EIP-AGRI Focus Group

'Pests and diseases of the olive tree'

Biological and Biotechnical methods for olive pest control

Marion Canale, Umberto Bernardo, Panagiotis Milonas, Sónia Santos

marion.canale@supbiotech.fr
umberto.bernardo@ipsp.cnr.it
p.milonas@bpi.gr
Sonia.Santos@estbarreiro.ips.pt

funded by European Commission

1. Introduction and description of the main problem related to the topic

The olive tree (*Olea europaea* L.) is a typical and emblematic tree in the Mediterranean countries where it has great relevance from economic to social and landscape aspects (Loumou & Giourga, 2003).
The olive tree is susceptible to the attack of different insect pests causing a decline of the olive production. Phytosanitary threats are the major cause of harvesting losses in olive groves. Each year, 15% of the production is damaged by insect pests (Bueno & Jones, 2002). Usually, olive growers use a range of pesticides to control pests that can have several negative impacts (e.g., loss of biodiversity, insecticide resistance and residues on olives) (Kombargi et al., 1998; Pereira-Castro et al., 2015). Notwithstanding, the European Union (EU) has established the adoption of environmentally-friendly farming practices through the agri-environment schemes and through the Directive 2009/128/EC for the sustainable use of pesticides. These measures bring the need to develop sustainable production systems where the application of biological and biotechnical methods can be promoted.
Biologically based methods for pest management use natural enemies of pests to reduce the population of the target-pest. Biotechnical methods are highly specific since they influence either the behaviour or the development of the target pest and do not have direct biocide activity. They include semiochemicals (attractants and repellents used for mass trapping, lure and kill and mating disruption), sterile insect technique, growth regulators and antibiotics.

2. Description of the main biological and biotechnical methods for olive pest control

2.1. Biological control methods

Biological pest control methods involve the use of living natural enemies (parasites, predators or pathogens) to reduce the impact of pests, causing their death. There are three strategies for biological pest control:
1 - Augmentation biological control – the population of a native or a non-native natural enemy temporarily increases with regular releases in the field (O'Neil & Obrycki, 2008).
2 - Conservation biological control – involves the manipulation of the habitat aiming at promoting the abundance and diversity of natural enemies already present in the area (Begg et al., 2017).
3- Classical biological control – a non-native natural enemy species is released in the field aiming at establishing their population in order to achieve the sustainable control of the target pest, usually also non-native (Van Driesch et al. 2008).
Different biological control strategies have been used in the olive grove with different outputs and a synthesis is presented in the following points.

a. The olive fruit fly, *Bactrocera oleae*

Classic biological control of *Bactrocera oleae* has a long history that started, in Italy, with the importation and the release of several species of **parasitoids** from Africa (Silvestri, 1913a, 1913b, 1914, 1915a, 1915b). The small releases of those parasitoid species did not give rise to its establishment in the new territories (Neuenschwander, 1982).
Until 2007, in southern Europe, five parasitoid species were commonly reared from *B. oleae*: the allochthonous braconid *Psyttalia concolor* and the chalcid wasps *Cyrtoptyx latipes* (Hymenoptera: Pteromalidae), *Eurytoma martellii* (Hymenoptera: Eurytomidae), *Eupelmus urozonus* (Hymenoptera: Eupelmidae), and *Pnigalio mediterraneus* (Hymenoptera: Eulophidae) (Hoelmer et al., 2011, Boccaccio & Petacchi, 2011, Bernardo & Guerrieri, 2011).
This species complex did not provide effective biological control of the olive fruit fly (Bigler et al., 1986, Johnson et al., 2012), and, some of the cited parasitoids can also behave as hyperparasitoids of the others ones. Moreover, recently *E. urozonus* resulted to be a complex of several species thus its large polyphagia should be revalued (Al Khatib et al., 2014).
A similar situation was found for *P. mediterraneus* whose synonymization with the congeneric *P. agraules* had suggested that the proximity of oaks to olive orchards could have promoted the parasitization of *B. oleae* because oak phytophagous could represent alternative hosts. With the definitive evidence that the

