	**46**	4.27 ± 1.88 c	1.55 ± 1.16 cd	28.03 ± 4.2 d	1.8 ± 0.8 d
11	5.24 ± 1.54 b	2.65 ± 0.95 bc	40.02 ± 7.41 c	2.2 ± 0.4 d	**47**	5.92 ± 0.29 b	**3.12 ± 0.30 a**	35.26 ± 3.82 cd	2.0 ± 0.1 d
12	5.22± 0.45 b	2.85 ± 0.47 ab	**56.24 ± 9.20 a**	1.0 ± 0.1 e	**48**	5.41 ± 0.99 b	2.77 ± 0.67 b	38.74 ± 8.88 c	1.4 ± 0.6 e
13	4.59 ± 1.21 c	2.32 ± 0.95 c	48.01 ± 6.86 b	2.0 ± 0.1 d	**49**	4.94 ± 1.40 bc	2.14 ± 1.01 c	27.63 ± 4.36 d	1.2 ± 0.4 e
14	4.27 ± 0.71 c	2.20 ± 0.48 c	34.05 ± 5.09 cd	2.4 ± 0.6 c	**50**	**6.18 ± 1.05 a**	2.58 ± 0.43 bc	35.14 ± 5.11 cd	2.2 ± 0.7 d
15	5.11± 0.51 b	2.54 ± 0.36 bc	39.53 ± 5.67 c	2.0 ± 0.1 d	**51**	5.80 ± 2.11 b	2.76 ± 1.24 b	29.63 ± 5.63 d	**3.0 ± 0.8 a**
16	5.82 ± 0.46 b	**3.38 ± 0.27 a**	50.86 ± 3.07 ab	**3.0 ± 0.7 a**	**52**	5.37 ± 1.43 b	2.48 ± 0.90 c	31.44 ± 7.70 d	1.8 ± 0.4 d
17	3.95 ± 0.46 c	1.84 ± 0.11 c	34.02 ± 1.89 cd	1.0 ± 0.5 e	**53**	4.97 ± 0.76 bc	2.34 ± 0.65 c	31.68 ± 5.69 d	1.6 ± 0.6 e
18	**6.12 ± 1.17 a**	**2.99 ± 0.68 a**	38.04 ± 4.77 cd	2.4 ± 0.7 c	**54**	4.20 ± 0.64 c	1.88 ± 0.62 c	42.94 ± 2.79 c	1.2 ± 0.4 e
19	5.31 ± 0.43 b	2.70 ± 0.41 b	33.64 ± 11.67cd	2.2 ± 0.4 d	**55**	5.19 ± 1.46 b	2.70 ± 0.95 b	36.65 ± 7.78 cd	1.4 ± 0.5 e
20	5.20 ± 0.71 b	2.75 ± 0.47 b	49.21 ± 3.20 b	1.8 ± 0.4 d	**56**	2.04 ± 0.63 d	0.16 ± 0.09 d	2.60 ± 3.03 g	0.4 ± 0.5 e
21	5.32 ± 2.14 b	2.65 ± 1.09 bc	47.83 ± 3.71 b	1.8 ± 0.4 d	**57**	4.07 ± 0.94 c	1.94 ± 0.60 c	33.68 ± 5.94 cd	1.0 ± 0.1 e
22	**6.28 ± 0.85 a**	**3.09 ± 0.39 a**	39.66 ± 6.53 c	2.2 ± 1.0 d	**58**	3.65 ± 1.02 c	1.61 ± 0.51 cd	35.41 ± 1.80 cd	1.6 ± 0.5 e
23	5.49 ± 0.26 b	**3.02 ± 0.31 a**	**53.84 ± 5.23 a**	1.8 ± 0.4 d	**59**	5.14 ± 0.62 b	2.23 ± 0.50 c	36.04 ± 6.82 cd	2.0 ± 0.7 d
24	4.73 ± 0.85 bc	2.35 ± 0.45 c	42,32 ± 4,79 c	1,6 ± 0,5 e	**60**	4,91 ± 1,03 bc	2,46 ± 0,95 c	41,22 ± 5,91 c	1,6 ± 0,5 e
25	4.69 ± 1.77 bc	2.12 ± 1.23 c	41.16 ± 23.14c	1.6 ± 0.9 e	**61**	5.82 ± 1.04 b	2.84 ± 0.69 ab	40.07 ± 3.70 c	2.6 ± 0.5 e
26	4.99 ± 0.49 bc	1.96 ± 0.13 c	**54.37 ± 3.22 a**	1.6 ± 0.6 e	**62**	5.07 ± 0.36 b	2.75 ± 0.26 b	45.96 ± 10.88 c	1.8 ± 0.4 d
27	4.93 ± 1.20 bc	1.86 ± 0.66 c	42.97 ± 7.69 c	1.8 ± 0.4 d	**63**	4.62 ± 1.04 bc	2.55 ± 0.80 bc	41.91 ± 2.83 c	1.6 ± 0.6 e
28	5.25 ± 0.95 b	2.35 ± 0.34 c	49.65 ±7.03 ab	1.6 ± 0.6 e	**64**	5.71 ± 1.09 b	**3.25 ± 0.80 a**	45.25 ± 7.41 c	2.4 ± 0.6 c
29	4.48 ± 0.53 c	2.13 ± 0.24 c	**54.32 ± 7.43 a**	1.2 ± 0.4 e	**65**	5.24 ± 0.62 b	2.84 ± 0.43 ab	41.43 ± 10.30 c	2.0 ± 0.1 d
30	5.40 ± 1.23 b	2.58 ± 0.41 bc	50.57 ± 5.63 ab	1.6 ± 0.5 e	**66**	4.46 ± 1.53 c	2.53 ± 0.36 bc	28.37 ± 10.19 d	1.6 ± 0.9 e
31	4.59 ± 1.61 c	1.89 ± 1.13 c	33.78 ± 18.81 cd	1.2 ± 0.8 e	**67**	5.43 ± 0.36 b	2.77 ± 0.38 b	34.10 ± 4.27 cd	2.6 ± 0.5 b
32	2.96 ± 0.60 d	0.96 ± 0.60 d	20.37 ± 1.73 e	1.2 ± 0.6 e	**68**	**6.49 ± 0.55 a**	**3.22 ± 0.26 a**	39.88 ± 15.29 c	**3.0 ± 0.1 a**
33	3.39 ± 0.94 d	1.59 ± 0.70 cd	28.15 ± 2.05 d	1.2 ± 0.5 e	**69**	5.54 ± 0.53 b	2.75 ± 0.31 b	42.17 ± 3.31 c	1.8 ± 0.5 d
34	5.16 ± 1.04 b	2.01 ± 0.84 c	28.93 ± 10.08 d	2.2 ± 0.4 d	**70**	4.13 ± 0.62 c	1.98 ± 0.45 c	42.20 ± 3.57 c	2.0 ± 0.6 d
35	3.37 ± 0.67 d	1.09 ± 0.15 d	19.07 ± 15.21 e	1.2 ± 0.5 e	**71**	4.33 ± 1.33 c	1.78 ± 0.47 c	31.44 ± 2.38 d	1.6 ± 0.5 e
36	4.30 ± 0.40 c	2.31 ± 0.46 c	51.72 ± 4.14 ab	1.0 ± 0.1 e	**72**	3.82 ± 0.62 c	1.25 ± 0.23 d	34.23 ± 8.92 cd	1.2 ± 0.4 e

Data are mean values of five replicates ± SD (standard deviation). Confidence intervals were calculated at the threshold of 5%. Acc: Accessions (1: Local variety Saida). Values within each column followed by the same letter are not significantly different according to Student-Newman Keuls test at $P < 0.05$.

Table 3. Matrix of the correlation coefficients of the parameters measured in the different accessions

Parameter	Dry weight (g)	Ear eight (g)	Tillers number	Weight of 1000 grains (g)
Epidermis thickness (mm)	0.091 ns	0.089 ns	0.104 ns	0.107 ns
Hair number	0.224 ns	0.268*	-0.007 ns	0.323*

ns: Not significant, *: Significant at $P \leq 0.05$.

DISCUSSION

In all tested barley accessions, the prolonged infestation with *D. noxia* caused a progression of chlorosis on leaves. It has been observed that the toxic saliva injected by the Russian wheat aphid at the time of feeding was responsible for the appearance of longitudinal chlorotic lines on leaves (Kiplagat 2005). In susceptible accessions, this leaf chlorosis evolves into necroses and the plants desiccate very early. This is the case of accessions 1 (the local variety Saida), 8, 32, 33, 35, 38, and 56 whose leaves were completely necrotic and desiccated. Studies have shown that this chlorosis can cause loss of photosynthetic pigments (chlorophyll a, chlorophyll b and carotene) due to the activity of degradation enzymes secreted by the aphid in the damaged parts of susceptible wheat accessions (Ni et al. 2002). However, in the resistant accessions, the high concentration of certain dissuasive substances (phenols) in the cells surrounding the stylet gives the sap a disagreeable taste (Belefant-Miller et al. 1994). In this study, the results showed that some accessions produced sterile ears, especially the accession 51. This phenomenon was explained by the imprisonment of the ears in the leaf sheath of the last leaf (Kiplagat 2005). As a result, the development of pollen grains cannot be accomplished. This sterility is frequently manifested by an abortion of stamens or grains of pollen, a delayed emergence of the ears and their deformation (Kiplagat 2005). Furthermore, the low grain production recorded in certain accessions can be explained, according to Gallais and Bannerot (1992), by the reduction of photosynthesis after flowering. These authors add that the last formed leaves participate with more than 50% in the filling of the grains. This is the case for accessions 1, 8, 32, 33, 35, 38, and 56 where the recorded low production in vegetative mass and in grain yield can be attributed to chlorosis which has affected more than 50% of their leaf area at the flowering stages. The resistance of plants to insects can be also attributed to the morphological traits of their leaves, in particular the epidermis thickness and the density of hairs. This study showed that, in general, the thickness of the epidermis was not correlated with the yield components (Table 3), whereas the high density of the hairs on the leaves has contributed to a certain level in the resistance to this aphid infestation. According to Andres and Connor (2003), the presence of hairs on the plant increases the time required for aphid feeding and reproduction. Other studies have pointed out that the strong presence of hairs on the leaves of certain accessions of wheat delays the movement of aphids compared with smooth leaf accessions (Lage et al. 2004). This is the case with accession 34 which is characterized by glabrous leaves on

which the Russian wheat aphid moves easily.

The current study revealed the resistance of certain accessions of barley to *D. noxia*. In addition to its resistance to chlorosis compared to other accessions, the yield of accession 23 in aerial biomass and grain was higher. Despite their susceptibility to chlorosis, production of accessions 16 and 68 in aerial biomass, weight of 1000 grains and number of tillers, was important. The accessions 1, 8, 32, 33, 35, 38, and 56, exhibiting the severest chlorosis symptoms, showed the lowest yield components. As for the epidermis thickness of leaves, no correlation between this morphological trait and yield components but interestingly, the hair density has limited the action of this aphid on weight of the ears and the weight of 1000 grains.

RESUME

Laamari M. et Benyahia L. 2018. Evaluation de la résistance de différentes accessions d'orge à l'égard du puceron russe du blé *Diuraphis noxia*. Tunisian Journal of Plant Protection 13 (si): 99-106.

L'étude menée a pour objet l'évaluation du niveau de résistance de 72 accessions d'orge au puceron russe du blé *Diuraphis noxia*. En plus du taux de chlorose limitée (11 à 20 %) enregistré chez l'accession 23, son poids d'épis (3,02 g) et son poids de 1000 grains (53,84 g) sont importants. Malgré le taux de chlorose compris entre 31 et 50 % noté sur son feuillage, la variété 68, a présenté un poids sec total (6,49 g) et un nombre de talles (3 talles) élevés comparativement au reste des accessions. Chez les accessions d'orge résistantes, la densité élevée des poils sur leurs feuilles a contribué à un certain niveau dans la réduction de l'action de ce puceron sur certaines composantes du rendement, en particulier, le poids des épis et le poids de 1000 grains. La variété locale « Saida » s'est révélée être la plus sensible au puceron russe du blé.

Mots clés: *Diuraphis noxia*, orge, puceron, résistance

ملخص

لعماري، مالك ولطفي بنيحي. 2018. تقييم مقاومة سلالات مختلفة من الشعير لمن القمح الروسي *Diuraphis noxia*. Tunisian Journal of Plant Protection 13 (si): 99-106.

أجريت هذه التجربة لتقييم مدى تأثير تواجد حشرات المنّ الروسي *Diuraphis noxia* على إنتاج 72 سلالة من الشعير. بينت النتائج أن السلالة 23 هي الأكثر مقاومة لاصفرار الأوراق والأكثر إنتاجية من حيث وزن 1000 حبة (53.84 غ) ووزن السنابل (3.02 غ). بالرغم من أن نسبة الاصفرار المسجلة عند السلالة 68 كانت من 31 إلى 50 %، إلا أن معدل وزنها الجاف (6.49 غ) وعدد السنابل التي أنتجتها (3) كانا مرتفعين مقارنة بباقي السلالات. أظهرت الدراسة عند هذه السلالات من الشعير أن كل زيادة في معدلات الأوبار التي تغطي الأوراق تقابلها زيادة نسبية في بعض عناصر الانتاج، خاصة وزن السنابل ووزن 1000 حبة. لوحظ أيضا أن الصنف المحلي "سعيدة" كان حساسا جدا لهذا النوع من المن.

كلمات مفتاحية: شعير، مقاومة، منّ، *Diuraphis noxia*

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Field Assessment of the Mass Trapping Technique for the Control of the Chickpea Leaf Miner *Liriomyza cicerina*

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ABSTRACT

Soltani, A., Amri, M., and Mediouni-Ben Jemâa, J. 2018. Field assessment of the mass trapping technique for the control of the chickpea leaf miner *Liriomyza cicerina.* Tunisian Journal of Plant Protection 13 (si): 107-112.

This work evaluated the chickpea leaf miner *Liriomyza cicerina* mass trapping technique as an alternative to insecticide spraying. A trap density of 2000 per ha was used. Trials were conducted in Beja during 2015 and 2016 using Nour variety. Leaves were sampled weekly from all treated and control plots and observed under binocular microscope. Regarding the reduction in infestation at harvest, results showed reductions of 20.11 and 18.13% respectively for chemical and mass trapping treatments compared to control. Efficacy also was assessed on the basis of captures and infestations reductions compared to control, the yield and 100-seeds weight. Results showed significant difference (at $P < 0.05$) between treatments, with 0.21 kg/m² grain yield for the control and 0.8 kg/m² for the chemical treatment and the mass trapping. Also, regarding the 100-seeds weight, it was 21.5g for the control and respectively 38.2 and 41.7 g with the chemical treatment and the mass trapping.

Keywords: Chickpea leafminer, deltamethrin, *Liriomyza cicerina*, mass trapping

The chickpea leafminer *Liriomyza cicerina* is an important insect pest attacking both spring and winter-planted chickpea (Bouhssini et al. 2008). It is widespread serious pest in Europe and North Africa, particularly Morocco and Tunisia (Çikman et al. 2008; Reed et al. 1987; Spencer 1976). The damage is caused by the larvae, which feed on the leaf mesophyll tissue, resulting in hole, galleries and premature leaf fall (Çikman 2006). Chickpea leaf miner causes yield reductions that can reach 40% (Reed et al. 1987). This insect pest can be controlled using various methods including insecticides (Çikman et al. 2011) and control practices like mass trapping (Çikman and Kaplan 2008). The aim of this research was to evaluate the impact of mass trapping technique used at the density of 2000 yellow sticky plastic traps/ha on the reduction of the infestation level and yield. Chemical treatment using Deltamethrin 25 ml/100 liters water and untreated plots served as control.

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MATERIALS AND METHODS

Study site and plant material.

This study was carried out during 2015 and 2016 in Beja site (North-west

Tunisia, 36°44'56.83"N 9°12'50.24"E). Trials were conducted in the experimental station of the Regional Center of Research on Field Crops (CRRGC). The experimental plan was identical for both seasons. Inside the field, 30 m² plots were randomly selected consisted of 30 rows each 4 m long. Each treated and untreated plot was replicated 3 times and trials were carried out during two years 2015 and 2016. Total experiment area was 270 m². There was no fertilization and no watering application during production period. The winter chickpea variety Nour (Pedigree: X96TH61-A3-W1-A2-W1-A1-W1-W1) was used for these trials. Chickpea has been sown on 25 December 2015 and 15 January 2016 at a density of 30 seeds per m² and no fertilization was applied during the season crop on both years.

Mass trapping trials.

A density of 2000 traps/ha was assessed. Traps were constructed from yellow plastic boards (20 × 15 cm) with a sticky coating. Traps were elevated 10 cm above the top of the plants as described by Çikman and Kaplan (2008) when plants height was 10 cm. Traps were checked once a week and changed weekly. Moreover, 6 traps were placed respectively in the field where 1 trap per 5 m² was placed in the middle (1 traps per 150 plants) to monitor *L. cicerina* adults. Trials were carried out in 3 plots of 30 m^2 each. Mass trapping efficacy was assessed on pest infestation means of larvae, emerged adults and chickpea yield. The traps were placed on 1st February on 2015 and 10 February on 2016. For treated plots with chemical spray and untreated plots, 1 trap was placed for the control of emerged adults' number.

Chemical treatment.

Deltamethrin (Decis® EC 50, Bayer Crop Science, France) was used at the dose of 25 ml/100 l water. Treatments were applied when the pest density reached a level of 2-3 larvae/leaf in 50% of plants in the field (Çikman and Kaplan 2008). Thus, three sprays were realized on 23 April, 20 May and 4 June in 2015, and 15 April, 15 May and 30 May in 2016. Untreated plots with no chemical sprays served as control. Infestation percentage, emerged adults and chickpea yield were noted.

Infestation assessment.

Thirty leaves were randomly sampled from each plot weekly starting from March to June. Samples were checked under binocular and the infestation percentage was determined according to the following formula (Toker et al. 2010):

$$Infestation\ (\%) = \frac{\text{Number of infested leaves}}{Total\ number\ of\ leaves} * 100$$

$$Reduction(\%) = 1 - \frac{\%\ infestation\ intreated}{\%\ infestation\ in\ control}$$

Yield assessment.

Grain yield per m^2 (GY/m^2) and 100-seed weight (100 SW) were determined in three replications for each plot.

Statistical analysis.

Statistical analyses were performed using the "SPSS statistical software version 20.0". Presented values were the average of three replications and were expressed as the mean ± standard deviation ($\bar{x}$ ± SD). Significant differences between the mean values ($P \leq 0.05$) were determined based on Duncan's Multiple Range test.

RESULTS

Effect of mass trapping and chemical treatments on chickpea leafminer infestation

Infestation was recorded weekly starting from the beginning of *L. cicerina* attacks on the 1st week of March during 2015 and 2016. Table 1 reports the average number of live larvae of *L. cicerina* on chemically treated plots, untreated plots and those with mass taping during March, April and May in 2015 and 2016 years.

As shown in Table 1, the highest infestations were recorded in control plots during May for both years. Infestations reached 50.6 and 57.3% for 2015 and 2016, respectively. However, plots treated with mass trapping and insecticide (Deltamethrin) showed lower infestations (Table 1). As it can be seen from these results, control plots' infestation level was 2-3 times higher than insecticide-treated plots and 1.5 times than mass trapping managed plots. Statistical analysis revealed significant differences between untreated (control) and treated plots (mass trapping and insecticide). Moreover, for results pointed out at the beginning of the infestation (March for both years), no significant differences were detected between mass trapping and insecticide-based treatment. However, when the insect populations increased during April and May, significant differences were thus observed between mass trapping and insecticide treatment. Best performances were achieved for the chemical control since infestations did not exceed 24% while they reached 42% for mass trapping treatment (Table 1).

Table1. Impacts of mass trapping and insecticide treatment on Nour chickpea variety infested by *Liriomyza cicerina* in Beja during 2015 and 2016 (Mean of larvae ± Standard Error/leaf)

Treatment	2015			2016		
	March	April	May	March	April	May
Control	8.9 ± 1.3 b	27.2 ± 1.2 c	50.6 ± 2.4 c	8.0 ± 0.0 b	28.3 ± 0.55 c	57.3 ± 0.57 c
Mass trapping	6.4 ± 0.7 a	22.0 ± 0.3 b	35.3 ± 1.1 b	5.3 ± 0.57 a	17.3 ± 0.75 a	42.0 ± 0.0 b
Deltamethrin	5.1 ± 0.6 a	15.0 ± 0.7 a	23.4 ± 0.5 a	5.3 ± 0.57 a	15.3 ± 0.57 a	22.3 ± 0.57 a

In each column, means followed by the same letter are not significantly different according to Duncan's Multiple Range test at $P < 0.05$.

Effect of mass trapping and chemical sprays on the reduction of *L. cicerina* populations.

Table 2 reports the results of the impact of mass trapping and insecticide treatment on the chickpea leafminer populations' reductions.

Table 2. Impact of mass trapping and insecticide treatment on reduction of *Liriomyza cicerina* infestation (%) on Nour chickpea variety in Beja during 2015 and 2016

Treatment	2015			2016		
	March	April	May	Marh	April	May
Mass trapping	2.52 a	5.18 a	15.32 a	1.79 a	6.97 a	15.04 a
Deltamethrin	2.23 a	12.25 b	29.44 b	2.22 a	15.03 b	34.92 b
Control	0 b	0 c	0 c	0 b	0 c	0 c

In each column, means followed by the same letter are not significantly different according to Duncan's Multiple Range test at $P < 0.05$.

Results showed interesting reductions in *L. cicerina* populations due to mass trapping and insecticide treatment. Furthermore, results indicated that the reduction varied according to the increase of the pest population. Indeed, during March, when the infestation is still at the beginning, reductions reached only 2.52 and 2.23% during 2015 and 1.79 and 2.22% during 2016, respectively, for mass trapping and insecticide treatment. However, reductions were more interesting at May for insecticide treatment with respective values of 29.44 and 34.92% during 2015 and 2016.

As shown in Table 2 for means separated based on Duncan's Multiple Range test, there was no significant difference on reduction percentage during March for both years 2015 and 2016 between plots with mass trapping and chemical spray. However, there was significant difference between mass trapping and Deltamethrin treatment during April and May.

Effect of mass trapping and chemical treatment on chickpea yield.

Effects of mass trapping and chemical treatment on *L. cicerina* adult density and chickpea yield (Grain yield per m^2 (GY/m^2) and 100-seed weight (100 SW)) are illustrated in Tables 3 and 4.

Table 3. Average number of *Liriomyza cicerina* adults by weekly count (lowercase letter) and Duncan groups (uppercase letter) during 2015 and 2016

Treatment	2015			2016		
	March	April	May	March	April	May
Control	17.7±1.5 aB	52.6±3.5 bB	78±2 cB	15.7±1.5 aB	52.6±6 bB	81.3±6 cB
Mass trapping	9±1 aA	31±1 bA	47.3±1.5 cA	9.3±0.6 aA	29±1 bA	48.3±1.5 cA
Deltamethrin	7.6±1.5 aA	25.7±4.9 bA	45.3±3 cA	8.7±0.6 aA	27±3 bA	45±2 cA

In each column, means followed by the same letter were not significantly different according to Duncan's Multiple Range test at $P < 0.01$.

During March, the mean number of fly adults was low in plots, and increased progressively in April to reach the peak in May for both years 2015 and 2016. As shown in Table 3 for Duncan groups, it was determined that there was no significant difference between years (df = 1, F = 0.14, $P > 0.05$). A significant difference was noted between months (df = 2, F = 1148.61, $P < 0.01$) and between treatments (df = 2, F = 345.9, $P < 0.01$). Number of adults per traps was not significantly different in mass trapping and Deltamethrin treated plots, and there was a significant difference between control and treated plots in both years.

Table 4. Impact of mass trapping and insecticide treatment on *Liriomyza cicerina* on Nour chickpea variety grain yield (GY) and 100-seed weight (100 SW) in Beja during 2015 and 2016

Treatment	2015		2016	
	GY (kg/m^2)	100 SW (g)	GY (kg/m^2)	100 SW (g)
Control	0.34 ± 0.02 a	21.56 ± 0.15 a	0.21 ± 0.02 a	23.13 ± 0.25 a
Mass trapping	0.80 ± 0.01 b	38.56 ± 1.33 b	0.81 ± 0.01 b	38.2 ± 0.41 b
Deltamethrin	0.83 ± 0.02 b	39.7 ± 0.55 b	0.82 ± b	41.7 ± 1.10 c

In each column, means followed by same letter were not significantly different according to Duncan's Multiple Range test at $P < 0.05$.

Results showed that yield values were higher for treated plots (mass trapping and insecticide treatment) compared to control. Statistical analysis showed significant differences between grain yield and 100-seed weight values of control and both treatment. It appears that mass trapping and insecticide treatment preserve grain weight during 2015 and 2016. In this respect, no statistical differences were observed between grain yield and 100-seed weight values between mass trapping and insecticide treatment. Results indicated that *L. cicerina* infestations had an effect on chickpea yield that could be reduced by more than 50% using both management methods.

DISCUSSION

L. cicerina is an important insect pest on chickpea plants (Çikman 2006). Adults emerged from March until June (Soltani et al. 2016). Previous works indicated that *L. cicerina* is a serious pest of chickpea in Tunisia (Soltani et al. 2016). Thus, control methods should be implemented. Bouhssini et al. (2008) reported that Deltamethrin had an impact in limiting *L. cicerina* populations. On the other hand, Arida et al. (2007) demonstrated that yellow sticky board traps could be incorporated in the management strategy against leaf miner adults under field conditions. The present study revealed that both mass trapping and Deltamethrin-based treatments significantly reduced *L. cicerina* damage on chickpea leaflets. However, Deltamethrin significantly reduced more the number of alive larvae compared to mass trapping and control. Yield losses are likely to appear due to damage caused by *L. cicerina* larvae and adults which could be eliminated by applying insecticides (Çıkman et al. 2011). This study pointed out that *L. cicerina* led to significant yield loss on chickpea winter crops (Nour variety). Additionally, this study showed that insecticide and mass trapping treatments displayed an important role to reduce pest losses.

Regarding the above results, mass trapping could well be used to control *L. cicerina* populations. Mass trapping should be taken into consideration in IPM studies and recommended for farmers to use when low pest populations densities occur.

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RESUME

Soltani A., Amri M. et Mediouni-Ben Jemâa J. 2018. Évaluation aux champs de la technique de piégeage de masse pour la lutte contre la mineuse du pois chiche *Liriomyza cicerina*. Tunisian Journal of Plant Protection 13 (si): 107-112.

Ce travail constitue une évaluation de la technique de piégeage de masse contre la mineuse du pois chiche *Liriomyza cicerina* comme une alternative à la pulvérisation d'insecticide. Les essais ont été menés à Beja en 2015 et 2016 en utilisant la variété Nour. Les feuilles ont été échantillonnées chaque semaine à partir de toutes les parcelles traitées et témoins et observées sous loupe binoculaire. En ce qui concerne la réduction de l'infestation à la récolte, les résultats ont montré des réductions de 20,11% et 18,13% respectivement pour les traitements chimiques et de piégeage de masse par rapport au contrôle. L'efficacité a également été évaluée sur la base des captures et les réductions d'infestations par rapport au témoin, le rendement et le poids de 100 grains. Les résultats ont montré une différence significative (à $P < 0,05$) entre les traitements avec un rendement de 0.21 kg/m² pour le témoin et 0.8 kg/m² pour le traitement chimique et le piégeage de masse. Egalement pour le poids de 100 graines, il

était de 23.1 g pour le témoin et respectivement 38.2 et 41.7 g pour le traitement chimique et le piégeage de masse.

Mots clés: Deltaméthrine, *Liriomyza cicerina*, mineuse du pois chiche, piégeage de masse

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ملخص

سلطاني، عبير ومعز عمري وجودة مديوني بن جماعة. 2018. تقييم حقلي لتقنية الصيد المكثف لمكافحة حشرة نافقة أوراق الحمص *Liriomyza cicerina* . Tunisian Journal of Plant Protection 13 (si): 107-112.

