



NEWSLETTER

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Periodical information bulletin for the Prometeo project co-financed by the European Union in the framework of the ENI Cross-Border Cooperation (CBC) Programme “Italy-Tunisia” 2014-2020

The ENI CBC Programme “Italy-Tunisia” 2014-2020 is a bilateral cross-border cooperation programme, co-financed by the European Union under the European Neighbourhood Instrument (ENI). With a budget of EUR 33.3 million, the programme, which joint management has been assigned to the Sicilian Region’s Programming Office, aims to promote fair, equitable and sustainable economic, social and territorial development in order to foster cross-border integration and enhance the territories and resources of the two participating countries. <https://www.italietunisie.eu/>

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SPECIAL EDITION: SCIENTIFIC ENTOMOLOGY PROTOCOLS OF THE PROMETEO PROJECT

This special issue of the Prometeo periodic Newsletter has a purely scientific character and is entirely dedicated to the publication of scientific protocols on Entomology, which are the result of research activities and discussions between scientific experts and stakeholders of the Prometeo project, with the objective of creating a cross-border network of collaboration between Italian and Tunisian researchers, compa-

nies and other actors to foster the development of innovative and sustainable technical solutions for the protection of Mediterranean tree crops against quarantine pathogens or emerging pests that threaten their profitability and survival.

These results will be useful to guide agricultural policies, strengthen phytosanitary services, increase production ef-

efficiency, competitiveness and sustainability of these sectors, and improve quality standards in food safety.

The next issue of the Newsletter, also with a purely scientific character, will instead be dedicated to the publication of the scientific Plant Pathology protocols of the Prome-teo project.

Enjoy your reading!



California red scale - Pou rouge de Californie - Cocciniglia rossa della California *Aonidiella aurantii*

Citrus species are among the most extensively cultivated tree fruit in the world. Nowadays, the citrus industry in the Mediterranean area accounts for 20% of the world's citrus industry. Worldwide, there are 345 species (belonging to Diaspididae, Coccidae, *Pseudococcidae* and *Monophlebidae* families) associated directly or indirectly with citrus, and some of them can sometimes pose a serious problem for production (Morales et al., 2017). The scale insects are obligatory plant parasites. Many of them are polyphagous, cosmopolitan, and able to build large populations when environmental parameters are favourable (Miller and Davidson, 2005; Pellizzari and Germain, 2010). Faunistic studies are important for documenting new distributional data and notifying researchers of potential exotic pests. The establishment of new pests can be costly owing to increased crop damage, control programs, and quarantine restrictions on trade (Jendoubi, 2018). Hence, accurate documentation of exotic pest species is required for pest control and research programs that provide quarantine treatments or other mitigation approaches to reduce or eliminate pest load in traded agricultural commodities. Scale insects are successful invaders of new territories, frequently introduced and acclimatized in all terrestrial zoogeographical regions. Around 129 scales are established in Europe, representing hence one of the major groups of alien insects to Europe (Pellizzari and Germain, 2010). Most of them (overall *Diaspididae* and *Pseudococcidae*) originate from tropical regions of Asia. The trade of fruit and ornamental trees appears to be the usual pathway of their transfer. The damage caused by scale insects is the ingestion of plant sap. Moreover, except for *Diaspididae* and *Asterolecaniidae*, they excrete honeydew that becomes a growth medium for a black sooty mold fungus and photosynthesis is reduced up to 70%, leading to early senescence and loss of aesthetic value. They also inject toxic saliva and, are vectors of closteroviruses (Morales et al, 2017).

Red scale damage

California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: *Diaspididae*) is among the major pests of citrus crops worldwide. It attacks all aerial parts of the tree including twigs, leaves,



Dégâts du Pou Rouge sur agrumes

branches, and fruit by sucking on the plant tissues. Severe infestations cause leaf yellowing and drop, dieback of twigs and limbs, and occasionally death of the tree.

In Tunisia, few years ago, Jendoubi (2018) reported that despite *A. aurantii* is a serious pest for citrus crops, it has a localized distribution and was rare (1 to 3% of the number of scale insects recorded). However, recently, outbreaks of *A. aurantii* occurred, and could represent a real threat for citrus cultivations in Tunisia. Consequently, PROMETEO aims to monitor this pest, study its distribution, evaluate its damage, and develop control alternatives.

1. Pest survey and monitoring

Effective monitoring and trapping of *A. aurantii* can be achieved using California red scale sticky trap (yellow) and impact boards (white) baited with pheromone lures. Traps will help to minimize damage and assist growers in gaining control. Monitoring for pests should be at the forefront of all pest management programs, as early diagnosis will minimize crop destruction and increase the likelihood.