two species are distinct and possess a different host range, also the possibility to use this association (oak-olive) for the conservation of biological control approach vanished (Gebiola et al., 2009).
More recently, in Italy, inadequate parasitization results were also found when an overall parasitism rate of about 12% was recorded during an evaluation of landscape effects on the parasitoid complex of *B. oleae* (Boccaccio & Petacchi, 2009).
In the last 20 years, there have been many attempts of biological control of this olive pest by either inoculative or inundative releases of different parasitoids (e.g. *P. concolor*) but the results were far from being considered satisfactory (Arambourg, 1986, Bernardo & Guerrieri, 2011).
In 2007, however, a new species of a gregarious pupal parasitoid of *B. oleae*, the eulophid *Baryscapus silvestrii* has been found and described for the first time (Viggiani et al., 2007).
This interesting species has expanded its range of diffusion, becoming predominant in some areas (Giacalone et al., 2011). The rearing difficulties of this promising parasitoid did not allow to improve its biological knowledge and to carry out an augmentative release program. However, a permanent rearing of *B. silvestrii* has been recently developed allowing also to evaluate some of its biological parameters. The first results confirm its high potential to control *B. oleae* (Sasso et al., 2018, Sasso et al., in prep.). Moreover, since the current legislation practically forbids the releasing of exotic parasitoids and therefore any classic biological control program, its natural widespread in Italy makes this species even more interesting.
The complex of parasitoids inhabiting in the Mediterranean area showed a low and non-specialized diversity compared to the parasitoid complexes observed in Africa and other countries (India). For this reason further studies have been recently carried out (Sime et al., 2007, Rugman-Jones et al., 2009, Daane, 2010). Much attention has focused in particular on the *P. concolor* species complex from sub-Saharan Africa (Rugman-Jones et al., 2009). Although the importance of the biological control is continuously increasing as pesticide use becomes more limited, the results obtained so far with parasitoids do not seem to guarantee an adequate control of *B. oleae* making the feasibility of managing the olive fruit fly solely by biological control questionable.

The action of parasitoids on the olive fruit fly can be complemented by **soil predators** during the overwintering period of the pest in the soil as pupae. Soil arthropods such as carabids, staphylinids, chilopods, dermapterans and spiders can predate the olive fruit fly in the field (Dinis et al 2016). Conservation biological control strategies, such as the introduction of stone walls that serve as shelter places for these arthropods, can be implemented in olive grove.

Moreover, contact and oral exposure to conidia of entomopathogenic agents such as *Mucor hiemalis*, *Penicillium aurantiogriseum*, *P. chrysogenum* and *Beauveria bassiana* revealed moderate to high mortality rates for the adults of the olive fruit fly (Konstantopoulou & Mazomenos, 2005) and, nowadays, commercial solutions based on *B. bassiana* can be used against the olive fruit fly.

b. The olive moth, *Prays oleae*

Biological control of the olive moth was implemented through the release of the mass-produced species *Trichogramma cacaeciae* (Hymenoptera: Trichogramatidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae). Results showed that *T. cacaeciae* generated a low level of egg parasitism and no control of the olive moth was achieved while *C. carnea* resulted in an increase of the percentage of predation of eggs from 32% in the non-treated plot to 50.1% in the treated plot (at a density of 180 larvae of *C. carnea* /tree) and 68% (at a density of 900 larvae of *C. carnea* /tree). In addition, fruit infestation significantly decreased from 36.6% in the non-treated plot to 28.4% and 17.2% (at densities of 180 and 900 larvae of *C. carnea* /tree, respectively) in the treated plot (Bento et al., 1999a). Environmental conditions (high temperatures and low humidity) and the occurrence of supplementary food could highly influence *Trichogramma* survival and reproduction and restrict their maintenance in the field.

In Portuguese olive orchards, parasitoids collected from larvae and pupae of the olive moth were described in several studies (e.g., Bento, 1999, Gonçalves et al., 2006, Villa et al., 2016). From those, four species, *Chelonus elaeaphilus*, *Ageniaspis fuscicollis*, *Apanteles xanthostigma* and *Elasmus flabellatus* have been the most representative for this pest (Bento, 1999, Gonçalves et al., 2006, Villa et al., 2016). Studies performed in the field showed that the parasitism rates can reach 21.0% for *A. xanthostigma*, 38.9% for *E. flabellatus*, 67.0% for *A. fuscicollis* and 88.6% for *C. elaeaphilus* (Bento, 1999). Among predators, *C.*

carnea, dermapterans and hemipterans such as *Orius* sp. have been listed as natural enemies of the olive moth. Further studies need to be developed using species adapted to the mediterranean climate in order to implement efficient biological control programmes.