يشكل هذا العمل تقييما لتقنية الصيد المكثف ضد نافقة أوراق الحمص *Liriomyza cicerina* كبديل للمداواة الكيميائية. أجريت التجارب في باجة في عامي 2015 و 2016 باستخدام صنف "نور". أخذت عينات من الأوراق أسبوعيا للمراقبة تحت العدسة المكبرة. أظهرت النتائج في ما يتعلق بالحد من الإصابة انخفاضا بنسبة 20,11% و 18,03% على التوالي في الحقل المُعامل كيميائيا والصيد المكثف. كما جرى تقييم الفعالية حسب عدد الحشرات المسجلة وانخفاض نسبة الضرر والانتاجية ووزن 100 حبة. بينت النتائج أن هناك فرق معنوي ($P < 0,05$) بين المعاملات حيث كانت انتاجية الحب 0.21 كغ/م² بالنسبة للشاهد و 0.8 كغ/م² بالنسبة للمعاملة الكيميائية والصيد المكثف. كذلك في خصوص وزن 100 حبة، كان 23.1 غ لدى الشاهد وعلى التوالي 38.2 و 41.7 غ بالنسبة للمعاملة الكيميائية والصيد المكثف.

كلمات مفتاحية: دلتامترين، صيد مكثف، نافقة أوراق الحمص، *Liriomyza cicerina*

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Rapid Ability Adaptation of *Callosobruchus maculatus* to a Novel Host *Vigna unguiculata*

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ABSTRACT

Haouel-Hamdi, S., Labidi, M., Hedjal-Chebheb, M., Aouji, A., Boushih, E., and Mediouni-Ben Jemâa, J. 2018. Rapid ability adaptation of *Callosobruchus maculatus* to a novel host *Vigna unguiculata.* Tunisian Journal of Plant Protection 13 (si): 113-121.

In Tunisia, the cowpea seed beetle *Callosobruchus maculatus* is the major and economic insect pest of stored chickpea. This work aims to study the adaptive behavior of Tunisian strain of *C. maculatus* exclusively reared on chickpea for 5 years on a novel host, the cowpea *Vigna unguiculata*. The relative aspects of the host adaptation tests consist of the assessment of the reproductive parameters and the demographic traits of the insect over six months of storage period on chickpea and cowpea seeds. Two types of bioassays free-choice and no-choice were performed. Comparison of reproductive and demographic parameters for *C. maculatus* showed that under no-choice situation, chickpea was the preferred host of *C. maculatus* along the first two months of storage. However, under free-choice, the results revealed that from the first month of storage, the reproductive and demographic parameters of *C. maculatus* shifted in favor of cowpea. Thus, through this work, we have demonstrated the rapid adaptive potential of *C. maculatus* toward its original host and its ability to recognize and adapt to it over a short period.

Keywords: Adaptive behavior, *Callosobruchus maculatus*, chickpea, cowpea

Beetles of the genus *Callosobruchus* are major storage pests of chickpea crops and cause considerable economic losses worldwide (Sharma and Thakur 2014). The cowpea seed beetle *Callosobruchus maculatus* is the principal field-carry-over storage pest of pulses including cowpea, chickpea, green gram, black gram, and red gram (Loganathan et al. 2011). The neonate larvae penetrate the grains causing serious damage such as grain weight loss, reduction in germination, weak seed viability, and nutritional low quality (Haouel-Hamdi et al. 2017a; Oke and Akintunde 2013).

It is well established that *C. maculatus* is among herbivorous insects characterized by the exploitation of novel food sources due to its rapid host diversification and its capacity of frequent

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host shifts (Fricke and Arnqvist 2007). Adaptation to one novel host may simultaneously affect an insect's performance on other hosts, including hosts that the population may never have encountered. For example, if a population has a means to detoxify a particular secondary compound in a novel host, it may be able to exploit closely related hosts that contain similar compounds (Agrawal 2000). Females of *C. maculatus* attach eggs to the surfaces of grain-legume seeds. Hatching larvae burrow into the seed directly beneath the oviposition site and complete development within a single seed. All suitable hosts for *C. maculatus* are in the subfamily Papilionoideae within the legume family Fabaceae. Within the Papilionoideae, most *C. maculatus* hosts belong to the tribe Phaseoleae, and the most severely infested crops are in the genus *Vigna* (Tuda et al. 2005). Lima et al. (2004) reported that alternation of hosts is an efficient strategy to avoid the development of *C. maculatus* populations.

Previous studies of Simmonds et al. (1989) reported that bruchids have already indicated that host size affects the number of oviposited eggs. Other factors that strongly affect egg laying are morphological parameters, such as texture (Johnson and Kistler 1987), chemical characteristics of the seed coat (Lale and Makoshi 2000), and nutritional quality (Janz and Nylin 1997). The texture of all the three host seeds was smooth, and probably only the relative quantity and/or quality of resource available inside the seeds was taken into account by females for deciding on the number of eggs oviposited on seeds (Cope and Fox 2003).

Therefore, this work aims to investigate population dynamics, reproductive parameters, and demographic traits of *C. maculatus* reared on two host legumes: chickpea and cowpea and to study its rapid ability adaptation to a novel host.

MATERIALS AND METHODS

Insect rearing and seed material.

The mass rearing method of *C. maculatus* used has been described by Haouel-Hamdi et al. (2017b). The stock cultures of the cowpea seed beetle were maintained in glass bottles of 1 liter of volume in a growth chamber at 30 ± 5ºC temperature, 65 ± 5% RH and 12:12 Light:Darkness photoperiod.

The strain of *C. maculatus* used for the experiments was isolated from chickpea infested seeds for five years. Ten mature couples of the flightless-form were transferred on seeds of two food legume hosts for one year by regular rearing: a chickpea (*Cicer arietinum*) Amdoun 1 variety and cowpea (*Vigna unguiculata*) Black eye variety. The study of the rapid ability adaptation was studied after three generations.

Free-choice and no-choice bioassays.

The free-choice bioassay aimed to evaluate the influence of the attractiveness of the food legume seeds used toward the cowpea seed beetle oviposition. The no-choice bioassay was carried out in order to assess the influence of each seed type on the oviposition without any interference by the other tested hosts.

Free-choice bioassay. The two-way device applied in this bioassay consisted of the following PVC parts:

1. A common release arena, got from a cylindrical box (11 cm diameter, 6 cm depth for 0.7 liter). Two circular holes (1 cm diameter), equidistant among them, were made on the sidewalls of this box, at 1 cm from its bottom;

2. Two oviposition arenas, got from smaller cylindrical boxes (5 cm diameter, 3 cm depth for 50 ml) and their intact lid. A circular hole (1 cm diameter) was made on the sidewall of each box at 3.5 cm from its bottom;
3. Two tubes (pipes 6 cm long and 1 cm for the external diameter) connected the release with the oviposition arenas.

Each oviposition arena was filled up 30 seeds of only one food legume hosts (chickpea and cowpea). No seed was placed in the common arena. Fifteen females and five males of *C. maculatus* adults (all emerged within the last 24 hours) were collected from the maintained culture and released in the common arena. They could freely come back from each oviposition arena and visit the other ones. The device was kept in a growth chamber at 30 ± 5°C and 65 ± 5% RH (Panzarino et al. 2012). The adults were removed just two days later, before first larval hatching, and the seeds of each arena were examined to count the eggs laid on their surfaces. The bioassay was thrice replicated with genotypes placed randomly into the device.

No-choice bioassay. The test was performed using glass bottles of 1 liter of volume. One bottle for each food legume host was filled up 30 seeds. Three females and one male of *C. maculatus* adults (all emerged within the last 24 hours) were released in each bottle according to the methods of Panzarino et al. (2012). Adults were collected from the maintained culture and three replicates per food legume host were performed. The bottles were kept in a growth chamber at 30 ± 5°C and 65 ± 5% RH (Haouel-Hamdi et al. 2017a) and the adults were removed just two days later, before first larval hatching, and eggs were counted on the seeds of each genotype.

Reproductive parameters and demographic traits study.

The reproductive parameters and demographic traits were assessed according to free-choice and no-choice bioassays. The total number of eggs laid and the emergence rate were determined according to Haouel-Hamdi et al. (2017b).

The population growth parameters namely: the mean growth rate (MGR), the mortality rate of eggs (MRE), the mortality rate of larvae (MRL), the sex-ratio (SR), the net reproductive rate (R_0), the generation time (GT), and the intrinsic rate of increase (r) were calculated according to Haouel-Hamdi et al. (2017a).

Statistical analyses.

To analyze the possible effects of the rearing substrates (chickpea and cowpea) on all biological parameters (reproductive and demographic traits) of *C. maculatus*, statistical analyses were performed using SPSS statistical software version 20.0. All values given were the mean of three replications and were expressed as the mean ± standard deviation. Significant differences between the mean values ($P \leq 0.05$) were determined by using Duncan's Multiple Range test. When necessary, data were transformed by common logarithm or square root to meet the assumptions of normality. Correlation analyses (Pearson's correlation coefficient) were established between demographic traits parameters and assays of choice, food host and generation.

RESULTS

Reproductive parameters.

Total number of laid eggs. The data recorded on total number of eggs laid under free-choice and no-choice conditions by *C. maculatus* for three generations on different hosts are presented in Fig. 1.

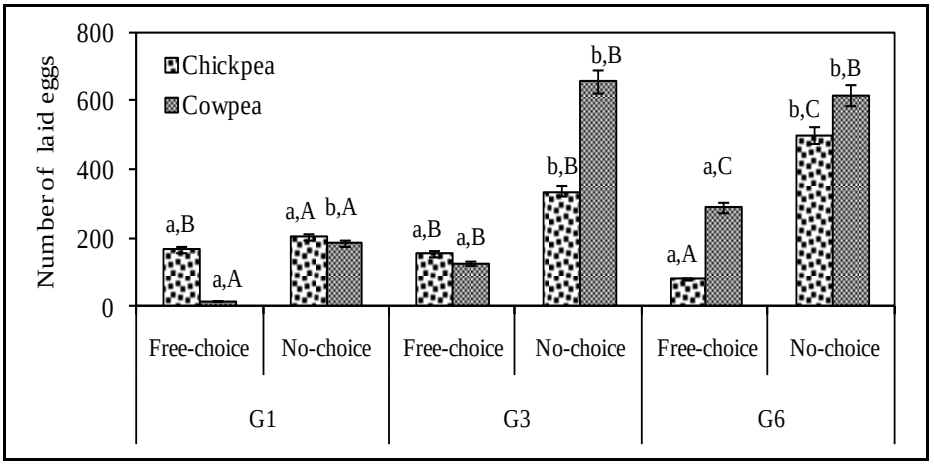

Fig. 1. Total number of laid eggs of *Callosobruchus maculatus* reared on different hosts for three generations under free-choice and no-choice assays. For each generation and each host, comparisons were made among free-choice and no-choice assays (lowercase letters) and for each assay (free-choice and no-choice) and each host comparisons were made among generations (uppercase letters). Bars having different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

For the first generation, under no-choice assays, females laid the highest number of eggs on chickpea, and the lowest number on cowpea. However, for the 3rd and 6th generation, under no-choice assays, females laid the highest number of eggs on cowpea, and the lowest number on chickpea (Fig. 1).

Results revealed that under free-choice condition, the number of eggs laid by *C. maculatus* on different food legume host ranged from 0 to 166, from 32 to 154 and from 18 to 290 for the 1st, 3rd and 6th generations, respectively. However, under no-choice condition, the number of eggs laid by *C. maculatus* on different food legume hosts ranged from 187 to 226, from 102 to 660, and from 164 to 616 for the 1st, 3rd and 6th generations, respectively.

In both bioassays (free-choice and no-choice), the total number of laid eggs was significantly dependent on host and generation number (For host F = 29.84, $P < 0.001$ and for generation F = 28.49, $P < 0.001$). Under free-choice condition, for the 1st generation, females laid significantly more eggs on chickpea (166 eggs) than on cowpea (12 eggs). For the 6th generation, the number of eggs laid was 290 eggs on cowpea against 81 eggs on chickpea (Fig. 1).

Emergence rate.

The data recorded on *C. maculatus* emergence rate under free-choice and no-choice conditions for 6 generations on different food hosts are presented in Fig. 2.

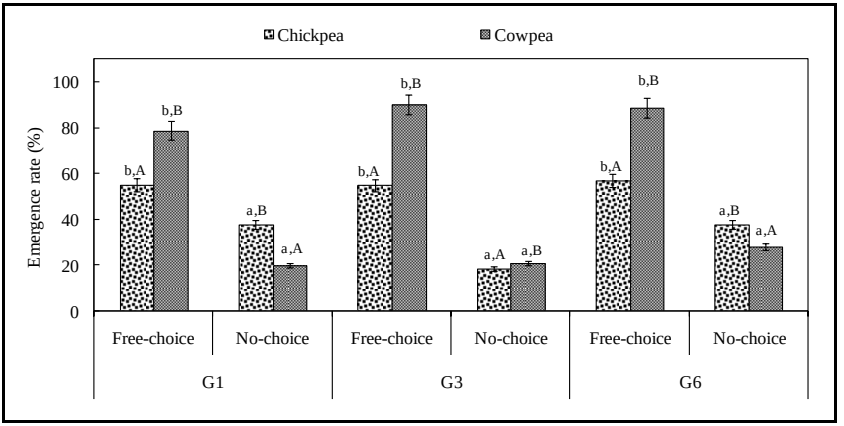

Fig. 2. Emergence rate (%) of *Callosobruchus maculatus* reared on various food hosts for the three generations under free-choice and no-choice assays. For each generation and each host, comparisons were made among free-choice and no-choice assays (lowercase letters) and for each assay (free-choice and no-choice) and each generation, comparisons were made among hosts (uppercase letters). Bars having different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Fig. 2 showed that under free-choice conditions, adults' emergence rate was higher than under no-choice condition for 6 generations. Maximum emergences were recorded on cowpea (78.61% for G1, 89.91% for G3 and 88.45% for G6) under free-choice condition but for the no-choice condition, maximum of emergences were observed on chickpea (37.66% for G1 and 37.61% for G6). In case of no-choice conditions, for the 1st generation, emergence rate of *C. maculatus* was higher when reared on chickpea (38%) than on cowpea (20%). In addition, for the 6th generation, the emergence rate was similar to the 1st generation when 38 and 28% were noted on chickpea and cowpea, respectively (Fig. 2).

When referring to the number of eggs laid and to the emergence rate under free-choice conditions, the most preferred host was cowpea followed by chickpea.

Demographic parameters.

Correlation analyses of demographic traits and assays of choice, food hosts and generation were recorded in Table 1. Results showed the importance of the type of bioassay (free-choice or no-choice) during the six generation. A highly significant and positive correlation was recorded between the assays of choice and sex-ratio ($r = 0.53$, $P < 0.001$) and generation time ($r = 0.46$, $P < 0.001$). Similarly, correlation studies were worked out between generation and mean growth rate, data showed a positive correlation ($r = 0.41$, $P < 0.001$). The correlation coefficient data indicated that when the generation increases the mean growth rate increases. However, a highly significant and negative correlation was observed between the assays of choice and mean growth rate ($r = -0.87$, $P < 0.001$), between generation and mortality rate of larvae ($r = -0.36$, $P < 0.001$) and between food legume hosts and intrinsic rate of increase ($r = -0.31$, $P = 0.008$).

Table 1. Correlation analyses between demographic traits of *Callosobruchus maculatus* and assays of choice, food host and generation

Correlation		MGR[a]	MRE[a]	MRL[a]	SR[a]	R_0[a]	GT[a]	r[a]
Assays of choice	r	-0.14	**-0.87****	-0.07	**0.53****	0.08	**0.46****	0.06
	P	0.25	<0.001	0.59	<0.001	0.50	<0.001	0.63
Food legume hosts	r	-0.09	-0.06	0.18	0.15	-0.21	0.08	**-0.31****
	P	0.47	0.61	0.14	0.21	0.08	0.51	0.008
Generation	r	**0.41****	0.01	**-0.36****	0.09	-0.10	-0.10	0.01
	P	<0.001	0.95	<0.001	0.45	0.41	0.39	0.91

[a] MGR = Mean growth rate, MRE = Mortality rate of eggs, MRL = Mortality rate of larvae, SR = Sex-Ratio, R_0 = Net reproductive rate, GT = Generation time, r = Intrinsic rate of increase.
** Significant at $P \leq 0.01$.

DISCUSSION

Results of the current investigation pointed out that the *C. maculatus* biology was largely affected by the free-choice and no-choice bioassays conditions, food host and generation number. In fact, under no-choice assays, *C. maculatus* female were highly attracted by chickpea and cowpea seeds. However, they showed a strong preference for cowpea seeds in free-choice assay. The differences in attraction of *C. maculatus* female to the food host was investigated by Rees (2004) who indicated that the bruchids have varied preferences for different food legume varieties.

C. maculatus is primarily a pest of cowpea but has many alternative hosts among leguminous seeds (Haouel-Hamdi et al. 2017a). However, little is known about the mechanisms of host location and preference. Being a field-to-store pest suggests that dispersing individuals are guided by specific cues to their preferred hosts (Ajayi et al. 2015).

The habitual behaviors of this insect in the search for the oviposition and feeding sites, can also be influenced by the perception of the colors and the shape of the host seed (Nicole 2002). Moreover, this author showed that the color, and the shape of host seeds as well as the chemicals they contain appear to play a very important role in the attraction of *C. maculatus* females to these host seeds. In this respect, Sankara et al. (2010) showed that *C. maculatus* females were able to recognize odors from their egg-laying substrates and to find their way to the sources of these odors. When these females have the choice between clean air and air containing seed odors, they are significantly more attracted to the smell of seeds. This would be explained by the volatile substances emitted by seeds. In addition, Ignacimuthu et al. (2000) confirmed that these chemicals would have an attractive effect on females of Bruchidae beetles in general and species of the genus *Callosobruchus* in particular.

Agosta (2008) indicated that in the free-choice test both the survival of offspring and the host seed size showed a positive relationship with the number of eggs laid on seeds. However, in the no-choice test, only the survival of offspring and the number of eggs laid showed a positive relationship, while there was a significant correlation between seed size and the number of eggs laid on the seed. Taken together, these results suggested that the females could choose appropriate hosts for their offspring both in free-

choice and no-choice host preference and survival of offspring.

The developmental plasticity is illustrated in this study by several biological indicators, which gave evidence of an important potential for a relatively rapid adaptation of *C. maculatus* on the three tested food legume hosts. In addition, according to Conord (2006) and Sankara et al. (2016), the preference of females of *C. maculatus* for *Vigna subterranea* and *Cajanus cajan* strains for the odor of cowpea seeds may be due to the difference in the chemical composition existing between used seeds of the host species. This difference in chemical composition is potentially the basis of specific olfactory discrimination by this insect in favor of cowpea. Indeed, Sankara et al. (2016) showed that *C. maculatus* females seem to remember the volatile signals from cowpea, the original host, even after three years of adaptation or development on the *Arachis hypogaea* and *C. cajan*. According to Sankara et al. (2012), in the case of a three-dimensional device, the insects of *V. subterranea* and *C. cajan* strains express their preference for *Arachis hypogaea* and *C. cajan*, suggesting that the ability to discriminate of *C. maculatus* led to different decision making in the case where the hosts are visible. Indeed, Nicole (2002) and Yang et al. (2006) showed that in the olfactometric studies based on olfactory stimuli and the other on the combination of two stimuli (visual and olfactory), *C. maculatus*, a cosmopolitan pest in storage is known for its ability to recognize its host.

Our results are in accordance with Wasserman and Futuyma (1981) who found a positive response to selection for ovipositional preferences after eleven generations of selection in the same species and Messina et al. (2009) who also demonstrated a change in oviposition preferences in seed beetle lines switched to new hosts.

RESUME

Haouel-Hamdi S., Labidi M., Hedjal-Chebheb M., Aouji A., Boushih E. et Mediouni-Ben Jemâa J. 2018. Adaptation rapide de *Callosobruchus maculatus* à un nouvel hôte *Vigna unguiculata*. Tunisian Journal of Plant Protection 13 (si): 113-121.

En Tunisie, la bruche du niébé *Callosobruchus maculatus* est un ravageur majeur d'importance économique du pois chiche conservé. Ce travail a pour but d'étudier le pouvoir adaptatif des souches tunisiennes de *C. maculatus* élevées exclusivement sur pois chiche pendant 5 ans à un nouvel hôte le niébé *Vigna unguiculata*. Les aspects relatifs du test d'adaptation au nouvel hôte consistent à l'évaluation des paramètres reproductifs et des traits démographiques durant six mois de stockage sur des graines de pois chiche et de niébé. Deux tests de choix et de non choix ont été effectués. La comparaison des paramètres reproductifs et démographiques de l'insecte a montré qu'en situation de non choix, le pois chiche constituait l'hôte préféré de *C. maculatus* durant les deux premiers mois de stockage. Cependant, en situation de libre choix, les résultats ont révélé qu'à partir du premier mois de stockage, les paramètres biologiques et démographiques de *C. maculatus* étaient en faveur du niébé. Ainsi, ce travail a montré la dynamique adaptative de cette bruche par rapport à son hôte d'origine et son aptitude à le reconnaitre et à s'y adapter au cours d'une courte durée.

Mots clés: *Callosobruchus maculatus*, niébé, pois chiche, pouvoir adaptatif

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ملخص

حوال-حمدي، سمية ومريم العبيدي ومريم حجال-شبهاب وعلي عوجي وآمنة بوصحيح وجودة مديوني-بن جماعة. 2018. التكيف السريع لخنفساء حب اللوبيا الجنوبية *Callosobruchus maculatus* على عائل جديد *Vigna unguiculata*. Tunisian Journal of Plant Protection 13 (si): 113-121.

في تونس، تعتبر خنفساء حب اللوبيا الجنوبية *Callosobruchus maculatus* واحدا من أكثر الآفات الضارة على الحمص أثناء الخزن. يهدف هذا العمل إلى دراسة القدرة على التكيف لمجتمعات خنفساء حب اللوبيا الجنوبية التي نمت حصريا على العائل الأصلي الحمص لمدة 5 سنوات وتكيفها على عائل جديد وهو اللوبيا الجنوبية. وتتألف الجوانب النسبية لاختبار التكيف مع العائل من متابعة الصفات الإنجابية والديموغرافية مدة ستة أشهر من التخزين للحمص واللوبيا الجنوبية. تم إجراء اختبارين للاختيار وعدم الاختيار للعائل من قبل الخنفساء. أثبتت مقارنة الصفات الإنجابية والديموغرافية للحشرة أنه في حالة عدم الاختيار كان الحمص العائل المحبذ للحشرة على طول الشهرين الأولين من التخزين. لكن في حالة الاختيار، أثبتت النتائج أنه منذ الشهر الأول من التخزين كانت الصفات الإنجابية والديموغرافية لصالح اللوبيا الجنوبية. هكذا، من خلال هذا العمل أثبتنا ديناميكية التكيف لهذه الحشرة فيما يتعلق بالعائل الأصلي وقدرتها على التعرف والتكيف معها في فترة قصيرة من الزمن.

كلمات مفتاحية: حمص، خنفساء حب اللوبيا الجنوبية، قدرة على التكيف، لوبيا جنوبية

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Combined Use of *Eucalyptus salmonophloia* Essential Oils and the Parasitoid *Dinarmus basalis* for the Control of the Cowpea Seed Beetle *Callosobruchus maculatus*

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ABSTRACT

Haouel-Hamdi, S., Abdelkader, N., Hedjal-Chebheb, M., Saadaoui, E., Boushih, E., and Mediouni-Ben Jemâa, J. 2018. Combined use of *Eucalyptus salmonophloia* essential oils and the parasitoid *Dinarmus basalis* for the control of the cowpea seed beetle *Callosobruchus maculatus.* Tunisian Journal of Plant Protection 13 (si): 123-137.

This work aims to evaluate the possible combined use of *Eucalyptus salmonophloia* essential oils and the ectoparasitoid *Dinarmus basalis* for the control of the cowpea seed beetle *Callosobruchus maculatus*, a serious pest of economic importance on stored legumes including chickpea. This study carried out first investigation on the insecticidal potential of *E. salmonophloia* grown in Gabès (South Tunisia). Fumigant toxicity of the essential oils was tested against pest adults and larvae (L1, L2 and L3 larval stages). The parasitoid was introduced respectively 3 and 6 days after oil application against the fourth instar larvae and nymphs of the target pest. Results reported the interesting insecticidal potential of *E. salmonophloia* essential oils against *C. maculatus* L1, L2 and L3 larvae and adults. Oils significantly inhibited the parasitism potential of *D. basalis*. Indeed, at the concentration 12.5 µl/l air, the emergence rate of *D. basalis* adults decreased from 93.33% for the control to 40 and 28.33%, respectively, at 3 and 6 days following oil application. Storage of seeds using plant-based insecticides and essential oils is not always compatible with biological control strategies. Thus, identifying components that have lower effects on natural enemies is very important for a successful IPM program.

Keywords: *Callosobruchus maculatus*, *Dinarmus basalis*, essential oils, *Eucalyptus salmonophloia*, parasitism

In Tunisia, a national chickpea improvement program has significantly contributed to yield increases during the last two decades (Amri et al. 2014; Khamassi et al. 2012). However, yield potential is seldom reached due to biotic and abiotic stresses (Solh et al. 1994). Chickpea seeds are vulnerable, particularly in storage, to attack by seed-beetles. Beetles of the genus *Callosobruchus* are major storage pests of

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chickpea crops and cause considerable economic losses worldwide (Sharma and Thakur 2014). The cowpea seed beetle *Callosobruchus maculatus* is the main field carryover storage pest of pulses including cowpea, chickpea, green gram, black gram, and red gram (Loganathan et al. 2011). In Tunisia, *C. maculatus* larvae feed on food legume seeds and cause major losses during storage (Haouel-Hamdi et al. 2017).

The efficient insect control in food commodities has long been the goal of producers and processors (El-Kady 1978). Chemical protection of stored food stuffs is the most available method. Fumigation treatments are the most common and economical tool for managing stored grain insect pests (Mediouni Ben-Jemâa et al. 2012; Mueller 1990). In addition, Tovignan et al. (2001) demonstrated that those insecticides acting as fumigants might be toxic for the users if not carefully handled. They could also have an adverse impact on arthropod predators or parasitoids of target pests (Van Huis 1991; Waage 1989) and it is well known that insecticide resistance may rapidly develop (Dales 1996; Georghiou 1990).

The use of plant materials can lead to the identification of new bioinsecticides. Essential oils for instance have been widely tested and have given promising results under laboratory conditions (Isman 2000; Regnault-Roger 1997). They have shown strong insecticidal activity against bruchid pests (Haouel-Hamdi et al. 2015; Mediouni Ben-Jemâa et al. 2012; Rahman and Schmidt 1999).

Eucalyptus, a large genus of the Myrtaceae family, has been subjected to various chemical and biological studies including insecticidal properties against stored product pests (Haouel et al. 2010, Mediouni Ben-Jemâa et al. 2013). In Tunisia, few references are available in the literature regarding the chemical composition of *Eucalyptus salmonophloia* growing in southern Tunisia. Previous study have demonstrated the antimicrobial (Ben Marzoug et al. 2010) and antioxidant (Haouel-Hamdi 2017b) properties of *E. salmonophloia* essential oils from southern Tunisia (Gabes).

Recently, stored pest control trends emphasize on the use of non-chemical procedures with the judicious use of pesticides. In this context, various researches demonstrated the efficacy of parasitoids and predators in controlling storage pests (Flinn and Schöller 2012; Titouhi et al. 2017). *Dinarmus basalis* is a notable parasitoid potentially used as biological control agent against *C. maculatus* (Dorn et al. 2002; Schmale et al. 2001). Besides, essential oils were found to be efficient replacement alternatives to synthetic pesticides (Mediouni Ben-Jemâa 2014). The complementary strategy of combining application of essential oils and use of natural enemies could be a better way to control bruchid development in storage conditions.