In the framework of PROMETEO project, a trapping network will be installed in various Tunisian citrus orchards in order to monitor *A. aurantii* and study its seasonal activities.

2. Pest distribution

The California red scale is widely distributed in many parts of the world, in particular the tropics and subtropics. In the Mediterranean basin, *A. aurantii* has been a serious pest for many years in eastern countries. In Italy, *A. aurantii* have been reported as citrus key pest (Elimem et al., 2022). In Tunisia, previous researches cited the limited distribution of this pest (Jendoubi, 2018; Limem et al., 2022).

3. Damage assessment

Aonidiella aurantii is one of the most important pests infesting citrus trees in different parts of the world (Claps et al. 2001; Abd-Rabou, 2009). It occurs on host plants belonging to at least 80 plant families (Moursi, 1991).

In the framework of PROMETEO project, the damage of *A. aurantii* will be assessed on different host plants in order to quantify losses.

4. Control

California red scale is one of the most dangerous pests on citrus around the Mediterranean basin. The damages by the California red scale are caused to all tree organs including fruit. Although there is no effective method available to eradicate the pest, scale control is mainly based on mineral oil and pesticide sprays in the field that are effective in reducing the incidence of the pest. Additionally, biological control using the natural enemies (parasitoids and predators) presented promising achievements. This method is now commonly used in citrus orchards in many countries. *Aphytis melinus* (Hymenoptera: Aphelinidae) is a specific parasitoid used with success against *A. aurantii* (Zappalà et al., 2012).

In the framework of PROMETEO project, treatments using some mineral oils and synthetic insecticides will be used. Additionally, the parasitoid *A. melinus* will be introduced from Italy. Biological trials using this parasitoid will be undertaken.

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The carob moth - La pyrale des caroubes - La falena del carrubo *Apomyelois* (= *Ectomyelois*) *ceratoniae*.

The moth *Apomyelois* (= *Ectomyelois*) *ceratoniae* Zeller (1839) (Lepidopteran *Pyralidae*), is commonly known as carob moth because of its infestation on pods of this plant. Other common names include “date moth”, “knot-horn moth”, “blunt-winged moth”, “locust bean moth” and “pomegranate neck worm” (Mirkarimi 2002; Botha and Hardie 2004; Massimino Cocuzza et al., 2016; 2021). As well as being a serious field pest of orchard crops, the carob moth is also known as a significant pest of stored products, including dried figs, dates, raisins, carobs, almonds and other nuts (Heinrich 1956; Higbee and Siegel 2009).

A. ceratoniae is a widespread pest of numerous commercial tree crops. In Tunisia, it attacks several hosts, such as citrus, pomegranate, date, and almond (Mediouni et al, 2004, 2012; Massimino Cocuzza et al., 2016; 2021). The carob moth can cause economic losses in the Mediterranean basin and Near East regions. Larvae feed on inner parts of the fruit and highly reduce its quality indices (Dhouibi, 1989).



Damage of *Ectomyelois ceratoniae* on stored almonds



Damage of *Ectomyelois ceratoniae* on orange Navel

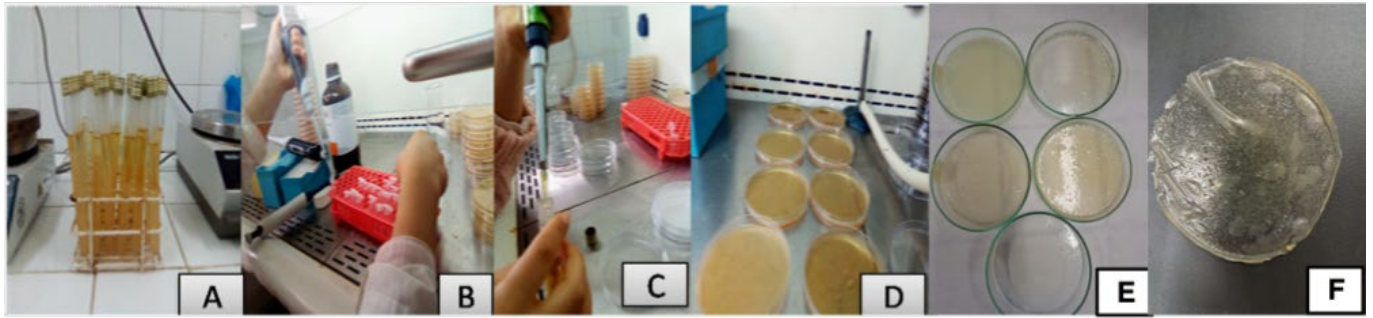
In fields, *A. ceratoniae* monitoring is mainly based on the use of sex pheromone traps (Mediouni, 2005). The control of the pest includes cultural practices like sanitation, removal and destruction of infested plant parts (including fruit) and fruit bagging (Dhouibi, 1989). In this regard, Gothilf (1970) cited that in carob plantations, unharvested pods have been noted to provide breeding sites and act as a population “reservoir” for carob moth. Indeed, in numerous crops, unharvested fruit remaining in orchards have been found to be a major breeding resource for the pest, and orchard hygiene has been recommended as a key pest management factor (Madge, 2015).