The **entomopathogen** ***Bacillus thuringiensis*** is the most wide and successful commercial biological control agent of lepidopterous pests. The use of this agent against *P. oleae* has been considered efficient. Bento et al. (1999b) found 60-80% of mortality rate of larvae of the antophagous generation collected in a plot treated with *B. thuringiensis* when compared with a non-treated plot. Moreover, the number of males captured on delta traps baited with pheromone was significantly lower in the *B. thuringiensis* plot as well as the percentage of attacked fruits (14% in the *B. thuringiensis* plot compared to 32% in the non-treated plot) (Cabanas et al., 2003).

c. The black-scale, *Saissetia oleae*

For the black-scale, most studies have been conducted in order to know the communities of natural enemies and promote their occurrence in the olive orchard through conservation biological control. Entomophagous insects of the black-scale include more than 10 species of **parasitoids** (Insecta: Hymenoptera) and around five species of **predaceous ladybugs** (Insecta: Coccinellidae). Among parasitoids, *Metaphycus lounsburyi*, *M. flavus*, *M. helvolus* (Hymenoptera: Encyrtidae), *Scutellista caerulae*, *S. obscura* (Hymenoptera: Pteromalidae), *Coccophagus lycimnia* and *C. semicircularis* (Hymenoptera: Aphelinidae) have been referred as frequent species emerging from the black-scale specimens (Pereira, 2004; Tena et al., 2008; Marrão, 2017). Parasitism rate reached 27.4% for *C. lycimnia* and 62.4% for *M. lounsburyi* in the study performed by Pereira (2004) and the parasitoids emerged from the black-scale were specific to one or two scale stages. Considering predaceous coccinellids, the most common species identified in olive orchards infested by the black-scale were *Chilocorus bipustulatus*, *Brumus quadripustulatus*, *Rhyzobius chrysomeloides* and *Scymnus interruptus* (Katsoyannos, 1992; Santos, 2007). The maintenance of these communities can contribute to keep the population of the black-scale under the economic threshold level. Biological control of the black-scale was implemented in France, by releasing *M. lounsburyi*, and in Greece, through the mass production of *B. quadripustulatus* (Katsoyannos, 1992) resulting in the reduction of the population of the pest.

2.2. Biotechnological control methods

Biotechnological control methods are promising techniques based on the understanding of pests' behaviour to manage infestations while contributing to the sustainability of modern agriculture (Attathom, 2002). These strategies include population inhibitors (with the sterile insect technique), growth regulators, attractants (pheromones), repellents and oviposition deterrents substances. For instance, semiochemical-based pest management provides environmentally friendly control through mass-trapping and/or mating disruption with sex pheromones (El-Sayed et al., 2006).

Knowledge gathered on the different interactions occurring in the olive orchard (among olive tree-microorganisms-natural enemies-non-crop plants) can be used to manage both pests and natural enemies for instance, under push-pull strategies. These strategies involve attempts to change the environment in order to decrease the pest population through a combined use of repellents placed in the olive orchards. Simultaneously, natural enemies are attracted into the crop in order to increase their action against pests. The identification of chemical compounds involved in these push-pull strategies can lead to the creation of new and more specific traps (to attract and kill pests) or devices (to attract and reward natural enemies) that could be used in both traditional and high density olive groves.

a. The olive fruit fly, *Bactrocera oleae*

The sex pheromone, identified by Baker et al. (1980), used to monitor *B. oleae* population is the 1,7-dioxaspiro [5.5] undecane compound and is secreted by virgin females to attract males. This pheromone has been integrated in an attract-and-kill trap in combination with ammonium phosphate (Mazomenos et al., 2002). Mass-trapping devices, such as the OLIPE trap, a bottle trap developed in Spain for organic olive groves by the Cooperative Olivarera de los Pedroches (Caballero, 2001), can capture high number of individuals of *B. oleae.* However, the high number of OLIPE traps needed to maintain low *B. oleae* population densities turns this method less interesting for the olive growers.

Growth regulators such as methoprene reduced the number of flies emerging from pupae and increased the incidence of sterility and malformation in adults. However, this substance was efficient during low density populations of the olive fruit fly (Delrio, 1985).

Infested fruits release specific volatiles that can have beneficial or negative impact on *B. oleae* (Lo Scalzo et al., 1994; Scarpati et al., 1996). Among others, kairomones are semiochemicals inducing beneficial interactions among pests and the plant, for instance in host finding process for oviposition. Germinara et al. (2006) listed few attractants, repellents or oviposition deterrents for *B. oleae*: Hexan-1-ol, (+)-ɑ-Pinene and D-(+)-Limonen are attractants whereas (E)-2-Heptanal and (E)-2-Hexanal are categorized as repellents. The Sterile Insect Technique (SIT) has also been tested to control *B. oleae* without a significant success, mainly because of a difficult and complex insect rearing process (Estes et al., 2012).