Therefore, this research aimed to assess the fumigant toxicity of *E. salmonophloia* essential oils from Métouia, Gabès (south Tunisia) and to evaluate its possible combined use with the ectoparasitoid *D. basalis* for the postharvest control of the cowpea weevil *C. maculatus*.

MATERIALS AND METHODS

Insect rearing.

Callosobruchus maculatus colony rearing. The *C. maculatus* colony was maintained in the laboratory. Insects were reared in chickpea, maintained at 27 ± 1 °C and 70 ± 5% RH in a 12:12 h cycle light:dark and renewed every three weeks according to the methods described by Haouel et al. (2017). Adult insects, 0-

1day old, were used for all bioassays. All trials were carried out under the same environmental conditions as the cultures.

Dinarmus basalis rearing. The *D. basalis* colony was maintained in the laboratory. One- to 2-day-old adults of *D. basalis* were introduced into transparent plastic boxes in the presence of chickpea seeds containing *C. maculatus* larvae (L4 and nymphs). The 4th larval instars were obtained 16-18 days after the bruchid oviposition period. After 2 days, the seeds containing bruchid larvae, whether parasitized or not, were removed from the cages and placed in Petri dishes under the standard rearing conditions.

Plant material.

Fresh *E. salmonophloia* leaves were collected in May 2015 from El-Metouia (Gabès, Tunisia). Specimens were identified and air-dried in a shady place at room temperature for 10 days. After air-drying, the plant material was subjected to a hydro-distillation using a Clevenger-type apparatus.

Chemical analysis.

GC analysis was carried out using an Agilant 6980 gas chromatograph equipped with a flame ionization detector and split-splitless injector attached to Hp-Innowax polyethylene glycol capillary column (30 m × 0.25 mm). One micro-liter of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. The constituents were identified by comparing their relative retention times with those of authentic compounds injected in the same conditions. The identification of the essential oils was performed using a Hewlett Packard HP5890 series II GC-MS equipped with a HP5MS column (30 m × 0.25 mm). The carrier gas was helium at 1.2 ml/min. Each sample (1 µl) was injected in the split mode (1:20), the program used was isothermal at 70°C, followed by 50-240°C at a rate of 5°C/min, then held at 240°C for 10 min. The mass spectrometer was a HP 5972 and the total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and mass spectra with the data from the library of essential oil constituents, Wiley, Mass-Finder and Adams GC–MS libraries.

Fumigant toxicity bioassays.

Toxicity bioassays against adults. A whatman filter paper (2 cm in diameter), impregnated with oils, was attached and hanged up to the screw caps of a 44 ml Plexiglas bottle with a support (2 cm length wire). Caps were screwed tightly on the vials, each of which contained 10 adults of *C. maculatus*. The tested oil doses were 0.5, 1, 2, and 3 µl providing equivalent concentrations of 12.5, 22.72, 45.45 and 68.18 µl/l air. Each treatment and check was repeated four times. Mortality was recorded hourly. Abbott correction formula (Abbott 1925) was applied to assess insect mortality. Probit analysis (Finney 1947) was used to determine lethal concentrations LC_{50} and LC_{95}.

Toxicity bioassays against larvae. The tests were carried out according Titouhi et al. (2017). The fumigant toxicity bioassays were applied against immature individuals developing inside the seed: neonate larvae L1 (3-day-old), second instar larvae L2 (5- to 6- day-old) and third instar larvae L3 (12- to 13-day-old). The different instars developing inside the seeds were determined subsequently by opening the seed and counting the number of exuvia (cephalic capsules) present inside. A sample of 20 seeds each containing one individual was removed from each culture of different

ages (3-, 5- and 12-day-old) and treated with four oil concentrations: 12.5, 22.72, 45.45, and 68.18 µl/l air. The treated individuals inside the seeds were exposed during 48 h and then incubated until adult emergence. The assessment of oil efficiency was measured by the number of emerged adults. Comparison was made with untreated stage.

Host treatments with oils and parasitoids.

This test was conducted to evaluate the effect of *E. salmonophloia* essential oils on parasitoids *D. basalis*. The immature individuals of *C. maculatus* (forth instar larvae L4) and parasitoid treatment with oils was carried out according Ketoh et al. (2005) and Titouhi et al. (2017).

Three treatments were administered, each replicated 4 times: the first designates the control where no protection measures against bruchids were applied (20 infested chickpea seeds). In the second trial, experiments were conducted with 16-day-old host larvae (corresponding to the L4 developmental stage which is the most easily controlled by *D. basalis* wasps); for that, 10 pairs of *D. basalis* adults were introduced on 20 *C. maculatus* L4 larvae. For the third experiment, a lot of 20 forth instar larvae (L4) of *C. maculatus* (on 20 chickpea seeds) were treated with oils followed by the introduction of 10 pairs of *D. basalis* after two periods of 3 and 6 days. This period was chosen to overcome all the parasitization period. All treatments were observed until the emergence of bruchids and/or parasitoids.

Statistical analyses.

Statistical analyses were performed using SPSS statistical software version 20.0. All values given were the mean of three replications and were expressed as the mean ± standard deviation. Significant differences between the mean values ($P \leq 0.05$) were determined by using Student test.

RESULTS

Essential oil yields and composition.

Oil yield based on dry matter weight was 1.63%. Results of the GC and GC-MS analysis were reported in Table 1 and Fig. 1.

Chemical composition of *E. salmonophloia* essential oils is reported in Table 1 and Fig. 1. A total of 14 compounds were identified constituting 99.26% of the oils. The main constituents were 1,8-cineol (62.78%), cryptone (9.34%), p-cymene (5.46%), verbenene (5.23%), eremophilene (3.5%), p-cumic aldehyde (3.16%) and α-pinene (2.84%).

Fumigant toxicity bioassays.

Toxicity bioassays against adults. Toxicity bioassays results were presented as mortality percentage of *C. maculatus* adults exposed for various periods of time to *E. salmonophloia* essential oils and showed in Fig. 2.

Results showed that fumigant toxicity depends on concentration and exposure period. For the lowest concentration (12.5 µl/l air), mortality attained 53.33, 60 and 100% after respectively 24, 48 and 78 h of exposure to *E. salmonophloia* essential oils. However, for other concentrations (22.72, 45.45, and 68.18 µl/l air), a total mortality of *C. maculatus* adults was recorded after only 18 h of exposure.

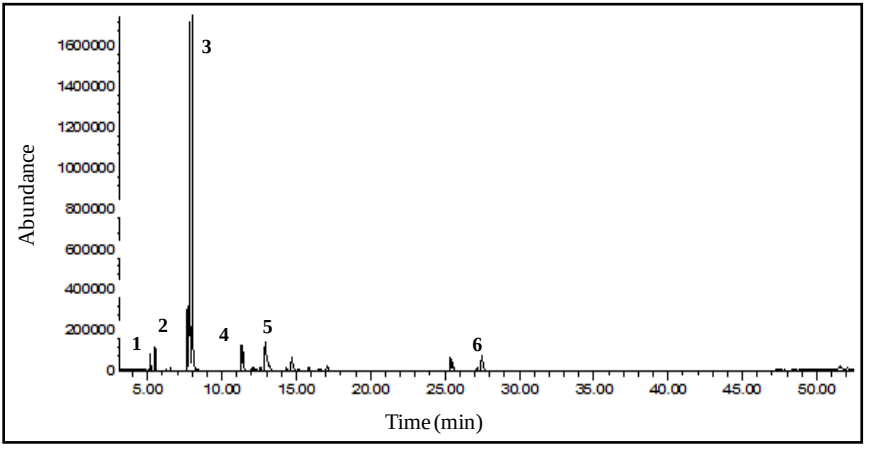

Fig. 1. GC-MS chromatogram of aerial parts of *Eucalyptus salmonophloia* essential oils on HP5MS column. *1 = α-pinene, 2 = p-cymene, 3 = 1,8-cineol, 4 = Verbenene, 5 = Cryptone, 6 = Eremophilene.

Table 1. Chemical fractions and total identified compounds (%) of essential oils obtained from *Eucalyptus salmonophloia* leaves

N°	Compound	TR	%
1	α-pinene	5.19	2.84
2	p-cymene	7.64	5.46
3	1,8-cineol	7.82	62.78
4	Verbenene	11.32	5.23
5	Pinocarvone	12.04	0.74
6	γ-Terpinene	12.59	0.37
7	Cryptone	12.86	9.34
8	p-Cumic aldehyde	14.71	3.16
9	Phellandral	15.84	0.73
10	o-cymen-5-ol	17.07	1.25
11	Isolongifolene	25.37	1.96
12	Aromadendrene	25.53	1.3
13	α-Amorphene	27.17	0.6
14	Eremophilene	27.47	3.5
	Total (%)	-	99.26

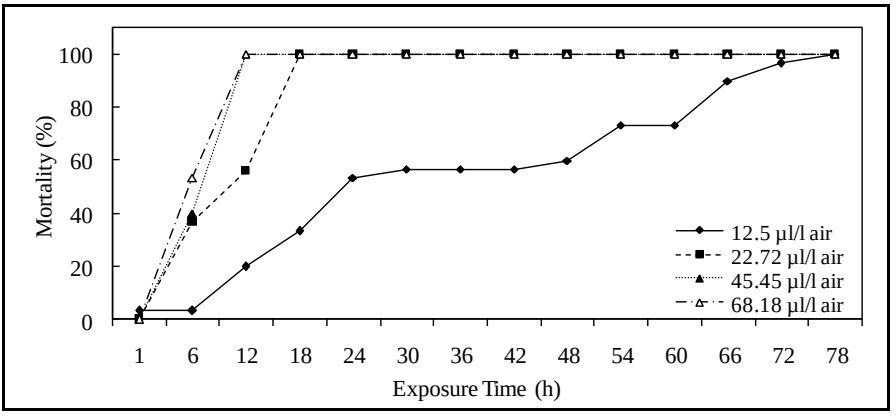

Fig 2. Mortality (%) of *Callosobruchus maculatus* adults exposed for various periods of time to different concentrations of essential oils from *Eucalyptus salmonophloia* leaves.

Results of the Probit analysis were given in Table 2. According to the median lethal concentration value, *C. maculatus* adults were very sensitive to *E. salmonophloia* essential oils. Probit analysis showed that LC_{50} and LC_{95} values were respectively 23.34 and 68.47 µl/l air. Results showed interesting potential toxicity of *E. salmonophloia* essential oils against target pest.

Table 2. LC_{50} and LC_{95} (µl/l air) values calculated for mortality within 24 h of exposure of *Callosobruchus maculatus* adults to various concentrations of *Eucalyptus salmonophloia* essential oils

essential oils	LC_{50}	LC_{95}	Chi square	d.f.	Slope
Eucalyptus salmonophloia	23.34 (9.40 - 64,07)	68.47 (45.57 - 228.34)	63.90	4	0.042

Toxicity bioassays against larvae (Immature stages).

Results of the toxicity bioassays against larvae (immature stages) were illustrated in Fig. 3 as percentage of mortality of *C. maculatus* larvae after 24 h of exposure to various concentrations of *E. salmonophloia* essential oils.

Results demonstrated that the effect of the essential oils on instars developing inside the seed was influenced by larvae age, developmental stage and essential oil concentration. Furthermore, results revealed the susceptibility of the three developmental stages (L1, L2 and L3) to *E. salmonophloia* essential oil fumigation.

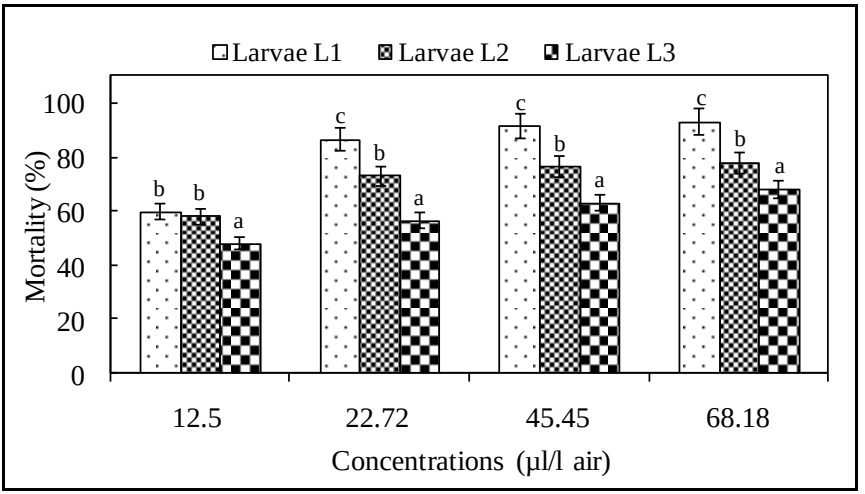

Fig. 3. Mortality (%) of *Callosobruchus maculatus* larvae (L1, L2 and L3) recorded after 24 h of exposure to various concentrations of *Eucalyptus salmonophloia* essential oils. * For each concentration, comparisons were made between the three larvae stage. Bars having different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Statistical analysis showed that the larvae age affects significantly its susceptibility to essential oil fumigation. The neonate larvae (L1) were the most susceptible, with a mortality rates reaching 60, 86.66, 91.66 and 93.33%, respectively, for the concentrations 12.5, 22.72, 45.45 and 68.18 µl/l air. The third instar larvae (L3) were the most tolerant. For the lowest concentration (12.5 µl/l air), *E. salmonophloia* essential oils induced a mortality of 48.33% for the third larvae of *C. maculatus*. For the concentrations 22.72 and 45.45 µl/l air, percentage of mortality of the third instar larvae were 56.66 and 63.33%, respectively.

Host treatments with oils and parasitoids.

The results of the effect of *E. salmonophloia* essential oils on the parasitoid *D. basalis* were illustrated in Fig. 4. Results showed that for the two insects (*C. maculatus* and *D. basalis*) and for the two periods (3 and 6 days), adult emergence decreased significantly as oil concentration increased. Statistical analysis showed that the concentration affect significantly the number of emerged adults. When the introduction of *D. basalis* occurred after 3 days of the treatment with essential oils, the parasitoids emergence rate was more important than with a delay of 6 days. For the lowest concentration (12.5 µl/l air), the emergence rates of parasitoids were 40 and 28.33% after 3 and 6 days, respectively. Furthermore, for the highest concentration (68.18 µl/l air), the emergence rates of parasitoids were 13.33 and 11.67% after 3 and 6 days, respectively.

When the oils were applied 3 or 6 days before the introduction of *D. basalis* adults, the emergence rates of *C. maculatus* were 66.66 and 68.33% respectively for 3 and 6 days at 12.5 µl/l air. In addition, at the concentration of 68.66 µl/l air, the respective emergence rates were 10 and 18.33% for 3 and 6 days.

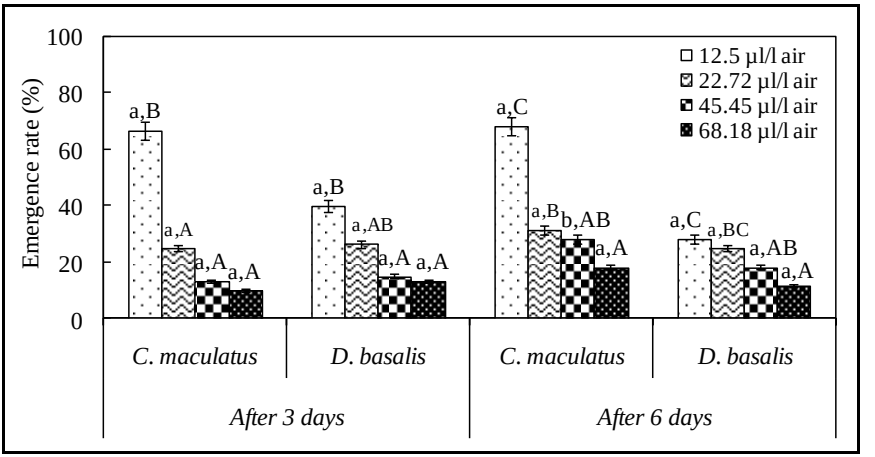

Fig. 4. Emergence (%) of *Callosobruchus maculatus* and *Dinarmus basalis* adults noted after exposure to various concentrations of *Eucalyptus salmonophloia* essential oils. * For each concentration, comparisons were made between the two periods (lowercase letters) and for each insect between concentrations (uppercase letters). Bars having different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Fig. 5 reported the application effect of *E. salmonophloia* essential oils after 3 and 6 days on the emergence rate of *D. basalis* adults. Results revealed that the application of essential oils affected the parasitoid potential. In addition, results pointed toward the high susceptibility of *D. basalis* adults to oil vapors and to the residual oil vapors activity after 6 days. Statistical analyses showed significant differences between the two periods (3 or 6 days).

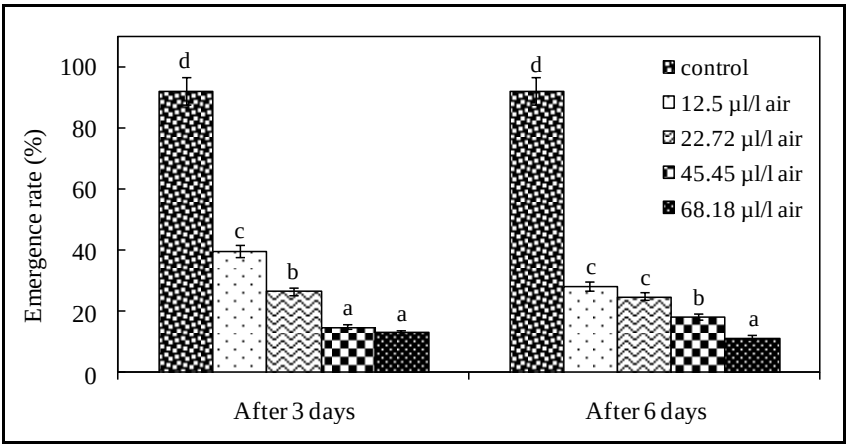

Fig. 5. Emergence (%) of *Dinarmus basalis* adults noted after exposure to various concentrations of *Eucalyptus salmonophloia* essential oils. * For each concentration, comparisons were made between the two periods. Bars having different letters are significantly different according to Student's test at $P \leq 0.05$.

DISCUSSION

Our results demonstrate that the success of the combination of *E. salmonophloia* essential oils from Métouia with the ectoparasitoid *D. basalis* was assured when the oil application is done at the beginning of the storage period and the introduction of the parasitoid was achieved after 6 days. In fact, White and Sinha (1990) observed that insecticides reduce the density and the diversity of natural enemies in stores. Similarly, Alzouma (1995) pointed out that *D. basalis* parasitism potential is affected when plant materials were added to stored cowpea. Previous related research reported that the use of *D. basalis* enabled effective control of *C. maculatus* population and limited weight losses of stored cowpea seeds at the beginning of the storage period (Sanon and Ouedraogo 1998; Sanon et al. 1998). Moreover, Corley et al. (2004) pointed out that parasitoids induced behavioral and physiological changes among their hosts. On the other hand, natural enemies play an important role in limiting potential pest populations and they are more likely to survive in case of application of eco-friendly biopesticides (Khater 2012). Actually, as an alternative pest control technology, essential oils have attracted particular attention (Liu et al. 2006). Caswell (1973) mentioned that in the presence of parasitoid wasps that parasitize the developing beetles, the percentage of damaged seeds does not exceed 60%. Additionally, Van Alebeek (1996) cited that in storage structures in West Africa, *D. basalis* induced 89% of parasitism on *C. maculatus* larvae. Furthermore, when *D. basalis* parasitoids were deliberately introduced into storage facility in large numbers, they effectively reduced the damage done by bruchid beetles (Sanon et al. 1998; Titouhi et al. 2017). Moreover, Boateng and Kusi (2008) reported that *D. basalis* adults were more susceptible to *Jatropha curcas* seeds' oil. Additionally, Aziz and Abbass (2010) showed the susceptibility of *C. maculatus* and its parasitoid *D. basalis* to essential oils of *Ocimum basilicum*, *Cymbopogon nardus* and *C. schoenanthus*. Contrarily, Ketoh et al. (2002, 2005) reported that *D. basalis* adults were more susceptible to essential oils from *Cymbopogon nardus*, *C. schoenanthus* and *Ocimum basilicum* than the adults of their host *C. maculatus*. They also indicated that the introduction of the essential oils into storage systems potentially could reduce density of parasitoid populations and increase seed losses.

In this study, the essential oil yields from *E. salmonophloia* leaves extracted by hydro-distillation and collected from south Tunisia was 1.63%. This results concord with those provided by Penfold and Willis (1961) who also observed that *E. salmonophloia* oil yield based on dry matter weight was 1.40%. However, according to Ben Marzoug et al. (2010), *E. salmonophloia* essential oil yield collected from south Tunisia was 4.6%. In the same context, Zrira et al. (1994) pointed out that extraction yield from *E. salmonophloia* oils collected from Morocco varied between 4.78 and 5% according the harvest area. Other studies have reported that the yield of *E. salmonophloia* from the mature leaves of plants native in Australia was 2.73% (Bignell et al. 1996). The variation of yield of some species growing in Tunisia could be attributed to the soil conditions and ecological and climatic conditions (Ben Marzoug et al. 2010).

Results of chemical composition of essential oils clearly demonstrated that *E. salmonophloia* essential oils were rich in 1,8-cineol (62.78%), cryptone (9.34%), p-cymene (5.46%), verbenene (5.23%), p-

cumic aldehyde (3.16%) and α-pinene (2.84%). These compounds were known to possess insecticidal activity against various insect species (Haouel-Hamdi et al. 2015). In addition, as reported by Ben Marzoug et al. (2010), the major components of *E. salmonophloia* essential oils were 1,8-cineol (59.30%), α-pinene (10.70%), cumicaldehyde (5.00%), p-cymene (2.80%), and trans-pinocarveol (4.10%). However, according to Bignell et al. (1996), *E. salmonophloia* essential oils of Australian origin was reported to contain α-pinene (5.1%), β-pinene (9.7%), 1,8-cineol (10.3%), p-cymene (16.9%), trans-pinocarveol (2.3%), cryptone (10.5%), and spathulenol (2.0%). Compared to our result, Australian essential oils have a very low percentage of 1,8-cineol. As reported by Hussain (2009), insecticidal proprieties were dependent upon oils since the major components of the essential oils determine their biological properties. In this respect, highest percentages of α-pinene, 1,8-cineol and γ-terpineol in *E. lehmani* oils conferred it best insecticidal potential against *Rhyzopertha dominica*, *C. maculatus*, and *Tribolium castaneum* adult (Haouel-Hamdi et al. 2015). Indeed, the pesticide activity of *Eucalyptus* oils is attributed to various components such as 1,8-cineol, α-pinene and γ-terpineol (Liu et al. 2008; Lucia et al. 2007). Besides, among the various components of *Eucalyptus* oils, 1,8-cineole is the most important one and, in fact, a characteristic compound of the genus *Eucalyptus*, and is largely responsible for a variety of its pesticide properties (Batish et al. 2008; Haouel-Hamdi 2017a). In this study, *E. salmonophloia* essential oils displayed fumigant toxicity to larvae and adults of *C. maculatus*. It showed strong species-specific toxicity that was highly dependent upon the tested concentrations and the exposure durations.

Previous studies reported the efficacy of various *Eucalyptus* species essential oils for bruchid beetles control (Haouel-Hamdi et al. 2015). On the other hand, regarding the combined treatment of essential oils fumigation and parasitoids releases were largely discussed. In this respect, Boeke et al. (2003) indicated that parasitoids were affected by the botanical insecticides and that the powders of *Azadirachta indica* and *Blumea aurita* may be compatible with biological control by *D. basalis*. In addition, Sanon et al. (2006) showed that volatiles from *Hyptis suaveolens* crushed leaves and essential oils reduced host location behavior and reproductive activity of *D. basalis*. Similarly, Ketoh et al. (2002) pointed out that sub-lethal doses of essential oils reduce the life duration of both *C. maculatus* and *D. basalis* adults and decrease fecundity of females. In addition, Titouhi et al. (2017) reported that the release of parasitoids *D. basalis* and *Triaspis luteipes* could be better if it was combined with *Artemisia campestris* essential oils, as these oils had more pronounced effects on the beetles than on their parasitoids. Our results demonstrated that *E. salmonopholia* essential oils were effective against the beetle host, but they also prevented successful parasitization when the oils were applied 3 or 6 days before the introduction of *D. basalis* adults. This agrees with Van Huis (1991) who stated that biological control of bruchids should receive more attention in particular research on the introduction and conservation of natural enemies.

To summarize, biological control is often an underutilized component of integrated pest management in storage commodities since industrials always tended to look for chemical alternatives to manage insect pests. Recently, essential oils have been appraised as alternatives to

chemical pesticides. However, seeds' storage using plant-derived insecticides and essential oils is not always compatible with biological control strategies. Thus, identifying components that have lower effects on natural enemies is a key feature for a successful IPM program.

RESUME

Haouel-Hamdi S., Abdelkader N., Hedjal-Chebheb M., Saadaoui E., Boushih E. et Mediouni-Ben Jemâa J. 2018. Utilisation combinée d'huiles essentielles d'*Eucalyptus salmonophloia* et du parasitoïde *Dinarmus basalis* pour la lutte contre la bruche du niébé *Callosobruchus maculatus*. Tunisian Journal of Plant Protection 13 (si): 123-137.

Ce travail a pour objectif d'évaluer la possibilité de combiner l'utilisation des huiles essentielles d'*Eucalyptus salmonophloia* et du parasitoïde *Dinarmus basalis* pour la lutte contre la bruche du niébé *Callosobruchus maculatus*, un ravageur d'importance économique sur les légumineuses stockées incluant le pois chiche. Cette étude présente la première investigation sur le potentiel insecticide des huiles essentielles d'*E. salmonophloia* poussant à Gabès (sud de la Tunisie). La toxicité par fumigation des huiles essentielles a été testée contre les adultes et les larves L1, L2 et L3 de *C. maculatus*. En outre, le parasitoïde a été introduit contre les larves L4 et les nymphes de son hôte 3 et 6 jours après l'application des huiles. Les résultats ont rapporté un potentiel insecticide très important des huiles essentielles d'*E. salmonophloia* à l'égard des larves L1, L2 et L3 et des adultes de *C. maculatus*. Toutefois, ces huiles ont inhibé d'une manière très remarquable le potentiel parasitaire de *D. basalis*. En effet, à la concentration 12,5 µl/l air, le taux d'émergence des adultes de *D. basalis* a passé de 93,33% pour le témoin sans huiles à 40 et 28,33% respectivement après 3 et 6 jours d'application des huiles. Le stockage des graines à l'aide d'insecticides à base de plantes et d'huiles essentielles n'est pas toujours compatible avec les stratégies de lutte biologique. Ainsi, l'identification des composants qui ont le moins d'effets sur les ennemis naturels est très importante pour un programme de lutte intégrée réussi.

Mots clés: *Callosobruchus maculatus, Dinarmus basalis, Eucalyptus salmonophloia*, huiles essentielles, parasitisme

ملخص

حوال-حمدي، سمية وندى عبد القادر ومريم حجال-شبهاب وعز الدين سعداوي وآمنة بوصحيح وجودة مديوني-بن جماعة. 2018. دمج استعمال الزيوت الروحية لنبتة *Eucalyptus salmonophloia* وشبه الطفيلي *Dinarmus basalis* في مكافحة خنفساء حب اللوبيا الجنوبية.

Tunisian Journal of Plant Protection 13 (si): 123-137.