On the other hand, methods of protecting stored fruits essentially involve the use of fumigants and synthetic insecticides. However, the application of these chemicals is controversial due to concerns about human and environmental effects (Ben Abada et al, 2019). Thus, alternative control methods are required.

In the framework of PROMETEO project, our work aims to investigate innovative options for managing the carob moth in stored almonds through i) the development of natural packaging films based on bioactive compounds with insecticidal properties (Djebbi et al., 2023) and ii) industrial-scale applications of packaging films.

1. Development of natural packaging films

Films will be produced from different natural matrix (such as, pectin, chitosan) with essential oils and their constituents as active biomolecules with insecticidal activities (toxic, repellent, attractant).



Preparation of packaging films with insecticidal potential for the control of carob moth (Djebbiet al, 2023).

2. Industrial scale application of packaging films

Some trials to assess the efficacy of the packaging films will be undertaken on an industrial scale to test the potential of such material in reducing the damage of *A. ceratoniae*.

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The olive fly - La mouche des olives – La mosca dell'olivo *Bactrocera oleae*

Olive (*Olea europaea* L.) is one of the main crops and fruit trees in the Mediterranean region, where wild, feral and domesticated forms are widespread (Fanelli *et al.*, 2022). Olive is the pillar of the Mediterranean agroecosystems, given its great economic, social and cultural importance (Besnard *et al.*, 2018; Famiani *et al.*, 2019). The crop is cultivated on more than 12 million hectares worldwide (Rugin *et al.*, 2016), and displays a wide variability, witnessed by more than 2600 different varieties for oil and/or table fruits (FAO, 2010). The olive tree, an iconic symbol of the Mediterranean basin, is the object of growing international interest in the production of olive oil for the world food market. In Tunisia, which is the fourth-largest producer of olive oil in the world, the production of olives and olive oil is of great socio-economic importance. Cultivation is widespread from north to south of the country (Debbabi *et al.*, 2022).

A major challenge has arisen in relation to the growing environmental impact of climate changes associated with abiotic stresses. They include cold, salinity and drought as well as new or re-surgent pests and diseases, such as the new disease, the Olive Quick Decline Syndrome (OQDS), caused by the bacterium *Xylella fastidiosa* (Montilon *et al.*, 2022) and the olive fly *Bactrocera oleae* (Debbabi *et al.*, 2022).

Bactrocera oleae (Rossi, 1790) (Diptera: Tephritidae), the olive fly, is monophagous and one of the primary threats to the olive tree causing serious economic losses (Ponti *et al.*, 2009). In field, olive flies damage fruit by punctures resulting from oviposition, and these sites can serve as the entry point for microorganisms of rot, compromising the organoleptic quality of olive and olive oil. Larvae feed on the olive mesocarp, causing galleries in the pulp and fruit drop, which result in the reduction of the commercial value of table olives and make it impossible to commercialize the olive oil because of the high levels of acidity (Zygouridis *et al.*, 2009, Daane and Johnson 2010). The estimation is that among the pest insects of the olive tree, *Bactrocera oleae* is responsible for up to 60% of the total damage (Cutierrez *et al.*, 2010).



Damage caused by the olive fly *Bactrocera oleae*

In the framework of PROMETEO project, the team intend to:

- Monitor the insect in different olive groves in North of Tunisia
- Development of baits and attractants,
- Identification of associated parasitoids and predators,
- Implementation of control trials

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The Mediterranean fruit fly - *La mosca mediterranea della frutta* - *La mouche Méditerranéenne des fruits* *Ceratitis capitata*

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), communally called Mediterranean fruit fly or Medfly, is among the major destructive pests of fruits worldwide, particularly in the Mediterranean regions (Aluja and Mangan, 2008). In Tunisia, Medfly is a key economic pest on citrus and various crops (Jerraya, 2003). Current control program is mainly based on applications of organophosphate insecticides (Boussabbeh et al., 2016). However, their use is subject to much controversy, which resides in the destruction of auxiliary fauna, the increase in residue levels in fruits, the resurgence of secondary pests and the development of resistant strains (Mediouni-Ben Jemâa et al., 2010). Therefore, the development of techniques that would provide more effective control against *C. capitata* without serious environmental effects is necessary.