Among **entomopathogens**, spinosad, a naturally derived insecticide produced by fermentation of the actinomycete Saccharopolyspora spinosa, can be an alternative to synthetic insecticides, in particular, when the pest population level is low. Results obtained in field surveys indicated that spinosad can be as effective as dimethoate under specific conditions (Gonçalves et al., 2012).

b. The olive moth, *Prays oleae*

Less is known about biotechnical methods for *Prays oleae*. The pheromone used to monitor *P. oleae* population is (Z)-7-tetradecenal and was identified by Campion et al. (1978). This compound is also involved on *P. oleae Trichogramma* parasitoïds attraction (Milonas et al., 2009), in mass-trapping systems as well as in mating disruption technique (Mazomenos et al., 1999). Considering mating disruption, although male disorientation was achieved, fruit damage level remained above the recommended economic threshold indicating that this technique needs to be optimized (Bento et al., 2005). Moreover, ethylene diffusers have been shown to reduce damage caused by the olive moth (Rosales et al., 2006).
Artificial food sprays (e.g., protein hydrolysate, yeast hydrolysate, acid-hydrolyzed L-tryptophan) were tested aiming at increasing the abundance of natural enemies in the olive grove. In field surveys, these substances were sprayed on olive foliage and results showed an increase of the number of parasitoids, Coccinellidae, Chrysopidae, Hemiptera, Diptera and Lepidoptera in the crop. Predation of olive moth eggs increased but no effect on flower and fruit infestation was observed (Bento et al., 2004).

c. The black-scale, *Saissetia oleae*

Currently no described biotechnological control methods are available to control the olive black-scale, *S. oleae*. This knowledge gap needs to be filled in the following years.

3. Research gaps and practical solutions

Research focusing on biological and biotechnical control methods is very expensive, needs highly qualified skills and a deep knowledge on the plant-microbiota-arthropod interactions and with the agroecosystem. Therefore, it is important to support research ideas aiming at developing these methods and/or filling the gaps. Currently, several research gaps can be listed, although some progresses have been made in the last decades on a few of them:

- The release of biological control agents to control olive pests had limited effects being questionable their efficacy. Therefore, conservation biological control strategies are needed to improve the communities of natural enemies; Practical solutions such as the conservation of field strips or buffer areas with spontaneous plants in traditional olive groves or the implementation of in-field buffer areas with herbaceous or woody plants in high density olive groves can create more heterogeneous landscapes that would supply food (nectar, pollen and honeydew) to natural enemies; moreover, for each specific geographical region, it is important to know which biological control agents can be enhanced and which strategies can be used to increase their populations. Networks including people working in this area should be created in order to gather data obtained in each country and generate guidelines that could be useful for farmers when they decide to manage the olive grove in a more sustainable way.

- The efficacy of several biological and biotechnical methods have been tested and most of them can only be used against low population levels or a specific generation of the pest due to its complex life cycles. Increasing the knowledge about potential available or new attractants that can be used for mass-trapping and study their efficacy in the field should be promoted.
- The identification of new attractants, deterrents and repellents compounds, based on plant-arthropod interactions, is needed and deciphering their action can only be possible by developing studies under laboratory conditions. These studies should combine analytical chemical, neurophysiological and behavioral studies in order to produce new practical devices that could be used in more sustainable production olive groves.

4. Priorities for further innovative actions and research

Olive pest management is a key tool to control pest infestations and maintain profitable economic earnings while promoting sustainable farming practices. Despite of the recent scientific accomplishments, further innovative actions and research on biological and biotechnical control methods need to be drawn up.
For instance, natural enemies of olive tree pests introduction is time-consuming and the observed results are often poor. Moreover, number of safeguards are necessary before implementing importation actions and release to ensure imported organism will not pose additional threats to non-target organisms.