يهدف هذا العمل إلى تقييم إمكانية دمج استعمال الزيوت الروحية لنبتة *Eucalytus salmonophloia* وشبه الطفيلي *Dinarmus basalis* لمكافحة خنفساء حب اللوبيا الجنوبية *Callosobruchus maculatus*. وقع اختبار الزيوت عن طريق التبخر ضد الطور البالغ وضد الأطوار اليرقية 1 و 2 و 3 للعائل. تم استعمال شبه الطفيلي ضد الطور اليرقي الرابع وضد العذارى. بينت النتائج أن الزيوت الروحية لنبتة *E. salmonophloia* تمتلك فعالية إبادة حشرية مرتفعة ضد الأطوار اليرقية 1 و 2 و 3 وضد الحشرة البالغة، غير أنها تعيق بطريقة بالغة القدرة التطفلية لشبه الطفيلي *D. basalis* حيث انه بالنسبة إلى الجرعة 12.5 مكل/لتر هواء، انخفضت نسبة ظهور شبه الطفيلي من 93.33 % بالنسبة للشاهد إلى 40 و 28.33% بعد 3 و 6 أيام على التوالي من استعمال الزيوت. إن تخزين البذور باستخدام المبيدات الحشرات النباتية والزيوت الروحية ليست دائما منسجمة مع استراتيجيات المكافحة البيولوجية. إن تحديد المكونات التي لها أقل تأثير على الأعداء الطبيعيين مهم جدا في برنامج المكافحة المتكاملة للآفات.

كلمات مفتاحية: تطفل، خنفساء حب اللوبيا الجنوبية، زيوت روحية، *Dinarmus basalis*، *Eucalyptus salmonophloia*

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Influence of Grapevine Vigor on the Dynamic and the Installation of the Invasive Pest *Jacobiasca lybica* in Mitidja, Algeria

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ABSTRACT

Bissaad, F.Z., Razi, S., and Bounaceur, F. 2018. Influence of grapevine vigor on the dynamic and the installation of the invasive pest *Jacobiasca lybica* in Mitidja, Algeria. Tunisian Journal of Plant Protection 13 (si): 139-145.

The vigor of six grapevine varieties was followed over three consecutive years in order to evaluate and analyze its impact on the installation and selection of egg laying sites by an invasive bio-aggressor in Algerian vineyards. The vigor was estimated by weighing the pruning during dormancy period from December during three years. A total of ten vines were randomly selected along the diagonal of the plot. Although the distribution of *Jacobiasca lybica* seems to be slightly in favor of some grape varieties compared to others, host vigor seems to be one of the major parameters affecting the repartition and distribution of this pest. The examination of the Principal Component Analysis (PCA) showed many similarities; group 1 included the less vigorous varieties (namely Cardinal, Syrah and Muscat of Alexandria), followed by group 2 including the Dattier of Beirut. On the other hand, group 3 with Cabernet Sauvignon and group 4 with Merlot varieties, are considered the most vigorous, but did not show a correlation between larval infestations and adult populations. Furthermore, from a numerical point of view, these grape varieties have the highest number of individuals as much as adults than larvae. In contrast, it was observed a very pronounced adult trend with grape vigor.

Keywords: Damage, grape variety, leafhopper, vigor, *Vitis vinifera*

The African leafhopper *Jacobisca lybica* is an endemic pest of some Africa regions. Bergevin and Zanon (1922) described this insect as a polyphagous species and its presence in vineyards is relatively recent. It is recently considered as one of the most harmful vineyard-pests in Algeria (Bounaceur 2010). Its importance as vineyard pest has been recently confirmed by Bounaceur et al. (2006).

J. lybica, named "green leafhopper" responsible for grapevine grilling, was reported as a pest of cotton in Egypt and Sudan. It was described the first time in Libya by Bergevin and Zanon (1922). It has spread from there to Egypt. It was also found in the eastern regions as well as in the Ethiopian region where it caused damages to South African vines (Della Guisttena 1989).

On vine, *J. lybica* performs three to four generations per year (Bounaceur

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et al. 2006; 2007). In Algeria, beyond the vine, this insect cannot find necessary factors for its hibernation and intermediate host plants. For all these raisons and given the extent of the damage some grape varieties, we found it useful to study some parameters of these grape varieties to explain the insect preference and to understand its behavior for a better management of its populations.

Therefore, this study was conducted in order to understand some bio-ecological aspects of this species, including the influence of vigor on its establishment and on the choosing site for egg laying. The data obtained may contribute to a better manage of this invasive species on vineyards.

MATERIALS AND METHODS

Characteristics and description of the study station.

The study was carried out in a vineyard of 145 ha, located at the west of the Mitidja (36° 36N 02° 24E). The observations were made in a grapevine vineyard grown on 1103P rootstock. Three grape varieties (Merlot, Cabernet Sauvignon, and Syrah) were studied. The table grape was composed of three cultivars: Muscat of Alexandria, Dattier of Beirut and Cardinal, of a grafted planted area grafted registry 41B in 2002.

These vineyards are trellised on three iron wires, trained as single Guyot, with a planting density of 3300 plants per hectare planted in East-West orientated rows.

Jacobiasca lybica populations monitoring.

A weekly sampling was carried out during the cropping years 2006, 2007 and 2008, by monitoring larvae and adults.

Monitoring of adults. Adults were monitored from March to November, using yellow traps coated with a sticky material, similar to INRA-type traps. It was fixed horizontally by a pair of pliers at the level of the lowest leaves. Five traps per plot were spread over five rows along the plots diagonal (Bastide 1989). Traps were weekly replaced during all the study period.

Monitoring of larvae. Larval populations were monitored in-situ weekly from March to November, by direct counting of larvae on 200 leaves per 50 trees per plots (at the rate of four leaves per vine), collected randomly according to Bastide (1989). These vines were distributed on five rows excluding the borders (Delbac 2000). Phytosanitary treatments consisted on a growth regulator Cascade (100 g/l of Flufenoxuron) applied by farmers from the first decades of May and July against green leafhopper. Other antifungal treatments were applied against down mildew and powdery mildew diseases based on Bordeaux mixture and wet table sulfur.

Effect of host vigor on the dynamics of *Jacobiasca lybica.*

The host vigor was estimated by weighing the pruning wood during grapevine dormancy of the study year in December. A total of ten vines were selected randomly along the plot diagonal (Bastide 1989; Goutouly et al. 2006). The host vigor was estimated according to the average weight of the strain per pruning (Regnier 2000).

Statistical analysis.

In the case of the study, the effects of grape and vigor and the distribution of adult and larval populations of *Jacobiasca lybica* green leafhopper on vines vats and tables in Mitidja, the multivariate relations are studied using a factorial analysis of principal component

correspondences (PCA) (PAST vers 1.37) (Hammer et al. 2001).

RESULTS

The host vigor effect on the dynamics of *Jacobiasca lybica.*

The distribution of *J. lybica* appeared to be slightly in favor of some grapevines varieties compared to others. The results showed that Merlot and Cabernet Sauvignon varieties are the most vigorous.

The Principal Component Analysis (PCA), as a powerful tool in statistical investigations, is reported in Fig. 1. It showed the groups 2 and 1 including the less vigorous varieties Cardinal, Syrah and Muscat of Alexandria, and the groups 3 and 2 composed of variety Dattier of Beirut, for the two cropping years 2006 and 2007 respectively. On the other hand, for 2006 data, groups 1 and 3 included the most vigorous varieties, without any correlation between larval infestations and adult populations. Although from numerical point of view, these grape varieties have the highest number of populations. The group 4 included the Merlot grape variety. In contrast to previous years, results of 2008 PCA analysis (Fig. 1) showed that the group 2 included the less vigorous grapevine varieties, such as Cardinal, Syrah and Dattier of Beirut followed by group 3 including Muscat of Alexandria. For this last year, a clear correlation was observed between the infestations degree and adult populations which was strongly related to groups 1 and 4 including Cabernet Sauvignon and Merlot, respectively.

DISCUSSION

This study showed that the distribution of *J. lybica* seems to be slightly in favor of some grape varieties compared to others. Vigor seems to be one of the major parameters that affect the distribution of this pest on vine (Bounaceur and Doumandji-Mitiche 2009).

During 2006 and 2007 monitoring, the PCA data showed a similarity between groups: group 1 including less vigorous varieties (namely Cardinal, Syrah and Muscat of Alexandria), followed by the group 2 including the Dattier of Beirut. Groups 3 and 4, on the other hand, were considered to be the most vigorous, but did not show any correlation between larval infestations and adult populations, despite the fact that these varieties had the highest number of populations as much as adults than larvae. I contrast, during 2007, there was a very strong adult trend with vigor. It is accepted that the most infested vines are the most vigorous, by being the most attractive for leafhoppers (Bacrot 1999; Chaboussou 1975; Decante 1999).

The characteristics of the vines (variety, vigor, phenology) together with other fields proprieties (presence of weeds, topography, exposure etc.), could exert the same effect on *J. lybica*. Indeed, grape varieties do not present the same sensitivity level. In the same context, Touzeau 1968 in Galet (1982) reported that tomentose grape varieties hosted less larvae than those with smooth leaves which is in agreement with our observations. In fact, Merlot and Cabernet Sauvignon have dense vegetation and large leaves, which could attract the leafhoppers seeking to lay eggs. Our data are also in accordance with those of Mayse et al. (1991), who showed that the intra-compartmental distribution of *Empoasca vitis* is close to *J. lybica* and is clearly associated with plants of high vigor.

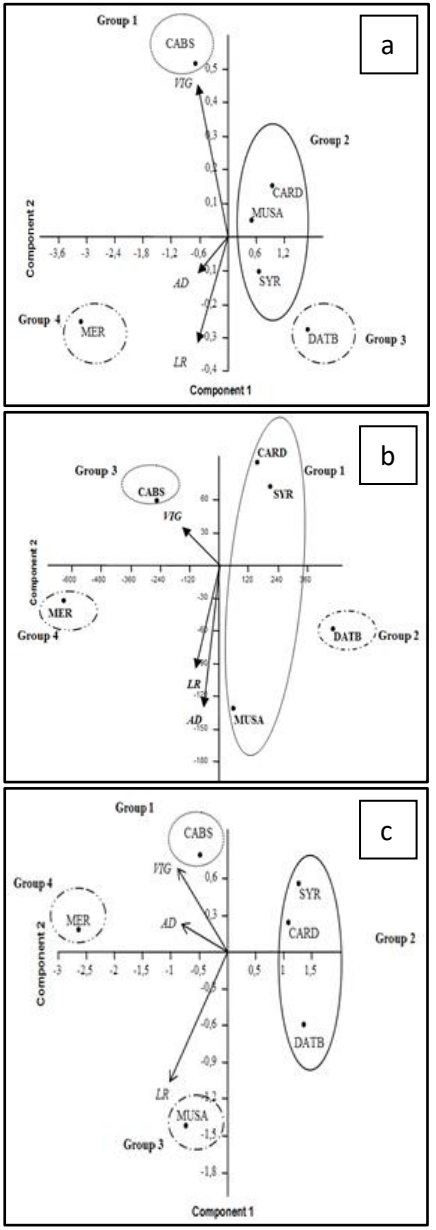

Fig. 1. Multivariate analysis "PCA" representing the effects of grape and vigor and the distribution of adult and larval populations of *Jacobiasca lybica* green leafhopper on vines vats and tables in Mitidja. a: 2006; b: 2007; c: 2008; CARD: Cardinal; SYR: Syrah; MUSA: Muscat of Alexandria; DATB: Dattier of Beirut; CABS: Cabernet Sauvignon; MER: Merlot.

It should be mentioned that other parameters may accentuate *J. lybica* distribution, like the effects of crowded leaves (Daane and Williams 2003; Fos et al. 1997; Genini 2000; Vidano et al. 1987), leaf chlorophyll concentration (Spring 1999; Spring and Zufferey 2000), and leaf structure (the plant support). All of these parameters are directly related to the vigor of the plant (Decante et al. 2006). The last authors showed that *Empoasca vitis* adults occurred in the areas of important vigor. This trait creates changes of microclimate, which becomes colder and moist and the plant is more protected against wind. All these conditions are preferred for adults (Daane and Williams 2003; Fos et al. 1997; Genini 2000; Vidano et al. 1987).

Besides, the larva aggregation zones correspond to a very strong vigor and thus reflecting the choice of laying sites made by adults (Decante et al. 2006). Eggs and larvae, sensitive to high temperatures (> 32°C) (Cerutti et al. 1991), are very likely to have a more favorable microclimate (Daane and Williams 2003; Fos et al. 1997; Genini 2000; Vidano et al. 1987) and/or sap of higher quality and quantity (Decante et al. 2006). The influence of vigor on the choice of egg laying sites is certain (Decante 2007). Additionally, nitrogen fertilization (Mayse et al. 1991) and water stress (Trichilo et al. 1990) in grapevine affect population levels of *Erythroneura variabilis*, a Typhlocybinae pest, of vine in North America. An accurate examination of distribution of *J. lybica* at plot scale seems necessary.

RESUME

Bissaad F.Z., Bounaceur F. et Doumandji-Mitiche, B. 2018. Effet de la vigueur de l'hôte sur la dynamique et l'installation d'un ravageur envahissant sur la vigne *Jacobiasca lybica* à Mitidja, Algérie. Tunisian Journal of Plant Protection 13 (si): 139-145.

L'analyse de la vigueur de six cépages a été suivie sur trois années consécutives afin d'évaluer l'incidence de cette dernière sur l'installation et la sélection des sites de ponte d'un bio-agresseur invasif sur les vignobles algériens. La vigueur a été estimée par la pesée du bois de taille au cours du repos végétatif à partir du mois de Décembre. Un total de 10 ceps a été pris au hasard selon la diagonale de la parcelle. Bien que la répartition de *Jacobiasca lybica* sur l'ensemble des cépages observés semble être légèrement en faveur de quelques cépages par rapport à d'autres, l'influence de la vigueur semble être un des paramètres majeurs qui caractérise la distribution et la répartition du ravageur. Les résultats de l'analyse en composante principale (ACP) a montré une très proche similarité; le groupe 1 formé par les cépages moins vigoureux (Cardinal, Syrah et Muscat d'Alexandrie), suivi par le groupe 2 formé par le Dattier de Beyrouth. En revanche, le groupe 3 Cabernet-Sauvignon et le groupe 4 Merlot sont considérés comme étant les plus vigoureux, mais n'ont montré aucune corrélation avec les infestations larvaires et les populations adultes, bien que du point de vue numérique, ces cépages ont marqué le nombre le plus élevé de populations autant d'adultes que de larves. En revanche, une tendance très marquée des adultes avec la vigueur a été observée.

Mots clés: Cépage, cicadelle, dégâts, vigueur, *Vitis vinifera*

ملخص

بساعد، فاطمة الزهرة وصباح رازي وفريد بوناصر. 2018 . تأثير قوة النبات العائل على حيوية واستقرار الآفة الغازية نطاط أوراق العنب *Jacobiaca lybica* في متيجة، الجزائر.

Tunisian Journal of Plant Protection 13 (si): 139-145.

تم تحليل قوة ستة أصناف من العنب على مدى ثلاث سنوات متتالية من أجل تقييم تأثيرها على اختيار مواقع تفريخ واحدة من الآفات الغازية لكروم العنب الجزائرية. تم تقدير القوة من خلال وزن خشب التقليم خلال مرحلة السبات النباتي ابتداء من شهر ديسمبر .تم أخذ مجموعة 10 الكروم عشوائيا على طول قطر المزرعة. على الرغم من أن توزيع نطاط أوراق العنب (*Jacobiasca lybica*) كان لصالح أصناف معينة من العنب مقارنة بأصناف أخرى، ويبدو أن تأثير قوة الصنف يعتبر واحد من المعالم الرئيسية التي تؤثر على توزيع وانتشار الآفة. أظهر فحص تحليل العنصر الرئيسي (PCA)، وجود تشابه كبير. فالمجموعة 1 هي التي شكلتها أصناف أقل قوة "كارديناال وسيراح ومسكة الإسكندرية"، تليها المجموعة الثانية التي شكلتها "تمري بيروت". من ناحية أخرى، تحتوي المجموعة الثالثة "كابيرنييت وسفينيون" والمجموعة الرابعة "ميرلوت" على الأصناف الأكثر قوة، ولكن لا تُظهر أي علاقة مع الإصابات باليرقات والأفراد البالغة، على الرغم من أنه من الناحية العددية هذه الأصناف القوية لديها أكبر عدد من المجموعات سواء من البالغين ومن اليرقات. في المقابل، لاحظنا اتجاه واضح جدا للأفراد البالغة نحو الأصناف القوية.

كلمات مفتاحية: أضرار، أنواع الكروم، قوة النبتة، نطاط أوراق العنب، *Vitis vinifera*

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Some Observations on the Predominance of *Aphis spiraecola* on Citrus in Northwestern Algeria

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ABSTRACT

Labdaoui, Z.E., and Guenaoui, Y. 2018. Some observations on the predominance of *Aphis spiraecola* on citrus in Northwestern Algeria. Tunisian Journal of Plant Protection 13 (si): 147-157.

Aphis spiraecola is the main aphid species found on citrus in Algeria. This study was carried out on *Citrus clementina* in northwestern Algeria, during a two year period (2016-2017) in the first flushing period (spring). The aphid fluctuation of the populations and their natural enemies, especially the parasitoids, were evaluated based on a weekly sampling of 100 leaves taken on 10 trees (10 leaves/ tree). *A. spiraecola* colonized citrus trees since the beginning of flushing. The density per young leaf reached a maximum of 78.8 ± 23.4 aphids in 2016 and 44.4 ± 13.0 aphids in 2017 with an average density of 6.0 ± 1.5 aphids/cm² and 4.4 ± 0.6 aphids/cm², respectively, where a significant difference between years ($P < 0.05$) was observed. The parasitism rate expressed in terms of number of *A. spiraecola* mummies remained very low, varying between 1.6% in 2016 and 3.0% in 2017 with no significant difference ($P > 0.05$) between years. Also, the emergence number of primary parasitoids was low for both years with 26.6% in 2016 and 10.8% in 2017. The primary parasitoids of *A. spiraecola* in 2016 were *Lysiphlebus testaceipes* and *Binodoxys angelicae* whereas only *L. testaceipes* was found in 2017. The total hyperparasitism rate varying between 16.7% in 2016 and 25.7% in 2017 did not differ significantly between years ($P > 0.05$). Mummies without adult emergence rate were found to be very high varying between 85 and 100%. This partial parasitic failure observed on *A. spiraecola* underlines many questions related with different factors (climate, ability of aphids to form winged populations to escape to their enemies, impact of hyperparasitoids). The new field of research is concerning the possible presence of endosymbiont organisms that could give to the aphid a defense reaction against its aggressors.

Keywords: *Aphis spiraecola,* citrus, hyperparasitoids, Northwestern Algeria, primary parasitoids

About twenty aphid species were recorded on citrus crops worldwide (Barbagallo and Patti 1985; Blackman and Eastop 2006) but the most harmful species are *Aphis spiraecola, A. gossypii, Toxoptera aurantii* and *T. citricidus* (Hermoso de Mendoza et al. 2006; Marroquin et al. 2004; Tena and Garcia-Marí 2011). The latest species has not been detected in Algeria yet.

A. spiraecola is one of the 14 aphid species of agricultural importance worldwide (Blackman and Eastop 2007). It is known to infest heavily citrus crops in all Mediterranean countries including Algeria (Ali-Arous et al. 2017; Barbagallo et al. 1996; Ben Halima-

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Kamel et al. 1994; Boukhris-Bouhachem et al. 1996, 2011; Hermoso de Mendoza et al. 2012; Labdaoui and Guenaoui 2015, 2017; Lebbal and Laamari 2016; Mostefaoui et al. 2014; Satar and Uygun 2008). This aphid species is known to be poorly controlled with parasitoids (Hermoso de Mendoza et al. 2006; Satar et al. 2014). In addition to leaf curling, flower falling and ants attracting (Satar and Uygun 2008), *A. spiraecola* is able to transmit the *Citrus tristeza virus* (CTV) with 23% of viral RNA detection (Marroquin et al. 2004). In Algeria, this virus is becoming epidemic in Chelif Valley known to be one of the most important citrus-growing region (Ali-Arous et al. 2017).

This aphid species is polyphagous and was recorded on more than 20 plant species (Blackman and Eastop 1984, 2006; Holman 2009). For Algerian private farmers, chemical control is the only issue to limit aphid infestation on citrus. The main objectives of this study were to explore the specific diversity of aphid populations and their parasitoids and to determine the causal relationship between different factors on the predominance of *A. spiraecola*.

MATERIALS AND METHODS

Study site.

This study was carried out in a *Citrus clementina* orchard, located at Mazaghran (35°53' 30.49"N - 0° 5'7.94"E, 1.12 ha) belonging to the university of Mostaganem. Trees were more than 30 years old and grafted on bigarade orange (*C. aurantium*) rootstock. The orchard was flood irrigated and was free from insecticide-based treatments since 2008.

Aphid sampling.

This study was conducted in 2016 and 2017 springs. The sampling dates started from the beginning of the flushing (from April, 14 to June, 02, 2016 and from March, 13 to May, 15, 2017). Aphid colonies were marked at the beginning of the spring leaf-flushing period and tracked weekly over the duration of the flush. One hundred young leaves were collected weekly from 10 trees (10 leaves/tree), and placed separately in plastic bags and brought to the laboratory for the monitoring of aphids and their associated parasitoids.

Leaf surface calculation.

The leaf area was calculated according to the formula proposed by Onillon et al. (1973): $s = 0.7707 \times a^{0.9392} \times b^{0.9893}$, where s: leaf area ($cm^2$), a: the largest length of the leaf (cm), and b: the largest width of the leaf (cm).

Aphid complex determination.

Samples were observed under a stereo-microscope "Zeiss". Parasitized aphids were left until mummification. New mummies were added to the total number. Aphids were identified according to several keys (Blackman and Eastop 1984, 2006; Leclant 2000).

Parasitoid complex determination.

Full mummies were smoothly taken and transferred separately in a gelatin capsule until the emerging of parasitoids. Emerged adults were immediately placed in 95% ethanol and stored at 4°C until identification. The percentage of parasitism was estimated by calculating the number of mummified aphids over the total number of aphids. Parasitoids were separated using several morphological keys (Barahoei et al. 2014; Gibson 2001; Hui and Da-wei 2000; Kavallieratos et al. 2001; Rakhshani et al. 2007; Stary et al. 2014; Tomanović et al. 2013). When needed, specialists were

asked for confirmation or correction of misidentification.

Statistical analysis.

The software IBM SPSS Statistics version 23 was used to analyze data.

RESULTS

Inventoried aphid species complex.

The aphid species recorded in the surveyed citrus orchards were *A. spiraecola* which was the most dominant species representing 80 to 100% of the total collected aphids (Fig.1). *A. gossypii*, *Toxoptera aurantii*, and *Myzus percicae* were found in a very limited numbers. *A. spiraecola* was recorded since the beginning of sampling on 91 and 51% of infested leaves in 2016 and 2017 monitoring, respectively. Its density reached a peak of 78.8 ± 23.4 individuals/ leaf representing 6.0 ± 1.5 aphids/cm² on May 16, 2016 and 44.4 ± 13.0 individuals/leaf corresponding to 4.4 ± 0.6 aphids/cm² on April 17, 2017. Aphids' density was found to be lower in 2017 than in 2016 with a highly significant difference between years (P = 0.0004). An earlier infestation was observed in 2017 probably related to the climate as expressed by the warmer temperatures occurring in March, 2017 (Fig. 1).

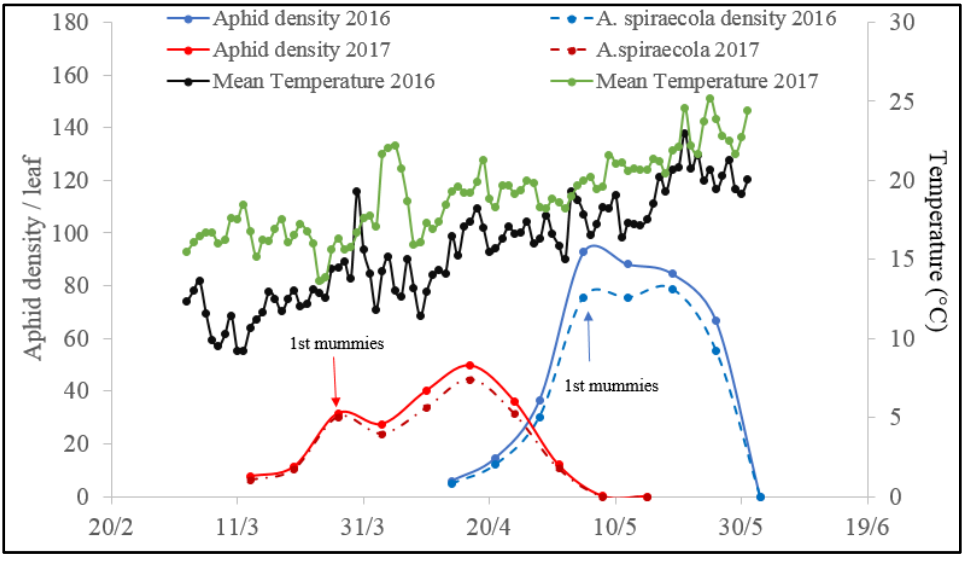

Fig. 1. Relative density of *Aphis spiraecola* over total aphids collected in 2016 and 2017 and the prevailing temperature data.

Primary parasitoids complex.

The parasitism rate on *A. spiraecola* expressed as mummies was very low, reaching a maximum of 1.6% in 2016 (Fig. 10) and 3.0% in 2017 (Fig. 11) with no significant difference noted between the two years (P = 0.535).

Only two primary parasitoids emerged from *A. spiraecola* which were *Lysiphlebus testaceipes* (Fig. 2) and *Binodoxys angelicae* (Fig. 3) while

interestingly *B. angelicae* was absent in 2017. The primary parasitoid emergence rate remained very low and did not exceed 26.6% in 2016 and 10.8% in 2017 but this difference was not significant (P = 0.937). In 2016, *L. testaceipes* reached a maximum of 10.8% ; however, in 2016, it did not exceed 9.4% of the total parasitism. *B. angelicae* was recorded only in 2016, with a rate of 17.0%.

A high rate of mummies without adult emergence was noted which varied between 70.8 and 87.5% in 2016 (Fig. 10) and between 71.4 and 91.0% in 2017 surveys (Fig. 11).

Fig. 2. *Lysiphlebus testaceipes*

Fig. 3. *Binodoxysangelicae*

Secondary parasitoids complex.

The rate of both hyperparasitoids reached 16.7% in 2016 and 25.7% in 2017 (Fig. 12) and did not show a significant difference between years (P = 0.232).

At least six secondary parasitoids were found namely *Pachyneuron aphidis* (Fig. 4), *Asaphes vulgaris* (Fig. 5), two *Alloxysta* species (Figs.6 and 8), *Phaenoglyphis* sp. (Fig. 9) and *Phaenoglyphis heterocera* (Fig. 7) which was recorded for the first time in Algeria on 2017 (Ferrer-Suay et al. 2017).

Fig. 4. *Pachyneuron aphidis*

Fig. 5. *Asaphes vulgaris*

Fig. 6. *Alloxysta* sp. 1

Fig. 7. *Phaenoglyphis heterocera*

Fig. 8. *Alloxysta* sp. 2

Fig. 9. *Phaenoglyphis* sp.

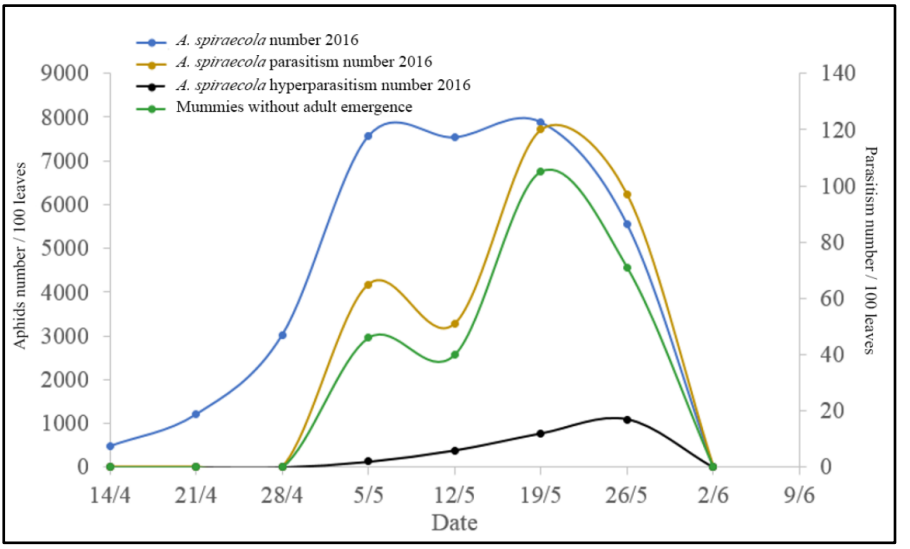

Fig. 10. *Aphis spiraecola* abundance and its parasitism rate in 2016 survey.