Damage caused by *Ceratitis capitata*

Numerous control approaches using attractants for the monitoring and the control of Medfly have been implemented (feeding and sexual attractants) (Figueroa Candia, 2018). On the other hand, Di-Ammonium Phosphate (DAP fertilizer) exhibited an attractive activity towards *C. capitata* adults. Indeed, traps baited with DAP solution were attractive to Medfly adults (Braham, 2013). Additionally, our recent work revealed the attractant potential of bear yeast toward Medfly adults.

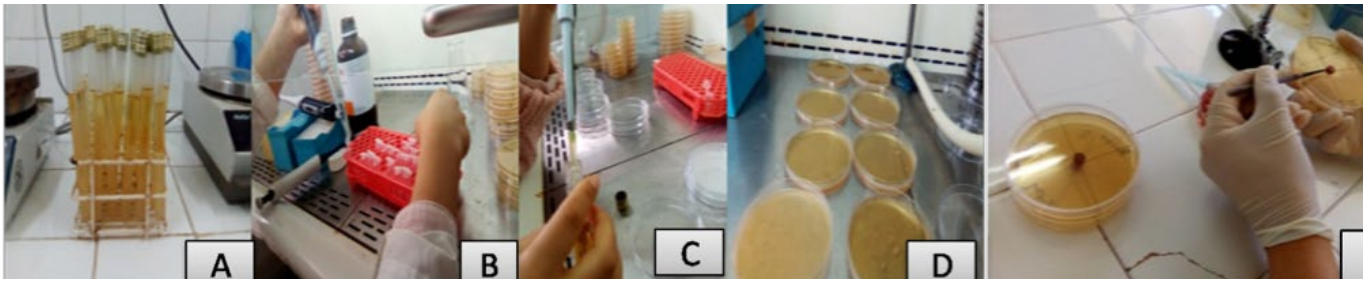


Trials to assess attractant potential of bear yeast under laboratory and field conditions.

In the framework of the PROMETEO project, we propose the following work plan and protocol:

1. Development of natural attractants

Our work will be focused on the development of natural attractant by adsorption onto DAP fertilizers of some plant extracts with attractant potential. Among the plant extracts, the use of



essential oil extracted from some plants (citrus fruit peels, Eucalyptus, ...) will be tested.
Different steps to prepare the attractant.

2. Field trials

The attractant developed and tested under controlled laboratory conditions will be tested and assessed under field conditions.

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Whitefly - L'aleurode des agrumes - La mosca bianca

Dialeurodes citri

Whiteflies (Hemiptera: *Aleyrodidae*) are important insect pests (Hodges and Evans 2005) able to cause damage by sucking plant sap inducing the weakening of plants and, for some species, through the transmission of viruses. The citrus whitefly, *Dialeurodes citri* (Ashmead) (Hemiptera: *Aleyrodidae*) is considered one of the most important citrus pests. However, in some environments, other species can cause damage to crops, and among these, the most important in the Mediterranean basin are *Aleurothrixus floccosus* (Maskell, 1896), *Parabemisia myricae* Kuwana, 1927, *Paraleyrodes minei* Iaccarino, 1990 and *Aleurocanthus spiniferus* (Quaintance, 1903), the latter of which is spreading rapidly in several European countries (Italy, Greece, Montenegro, Bulgaria and Croatia).

The whitefly consumes large quantities of sap and this feeding activity can weaken plants, especially young ones. Moreover, sooty mould fungi, belonging to the genera *Cladosporium*, *Capnodium* and *Alternaria*, grows over fruit and foliage due to the copious amount of honeydew produced by the whitefly. It may cover the leaves and fruit and can interfere with the physiological activities of the plants. Heavily-infested trees become weak and produce small crops of insipid fruit. As mentioned above, fruit covered with sooty mould will be retarded in ripening and late in colouring, especially the upper part, which may remain green after the lower portion has assumed the colour of ripe fruit. In Tunisia, recently, *D. citri* was detected with very high population densities in some citrus orchards in the Cap-Bon region (main area of citrus production in Tunisia). These observations confirmed its change of status and potential widespread in Tunisia (Boulahia-Kheder, 2021). Thus, in the framework of the PROMETEO project, we propose the following work plan and protocol:

1. Monitoring of *D. citri* using sticky trap (Surveillance moyennant les pièges englués)
2. Study of *D. citri* distribution in Tunisia (Etude de la distribution de l'insecte en Tunisie)
3. Damage assessment
4. Establishment of an IPM program



Dialeurodes citri: Adults, larvae and damage (Photograph by University of Florida)

1. Monitoring

In recent years, yellow sticky traps have been widely used in pest control and monitoring (Tariq et al, 2016). Coloured sticky traps have been used for monitoring the population of whiteflies, leafminers, thrips and other insects in greenhouses and fields (Qiu and Ren, 2006).

2. Distribution

The citrus whitefly *Dialeurodes citri* has a wide range of distribution in different regions around the world (Bellows and Meisenbacher, 2007). *Dialeurodes citri* is one of the most widely distributed whitefly pests of citrus. It spreads rapidly throughout the citrus-growing area. In Tunisia, *D. citri* distribution was not studied.

In the framework of PROMETEO project, we intend to carry out the following activities:

- Surveys over citrus orchards,
- Study of *D. citri* distribution in Tunisia,
- Identification of the main infested areas (hot spots)

3. Management

Traditional chemical controls are the most used methods to manage citrus whiteflies. However, the heavy use of chemical pesticides has generated negative side-effects, especially insecticide resistance. Therefore, it is important to search for alternative methods for whitefly control (Kunimi, 2007).

3.1. Biological control

Biological control is the control of insect pests using predators and parasitoids and should be adapted at a large scale to avoid the unnecessary use of insecticides. There are various predators and parasitoids of *D. citri* in the citrus crop. The primary source of generalist predators is syrphid flies, ladybird beetles, lacewings, predatory mites, and ants (Yang et al, 2006). The first effective control method is to restore a biological balance in the agrosystem. This can allow the natural enemies of aleyrodids to develop and keep the whiteflies population below the economic threshold of damage.

In the framework of PROMETEO project, we intend to study:

- Sampling and identification of natural enemies associated to *D. citri*
- Assessment of control potential of natural enemies.

3.2. Cultural control

All the practices in citrus orchards that enhance the passage of airflow through the canopy of citrus trees come under cultural control, these practices include the following: maintaining a proper plant to plant and row to row distance, weed control, moderate pruning, and optimum application of irrigation and fertilizer. These cultural practices do not allow humidity among the trees to increase significantly and thus keep the population under check (Uygun and Satar, 2008).

In the framework of PROMETEO project, we intend to:

- Experiment with some cultural control methods
- Determine the impact of cultural control methods on *D. citri* infestations.

3.3. Chemical control

Chemical control using inorganic compounds, botanicals, and synthetic insecticides is an integral part of IPM for the control of citrus whiteflies; however, it should be used judiciously and only when required.

In the framework of PROMETEO project, we intend to:

- Application of summer oil or white oil emulsion,
- Application of some synthetic insecticides and plant extracts,
- Study the impact of chemical application on the natural enemies

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General information on PROMETEO

Main beneficiary

Università degli Studi di Catania (UNICT)

Partners

P2: Université de Tunis El Manar (UTM)

P3: Centre Technique des Agrumes (CTA)

P4: Institut National de la Recherche Agronomique de Tunisie (INRAT)

P5: Agence Nationale de Promotion de la Recherche scientifique (ANPR)

P6: Comune di Palazzolo Acreide (PALAZZOLO)

P7: Centro di Ricerca per l'Innovazione e Diffusione della conoscenza (CERID)

P8: Expergreen S.R.L. (EXPERGREEN)

PROMETEO PROJECT IN NUMBERS

Duration	24 months
Project start-up	29/10/2021
Date of completion	28/10/2023
N. of project Partners	8
Overall budget	1.459.103,08 €
EU Contribution	1.291.659,13 €

PROMETEO PROJECT ACTIVITIES

Number of dissemination events and thematic meetings	5
Involved participants	450+
Project website	1
Social media channels	4

OUR CONTACTS

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E-mail address: info@prometeo-italietunisie.eu

Facebook: <https://www.facebook.com/Prometeo.ItalieTunisie>

Instagram: https://www.instagram.com/prometeo_italietunisie/

Twitter: https://twitter.com/prometeo_ItaTun

Youtube: <https://www.youtube.com/@prometeoitalietunisie4919>



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