An overall understanding of the olive agroecosystem and how the pests, the olive crop and the environment interact together would give new research axes to develop innovative non chemical control methods. At the olive grove scale, semiochemicals or other compounds influence the behaviour of pests and their associated natural enemies. The conditions and consequences of these molecular interactions either attractive or repulsive remain unclear for the majority of olive tree pests. Accordingly, to study the chemical ecology of olive pests may be an interesting path to improve their management on the field.
On this same idea, farmers and scientists can elaborate a trophic network depicting all the trophic interactions existing between all organisms naturally present in an orchard. The obtained overview of the local biodiversity will widely be necessary to figure out what kind of management or technique to apply or how these trophic interactions can be modified when introducing an exogenous component. For instance, a trophic map could either preconize augmenting natural enemies through rearing and periodic releases, or by using biotechnical or physical (mechanical) control methods. In some cases, the use of specific entomopathogens can be interesting as they normally target only one pest. The agroecosystem study could also highlight the present of these entomopathogens on an orchard leading to their population management to control the targeted pest. However, this strategy is poorly studied although it appears to be an interesting management tool for farmers and farmer's associations.
Recently, the FAO guidelines promoted biological and biotechnical control methods to manage pest infestations. Additional research priority would be to find new substances based on plant extracts that could act as repellent for *B. oleae* to implement for instance push-pull strategies in the field to have a more effective control method. Some attractive substances are already known (Lo Scalzo et al., 1994) but there is a lack of knowledge about the field application or their efficacy on grove conditions. Innovative trapping systems can be set up to overcome environmental constraints (temperature, wind or rain, ...) and ensure the effectiveness of a treatment over a longer period. This would be beneficial for farmers.
The lack of applied research is evident and efforts must be done for further attractive or repellent systems development.

5. Links to potential practical operational groups

There are different options that can be made in the framework of a pest control program. In this paper, we reviewed the potential use and the research gaps related with biological and biotechnical methods against olive pests. Thus Operational Group (OP) Proposals related to olive orchard, biological control or biotechnical control of pests (tephritid pests or lepidopterous pests) linked to the subject presented in the paper should be promoted. Investments on the development of new products based on the knowledge obtained from plant-microorganism-pest-arthropod interactions should be promoted. In this context, attractive volatile substances can be used to pull natural enemies into the crop and repellent volatile substances can be used to reduce olive pest infestations. Biotechnological based knowledge can be obtained through the identification of specific volatile substances using GC analyses followed by behavioural and olfactory experiments and electroantennography with natural enemies and pest species. Innovative solutions (e.g., food attractants, new and more efficient traps) could be created after these studies in order to turn the olive grove more resilient and more sustainable, mainly those olive groves where insecticides are frequently sprayed.

6. Dissemination of results to stakeholders

Based on the knowledge resulting from several studies in the domain of biological and biotechnical control, there is a need to inform farmers about the existence of alternative methods to the use of pesticides, how they can introduce these methods in the olive grove and the importance of promoting functional biodiversity in the agroecosystem. Practical workshops can be arranged as a means to disseminate information and train and educate farmers as well as farmers associations to the use of more sustainable strategies, such as the maintenance of spontaneous vegetation and creation of stone walls, improving landscape heterogeneity. For policy makers, it is important to inform for the need to conserve biodiversity and promote their action in the olive agroecosystem and pest control can be achieved through the implementation of biological and biotechnical methods.

7. Conclusions

Pests have been considered as the most economically damaging cause of production loss worldwide. Among them, the olive fruit fly poses a serious threat for all olive growers in most olive producing areas. Native from eastern Africa, there are records of infestations in fruit going back to the third century BC.

Current legislation tends to limit the use of unsafe chemical solutions to control pests of the olive tree. On this regard, biological or biotechnical methods seem to be an effective way for orchard protection and obtain interesting profits. Indeed, several species of parasitoids are known for *B. oleae* (*Psyttalia concolor* and the chalcid wasps *Cyrtoptyx latipes* allowing a good management of this fly in some regions. Moreover, entomopathogens such as *Bacillus thuringiensis* targeting the olive moth is an additional favourable pest control strategy that can overcome chemical products drawbacks.
Concerning biotechnical innovations, chemical ecology is considered to be the most promising option to control olive tree pests. However, the technical limitations are significant due to attractant or repellent molecule volatility under natural environmental conditions.

On a final note, it is necessary to highlight all the promising techniques and solutions mostly available on the market. These innovations or biological strategies, supported by the FAO, tend to control pests infestations and maintain sustainable profits especially for organic farming. Even though research gaps are still noticed (effective repellent, lack of local biodiversity knowledge, etc…), olive pests management by using these approaches will face an important progress within the next few years.

8. References

Al Khatib, F., Cruad, A., Fusu, L., Borowiec, N., Gibson, G., Rasplus, J.-Y., Ris, N., Delvare, G. (2014) An integrative approach to species discrimination in the *Eupelmus urozonus* complex (Hymenoptera, Eupelmidae), with the description of 11 new species from the Western Palaearctic. Systematic Entomology, 39, 806–862.

Arambourg, Y. (1986) Curculionidae. In: Arambourg, Y. (ed.) Traité d'Entomologie Oléicole. Conseil Oléicole International, Madrid, pp.