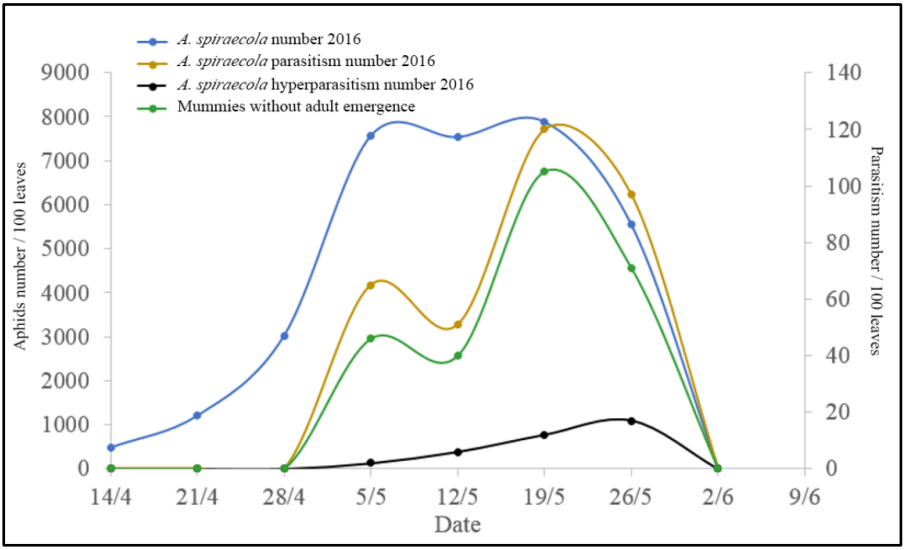

Fig. 10. *Aphis spiraecola* abundance and its parasitism rate in 2016 survey.

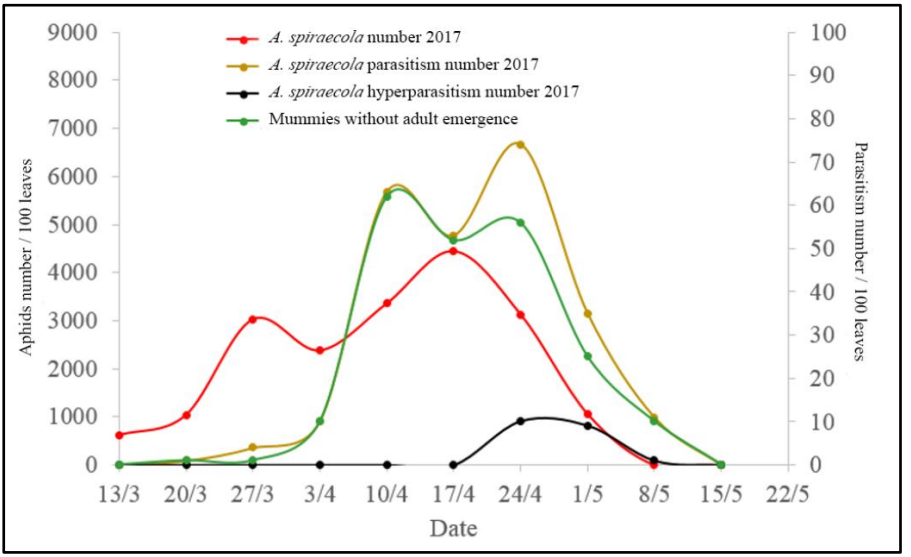

Fig. 11. *Ahis spiraecola* abundance and its parasitism rate in 2017 survey.

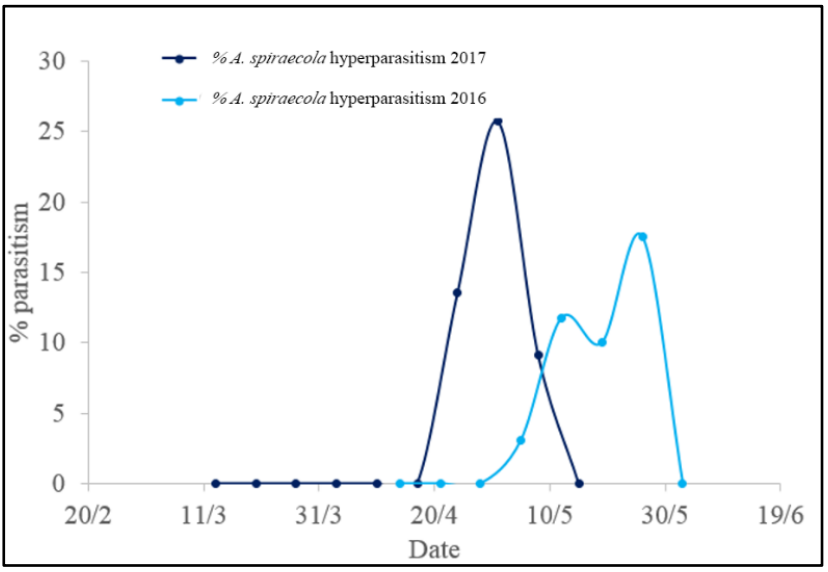

Fig. 12. Hyperparasitism rate on *Aphis spiraecola* (2016-2017)

DISCUSSION

In the present study, we have contributed to a better knowledge regarding specific variation in aphids, primary and hyperparasitoids in a representative citrus growing area of the Mostaganem region (Algeria). This study confirmed the predominance of *A. spiraecola* on citrus in this site. Only few colonies of *A. gossypii*, *T. aurantii* and *M. persicae* were observed which is in accordance with Hermoso de Mendoza et al. (2006) findings where the effect of these aphid species was also negligible. The relatively high density of *A. spiraecola* may be probably attributed to the prevailing favorable temperatures (Fig. 1). A climate conditions corresponding to the optimal range of temperature for *A. spiraecola* population growth reported in Wang and Tsai (2000) study. The density of infested leaves always exceeded the intervention threshold which was established between 5 and 10% according to Barbagallo et al. (2007).

The impact of parasitoids on *A. spiraecola* populations was very limited (below 3%) without significant difference between the two years. This in agreement with Gómez-Marco (2016) finding reporting that in Spain this parameter did not exceed 5%.

In this study, *L. testaceipes* was detected in 2016 and 2017 surveys. As for its historical introduction to the Mediterranean basin, this parasitoid originated from Cuba was introduced in the south of France in 1973 for controlling citrus aphids (Starý et al. 1988). However, it was rarely able to achieve its development on *A. spiraecola* (Boukhris-Bouhachem 2011; Costa and Starý 1988; Michelena and Sanchis 1997; Tremblay and Barbagallo 1983) which could explain the high rate of mummies without adult emergence noted in the current study (Figs. 10 and 11). This failure may be due to a physiological parasitic inadequacy (Starý et al. 1988). Endocrine effects could arrest the development of many parasitized insects

and prevent them reaching the adult stage (Beckage 1985). This low rate of *A. spiraecola* parasitism could probably be attributed to defensive symbiosis in aphid. In fact, as firstly demonstrated by Oliver et al. (2003), the pea aphid *Acyrthosiphon pisum* was found to harbor heritable infections with bacterial endosymbionts that are involved in their increased resistance to the parasitoid *Aphidiuservi* commonly employed as a biocontrol agent (Vorburger 2017). More recently, a study on intraspecific variation in facultative symbiont infection among native and exotic aphid populations showed the presence of the bacterium *Arsenophonus* sp. in native *A. spiraecola* infesting citrus and interestingly the main endosymbionts bacteria found in most aphid species were absent from *A. spiraecola* (Desneux et al. 2017).

As demonstrated in the current study, *B. angelicae* was not found in 2017. It was also absent in Tunisia (Sellami et al. 2013). Interestingly, in Spain, it was the only primary parasitoid of *A. spiraecola* (Gómez-Marco 2015). The variation of specific richness of primary parasitoid is not clear because of the complexity of the relationship between primary and secondary parasitoids. Other factors could be implicated. The hyperparasitism rate (25%) including 6 species is one of factors making ineffective the control by primary parasitoids. It remains also a problem in Spain with a hyperparasitism of 40% (Gómez-Marco 2015).

Our data are yet insufficient to reach any definitive conclusion concerning the dominance of *A. spiraecola* populations occurring in citrus crops; this study strongly suggested that multiple factors and processes are involved over the season, location and years. Citrus producers should have a good knowledge on IPM-based protection because their basic education is not sufficient to achieve higher efficiency in citrus production.

ACKNOWLEDGMENTS

This study was partially supported by the University of Mostaganem. We would like to express our gratitude to Dr. Mar Ferrer-Suay from the Universitat of Valencia, Faculty of Biological Sciences (Spain) for the identification of hyperparasitoids from Algeria. Thanks to Dr. Bernard Chaubet from INRA Rennes (France) for his help in confirmation of the primary parasitoids.

RESUME

Labdaoui Z.E. et Guenaoui Y. 2018. Quelques observations relatives à la dominance du puceron *Aphis spiraecola* sur agrumes dans le nord-ouest de l'Algérie. Tunisian Journal of Plant Protection 13 (si): 147-157.

Aphis spiraecola est le puceron le plus dominant dans les vergers d'agrumes en Algérie. La présente étude a été réalisée en verger de clémentinier *Citrus clementina* dans la région nord-ouest d'Algérie, durant deux années (2016-2017), pendant la première poussée de sève. Les fluctuations des populations des pucerons et de leurs ennemis naturels en particulier, les parasitoïdes, ont été évaluées sur la base d'un échantillonnage hebdomadaire, effectué sur 100 feuilles prélevées sur 10 arbres à raison de 10 feuilles/arbre. *A. spiraecola*a colonisé les arbres du clémentinier dès l'apparition des premières pousses. La densité moyenne maximale a atteint 78,8 ± 23,4 pucerons par feuille en 2016 et de 44,4 ± 13,0 pucerons par feuille en 2017, ce qui représente respectivement une densité d'environ 6,0 ± 1,5 pucerons et 4,4 ± 0,6 pucerons/cm² montrant une différence significative entre les deux années (P <0,05). Le taux de parasitisme exprimé en termes de nombre de momies d'*A. spiraecola* a été très faible, variant entre 1,6% en 2016 et 3,0% en 2017 mais aucune différence significative entre les deux années n'a été enregistrée (P >0,05). L'émergence des parasitoïdes primaires a été faible pour les deux années avec un pourcentage de 26,6% en 2016 et de 10,8% en 2017 ; cette différence n'est pas

significative entre les deux années ($P > 0,05$). *Lysiphlebus testaceipes* et *Binodoxys angelicae* sont les seules espèces de parasitoïdes primaires trouvées en 2016. Or, seulement *L. testaceipes* a été trouvé en 2017. Le pourcentage des hyperparasitoides a varié entre 16,67% en 2016 et 25,71% en 2017 sans aucune différence significative ($P >0,05$). Le taux de momies sans émergence d'adultes a été très élevé variant entre 85 et 100%. L'échec partiel parasitaire observé sur *A. spiraecola* suscite de nombreuses questions liées à la fois au climat, à la faculté du puceron à former des populations ailées et à la présence précoce des hyperparasitoïdes. On peut également évoquer une possible présence d'organismes endosymbiontes qui peuvent conférer au puceron une réaction de défense contre ses agresseurs.

Mots clés: Agrumes, *Aphis spiraecola*, parasitoïdes primaires, parasitoïdes secondaires, nord-ouest de l'Algérie

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ملخص

لعبداوي، زين الدين ويمينة قناوي. 2018. بعض الملاحظات حول هيمنة المن *Aphis spiraecola* على القوارص/الحمضيات في منطقة الشمال الغربي للجزائر

Tunisian Journal of Plant Protection 13 (si): 147-157.

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تعتبر حشرة المن *Aphis spiraecola* النوع المهيمن على بساتين القوارص/الحمضيات في الجزائر. أجريت هذه الدراسة على ديناميات مجموعات المن في بستان كليمنتين *Citrus clementina* بمستغانم بشمال غرب الجزائر وذلك على مدار عامين 2016-2017 في الفترة الأولى للدفع النسغي. أجريت معاينة أسبوعية على أساس 100 ورقة مأخوذة من 10 أشجار (10 أوراق/ شجرة). لوحظ استيطان *A. spiraecola* على أوراق أشجار الكليمنتين منذ بداية خروج البراعم الصغيرة. سجلنا معدل حوالي 78,8 ± 23,4 من/ورقة في 2016 و44,4 ± 13,0 من/ورقة في 2017 والذي يمثل على التوالي 6,0 ± 1,5من/سم^2 و4,3 ± 0,6من/سم^2 مما يظهر فرقا معنوي بين السنتين ($p<0,05$). كان معدل التطفل الذي عبر عنه من حيث عدد المومياوات *A. spiraecola* ضعيفا جدا يتراوح بين 1,6 % في 2016 و 3,0 % في 2017، ولا يوجد فرق بين السنتين ($p>0,05$). كان بزوغ أشباه الطفيليات الأولية ضعيفا في كلتا السنتين بمعدل 16,7 % في 2016 و 25,7 % في 2017، ولم يبين التحليل الاحصائي اختلاف بين السنتين ($p> 0,05$). تم العثور على نوعين من أشباه الطفيليات الأولية *Lysiphlebus testaceipes* و *Binodoxys angelicae* في 2016. كان شبه الطفيلي الأولي *L. testaceipes* الوحيد الذي تم العثور عليه في 2017. تراوحت نسبة أشباه الطفيليات الثانوية بين 16,7% في 2016 و25,7% في 2017، ولم يوجد اختلاف بين السنتين ($p<0,05$). كان معدل المومياوات بدون بزوغ أشباه الطفيليات مرتفعا جدا وتراوح بين 85-100%. يطرح الفشل الجزئي للتطفل على *A. spiraecola* عدة أسئلة متعلقة بالمناخ وقدرة المن على تشكيل مجموعات مجنحة وربما وجود كائنات تكافل داخلي تعطي للمن مقاومة ضد مهاجميه.

كلمات مفتاحية: أشباه الطفيليات الأولية، أشباه الطفيليات الثانوية، حماية متكاملة من الآفات، شمال غرب الجزائر، قوارص/حمضيات، *A. spiraecola*

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Contribution to the Study of a New Date Palm Pest *Oryctes agamemnon* in the Palm Groves of El-Oued, Algeria

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ABSTRACT

Chouia, A., Guerfi, Z., and Sadine, S.E. 2018. Contribution to the study of a new date palm pest *Oryctes agamemnon* in the palm groves of El-Oued, Algeria. Tunisian Journal of Plant Protection 13 (si): 159-170.

A survey concerns *Oryctes agamemnon* in El-Oued governorate (Algeria) next to the border of Tunisia. Its aim is to study the spread and the damages caused by this pest, in three stations: Taleb Larbi, Ben Guecha, and Douar El-ma. In all 60 prospected farms, the insect damage was estimated at an average of 53.75%. The highest level of infestation was recorded in Taleb Larbi and Douar El-ma with 75 and 65%, respectively. However, Ben Guecha station was marked by the lowest rate of infestation and this was probably due to the presence of the Ghouts-type farms and/or the sandy and relatively wet soils increase juvenile stages proliferation. It has been also noted that the third larval stage was the most harmful stage due to its long development duration and its voracity. The difference in the recorded infestation rate can be explained by the geographical location nearby the Tunisian infested palm groves, soil nature, date palm seedlings origin and / or the poorly maintained palm groves. Concerning the varietal sensitivity, Deglet Nour exhibited the most serious damage in comparison with Ghars variety with severe infestations on roots and trunks. Therefore, the real threat of this insect will be the infestation expansion to new palm groves where the damage can be severe.

Keywords: Algeria, date palm, infestation, *Oryctes agamemnon*, survey

Rhinoceros beetles constitute a group of medium to large sized scarabs of the Dynastinae (Rochat et al. 2004). Among rhinoceros beetles, the genus *Oryctes* includes about 40 species (Endrodi 1985) but only some of them have a real impact on the development of palm trees (Balachowsky 1962).

In certain Gulf countries (United Arab Emirates, Sultanate of Oman and Kingdom of Saudi Arabia), several species develop within oases (Al-Sayed and Al-Tamiemi 1999).

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However, damages caused by *Oryctes* to palm trees are economically secondary and of less importance alone but it attracts the xylophagous pest *Rhynchophorus ferrugineus* for oviposition which leads to the palm dying (Soltani and Ben Hamouda 2015).

In the Maghreb countries, *Oryctes agamemnon arabicus* was accidentally introduced during the last three decades in Djerid region (Tunisia), via off-shoots of new varieties imported from United Arab Emirates oases (Khoualdia and Rhouma 1997). In Algeria, *O. agamemnon* was discovered for the first time in 2013 in some oases of Taleb Larbi belonging to El-Oued governorate (INPV 2014). The present study aimed to

determine the current situation of the attack relative to this pest in the region of Taleb Larbi (El-Oued) which represented the primary focus in Algeria, to monitor its damage and impacts on date palm trees, and to determine the infestation rates of different infested parts by station and by grown varieties.

MATERIALS AND METHODS

Study area.

The study occurred in the region of Taleb Larbi, El-Oued governorate. It is located in the South East of Algeria and bordered with Tunisia by Tozeur and Kebili governorates. The concerned frontier with Tunisia extends for about 300 km (Fig. 1).

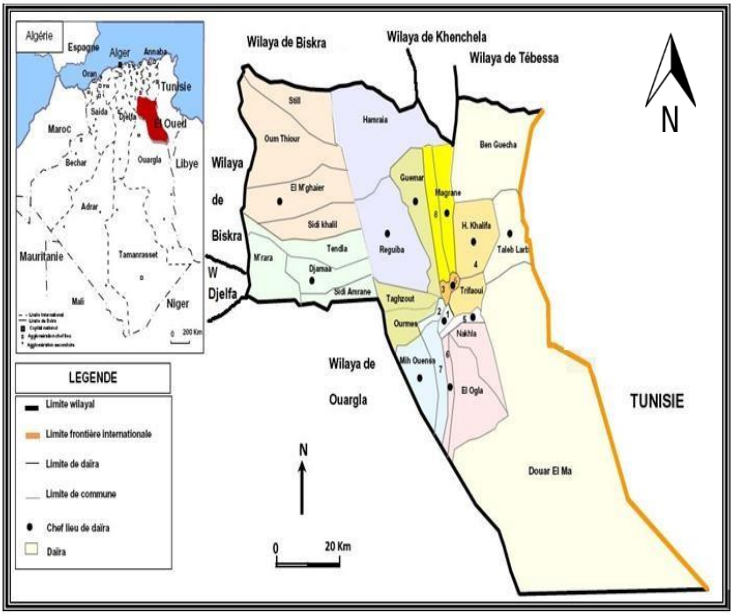

Fig. 1. Administrative limits of the region of Taleb Larbi (Anonymous 1997)

The survey was carried out in three stations: Douar El-ma, Taleb Larbi, and Ben Guecha which are located on the frontier with Tunisia on the side of Rjim Maatoug and El-Matrouha (Kebili governorate) and Hezoua (Tozeur governorate).

Plant survey.

In the three stations of the Taleb Larbi area, the study was carried out in 60 farms. In each farm, four palm trees were chosen randomly taking into account the observed symptoms either on the trunk or

on roots. The work was focused on the two most dominant varieties Deglet Nour and Ghars. The selected samples of palm trees in each grove were equitably divided between them.

Insect sampling.

Sampling of different stages of *O. agamemnon* was carried out on crowns, trunks and roots of date palm trees with different infestation levels. Insect survey concerned its different developmental stages and was performed as follow. At the palm tree bases, the sand surrounding the trunk was moved using a shovel until the appearance of the superficial roots. Searching operation for the different insect stages was carried out between the root biomass using a pickaxe to confirm their existence or not at 40 cm deep. Once observed, the extraction and the collection of insect samples were achieved using a large forceps. At the trunk level, the searching operation was carried out manually by eliminating the fibrillium layers and lifting petioles using a pickaxe. For the inspection of the crown, presence or absence of insect was checked between palms.

Estimation of infestation rate.

In order to determine the infestation rate (I.R) by *O. agamemnon* in the surveyed date palm oases, the two following formulas were used. In fact, to calculate the general rate per region (for 240 palm trees) and per station (80 palm trees): IR (%) = (Number of infested palm trees/Total number of surveyed palm trees) × 100.

For each studied variety, it is calculated as: IR = (Number of infested palms/Total number of surveyed palms) × 100.

Statistical analysis.

Data collected from the oases were presented as means ± SD (Standard Deviation) of percentage. They were also subjected to ANOVA analysis. Means were compared using a Least Significant Difference (LSD) test at $P \leq 0.05$.

RESULTS

Surveys made at the different prospected areas showed that *O. agamemnon* is a monophagous species surviving strictly linked to date palm trees.

Infestation rate per surveyed site.

The general rate of infestation registered in the different surveyed sites of the region of Taleb Larbi was 53.75%. Fig. 2 indicates that the highest rate of infestation was recorded in Taleb Larbi (with 75 ± 38%) and Douar El-ma (with a rate of 65 ± 18.62%). The lowest rate (21.25 ± 30.65%) was noted in Ben Guecha. Statistical analysis using ANOVA, revealed the existence of a significant difference between the study stations ($df = 2$, $F = 27.23$, $P \leq 0.05$) as indicated by the two groups shown in Fig. 2.

Infestation rate per variety.

The results of infestation rates by variety for the three surveyed sites are summarized in Fig. 3. In fact, the comparison between the studied varieties in different surveyed sites showed that *O. agamemnon* had a marked preference to Deglet Nour variety in comparison to Ghars. Also, as given in Fig. 3, Deglet Nour and Ghars grown at Taleb Larbi site showed the highest rate of infestation with 90 ± 26.16% followed by Douar El-ma with 77.50 ± 25.52%.

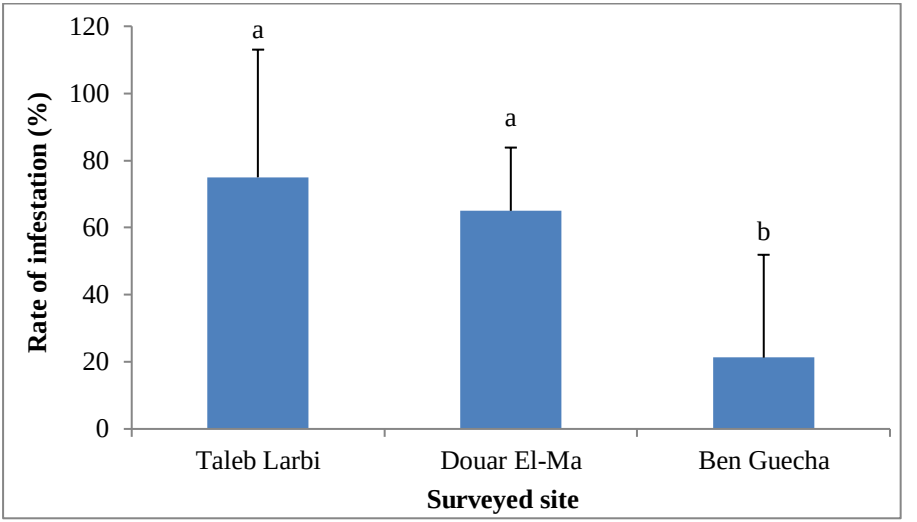

Fig. 2. Estimated rate of infestation with *Oryctes agamemnon* in the surveyed sites. Bars sharing the same letter are not significantly different based on LSD test at $P \leq 0.05$.

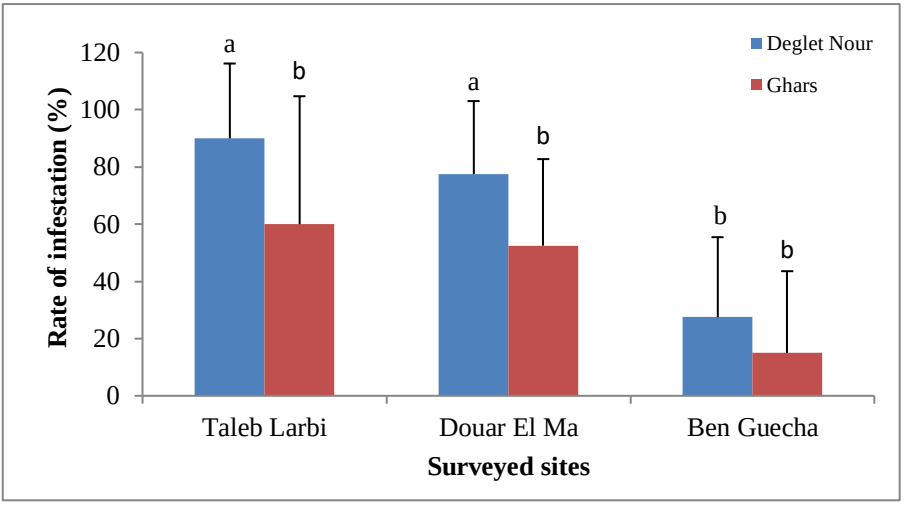

Fig. 3. Infestation rates of two date palm varieties in the three surveyed sites. For each surveyed site, bars sharing the same letter are not significantly different based on LSD test at $P \leq 0.05$.

It can be deduced that Deglet Nour trunk is strongly attacked by *O. agamemnon* compared to that of Ghars variety. This difference of attacks can be explained in part, by the circumference of Ghars trunk which is very large in comparison to that of Deglet Nour. Additionally, woods of both petioles and trunk of Ghars were harder than those of Deglet Nour (Fig.4).

Fig. 4. Variation in the infestation level of date palms trees by *Oryctes agamemnon* depending on varieties.

Infestation rate per organ.

The damage caused by *O. agamemnon* was invisible and very difficult to detect in the beginning of attacks because larvae grown in hidden places. Based on surveys made on date palm trees in the different sites, infestations were localized at different levels throughout the plant starting from roots to the trunk but no infestation was recorded on the crown.

Trunk symptoms.

In general, attacks of the trunk concerned dead tissues such as fibrillium, the bark of the trunk and the base of dry petioles. The trunk attacks were easily identified by the presence of superficial cavities on the dry petiole that did not exceed 2 cm deep commonly caused by adults (Fig. 5).

Fig. 5. Cavities induced by *Oryctes agamemnon* on a date palm trunk.

Statistical analysis using ANOVA, shows the existence of significant difference between study stations (df = 2, F = 190.44, $P \leq 0.05$). In fact, according to Fig.6, the highest rate of infestation in Taleb Larbi was estimated to 93.75 ± 15.97% followed by Douar El-ma with 78.75±14.68% whereas the lowest infestation rate (10 ± 12.56%) was recorded in Ben Guecha.

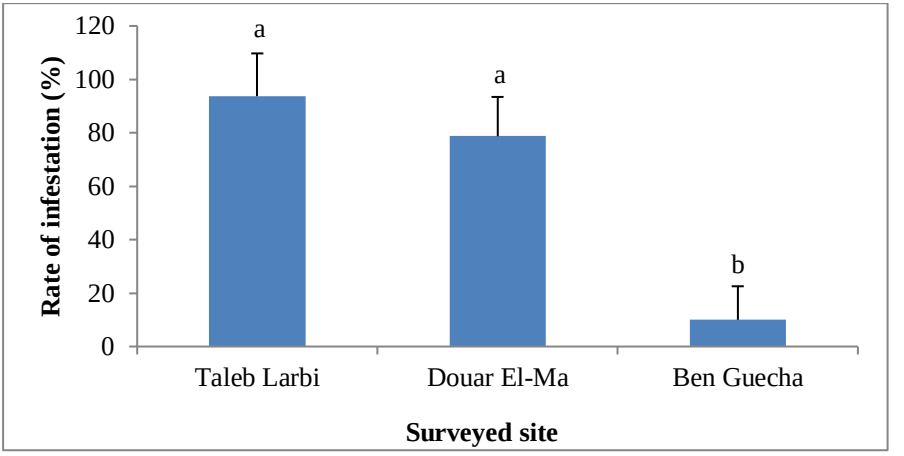

Fig. 6. Infestation rate of the date palm trunks in the surveyed sites. Bars sharing the same letter are not significantly different based on LSD test at $P \leq 0.05$.