Attathom, T. (2002) Biotechnology for insect pest control. Proc. Sat. Forum, "Sustainable Agricultural System in Asia" Nagoya Japan. p. 73-84

Baker, R., Herbert, R.H., Howse, P.E., Jones, O.T., Franke, W., Reith, W. (1980) Identification and synthesis of the major sex pheromone of the olive fly (*Dacus oleae*). Journal of Chemical Society, Chemical Communications, 1106, 52-53.

Begg, G.A., Cook, S.M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G.L., Mansion-Vaquie, A., Pell, J.K., Petit, S., Quesada, N., Ricci, B., Wratten, S.D., Birch, A.N.E. (2017) A functional overview of conservation biological control.Crop Protection.97: 145-158

Bento, A. (1999) Contribuição para o estabelecimento de um programa de proteção integrada contra a traça-da-oliveira, *Prays oleae* (Bern.) em Trás-os-Montes. PhD Thesis. Universidade de Trás-os-Montes e Alto Douro, Vila Real. Portugal.

Bento, A. Torres, L., Lopes, J. (1999a) Results from *Trichogramma cacoeciae* March. Releases to control the carpophagous generation of *Prays oleae* (Bern.) in Trás-os-Montes. Revista das Ciências Agrárias, 21, 207-211.

Bento, A. Torres, L., Pereira, J.A., Lopes, J. (1999b) Avaliação da eficácia de *Bacillus thuringiensis* (Berliner) contra a geração antófaga de *Prays oleae* (Bern.) em condições de campo. Proceedings of the V Encontro Nacional de Proteção Integrada. Bragança, pp. 239-244.
Bento, A., Cabanas, J.F., Pereira, J.A, Torres, L. Herz, A., Hassan, S.A. (2004) Effects of different attractive sources on the abundance of olive predatory arthropods and possible enhancement of their activity as predators on eggs of *Prays oleae* Bern. 5th International Symposium on Olive Growing. Izmir, p.73.
Bento, A., Pereira, J.A, Cabanas, J.F., Konstantopoulou, M., Torres, L., Mazomenos, B. (2005). Mating disruption trials for the olive moth, *Prays oleae* (Bern.), (Lep.:Yponomeutidae) in Trás-os-Montes olive groves (Northeast of Portugal). V Congresso Ibérico de Ciências Hortícolas, IV Congresso Iberoamericano de Ciências Hortícolas, Porto, Portugal.
Bernardo, U., Guerrieri, E. (2011) Controllo eco-sostenibile della mosca dell'olivo: recenti acquisizioni. Acta Italus Hortus, 1, 351–354.
Bigler, F., Neuenschwander, P., Delucchi, V., Michelakis, S. (1986) Natural enemies of preimaginal stages of Dacus oleae Gmel. (Dipt., Tephritidae) in Western Crete II. Impact on olive fly populations. Bollettino Laboratorio di Entomologia Agraria Filippo Silvestri, 43, 79–96.
Boccaccio, L., Petacchi, R. (2009) Landscape effects on the complex of *Bactrocera oleae* parasitoids and implications for conservation biological control. BioControl. 54:607–616.
Bueno, A.M., Jones, O. (2002) Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. IOBC wprs Bulletin, 25, 1-11.
Caballero, J.A., 2001. Sistemas de control de la mosca del olivo (*Bactrocera oleae*) en olivar ecológico. Experiencias en "Los Pedroches". Actas X Simposium científico-técnico. Foro del olivar y el medio ambiente. Comunicación nº 12.
Cabanas, J.E., Pereira, J.A., Bento, A.A., Pereira, S., Torres, L. (2003) Efficacy of *Bacillus thuringiensis* to control the olive moth, *Prays oleae* (Bern.) and side effects on the olive tree arthropodofauna. 1st European Meeting of the IOBC/WPRS Study Group-Integrated Control in Olives. Chania, 70.
Campion, D.G., I. Polyrakis, S. Michelakis and B.F. Nesbitt. (1978) The sex pheromone of the olive moth *Prays oleae* Bem. and its uses in the field. Olea (FAO): 42-47.
Daane, K., Johnson, M. (2010) Olive fruit fly: managing an ancient pest in modern times. Annual Review of Entomology, 55, 155–169.
Delrio, G. (1985) Biotechnical methods for olive pest control. In Cavalloro, R., Crovetti, A. (Eds) Integrated pest control in olive groves. Proceedings of the CEC/FAO/IOBC International Joint Meeting. 3-6 April 1984, Pisa, Italy.
Dinis, A.M., Pereira, J.A., Pimenta, M.C., Oliveira, J., Benhadi-Marín, J., Santos, S.A.P. (2016) Suppression of *Bactrocera oleae* (Diptera: Tephritidae) pupae by soil arthropods in the olive grove. Journal of Applied Entomology, 140, 677–687.
Estes, A.M., Nestel, D., Belcari, A., Jessup, A., Rempoulakis, P., Economopoulos, A.P. (2012) A basis for the renewal of sterile insect technique for the olive fly, *Bactrocera oleae* (Rossi). Journal of Applied Entomology, 136, 1-16.
Gebiola, M., Bernardo, U., Monti, M., Navone, P., Viggiani, G. (2009) *Pnigalio agraules* (Walker) and *Pnigalio mediterraneus* Ferrière and Delucchi (Hymenoptera: Eulophidae): two closely related valid species. Journal of Natural History, 43, 2465–2480.
Giacalone C, Maltese M, Caleca V. (2011) Parassitizzazione di *Bactrocera oleae* su olivo coltivato e selvatico in sicilia e nel western cape (Sud Africa). In: XXIII CNIE Genova, 13-16 giugno 2011. p. 338.
Gonçalves, F., Oliveira, A., Herz, A., Torres, L. (2006) Contribuição para o conhecimento da bioecologia da traça-da-oliveira, *Prays oleae* (Bernard) em Trás-os-Montes. IV Simpósio Nacional de Olivicultura, Elvas, 22 a 24 de novembro, Melhoramento, 41, 146.
Gonçalves, F., Santos, S.A.P., Torres, L. (2012) Efficacy of spinosad bait sprays to control *Bactrocera oleae* and impact on non-target arthropods. Phytoparasitica, 40, 17 – 28.
Hoelmer, K.A., Kirk, A.A., Pickett, C.H., Daane, K.M., Johnson, M.W. (2011) Prospects for improving biological control of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), with introduced parasitoids (Hymenoptera). Biocontrol Science & Technology, 21, 1005–1025.
Johnson, M.W., Daane, K.M., Wang, X-G., Yokoyama, V., Pickett, C.H., Hoelmer, K.A., Kirk, A.A.. (2012) Biological control of olive fruit fly. Integrated Protection of Olive Crops IOBC- WPRS Bulletin Vol 79 2012. 79:63–69.
Katsoyannos, P. (1992) Olive Pests and Their Control in the Near East. FAO Plant Production and Protection Paper, 115- 178 pp.
Kombargi, W.S., Michelakis, S.E., Petrakis, C.A. (1998) Effect of olive surface waxes on oviposition by *Bactrocera oleae* (Diptera: Tephritidae). Journal of Economic Entomology, 91(4), 993–998.
Konstantopoulou, M.A., Mazomenos, B.E. (2005) Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. BioControl, 50, 293
–305
Lo Scalzo, R., Scarpati, M. L., Verzegnassi, B., Vita, G. (1994) *Olea europaea* chemicals repellent to *Dacus oleae* females. Journal of Chemical Ecology, 20 (8), 1813–1823.
Loumou, A., Giourga, C. (2013) Olive groves: "The life and identity of the mediterranean". Agriculture and Human Values, 20, 87-95.
Marrão, R.M. (2017) Effect of parasitoid competition, ant exclusion and carbohydrate sources on biological control of *Saissetia oleae* on olive trees. PhD Thesis. Universidad de León, León, Spain.
Mazomenos, B.E., Ortiz, A., Mazomenos-Pantazi, A., Stefanou, D., Stavrakis, N., Karapati, C., Fountoulakis, M. (1999). Mating disruption for the control of the olive moth, Prays oleae (Bern) (Lep., Yponomeutidae) with the major sex pheromone component. Journal of Applied Entomology, 123, 247–254.
Mazomenos, B. E., Pantazi-mazomenou, A., Stefanou, D. (2002). Attract and kill of the olive fruit fly *Bactrocera oleae* in Greece as a part of an integrated control system, Use of pheromones and other semiochemicals in integrated production. IOBC wprs Bulletin. *25*, 1–11.
Milonas, A. P. G., Martinou, A. F., Kontodimas, D. C. H., Karamaouna, F., Konstantopoulou, M. A. (2009) Attraction of different *Trichogramma* Species to *Prays oleae* sex pheromone. Annals of the Entomological Society of America, 102 (6), 1145–1150.
Neuenschwander, P. (1982) Searching Parasitoids of *Dacus oleae* (Gmel.) (Dipt., Tephritidae) in South Africa, Zeitschrift fur Angewandte Entomologie, 94, 509-522.
O'Neil, R., Obrycki, J. (2008). Introduction and augmentation of biological control agents. In E. Radcliffe, W. Hutchison, & R. Cancelado (Eds.), Integrated Pest Management: Concepts, Tactics, Strategies and Case Studies (pp. 107-115). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511626463.010
Pereira, J.A. (2004) Bioecologia da cochonilha-negra, *Saissetia oleae* (Olivier), na oliveira em Trás-os-Montes. PhD Thesis. Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal.
Pereira-Castro, I., Van Asch, B., Rei, F.T., Costa, L.T. (2015) *Bactrocera oleae* (Diptera : Tephritidae) organophosphate resistance alleles in Iberia : Recent expansion and variable frequencies. European Journal of Entomology, 112, 20–26.