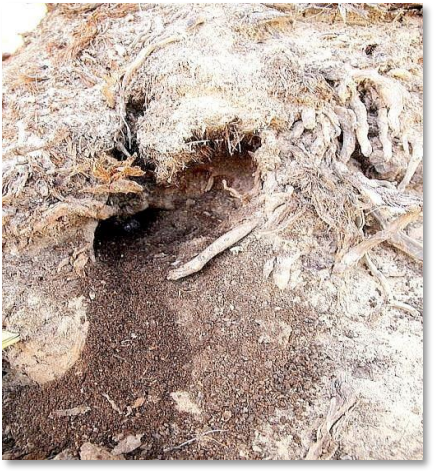

Fig. 7. Traces of brown powder on the ground and around date palm roots infested with *Oryctes agamemnon*.

Root symptoms.

The respiratory roots constitute the main environment for the survival and development of the insect. This part was a source of food for the offspring of the insect because it is composed of soft and moist root hair. The larvae infest the aerial roots at the base of the trunk as indicated by the traces of brown powder, later leading to yellowing and drying all the palms of the crown.

The repetition of root attacks for many years is a potential hazard that can lead to imbalance by weakening its basal support and then total palm drying (Fig. 7).

Statistical analysis revealed the existence of a significant difference between the surveyed sites ($df = 2$, $F = 27.23$, $P \leq 0.05$). Date palm trees grown in Taleb Larbi and Douar El-ma sites were the most heavily attacked at the root level as expressed by the infestation rates of 75 ± 38 and 65 ±18.62%, respectively (Fig. 8). However, those of Ben Guecha showed the lowest infestation (21.25 ± 30.65%).

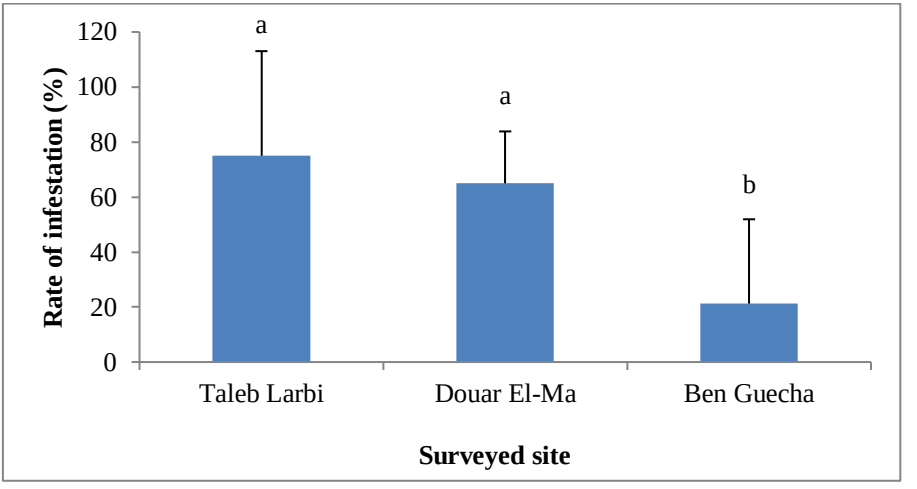

Fig. 8. Infestation rate of the date palm roots in the surveyed sites. Bars sharing the same letter are not significantly different based on LSD test at $P \leq 0.05$.

Off-Shoot symptoms. The off-shoots are the main point of entry above all in the basal zone of the roots before the weaning operation (Fig. 9).

Fig. 9. The total fall of a date palm tree due to a severe *Oryctes agamemnon* infestation.

Off-shoots are grown naturally in tuft at the base of the palm tree and they are rarely maintained by the farmer. For this reason, they are considered the most targeted places by the insect because these tufts are dirty, wet, dark, and hard to reach by both farmer and other insect species.

Tuft of off-shoots non maintained | Off-shoot weaning operation | Dry palms and death of the off-shoots

Fig. 10. Stages of off-shoots infestations by *Oryctes agamemnon*.

Advanced stage of offshoots attacks led to the deformation and the stunting of the green palms but in case of total consumption of its internal basal part, date palms turn yellow, dry out and perish (Fig. 10). Effectively, the heavy attacks were localized in the new plantations as noted in the zone of Ben Guecha (Fig. 11).

Fig. 11. Stages of infestation of off-shoots by *Oryctes agamemnon* larvae.

DISCUSSION

O. agamemnon is a new pest recently introduced in date palm oases in the South East of Algeria. During field surveyed undertaken in different sites where 240 date palms were sampled, 129 trees showed symptoms of attack by this pest. Thus, the infestation rate of the surveyed areas was estimated at 53.75%.

The high rates of infestation recorded in the oases of Taleb Larbi and Douar El-ma can be explained by the type of sampled palm groves called Ghouts. This specific culture system was characterized by a relatively deep soils and sandy physiognomy. This environment is characterized by its favorable conditions of humidity, darkness and moderate temperature which attracted this insect species and offered suitable environment for the development of its all stages mainly larvae. However, the low rate of infestation found in the oases of Ben Guecha can be explained, in part, by the clay texture of soil, hard and compact, that does not facilitate the survival of this insect.

As for the variation of *O. agamemnon* attacks per organs and especially at the crown level, no attack was recorded in different sites although the occurrence of favorable environmental conditions and the sufficient amounts of food. These findings are in agreement with those of same Khalaf et al. (2013) in Iraq. In general, attacks at this level interested dead tissue such as the inner part of the dry petioles and the fibrillium.

Bedford et al. (2015) reported that *O. agamemnon* adult generally dig the trunks. Khalaf et al. (2013) also demonstrated that the insect infests the middle of the trunk in the young ages and old palms. These cavities provide access to subsequent generations of adults for entry and oviposition. Groups of adults, mainly ovipositing females, can coexist in these cavities, along with larvae, and the continuous feeding and tunneling activity by larvae can lead to collapse of the palm within few years (Ehsine et al. 2009; Soltani 2009).

The larval stages infest the aerial roots at the base of the trunk due to the presence of frond bases near the soil surface. However, in Iran, larvae of the *Oryctes* rhinoceros live in the crown and

trunk, feeding on the petioles and on the palms, and this injury can allow the entry of fungi and secondary insect pests (Bedford et al. 2015).

Larval feeding activity is known to cause damage to aerial roots and dry petioles (Soltani 2009, 2010). In the same context, Surany (1960) reported that *Oryctes* spp. are generally not very active and do not go out and that most of them do not feed in their relatively short adult lives (Balachowsky 1962, Howard et al. 2001).

As for varietal preferences, this study revealed different degrees of attack by this pest between the variety Deglet Nour, which is the most attractive, compared to Ghars. This difference is probably due to the vigorous characteristics and the trunk girth which is very important in comparison with Deglet Nour. In fact, the wood of the petioles of Ghars is harder than that of the Deglet Nour.

According to Soltani (2004), a similar study was carried out in the date palm oases of Mrah Lahouar (South-Tunisia) revealed similar results with a rate of 44.45% among the pollinators (Dokkar) and 30% for Deglet Nour.

However, the irremediable danger of this species is the infestation of new plantations where the risk can reach very important percentages (Soltani 2009).This is the case of groves of Ben Guech a site where the novel plantations are imported from an infested area.

RESUME

Chouia A., Guerfi Z. et Sadine S.E. 2018. Contribution à l'étude d'un nouveau ravageur de palmier dattier *Oryctes agamemnon* dans les palmeraies d'El-Oued, Algérie. Tunisian Journal of Plant Protection 13 (si): 159-170.

Cette étude concerne l'*Oryctes agamemnon* dans la gouvernorat d'El-Oued (Algérie) avec la frontière tunisienne. Le but est d'étudier la propagation et les dégâts causés par ce ravageur dans trois stations d'études sont Taleb Larbi, Ben Guecha et Douar El-ma. Dans la totalité des 60 exploitations prospectées, le degré d'infestation a été estimé à 53,75%. Les plus élevés ont été enregistrés à Taleb Larbi et Douar El-ma avec 75 et 65%, respectivement. Cependant, la station de Ben Guecha s'est distinguée par le plus faible taux d'infestation. Cette différence peut être expliquée par la présence des exploitations des systèmes Ghouts et/ou les sols sablonneux et relativement humides favorisant la prolifération des stades juvéniles. Il a été aussi noté que la larve du troisième stade est la plus dommageable en raison de sa voracité et de la durée de son développement. La différence enregistrée dans le taux d'infestation peut être expliquée par la position géographique par rapport aux foyers tunisiens infestés, la nature du sol, l'origine des rejets et/ou le manque d'entretien des palmeraies. Concernant la sensibilité variétale, la variété Deglet Nour a présenté les dommages les plus graves par rapport à la variété Ghars avec une infestation sévère au niveau des racines et des troncs. Par conséquent, la véritable menace de cet insecte sera l'expansion de l'infestation vers les nouvelles plantations où les dégâts peuvent être plus graves.

Mots clés: Algérie, infestation, *Oryctes agamemnon*, palmier dattier, prospection

ملخص

شوية، عبد الواحد وقرفي الزبير وسعدين صلاح الدين .2018. مساهمة في دراسة آفة خنفساء وحيد القرن *Oryctes agamemnon* على النخيل التمر بولاية الوادي، الجزائر.

Tunisian Journal of Plant Protection 13 (si): 159-170.

هذا البحث يهتم بدراسة آفة خنفساء وحيد القرن (*Oryctes agamemnon*) بولاية الوادي (الجزائر) مع الحدود التونسية. الهدف هو دراسة انتشار هذه الآفة مع الخسائر التي تسببها بمناطق الدراسة الثلاث وهي الطالب العربي وبن قشة ودوار الماء. من خلال 60 مزرعة مستكشفة، تبين أن الضرر الإجمالي الناجم عن هذه الافة مقدر بـ 53.75%، حيث سجل أعلى معدلات الإصابة في منطقة الطالب العربي ودوار الماء على التوالي 75 و65% ولكن بالمقابل سجل أدنى معدل إصابة في منطقة بن قشة. هذا الاختلاف يمكن تفسيره بوجود مزارع من نوع الغوط الذي يتميز بالتربة الرملية والرطوبة النسبية التي تساعد على تكاثر وانتشار أطوار هذه الحشرة. إن الطور الثالث للحشرة هو الأكثر ضرر بسبب شراهته ومدة حياته المعتبرة. كما يعزى هذا الاختلاف في الإصابات المسجلة إلى الموقع الجغرافي للمنطقة وقربه من الحدود التونسية المصابة، طبيعة التربة، مصدر فسائل النخيل المجلوبة من المناطق المصابة أو إلى عدم القيام بعمليات التنظيف والصيانة لمزارع النخيل. فيما يخص حساسية الأصناف المدروسة لهذه الآفـة، يلاحظ ان الضرر الأكثر قد سجل على صنف دقلة النور مقارنة بصنف الغرس مع تسجيل درجة إصابة مرتفعة وخطيرة على مستوى الجذور والجذع. من ناحية أخرى إن مجمل فسائل النخيل المغروسة حديثا في المنطقة تزداد فيها نسبة الإصابة بهذه الآفة إلى درجة خطيرة.

كلمات مفتاحية: استكشاف، إصابة، الجزائر، نخيل التمر، *Oryctes agamemnon*

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Oak Forests Infestation by *Tortrix viridana* and its Performance on Three *Quercus* Species

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ABSTRACT

Mannai, Y., Ezzine, O., and Ben Jamâa, M.L. 2018. Oak forests infestation by *Tortrix viridana* and its performance on three *Quercus* species. Tunisian Journal of Plant Protection 13 (si): 171-181.

The Tunisian oak forests are susceptible and sensitive to invasion of various pests, among them, the green oak leaf roller, *Tortrix viridana*, which is considered one of the most destructive defoliators of oak trees in the western Palearctic region. Important defoliations caused by this pest have been observed in different cork oak forests of north-western Tunisia. The study was carried out in five native cork oak (*Quercus suber*) forests (Ain El Baya, Bellif 1, Bellif 2, Bellif 10, and El Jouza), one zeen oak (*Q. canariensis*) forest Mzara and the Ain Zena forest which is a mixed cork oak, zeen oak and afares oak (*Q. afares*) located in the north-west of Tunisia. The average number of larvae per branch varied significantly between sites (6.6 ± 0.77 larvae/branche in El jouza and 0.11 ± 0.05 larva/branche on *Q. afares* in Ain Zena forest). Budburst timing did not differ between *Q. canariensis* and *Q. afares* whereas that of *Q. suber* occurred about 3 weeks later than the other two species. As the host plant phenology differs from species to another, the larval survival and their development are likely different across the host plant species. Feeding performance of the green oak leaf roller was determined on three host plants including *Q. suber*, *Q. canariensis* and *Q. afares* under laboratory conditions. Larvae grew best on *Q. suber*. Total larval development time from the 1st to the 5th instar was shorter on *Q. suber* (24.92 ± 0.26 days) than on *Q. canariensis* (26.69 ± 0.29 days) and *Q. afares* (29.03 ± 0.39 days). Larval mortality of the three host plants did not differ significantly while for the pupal weight, data showed a significant difference where 35.52 ± 9.44, 30.43 ± 8.25, and 22.95 ± 5.34 mg of pupae from the larvae reared on *Q. suber*, *Q. canariensis* and *Q. afares* were noted, respectively.

Keywords: Infestation, oaks, performance, *Tortrix viridana*, Tunisia

Early spring feeding Lepidoptera demonstrate synchronization of larval emergence with host leaf flush (Jones and Despland 2006). They can show reduced survival and performance if they miss the phenological window of young foliage (Scriber and Slansky 1981). In Tunisia, oak forests are subjected of various aggressions, mainly by insect attacks. The early spring green oak moth, *Tortrix viridana*, is one of the most important defoliators of oaks in the western Palearctic region (Du Merle 1999a). Their larvae can cause complete defoliation during outbreaks (Rubtsov and Utkina 2003). Significant defoliations caused by this insect have been observed in different

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cork oak forests of north-west of Tunisia (Mannai et al. 2010). *T. viridana* has one generation per year (Mannai et al. 2010). Females laid their eggs preferentially on the twig tips and buds of oak trees during the spring. The insect overwinter as eggs on branches, and they hatch in mid-March (Du Merle 1999a; Hunter 1990; Ivashov et al. 2002). Budburst timing varies among and within tree species (Van Dongen et al. 1997). The first larval stage needs newly opened buds for successful development (Hunter 1990). Advances or delays in leafing are important for insect life cycles (Foster et al. 2013). Many studies were investigated on the close coincidence that occurred between the budburst and *T. viridana* larval hatching (Du Merle 1983; Hunter 1992; Ivashov et al. 2002).

Study of the effect of food on the biology of insects is critical for understanding host plant suitability for herbivore species (Yazdanfar et al. 2015). *T. viridana* is oligophagous feeding on oak species (Du Merle 1999b). The quality of trees varies greatly within this plant genus (Kapeller 2009). Information on the process of oak leaf roller infestation in forests is critical for forest management activities (Gooshbor et al. 2016). In Tunisia, Mannai (2017) showed that cork oak (*Quercus suber*), zeen oak (*Q. canariensis*), and afares oak (*Q. afares*) were attacked by *T. viridana* larvae. Besides, there is very little information on the outbreaks of this pest in these forests. Therefore, to estimate the risk of defoliation, we studied the infestation and compared the performance of the oak leaf roller larvae fed on leaves of three oak species of ecological and economic importance. In this research, we studied the larvae density on host plants and their performance and monitored their development time, mortality and pupal weight on the three host species namely *Q. suber*, *Q. canariensis*, and *Q. afares*.

MATERIALS AND METHODS

Study area.

The study was carried out in five native cork oak forests (Ain El Baya, Bellif 1, Bellif 2, Bellif 10 and El Jouza), one zeen oak forest Mzara and the Ain Zena forest which is a mixed cork oak, zeen oak and afares oak located in the north-west of Tunisia (Table 1).

Table 1. Ecological characteristics of the different studied forests

Site	Type of forest	Long.	Lat.	Alt	P	T	Bioclimatic stage
Ain El Baya	cork oak	8°938'	36°65'	380	600	18.8	Sub-humid to temperate winter
Bellif 1	cork oak	9°538'	37°152'	73	1000	18	Wet, sub-floor to mild winter
Bellif 2	cork oak	9°074'	37°029'	162	1000	18	Wet, sub-floor to mild winter
Bellif 10	cork oak	9°014'	37°04'	199	1000	18	Wet, sub-floor to mild winter
El Jouza	cork oak	9°015'	36°513'	550	1222	16	Wet inferior than temperate and mild winter
Mzara	zeen oak	8°72'	36°769'	653	950	16.75	Fresh wet
Ain Zena	cork oak, zeen oak, afares oak	8°515'	36°435'	924	1780	16.1	Wet at mild and temperate winter

Long.: Longitude; Lat.: Latitude; Alt.: Altitude (m), P.: Precipitation (mm), and T.: Temperature (°C)

Budburst phenology and larvae collect.

In 2010, 2011 and 2012, samples of branches were taken weekly from mid-March to the end of April, for six weeks (W1-W6) to collect larvae and nine weeks for budburst, until the first of May (W1-W9). Every week and per host species, two branches from 10 mature trees, one low-level branch (2.5 to 5 m) and one from crown height level (> 5 m) were randomly monitored (Mannai et al. 2017).

Larvae density.

From 2009 to 2013, samples were taken weekly from the beginning of spring to the beginning of summer. Every week, one branch from 10 mature trees per host species was randomly monitored. The branches were carefully cut and conserved in a large plastic bags to prevent losing larvae. In the laboratory, larvae were counted and set in breeding (Mannai et al. 2017).

Laboratory feeding trials.

The performance of *T. viridana* on the three hosts: *Q. canariensis*, *Q. afares* and *Q. suber* was compared in laboratory by feeding trials. Experiments were performed in the spring of 2010, coinciding with the budburst of the host plants. Buds hosting neonate larvae were collected in the field from the three host plants in March 2010. A total of 90 larvae were used for each tested plant. Larvae (n = 270) were individually placed in Petri dishes, kept at 25 ± 2°C in light regime of 12:12 h L:D as in natural conditions and they were reared ad libitum on leaves of the three tested plant species. Young still-expanding leaves were collected daily from plantlets of each species, planted in the same conditions at the nursery of *the Institut National de Recherches en Génie Rural, Eaux et Forêts*, Tunisia. Larvae were checked daily and the number of molted larvae was recorded. Larval development time for each tested oak species was reported. To evaluate larval performance, development time, larvae survival and pupal weight of the 5th instar larvae were assessed on each tested species as biological parameters (Mannai et al. 2017).

Statistical analysis.

The statistical analysis was performed using the SPSS-10.0 software package for Windows. Generalized linear models (GLMs) were applied to the following dependent variables: (1) the Julian day when 50% of budburst occurred (2) the number of larvae per branch, considering the factors host species and year; (3) larvae development (number of days spent in each instar), considering the factor plant species. A normal distribution model best fitted the Julian day when 50% of budburst occurred and larvae development. A Poisson distribution model best fitted the number of larvae per branch. The effect of each tested oak species on the larval development time and the pupal weight were assessed with an analysis of variance (ANOVA) and complemented by multiple comparisons of means by the Student-Newman-Keuls (SNK) test and was expressed as mean ± SE of mean (MSE).

The proportion of dead larvae among the total individuals obtained in the feeding experiments was analyzed by GLM using a Binomial model with log-link function, considering the factor plant species. Results are presented in the form of the Wald's chi-square test value (χ^2), parameter estimates and the respective *P* value.

RESULTS

Budburst variation.

The Julian day when 50% of budburst occurred varied between forests ($\chi^2_2 = 2163.043$, $P < 0.001$) and years ($\chi^2_2 = 2813.4578$, $P < 0.001$). The interaction term was also significant ($\chi^2_4 = 5418.789$, $P < 0.001$). It varied also in Ain Zena forest between host plants ($\chi^2_2 = 13.03$, $P < 0.001$) and years ($\chi^2_2 = 13.78$, $P < 0.001$). The interaction term was also significant ($\chi^2_4 = 18.89$, $P < 0.001$). In the three Bellif forest sites, budburst began in early April, while it started in mid-April and end of April, depending on the year, in Ain El Baya. Budburst in El Jouza occurred about 4-5 weeks later than Mzara (Table 2). In Ain Zena forest, budburst of *Q. afares* and *Q. canariensis* began in late March, but budburst of *Q. suber* occurred about 3 weeks later (Table 3). For all years, *Q. canariensis* and *Q. afares* budburst was before *Q. suber*.

Larval density on host plants.

Larvae in the Bellif 1, Bellif 2, Bellif 10, Mzara and Ain Zena sites were observed between the end of March and the end of May, whereas they were abundant from late spring until summer in Ain El Baya and El Jouza forests. In Ain Zena, no larva was collected on *Q. suber*. The average number of larvae per branch varied between sites ($\chi^2_5 = 433.888$, $P < 0.001$) and years ($\chi^2_4 = 1.081$, $P = 0.01$). The interaction between the two variables was also highly significant ($\chi^2_{20} = 74.455$, $P < 0.001$). El Jouza forest was the most infested in 2009. This rate was also important at Bellif 10. In 2010, the number of larvae decreased progressively in all the oak forests. This rate was also quite high in Bellif 10 and Bellif 2 sites between 2009 and 2011. The number of larvae was low in the Ain El Baya, Mzara and Ain Zena forests (Table 4).

Larval development and mortality.

This investigation highlighted that the total larval development time from the 1st to the 5th instar was shorter on *Q. suber* (24.92 ± 0.26 days) than on *Q. canariensis* (26.69 ± 0.29 days) and *Q. afares* (29.03 ± 0.39 days). The host species had a significant effect on larval development ($F_{(2,206)} = 245.84$, $P < 0.001$); for the 1st instar ($F_{(2,267)} = 4.25$, $P = 0.015$), 2nd instar ($F_{(2,254)} = 5.77$, $P = 0.004$), 3rd instar ($F_{(2,252)} = 6.28$, $P = 0.002$), 4th instar ($F_{(2,249)} = 11.99$, $P < 0.001$) and the 5th instar ($F_{(6,241)} = 4.67$, $P < 0.001$). For each instar larva, the development was faster on *Q. suber* than on *Q. afares* and *Q. canariensis* (Fig. 1).

Mortality of larvae reared on the three host plants was higher on *Q. canariensis* for the 1st, 2nd and 3rd instars of larvae than the other host species. For the 4th and 5th instars, mortality was higher on *Q. afares* than the other host species (Fig. 2).

Pupal weight.

Pupal weight varied significantly among host species ($F_{(2,233)} = 75.957$, $P < 0.001$). It was higher for pupae coming from larvae reared on *Q. suber* with an average of 35.52 ± 9.44 mg than for *Q. canariensis* (30.43 ± 8.25 mg) and *Q. afares* (22.95 ± 5.34 mg) (Fig. 3).

Table 2. Results of budburst monitoring (%) in native oak forests performed in 2010, 2011 and 2012

Time	Mzara			Bellif 1			Bellif 2			Bellif 10			Ain El Baya			El Jouza		
	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
S1	11. 2	19.3	15.07	0	0	0		0	0	0	0	0	0	0	0	0	0	0
S2	28.3	31.5	21.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3	49.2	40.14	42.1	14.33	14.17	22.91	0	0	0	0	0	7.51	0	0	0	0	0	0
S4	55.6	61.4	51.35	24.25	31.18	36	3.55	5.65	17.87	2.56	11.02	27.23	0	0	0	0	0	0
S5	67.1	67.3	63.3	43.68	52.55	41.3	19.74	22.81	37	13.19	27.77	40.56	0.85	1.94	20	0	0	1.54
S6	69.15	71.1	68.1	55.65	59.89	62.84	40.33	31.01	47.3	32.39	32.93	56..88	26.64	17.79	31.77	17.23	22.5	16.51
S7	74.02	72.78	69.4	61.46	64.5	64.47	52.67	45.68	51.3	50.04	57.94	60.31	45.79	23.77	47.46	21.68	25.5	31.77
S8	75.32	77.1	71.4	68.86	73.98	72.49	61.43	63.7	58.6	60.9	63	67.93	58.58	42.62	54.28	40.27	38.67	49.2
S9				71.64	75	72.7	62.66	69.76	62	63.29	70.5	69.8	65.96	57.69	62.3	52.82	57.88	57.27
S10				73.15	77.2	79.88	74.74	75.21	65	75	75	74.16	64.69	61.65	69.41	63.86	68	63.33
S11							76	76	70	78	79		70	77.1	71.39	69.1	71	67.51
S12													71	81.82	81.71	70.45	77	73.2
S13																73.4	78.21	

S1: 3rd week of March; S2: 4th week of March; S3: 1st week of April S4: 2nd week of April; S5: 3rd week of April; S6: 4th week of April; S7: 1st week of May; S8: 2nd week of May; S9: 3rd week of May; S10 :4th week of May; S11: 1st week of June; S12: 2nd week of June; S13: 3rd week of June.

Table 3. Results of budburst monitoring (%) of three *Quercus* species performed in Ain Zena in 2010, 2011 and 2012

Time	2010			2011			2012		
	Q. canariensis	*Q. afares*	*Q. suber*	*Q. canariensis*	*Q. afares*	*Q. suber*	*Q. canariensis*	*Q. afares*	*Q. suber*
S1	4.78	2.78	0	1.5	0.54	0	0	3.06	0
S2	11.53	6.14	0	2.38	8.52	0	1.74	10.7	0
S3	26.18	14.06	0	21.6	16.31	0	8.5	19.85	0
S4	44.68	34.28	0	53.77	42.36	0	22.12	30.7	0
S5	59.82	45.25	8.62	71.6	61.16	20.79	34.26	49.8	10.16
S6	72.12	61.67	24.36	76.33	70.78	40.68	45.1	59.84	18.08
S7	75.27	67.87	46.95	79	77.21	52.98	55.7	68.45	37.99
S8	78.48	77.14	53.82	81.77	79.1	61.83	68.39	73.64	48.8

S1: 3rd week of March; S2: 4th week of March; S3: 1st week of April S4: 2nd week of April; S5: 3rd week of April; S6: 4th week of April; S7: 1st week of May; S8: 2nd week of May.