Rosales, R., Garrido, D., Ramos, P, Ramos, J.M. (2006) Ethylene can reduce *Prays oleae* attack in olive trees, Crop Protection, 25, 140-143.
Rugman-Jones, P., Wharton, R., Noort, T., Stouthamer, R. (2009) Molecular differentiation of the *Psyttalia concolor* (Szépligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Africa. Biological Control, 49, 17–26.
Santos, S.A.P. (2007). Action of predators against the black-scale, *Saissetia oleae* (Oliv.) in Trás-os-Montes olive groves. PhD Thesis. Universidade de Aveiro. Aveiro.
Sasso R., Gualtieri L., Russo E., Nugnes F., Gebiola M., Bernardo U. (2018) Biology, ecology and behaviour of *Baryscapus silvestrii* (Hymenoptera: Eulophidae), a gregarious pupal parasitoid of fruit-infesting flies. XI European Congress of Entomology 2-6 July 2018 Naples, Italy; PO145 p. 196.
Sasso R., Gualtieri L., Russo E., Nugnes F., Gebiola M., Bernardo U. The establishment of a rearing technique for the fruit fly parasitoid *Baryscapus silvestrii* (Hymenoptera: Eulophidae) increases knowledge of biological, ecological and behavioural traitsIn prep.
Scarpati, M.L., Lo Scalzo, R., Vita, G., Gambacort (1996) Chemiotropic behavior of female olive fly (Bactrocera oleae GMEL.) on Olea europaea L. Journal of Chemical Ecology 22 (5), 1027–1036.
Silvestri F (1913a) Viaggio in Africa per cercare parassiti di mosche dei frutti. Boll Lab Zool Gen Agr R, Portici 8:3-164.
Silvestri F (1913b) Notizie preliminari di un *Tetrastichus* (Imenottero Calcidide) parassita di *Ceratitis* e *Dacus* nell'Africa occidentale. R Accad Linc 22:205-206.
Silvestri F (1914) Viaggio in Eritrea per cercare parassiti della mosca delle olive. Boll Lab Zool Gen Agr R, Portici 9:186-226.
Silvestri F (1915a) Contributo alla conoscenza degli insetti dell'olivo dell'Eritrea e dell'Africa meridionale. Boll Lab Zool Gen Agr, Portici, 9:240-344.
Silvestri F (1915b) Descrizione di nuovi Imenotteri Calcididi africani. Boll Lab Zool Gen Agr, Portici 9:337-377.
Sime, K.R., Daane, K.M., Kirk, A., Andrews, J.W., Johnson, M.W., Messing, R.H. (2007) Psyttalia ponerophaga (Hymenoptera: Braconidae) as a potential biological control agent of olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) in California. Bulletin of Entomological Research, 97, 233-242.
El-Sayed, A.M., Suckling, D.M., Wearing, C.H., Byers, J.A. (2006) Potential of mass trapping for long-term pest management and eradication of invasive species. Journal of Economic Entomology, 99(5), 1550-1564.
Tena, A., Soto, A., Garcia-Marí, F. (2008) Parasitoid complex of black scale *Saissetia oleae* on citrus and olives: parasitoid species composition and seasonal trend. Biocontrol, 53, 473-487.
Van Driesche R, Hoddle M, Center T (2008) Control of pests and weeds by natural enemies. Blackwell Publishing Limited, Malden
Viggiani, G., Bernardo, U., Sasso, R. (2007) Description of *Baryscapus silvestrii,* n. sp. (Hymenoptera: Eulophidae), a new gregarious parasitoid of the olive fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) in southern Italy. Bollettino del laboratorio di Entomologia Agraria Filippo Silvestri, 61, 63–70.
Villa, M., Santos, S.A.P., Mexia, A., BentoA., Pereira, J.A. (2016) Ground cover management affects parasitism of Prays oleae (Bernard). Biological Control, 96, 72–77.