Table 4. Larvae density (Main number of larvae ± SE per branch) in the studied sites between 2009 and 2013

Site	Quercus species	Year				
		2009	**2010**	**2011**	**2012**	**2013**
El Jouza	*Q. suber*	6.6 ± 0.77	5.53 ± 0.7	4.96 ± 0.83	2.43 ± 0.45	3.61 ± 0.57
Bellif 1	*Q. suber*	2.78 ± 0.41	2 ± 0.35	1.88 ± 0.28	1.65 ± 0.27	1.68 ± 0.29
Bellif 2	*Q. suber*	0.81 ± 0.18	1.28 ± 0.32	1.55 ± 0.31	1.31 ± 0.28	0.76 ± 0.17
Bellif 10	*Q. suber*	3.46 ± 0.5	2.76 ± 0.42	1.6 ± 0.33	1.1 ± 0.23	0.55 ± 0.15
Ain El Baya	*Q. suber*	0.56 ± 0.14	0.51 ± 0.16	0.85 ± 0.24	0.88 ± 0.24	0.78 ± 0.18
Mzara	*Q. canariensis*	0.23 ± 0.11	0.03 ± 0.03	0.13 ± 0.06	0.25 ± 0.09	0.21 ± 0.11
Ain Zena	*Q. canariensis*	0.15 ± 0.06	0.36 ± 0.1	0.51 ± 0.17	0.86 ± 0.22	0.75 ± 0.22
	Q. afares	0.05 ± 0.02	0.13 ± 0.05	0.13 ± 0.06	0.11 ± 0.05	0

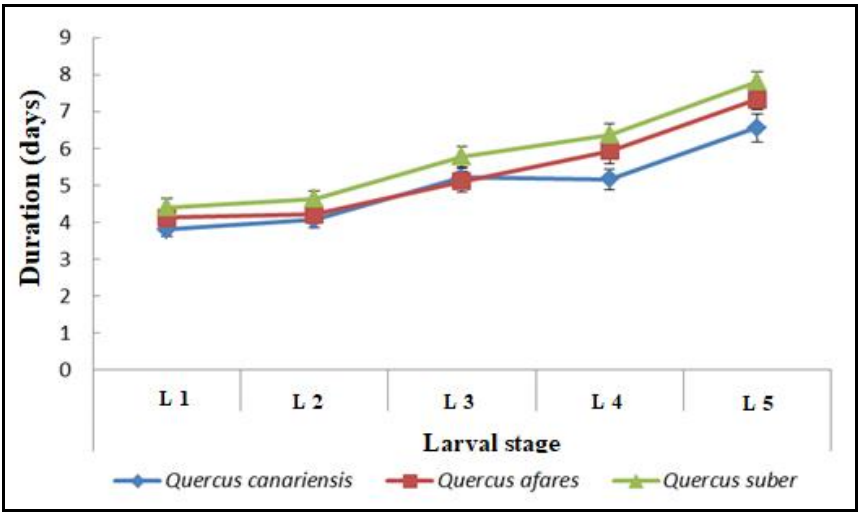

Fig. 1. Development time of *Tortrix viridana* in days (± SE) from 1st to 5th instar larvae on the tree *Quercus* species.

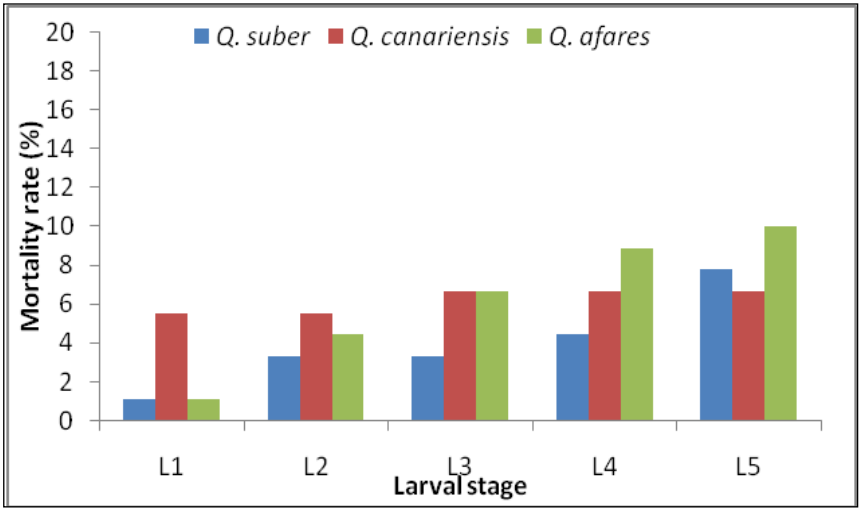

Fig. 2. Mortality rate of each larval stage from 1st to 5th instar larvae reared on the three *Quercus* species.

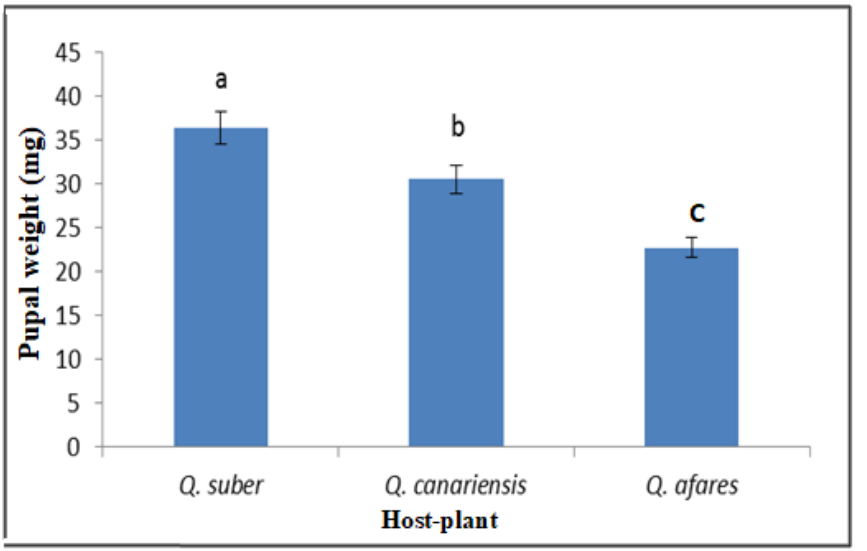

Fig. 3. Mean pupal weight in mg (± SE) of *Tortrix viridana* reared on the three *Quercus* species. Bars with different letters are significantly different based on SNK test at $P \leq 0.05$.

DISCUSSION

Development of many phytophagous insects is closely related with host plant phenology (Bale et al. 2002). Early or late budburst can directly affect both the quantity and quality of suitable food available to herbivores at specific times (Lawrence et al. 1997). Many studies focused on the interactions between budburst variation and the ecological consequences for trees and their associated phytophages (Du Merle 1983; Holliday 1985). The variation of altitude effects the leafing. In the three sites of Bellif forest, budburst began in early April and it was late in the sites in higher altitude (El Jouza and Ain El Baya forests) where the temperature was low. Budburst also varied depending on tree species. Our observations highlighted that in the Zen and Afares oaks budburst was observed before the cork oak. In the mixed forest of Ain Zena, budburst timing did not differ between *Q. canariensis* and *Q. afares* but for *Q. suber*, it occurred about three weeks later. Said (1993) and Mannai et al. (2017) reported that the budding of *Q. canariensis* and *Q. afares* precedes that of *Q. suber*. There was synchronization between budburst and larval hatching of *T. viridana* which can influence the number and intensity of outbreaks of forest pests (Forkner et al. 2008). Larvae in the Bellif 1, Bellif 2, Bellif 10, Mzara and Ain Zena sites were observed between the end of March and the end of May, whereas they were abundant from late spring until summer in Ain El Baya and El Jouza forests, because of the bursting of the oaks which is realized with a shift according to the geographical conditions mainly the altitude (Du Merle 1983) and the host plant (Mannai et al. 2017; Said 1993). Our results corroborate with several studies conducted on this subject that proved the variation of phenology of trees which is considered as the major determinant factor of the track

dispersal behavior (Du Merle 1983; Hunter 1990; 1992; Mannai et al. 2017).

Host plants affect development, survival and reproduction of phytophagous insects (Marchioro and Foerster 2014). In many tree species, there is considerable variation in the phenology of budburst which has profound effects on insect performance (Hunter 1992). Feeding performance of the green oak leaf roller was determined on three host plants including *Q. suber*, *Q. canariensis* and *Q. afares* under laboratory conditions. The shortest larval development time was recorded for larvae feeding on *Q. suber* which was the most infested host, while the longest development time was recorded for those feeding on *Q. afares* (Fig. 1). Yazdanfar et al. (2015) found that larval development of *T. viridana* was shorter for larvae fed on *Q. libani* and longer for those fed on *Q. brandi* and *Q. infectoria*. Larval development was clearly better on some hosts than on others (Kirsten and Topp 1991). This may be explained by the low density of *T. viridana* in Ain Zena and Mzara because of the absence or the low abundance of their main host (*Q. suber*). Larval mortality was not significantly different on host plants.

Pupal weights of *T. viridana* on *Q. canariensis*, *Q. afares*, and *Q. suber* were compared to investigate the suitability of these hosts for the green roller moth larvae. On *Q. suber*, pupae were the heaviest one with an average weight of 35.52 mg. Mannai et al. (2010) found that the mean pupal weight on *Q. suber* ranged from 24.07 mg to 32.15 mg and suggested that the host plants have a significant effect on the resulting size of pupae. *Q. canariensis* and *Q. afares* are relatively new host plants for this defoliator. There may not have been a selection for the time being for a match between the phenology of oak zen and oak afares and the larvae hatching. Our laboratory rearing also confirmed the low weight of pupae on these two host species.

Studies of Foss and Rieske (2003) on the feeding preferences of gypsy moth in North America indicate that oaks differ greatly in their suitability for this insect. It is also the case of Magnoler and Cambini (1997) findings on European oak and those of Schopf et al. (1999) on sessile and Turkey oaks who indicated differences in their suitability as a food source for *Lymantria dispar*. It will be interesting to study the preference of *T. viridana* to these three host species.

RESUME

Mannai Y., Ezzine O. et Ben Jamâa, M.L. 2018. Infestation des forêts de chênes par *Tortrix viridana* et sa performance sur trois espèces de *Quercus*. Tunisian Journal of Plant Protection 13 (si): 171-181.

Les forêts de chênes sont soumises à des agressions diverses, principalement par les attaques des insectes. La tordeuse verte, *Tortrix viridana*, est un défoliateur de diverses espèces de chênes. Cet insecte a provoqué depuis plusieurs années des défoliations considérables dans différentes subéraies du Nord-Ouest de la Tunisie. L'étude a été conduite entre 2009 et 2013 dans cinq forêts de chêne-liège (*Quercus suber*), une forêt de chêne-zeen (*Q. canariensis*) et une forêt mixte de chêne-liège, chêne-zeen et chêne-afares (*Q. afares*). Le nombre moyen de chenilles par branche variait significativement d'un site à un autre (6,6 ± 0,77 chenilles / branche à El jouza et 0,11 ± 0,05 chenilles / branche sur *Q. afares* dans la forêt d'Ain Zena). La date du débourrement n'a pas différé entre *Q. canariensis* et *Q. afares* alors que celui de *Q. suber* s'est produit environ 3 semaines plus tard que pour les deux autres espèces. Comme la phénologie de la plante hôte diffère entre les essences hôtes, il est évident que la

survie et le développement larvaire ne sont pas les mêmes pour toutes les espèces de plantes hôtes. Le développement des chenilles reste plus important sur *Q. suber*. La durée de développement larvaire du 1er au 5ème stades larvaires est plus réduite sur *Q. suber* (24,92 ± 0,26 jours) que sur *Q. canariensis* (26,69 ± 0,29 jours) et sur *Q. afares* (29,03 ± 0,39 jours). La mortalité larvaire n'a pas montré une différence significative entre les plantes hôtes. La comparaison des poids des chrysalides issues de l'élevage des chenilles sur les essences de chênes hôtes a montré un effet significatif. Les chenilles ayant consommé des feuilles de chêne-liège ont donné des chrysalides plus pesantes avec 35,52 ± 9,44 mg que celles élevées sur le chêne-zeen (30,43 ± 8,25 mg) et le chêne-afares (22,95 ±5,34 mg).

Mots clés: Chênes, infestation, performance, *Tortrix viridana*, Tunisie

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ملخص

مناعي، يسرى وألفة الزين ومحمد لحبيب بن جامع. إصابة غابات البلوط بواسطة حشرة *Tortrix viridana* ودراسة كفاءتها على ثلاثة أنواع من جنس *Quercus*.

Tunisian Journal of Plant Protection 13 (si): 171-181.

تتعرض غابات البلوط لهجمات مختلفة من الحشرات. تعتبر حشرة *Tortrix viridana* من أهم آفات البلوط وقد تسببت هذه الحشرة لعدة سنوات في إزالة الاوراق في مختلف مناطق شمال غرب تونس. تم تنفيذ هذا العمل بين 2009 و 2010 في خمسة غابات بلوط فلين (*Quercus suber*)، غابة بلوط زان (*Q. canariensis*) وغابة مختلطة من بلوط الفلين وبلوط الزان وبلوط الزان المقلوب (*Q. afares*). سجل متوسط عدد اليرقات في الغصن اختلافا كبيرا بين المواقع (6,6± 0,77 يرقة / غصن في الجوزة و0,11 ± 0,05 يرقة / غصن على *Q. afares* في غابة عين الزانة). لم يختلف وقت ظهور براعم *Q. canariensis* و *Q. afares* بينما ظهرت البراعم على *Q. suber* بعد حوالي 3 أسابيع من النوعين الأخرين. بما أن فينولوجيا النبات العائل تختلف بين الانواع المضيفة فإن بقاء اليرقات على قيد الحيات وتطورها يختلفان من المؤكد حسب أنواع النباتات العائلة. تم تحديد مدة تغذية اليرقات في ظروف المختبر على *Q. suber* و *Q. canariensis* و *Q. afares*. نمت اليرقات بطريقة أسرع على *Q. suber* حيث كان الوقت الاجمالي لنمو اليرقات (من المرحلة الأولى الى الخامسة) أقصر على *Q. suber* (24,92 ± 0,26 يوم) مقارنة بـ *Q. canariensis* (26,69 ± 0,29 يوم) و *Q. afares* (29,03 ± 0,39 يوم). لم تكن وفيات اليرقات مختلفة بشكل كبير على النباتات المضيفة. بينما اظهرت البيانات اختلافا كبيرا بالنسبة لوزن الشرانق حيث ان شرانق اليرقات التي استهلكت أوراق *Q. suber* أثقل (35,52 ± 9,44 مغ) من التي استهلكت *Q. canariensis* (30,43 ± 8,25 مغ) و *Q. afares* (22,95 ± 5,34 مغ).

كلمات مفتاحية: إصابة، بلوط، تونس، كفاء ة، *Tortrix viridana*

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Performance of *Anacampsis scintillella* in Tunisia

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ABSTRACT

Ezzine, O., Hammami, S., Boudhina, S., and Ben Jamâa, M.L. 2018. Performance of *Anacampsis scintillella* in Tunisia. Tunisian Journal of Plant Protection 13 (si): 183-198.

In Tunisia, larvae of *Anacampsis scintillella* were observed for the first time in 2010, on *Halimium halimifolium*. Preliminary investigations on biological behavior of *A. scintillella* were carried out in Bizerte (Sejnane) in 2017. To estimate the level of host plant infestation, the number of *A. scintillella* shelters found was counted and the collected individuals were subjected to biological study. The mean number of shelters was 5.4 per plant. Larvae of *A.scintillella* stick leaves (4-34, mean of 13 leaves) together by means of silkthreads to form a distinctive pouch like shelter within which the larva feeds.Larvae were observed from April to early May. Chrysalis was observed from early May to mid-May. Adult flight occurs from late May to early June.

Keywords: *Anacampsis scintillella, Halimium halimifolium*, shelter, Tunisia

The Gelechiidae is a very large family of microlepidoptera including species which are important pests of evergreens, agriculture and forestry as well as species of biological and ecological interest (Lee et al. 2009). Larvae of the majority of species in the genus *Anacampsis* are monophagous or oligophagous (Harrison and Berenbaum 2013).

Anacampsis scintillella is widespread in most of Europe (Aberlenc 2011). It is considered to be polyphagous. It attacks one of the most important families of the Medittereeanean flora including Cistaceae (Talavera et al. 1997). This insect feeds on *Helianthemum vulgare* (Stainton et al. 1865), *Cistus psilosepalus*, *C. crispus* and *C. Salvifolius* (Huertas Dionisio 2007), *Halimium halimifolium* (Ezzine et al. 2015; Huertas Dionisio 2005) and *H. lasianthum* (Corley et al. 2008). *A. scintillella* larvae were protected between the host plant leaves. They tie the terminal leaves and the other leaves together, formed holes in them and feed from their entire thickness (Stainton et al. 1865). Larvae of this insect can cause a significant damage. In fact, an important defoliation was recently observed in Tunisia where a total infestation was recorded at early April 2010 (Ezzine et al. 2015). *H. halimifolium* is a shrub occurring on sandy substrates of the south and the west of the Iberian Peninsula, Italy, Greece and north of Morocco (Zunzunegui et al. 2002) with standing a high environmental variability, thanks to its greater plasticity, compared with co-

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existing shrub species (Zunzunegui 2009). In Tunisia, *H. halimifolium* is distributed in Cap Bon where it is associated with *Quercus coccifera* (Guillerm et al. 1965), in the forest of Ain Draham and in the dunes of Tabarka (Debazac 1959). It was also observed in Cap Sirrat (Sejnane) by Pottier-Alapetite (1979). This species exhibits a highly plastic character with the ability to modify its morphological and physiological features in response to environmental variations in its habitat (Díaz Barradas et al. 1999).

H. halimifolium is a common medicinal plant in the Mediterranean regions (Kerbab 2017) and it is used for its antispasmodic (Zunzunegui et al. 2000) and antimicrobial activities (Rebaya et al. 2016). It is used in folk medicine in the form of an infusion as an anti-contraction and treat gastrointestinal pains (Zaiter et al. 2012) and as a preventative remedy from animal diseases (Mezghani 1992).

The main objective of this study is to determine the infestation of *H. halimifolium* by *A. scintillella*. The study aimed also to define the defoliation degree based on the number of shelters found in the host plant.

MATERIALS AND METHODS

Study area.

The investigations were carried in north-western Tunisia (Sejnane, Barrage Ziatine (latitude 37°11´N, longitude 9°11´E, elevation 48m). The climate of the region is wet Mediterranean climate characterized by two seasons: cool and wet winters, and dry summers. Current precipitations vary between 700 and 1000 mm. The maximum temperature of the hottest month is about 45°C and the average of the coldest month is of 10°C (Anonymous 1996). The study area includes Mediterranean maquis and consists mainly of *Quercus coccifera*, *Pistacia lentiscus*, *Myrtus communis*, *Erica arborea*, *Arbutus unedo*, *Daphne gnidium* and *H. halimifolium* (Anonymous 1996).

Two orthogonal transect lines were considered (Nsibi et al. 2006). Each transect consisted of 12 plots, about 300m in length each, and were 50 m spaced. The plot was square shaped (25m²) (Wikum and Frederick Shanholtzer 1978).

Host plant infestation.

In April 2017, in each plot, the number of shelters of *A. scintillella* was counted for 30 *H. halimifolium* samples. The largest and smallest diameters of the crown of each plant were measured and the mean diameter per plant was calculated (Ezzine et al. 2015).

Shelters collection and examination.

In April and May 2017, shelters of *A. scintillella* (Fig. 1) were collected, placed in cylindrical plastic boxes (7 cm height × 3 cm Ø), and kept in the laboratory at 25°C. Length and width of the shelters were measured (Urban 2010) (Fig. 2). Agglomerated leaves were separated and counted.

Statistical analysis.

The statistical analysis was performed using the SPSS-10.0 software package for Windows. Average of length and width were reported as mean ± standard error of mean (MSE), using analysis of variance (ANOVA) and complemented by multiple comparisons of means by the SNK test (Student-Newman-Keuls). Results are presented in the form of the Wald's chi-square test value (χ^2), parameter estimates and the respective *P* value. Pearson's correlation coefficient was calculated using Microsoft Excel.

Fig. 1. Shelter of *Anacampis scintillella*.

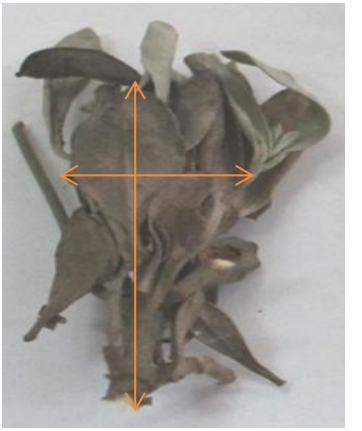

Fig.2. Measurement of length and width of the shelter.

RESULTS

Infestation.

In this investigation, we found that about 90% of *H. halimifolium* were infested in the studied site. Shelters number ranged between 1 and 17 (average 5.4) shelters per plant. Mean crown diameter varied between 53 and 198.5 cm (average 115.46 cm) (Table 1). The correlation coefficient between the mean crown diameter and the number of shelters showed a positive and important relationship (*P*=0.328; r=0.185).

Table1. Descriptive statistics of measured parameters

Parameter	N	Minimum	Maximum	Mean	SE
Mean crown diameter (cm)	30	53	198.5	115.466	6.403
Shelters number	30	0	17	5.4	0.960
Length (cm)	37	2.1	7	4.662	0.203
Width (cm)	37	0.6	2	1.232	0.050
Number of leaves/shelter	37	4	34	12.864	1.127

Shelters density.

In total, 37 shelters were collected. The shelter length ranged from 2.1 to 7 cm (average 4.66 cm), while the shelter width varied between 0.6 and 2 cm (average 1.23 cm). The correlation coefficient between the shelter length and the number of leaves/shelter showed low positive correlation (*P*=0.046; r=0.33). The same link was also noted for the shelter width and the number of leaves/shelter (*P*=0.166; r=0.23). In fact, the insect does not present any preference for leaves. The larvae of *A. scintillella* are

cryptic and they feed within folded leaves. *A. scintillella* larva folds between 4 and 34 leaves per shelter longitudinally in a tube.

Length of the shelter differed significantly between classes ($F_{(2, 34)}$=5.284; P =0.01) whereas the width did not ($F_{(2, 34)}$=1.409; P=0.258). Mean length of C_1, C_2 and C_3were respectively 4, 5.28, and 4.78 cm (Fig. 3). Mean width of C_1, C_2 and C_3were respectively 1.18, 1.21, and 1.44 cm (Fig. 3). The average number of leaves was 12.86 leaves/shelter. The number of leaves/shelter was divided in three classes (C_1=4-10 leaves; C_2=12-17 leaves and C_3=23-34 leaves).Overall, 43.24% of *A. scintillella* shelters belongs to C_1 and C_2 classes and the rest (13.52%) belong to C_3.

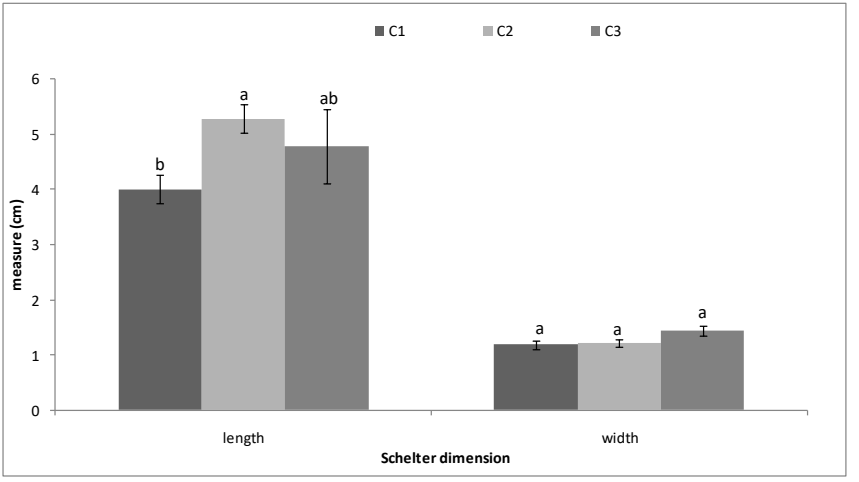

Fig. 3. Length and width of shelters of *Anacampis scintillella* between classes.C_1=4-10 leaves; C_2=12-17 leaves and C_3=23-34 leaves. Values labeled with different superscript letters are significantly differentbased on SNK test ($P < 0.05$).

DISCUSSION

Living in a leaf shelter is a common behavior among the order Lepidoptera (Lill and Marquis 2007).It allows a refuge from natural enemies (Eubanks et al. 1997), protection against adverse microclimatic conditions (Larsson et al. 1997) and acquisition of high-quality food (Fukui et al. 2002). *A. scintillella* spends the whole larval stage protected without changing its feeding habits. Shelters can take different shapes, from a simple folded leaf fragment to a complex silk tunnels and structures involving multiple leaves sewn together (Huertas Dionisio 2006). *A. scintillella* folds 4-17 leaves, rarely 23-34 leaves of *H. Halimifolium* of 1- 4 cm in length and 0.5- 2 cm in width. Other Gelechiidae feed on *Populus* sp. (leaves with 4-15 cm in length) as *A. populella* folds leaves (1-5, but usually 2-3 leaves) longitudinally in a tube and *Gelechia turpella* usually folds only one leaf (Georgiev and Beshkov 2000). *A. Scintillella* does not choose the host plant for its height. The

correlation coefficient between the mean crown diameter and the number of shelters (r = 0.185) was low.

Larvae of *A. scintillella* feed during June to the end of the month or early July, while the adults appear from the mid-July to late July (Stainton et al. 1865).

The outbreak of *A. Scintillella* was recorded in Sejnane where this insect was observed for the first time in 2010 and its adults detected in June. In 2017, the emergence of the first adult under laboratory conditions occurred in mid-May. *A. scintillella* life cycle could be influenced by temperature variation. Moreover, Denno et al. (1995) showed that herbivorous insects sharing a host plant often affect one other considerably by changing the quality or quantity of the host plant. In fact, in April 2010, *A. scintillella* was observed on *H. halimifolium* competing with *Orgyia trigotephras* for the same part of the host-plant. Larvae of *O. trigotephras* were also observed on *Q. coccifera* and *Pistacia lentiscus* and defoliation was mainly caused by *O. trigotephras* (Ezzine et al. 2015). In April 2017, the opposite scenario was observed. In fact, no *O. trigotephras* larvae were observed on all shrub species hosting its eggs and larvae. On *H. halimifolium*, only *A. scintillella* was observed. It will be interesting to study the host-plant infestation by *A. scintillella*, effect of temperature variation on its population dynamics during the next years and the shelter-building behavior and life cycle of this insect.

RESUME

Ezzine O., Hammami S., Boudhina S. et Ben Jamâa M.L. 2018. Performance d'*Anacampsis scintillella* en Tunisie. Tunisian Journal of Plant Protection 13 (si): 183-189.

En Tunisie, les chenilles d'*Anacampsis scintillella* ont été observées pour la première fois en 2010 sur *Halimium halimifolium.* Des investigations préliminaires sur le comportement biologique d'*A. scintillella* ont été réalisées à Bizerte (Sejnane) en 2017. Pour estimer le taux d'infestation de la plante-hôte, le nombre des abris d'*A. scintillella* trouvés a été compté et les abris collectés ont fait l'objet d'une étude biologique. Le nombre moyen d'abris par arbuste est égale à 5,4. Les chenilles d'*A. scintillella* colle les feuilles (4-34, 13 feuilles en moyenne) entre elles par un fil de soie formant une poche dans laquelle la chenille se nourrit. Les chenilles ont été observées entre avril et début mai. Les chrysalides ont été observées à partir du début mai jusqu'à mi-mai. L'envol des adultes se produit à la fin du mois de mai jusqu'au début juin.

Mots clés: *Anacampsis scintillella, Halimium halimifolium,* abri, Tunisie.

ملخص

الزين، ألفة وسنية الهمامي وسارة بوذينة ومحمد لحبيب بن جامع. 2018. كفاءة حشرة *Anacampsis scintillella* في تونس. Tunisian Journal of Plant Protection 13 (si): 183-189.

شوهدت يرقات *Anacampsis scintillella* للمرة الأولى في تونس،في عام 2010 على *Halimium halimifolium*. أجريت دراسات أولية للسلوك البيولوجي لحشرة *A. Scintillella* في بنزرت (سجنان) في 2017. لتقدير إصابة النبات العائل،تم احتساب عدد مخابئ *A. scintillella* الموجودة وكانت المخابئ التي جمعت موضوع دراسة بيولوجية. يساوي متوسط عدد المخابئ في الشجيرة 5.4. تلصق يرقات *A. scintillella* الأوراق (4-34، 13 متوسط العدد) بينها بخيوط الحرير لتشكل الجيب الذي تتغذي بداخله. شوهدت اليرقات بين شهريْ افريل وماي. وشوهدت الشرنقات من بداية شهرماي إلي منتصفه. يتم تحليق الفراشات من أواخر شهر ماي إلي بدايات شهر جوان.

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TUNWeeds: A Smart Identification Tool for Brassicaceae Weeds in Rapeseed (*Brassica napus*) Crop in Tunisia

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ABSTRACT

El-Waer, N.E-H., Medimagh, S. Mekki, M., and El-Waer, S. 2018. TUNWeeds: A smart identification tool for Tunisian Brassicaceae weeds in rapeseed *(Brassica napus*) crop. Tunisian Journal of Plant Protection 13 (si): 191-198.

Rapeseed is the second oilseed in the world. Its cultivation requires successful weed control. Weed identification is critical in weed management programs. Two surveys of common weeds in rapeseed fields allowed us to identify a wide specific diversity of weed flora and a great identification difficulty of certain specimens. To facilitate this task, we have developed a web application (TUNWeeds) for Tunisian farmers and students. At first, we limited our database to 44 Brassicaceae weeds. Subsequently, we plan to extend this application to other botanical families. TUNWeeds can help farmers in chemical weed control, manage herbicide resistance problems, and monitor weed flora.

Keywords: Brassicaceae, identification, web application, weed flora

The production of vegetable oils (excluding olive) in Tunisia is a national concern. Rapeseed, the second oilseed in the world, has the advantage of diversifying field rotations; it improves yields of cereal crops and minimizes several phytosanitary problems (Ben Salah 2008). The Tunisian experience in the production and processing of oilseed rape dates back to the 1980s. However, according to the Tunisian Ministry of Agriculture, its acreage in 2016 is about 1400 ha.

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Successful weed control in rapeseed crop is crucial. Weed identification is critical for successful weed management programs. Traditional weed identification tools present difficulties for farmers and technicians (too technical and inefficient for juvenile specimens). New computer tools for weed identification allow users to freely choose identification characters based on the specimen or user and tolerate user's observation errors. These tools can be used with PC, Tablets, or Smartphones off line or on line. They are easily updatable and extensible (illustrations, additional information, etc.) (Grard et al. 2012).

The main goal of our graduation project was to conceive a smart weed identification tool for farmers and students to improve weed management. Our specific objectives were: (i) to assess the difficulties of the conventional tools of weed identification and (ii) to design a smart tool to facilitate identification of Brassicaceae weeds.

MATERIALS AND METHODS

Weed scooting in rapeseed was carried out at two geographical locations (OTD-Borj Essebeii-Mateur and Smenja-Zaghouan), view the lack of time and the huge workload. At each location, we conducted two floristic samplings at 02-03 March 2017 and 06-10 May 2017. The weeds were identified using the printed flora of Pottier-Alapetite (1979) and Lenten catalog (1990).

The main reference used for compiling our database was the flora of Pottier-Alapetite (1979). Updating of species nomenclature has been done according to Dobignard and Chatelain (2011). The List of Brassicaceae weeds was based on national and international bibliographic references (Jauzein 1995; Lent 1990; Stephen 2003; Taleb et al. 1998; Tanji 2005). The database gathers in a well-structured way all the data needed for fast and reliable identification of 44 Brassicaceae weeds. It includes:

- Species nomenclature: official scientific names (Jauzein 1995; Lent 1990; Stephen 2003; Taleb et al. 1998; Tanji 2005), EPPO codes, Synonyms, and Common names.
- Species descriptors of whole plants and their main organs (leaves, flowers and fruits) as described by Pottier-Alapetite (1979).
- Species sheets including detailed information on each taxon.
- Glossary of botanical terms (Boullard 1988; Danjon et al. 2007; Douzet 2007; Gadrat2003; Reille 2012, 2013; Thebault 2017).

TUNWeeds is designed as a website requiring perfect mastery of several coding languages such as PHP, HTML, CSS, and Javascript.

RESULTS

The weed flora in rapeseed crop revealed a great weed species diversity, depending on locations and sampling dates (Table 1). Weed identification owing to the printed flora of Pottier-Alapetite (1979) revealed major difficulties with several specimens, especially at the first sampling date.

TUNWeeds home page offers three possibilities for weed specimen identification (Fig.1):

(i) Search by name (scientific or common) of a species to access to its information sheet (Fig. 2);

(ii) Search by displayed images to access to the information sheet of the selected species; and

(iii) Search with the engine by answering simple questions about the descriptors of the specimen to be identified (Figs. 3 and 4).

DISCUSSION

Weed control is a major challenge for sustainable development of agriculture. It requires easy and reliable identification of weed flora in order to monitor its dynamic and favor ecological management of the most noxious species. Correctly identifying, major weeds is an important step toward effective weed control but identifying weeds on a farm is not easy.

Table 1. Weed flora in rapeseed at two Tunisian locations during two sampling dates

Location	**Location 1 : Mateur**		**Location 2 : Zaghouan**	
Sampling date (2017)	**March 02**	**May 10**	**March 03**	**May 06**
Taxa count	14	25	29	33
Families count	7	11	14	11
Most abundant weeds				
Arum italicum	x			
Lolium rigidum	x		x	x
Medicago ciliaris	x			
Glebionis coronaria	x		x	
Sonchusa rvensis		x		
Fumaria parviflora		x		
Anagallis arvensis		x		
Diplotaxis erucoides		x		
Papaver hybridum			x	x
Papaver rhoeas			x	
Avena sterilis				x
Bupleurum lancifolium				x
Unidentified taxa	5 (36%)	4 (16%)	2 (7%)	4 (12%)
Brassicaceae taxa frequency (%)	14	20	10	10

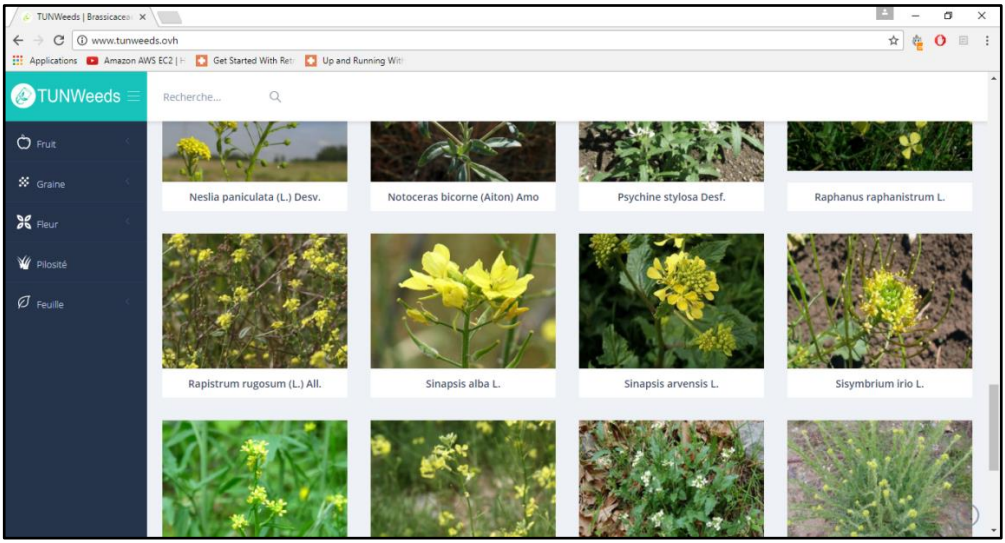

Fig. 1. TUNweeds homepage: three recognition possibilities: (i) Name of a species, (ii) Selection of an image from the displayed list and (iii) Web engine.

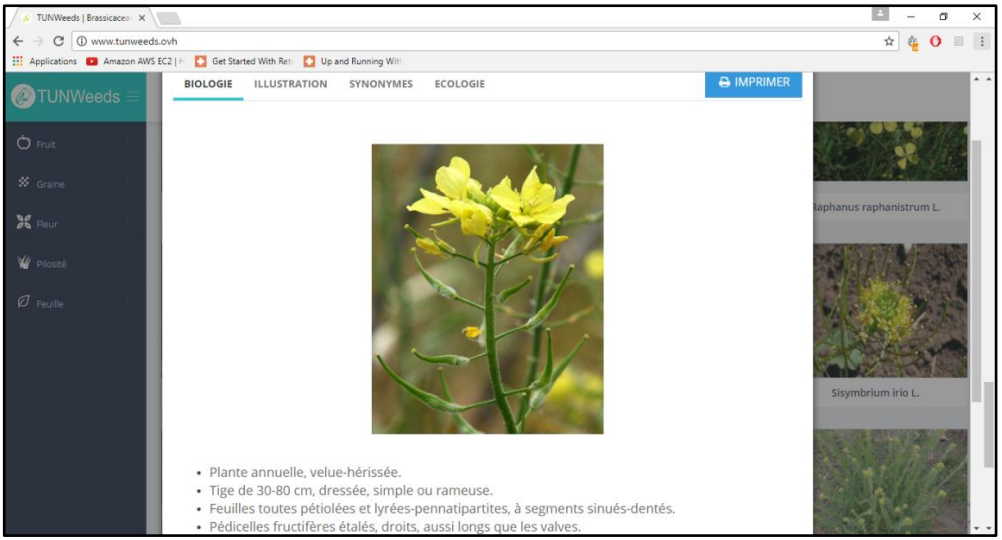

Fig. 2. Species information sheet including detailed information on each taxon: (i) Biology, (ii) illustrations, (iii) Synonyms, and (iv) Ecology.

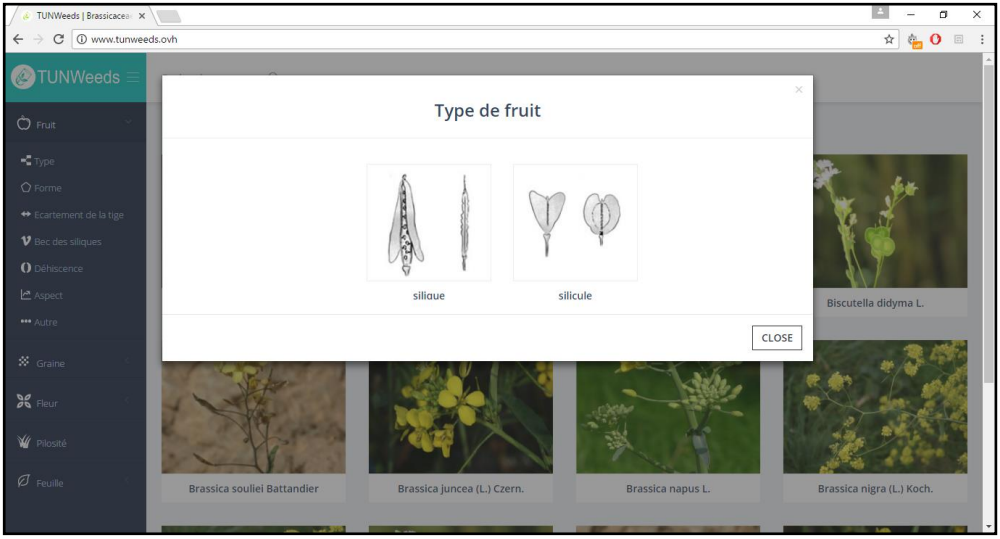

Fig. 3. Search with the web engine: e.g. fruit type.

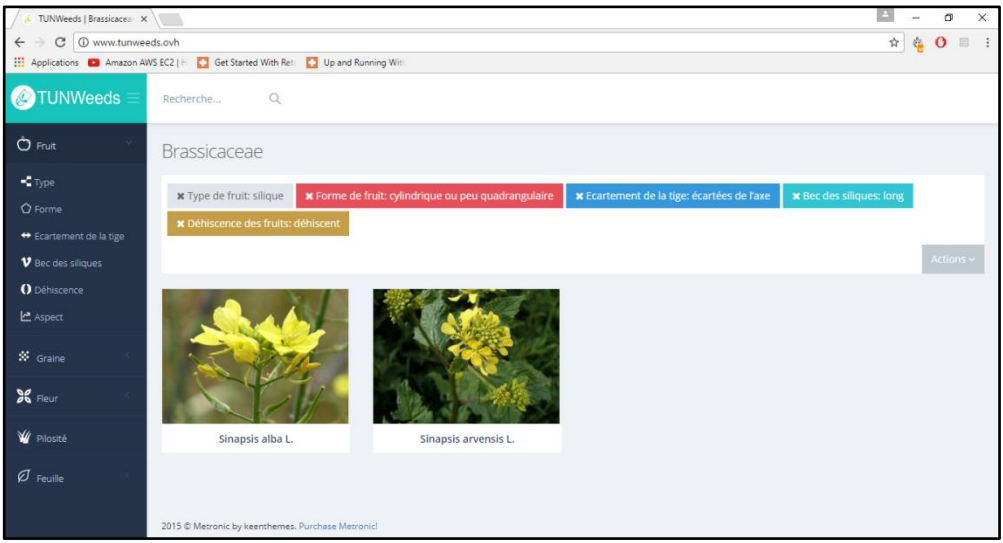

Fig. 4. Output of a search with five descriptors.

Conventional weed identification is based on field guides, manuals, or dichotomous keys. The grower should collect and examine representative specimens. Therefore, he should be familiar with the jargon used in field guides or keys. Plants are identified by visible characteristics but several characteristics are variable. Flowering specimens are easiest to identify to the species level. However, growers often want to identify major weeds in earlier growth stages. A dichotomous key gradually narrows possibilities down to one or a few species. However, an interactive key, usually available on line or on a computer CD-ROM, allows you to start with the most readily observable characteristics of the specimen at hand. It is usually better than the dichotomous key for identifying a weed in a vegetative (non-flowering) stage of development. Several weed scientists have developed interactive keys, based on computer databases that catalogue main agricultural weeds.

TUNweeds is a web application dedicated to the identification and management of weeds and invasive alien plants in Tunisia. A pilot project has been carried out to identify 44 weeds of the family Brassicaceae (Table 2) and we plan to continue the development of this application and extend it to other botanical families.

Table 2. List of TUNweeds' 44Brassicaceae weeds

Scientific names of the Brassicaceae taxa within the TUNweeds	
Arabidopsis thaliana	*Hirschfeldia incana*
Barbarea vulgaris	*Hornungia petraea*
Biscutella auriculata	*Iberiso dorata*
Biscutella didyma	*Lepidium sativum*
Brassica souliei	*Lobularia libyca*
Brassica juncea	*Lobularia maritima*
Brassica napus	*Malcolmia africana*
Brassica nigra	*Matthiola parviflora*
Brassica rapa subsp. *sylvestris*	*Moricandia arvensis*
Brassica tournefortii	*Neslia paniculata*
Bunias erucago	*Notoceras bicorne*
Camelina sativa	*Psychi nestylosa*
Capsella bursa-pastoris	*Raphanu sraphanistrum*
Cardamine hirsute	*Rapistrum rugosum*
Cardaria draba	*Sinapsis alba*
Clypeo lejonthlaspi	*Sinapsis arvensis*
Conringia orientalis	*Sisymbrium irio*
Lepidium didymum	*Sisymbrium officinale*
Lepidium squamatum	*Sisymbrium orientale*
Diplotaxis erucoides(	*Sisymbrium polyceratium*
Diplotaxis muralis	*Sisymbrium runcinatum*
Eruca vesicaria	*Teesdalia coronopifolia*

RESUME

El-Waer N.E-H., Medimagh S., Mekki M. et El-Waer S. 2018. TUNWeeds: Un outil didactique d'identification des adventices *Brassicaceae* de la culture du colza *(Brassica napus*) en Tunisie. Tunisian Journal of Plant Protection 13 (si): 191-198.

Le colza est la deuxième graine oléagineuse la plus produite dans le monde. Son introduction en Tunisie nécessite une bonne maîtrise de son désherbage. Des relevés floristiques de la flore adventice de cette culture à deux sites tunisiens nous a permis de constater une large diversité des adventices et une difficulté de les identifier. Pour cette raison et afin de mieux gérer le désherbage, une application web (TUNWeeds) est conçue et développée dans l'objectif de faciliter cette identification par les agriculteurs. TUNWeeds peut aider les agriculteurs à mieux raisonner le désherbage chimique de leurs cultures, gérer les problèmes de résistance des plantes indésirables aux herbicides, et surveiller la dynamique de la flore adventice. Cette application informatique peut servir comme outil didactique de reconnaissance des adventices pour les étudiants, les techniciens et les agriculteurs. Dans un premier temps, TUNweeds s'est limitée à la reconnaissance des adventices de la famille des *Brassicaceae*. Ultérieurement, nous envisageons d'étendre cette application à d'autres familles botaniques et de la développer pour les Smartphones.

Mots clés: Application web, *Brassicaceae*, flore adventice, identification

ملخص

الواعر، نور الهدى وسناء مديمغ ومنير المكي وسيف الدين الواعر. 2018. "TUNWeeds": أداة توجيهية لتشخيص الأعشاب الضارة من فصيلة الكرنبيات في زراعة السلجم في تونس.

Tunisian Journal of Plant Protection 13 (si): 191-198.

تعتبر حبوب السلجم ثاني أهم الحبوب الزيتية إنتاجا في العالم، وعملية إدراج زراعة السلجم في تونس تتطلب قدرة فائقة على التحكم في الأعشاب غير المرغوب فيها بهذه الزراعة. مكنتنا المتابعة المتواصلة لهذه الأعشاب في موقعيْن لزراعة السلجم من إدراك تنوع كبير في أصناف الأعشاب الضارة المتواجدة بهما مع صعوبة في التشخيص. لهذه الأسباب وسعيا إلى إدارة أفضل لهذه الاعشاب الضارة داخل المزارع قمنا بإعداد تطبيقة واب تحت تسمية "TUNWeeds" قادرة على تبسيط طريقة التشخيص لدى الفلاحين. تساعد هذه التطبيقة الفلاح على ترشيد المكافحة الكيميائية لمزارعهم وإدارة مشاكل مقاومة الأعشاب غير المرغوب فيها بالمبيدات العشبية كما تسهل مراقبة ديناميكية الفلورا الضارة. يمكن استعمال هذه التطبيقة كأداة توجيهية لتشخيص الأعشاب الضارة من طرف الطلبة والتقنيين والفلاّحيين. كبداية، اقتصرنا على الأعشاب الضارة لفصيلة الكرنبيات. مستقبلا، سنعمل على توسيع هذه التطبيقة لتضم فصائل نباتية أخرى وعلى تطوير استعمالها على الهواتف الذكية.

كلمات مفتاحية: تشخيص، تطبيقة واب، فلورا ضارة، كرنبيات

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First Report of *Lobesia botrana* on *Daphne gnidium* in North of Tunisia

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ABSTRACT

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In Tunisia, *Lobesia botrana* is a pest of grapevine. Larvae of *L. botrana* were observed for the first time in 2014 in Sejnane (Northwestern Tunisia) on *Daphne gnidium* and more recently, in 2017, in Delhiza (Northeastern Tunisia). Larvae need to enter into a bud at budburst to feed on young leaves. Pupae were observed from the end of May to early June on *D. gnidium* buds and adults emerged in June. After mating, female lays eggs by mid-June. In this paper, we present a first report of *L. botrana* on *D. gnidium* in Tunisia.

Keywords: *Daphne gnidium, Lobesia botrana,* North of Tunisia

Lobesia botrana is mainly known as a pest of grapevine (*Vitis vinifera*). The moth is polyphagous, it attacks a large diversity of host plants (Masante-Roca et al. 2007) and sometimes achieves its development on toxic plants such as *D. gnidium* (Ladhari et al. 2011). This evergreen shrub grows in the Mediterranean area and is used in traditional medicine (Mezghani 1992) as a diuretic agent to treat toothache (Borris et al. 1998) and against hepatitis (Bellakhdar et al. 1991). It also has an antiproliferative effects (Chaouki et al. 2009). *D. gnidium* is a main host for *L. botrana* that would have spread in the vine (Marchal 1912). Larvae were observed feeding on other host species (more than 30) such as: *Actinidia chinensis*, *Arbutus unedo*, *Berberis vulgaris*, *Clematis vitalba*, *Cornus mas*, *Cornus sanguinea*, and *D. Laureola* (Bovey 1966; Thiéry and Moreau 2005).

L. botrana was firstly described and reported by Denis and Schiffermüller (1776) from Australia under the name of *Tortrix botrana*. The species is included in the genus *Lobesia* by Guenée (1845).

L. botrana is a Palaearctic insect. It was widespread in Central and Southern Europe (Noma et al. 2010), in North and West Africa and in Egypt

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(Abdel-Lateef et al. 1978; Ali et al. 1978; EPPO 2013; Nasr et al. 1995). It occurs in the Middle East, the Central of Asia, Japan, Thailand and America (EPPO 2013).

In Tunisia, this insect was reported for the first time on grapevine in Cap-Bon by Bovey (1966). Moreover, in 2014 we observed *L. botrana* for the first time on *D. gnidium* in Sejnane in Northwestern Tunisia (latitude 37°11'N, longitude 9°11´E, elevation 48 m). More recently, in 2017, we observed this pest in Delhiza (Cap-Bon) in Northeastern Tunisia (latitude 36°51'N, longitude 10°47´E, elevation 401 m). Morphological identification was done by Dr. Pasquale Trematerra (General and Applied Entomology, University of Molise, Campobasso, Italy) using the Razowski (2003) key.

In European vineyards, *L. botrana* has two to four generations per year on vine and it is active from early spring to late summer (Noma et al. 2010). The larvae go through five instars like all Tortricidae. Adult males emerge about a week before females (Valeria et al. 2011). Eggs are laid singly and more rarely in small clusters of two or three on or near buds, pedicels, flowers or fruits of host plants (Noma et al. 2010).

In this study, host plant infestation was estimated in April 2017 in Sejnane and Delhiza, by counting the number of infested buds per plant on 30 *D. gnidium* trees. In the laboratory, infested buds (Fig. 1) were observed under a binocular microscope (Leica, S42) to raise the eggs, different larval stages and pupae. A total of 12 infested buds was collected from Delhiza (0 and 3; average 0.4 buds/plant) and a total of 148 infested buds from Sejnane (0 and 21; average 4.9 buds/plant). Host plant infestation was higher in Sejnane (76%) than in Delhiza (26%).

The first and the second instars larvae were observed from the end of March until the beginning of April on buds (Fig. 2.b). Mature larvae (Fig. 2.c) were observed from mid-April to mid-May feeding on budburst and young leaves. Pupa was observed from the end of May to early June (Fig. 2.d). Adults (Fig. 2.e) flight was observed in June. After mating, female laid eggs (Fig. 2.a) by mid-June.

This work is the first report of *L. botrana* on the medicinal wild species *D. gnidium* in Tunisia and it will be continued by the study of its bio-ecology and its natural enemies.

Fig. 1. *Daphne gnidium*, a: Infested buds; b: Healthy buds.

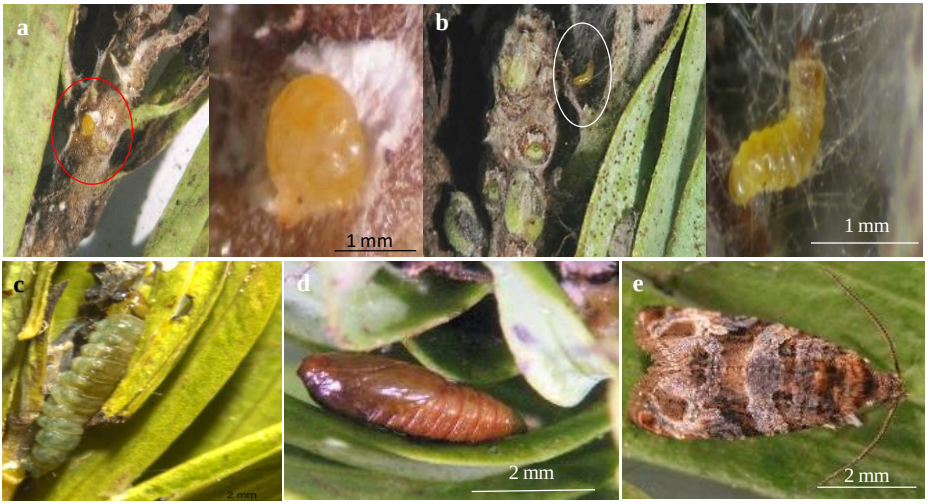

Fig. 2. *Lobesia botrana*, a: Egg; b:Young larva (L1); c: Mature larva (L5); d: Pupae and e: Adult.

RESUME

Hammami S., Ezzine O., Dhahri S. et Ben Jamâa M.L. 2018. Premier signalement de *Lobesia botrana* sur *Daphne gnidium* au Nord de la Tunisie. Tunisian Journal of Plant Protection 13 (si): 199-202.

En Tunisie *Lobesia botrana* est connu comme ravageur de la vigne. Les chenilles de *L. botrana* ont été observées pour la première fois en 2014 dans la région de Sejnane (Nord-Ouest de la Tunisie) sur *Daphne gniduim*, et plus récemment, en 2017, à Delhiza (Nord-Est de la Tunisie). Les larves pénètrent dans le bourgeon pour se nourrir des jeunes feuilles. Les chrysalides ont été observées entre fin mai et début juin dans les bourgeons de *D. gnidium* et les adultes en juin. Après l'accouplement, la femelle dépose les œufs en mi-juin. Ce travail est un premier signalement de *L. botrana* sur *D. gnidium* en Tunisie.

Mots clés: *Daphne gnidium*, *Lobesia botrana*, Nord de la Tunisie

ملخص

همامي، سنية وألفة الزين وسمير الظاهري ومحمد الحبيب بن جامع. 2018. أول تقرير حول حشرة *Lobesia botrana* على نبتة *Daphne gnidium* في شمال تونس.

Tunisian Journal of Plant Protection 13 (si): 199-202.

لأول مرة في سجنان تعرف حشرة *Lobesia botrana* في تونس كآفة على العنب. شوهدت يرقات *L. botrana* (شمال غربي تونس) على نبتة Daphne *gnidium* سنة 2014 ومؤخرا بدلهيزة (شمال شرقي تونس) سنة 2017. تخترق اليرقات البراعم لتتغذى من الأوراق الصغيرة. لوحظت الشرانق بين أواخر شهر ماي إلى أوائل شهر جوان داخل براعم *D. gnidium* والفراشة البالغة في جوان. بعد التزاوج، تبدأ الأنثى بوضع البيض في منتصف شهر جوان. هذا العمل هو أول تقرير لحشرة *L. botrana* على نبتة *D. gnidium* في تونس.

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Plant Protection Events

Report on

The First Maghreb Symposium on Integrated Plant Protection (SYMPIP-2017)

30 October-1 November, 2017, Sousse, Tunisia

The first Maghreb Symposium on Integrated Plant Protection (SYMPIP-2017) was organized at the Hotel Marhaba Palace in Sousse, Tunisia, from October 30 to November 1, 2017. The symposium was co-organized by the Regional Research Center on Horticulture and Organic Agriculture (CRRHAB), the Technical Center of Organic Agriculture (CTAB) and the Tunisian Association for Sustainable Agriculture (ATAD).

This symposium followed the first National Symposium on Integrated Plant Protection, which was held from April 20 to 21, 2015, and was a great success. The symposium aimed to provide an

opportunity for researchers, students, technical staff and professionals from the Maghreb countries to meet and exchange information, research results and experience dealing with integrated plant protection.

The program of the symposium began with an opening ceremony chaired by Prof. Messaoud Mars, the Director General of CRRHAB. A total of 9 sessions of oral presentations and posters preceded by 4 plenary lectures of eminent scientists were presented. The topics covered in SYMPIP-2017 are in relation with Emerging pests and invasive species, Plant biological protection, Alternative methods for pest management, Biotechnologies and plant protection, Plant-pest interactions, Pesticides and the environment.

About 200 participants from several countries (Tunisia, Algeria, Morocco, Germany, and Moldavia) have attended this symposium. In total, 48 oral communications and 67 posters were presented during the symposium. Most aspects related to integrated plant protection were discussed including different disciplines such as entomology, mycology, bacteriology, virology, weed science, allelopathy, biological control, phytopharmacy,... Two poster sessions were planned during which a jury was formed to choose the best posters presented by young researchers.

The various presentations, discussions and contacts were beneficial for all participants who expressed their satisfaction with the scientific content and the quality of the organization and showed an interest and need to renew this kind of events in Maghreb regions and why not in other Mediterranean regions.

Dr. Ikbal Chaieb
President of ATAD

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Photo of the cover page: The hyperparasitoid *Alloxysta* sp. (Courtesy *Zine Eddine Labdaoui*)

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