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OILB-SROP

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Dionyssios Perdikis, Jelena Latinović, and Andrea Lucchi

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Preface

This IOBC-WPRS Bulletin contains the proceedings of the 6th meeting of the IOBC-WPRS Working Group "Integrated Protection of Olive Crops" that was held in Bečići, Budva, Montenegro, 12th to 15th May 2013.

In the meeting 27 presentations were given on topics related to the integrated management of pests, diseases and weeds of the olive crops. Emphasis was given to the new developments in pest and disease management, molecular biology of olive fruit fly, parasitoids and predators of olive fruit fly and the olive moth, entomopathogenic fungi and nematodes, bait sprays wide area olive fly control, electroantennographic responses, bacterial symbiosis, biodiversity and biological control of olive pests, control of olive leaf spot and olive fruit rot and other topics.

In the meeting, 3 invited lectures were given that very nicely elaborated the state of the art and the needs for future research in ad-hoc topics. The high standard presentations and the intensive discussions underlined that this group provides a forum for integration of research and extension of protection measures in olive crops.

This meeting was organized by Jelena Latinović, Snježana Hrnčić, Tatjana Perović from the University of Montenegro and Zorka Prljević from the Phytosanitary Directorate of Montenegro. The organizers did excellent work to ensure us a scientifically very fruitful and inspiring staying. This meeting was characterized by a very friendly atmosphere that very soon created among the participants, the ample opportunity for sharing ideas and findings that led to the establishment of cooperation activities of the WG.

Many sincere thanks are expressed to all the members of the organizing committee for a really excellent, professionally organized and enjoyable conference. Many thanks are also due to the sponsors of the meeting and also to the IOBC-WPRS. Finally, I would like to thank the co-editors Jelena Latinović and Andrea Lucchi for their contribution to the review of the papers of this bulletin.

Dionyssios Perdikis
(Convenor)

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Opening Session

Integrated Protection of Olive Crops
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Olive growing in Montenegro – current state and perspectives

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Abstract: Montenegro is a small country bordering the Adriatic Sea with the coastline of 293.5 km, and topography characterized by large hilly mountainous areas with distinctive relief and only small areas of lowland. The terrains of olive growing areas are either sloped with very high inclination or foothills of the mountain massifs of Orjen, Lovcen and Rumija. Favourable environmental conditions prevail on the Montenegrin seaside enabled the olive to become the leading species in the coastal area. With about 450,000 productive trees is spread at around 3,200 ha. It is dominantly traditional culture (70%) grown on family farms. New olive groves present approximately 10% of total olive growing and are increasing. The olive assortment is composed of autochthonous varieties in which variety Zutica dominate, approximately 65%. There are around 5% of introduced foreign varieties, Picholine, Coratina, Leccino, in the new groves, and Arbequina variety for high density orchards. The production of olive oil is relatively low (below 500 t), making Montenegro importing country for additional 300 t per year. There are much bigger capacities for oil production, of around 2,000 t, which can significantly reduce the import by increasing of domestic production and utilization of existing capacities. Consumption per capita is low, except in the coastal area where every day diet is based on olive oil, also due to the price of 8-15 Euro per liter. There is also a limited production of organic olive oil. Plant protection influences very much the olive growing, olive production and olive oil quality. However, the olive protection is conditioned by the terrain structure, traditional olive groves and small properties. Monitoring of major pest, olive fly, is performed by Plant protection department in collaboration with olive growers Association and Extension service. The olive oil processing technology has been changed significantly in the last decade. Nowadays there are only 11 traditional mills active and 15 two-phase system plants have been introduced. The processing facilities improvement is followed by improvement of the condition for olive oil storing. The olive oil quality control is based on determination of the basic quality parameters. However, recent equipping of the laboratory at Biotechnical Faculty with GS and HPLC, extended the range of analyses for the market, as well as for scientific purpose as in characterization of olive oil of local varieties. Montenegro is very rich in agricultural genetic resources and olive germplasm as well, considering the small area covered. There are the oldest exemplars of the old trees in the region and beyond, the 'Old Olive' in area of Bar, estimated to more than 2,000 years old and protected by law, and the 'Velja maslina – 'Great olive' in area of Budva, and numerous other exemplars. Characterization of local varieties on morphological and molecular level, clone selection of the major variety and preservation of olive genetic resources are big challenges for Biotechnical Faculty. Furthermore, the area of Montenegro coast as the main olive habitat is threatened by the construction of tourism facilities. From the other side, the olive is the basis for the coastal rural tourism. The important ways to market olive oil are through the tourism, and through organized Mediterranean diet, providing great opportunities and challenge for the olive oil sector.

Key words: olive production, genetic resources, tradition, Montenegro

Introduction

Montenegro is a small country bordering the Adriatic Sea with the coastline of 293.5 km. The general topography of Montenegro is characterized by large hilly mountainous areas with distinctive relief and only small areas of lowland. Only 10% of the territory is below 200 m above sea level, 35% from 200 to 1,000 m, and 40% from 1,000 to 1,500 m.

Thanks to the topography, agriculture is quite diversified, from olive and citrus growing in the coastal region, early vegetables and tobacco in the central part to extensive sheep breeding in the northern part.

The terrains of olive growing areas are either sloped with very high inclination or foothills of the mountain massifs of Orjen, Lovcen and Rumija (85%), (Figure 1). To smaller extent the olive is present in the areas of Scadar Lake and of Podgorica, where planting of olive trees has been intensified in the last 5 years. Influence of costal climate can be effective up to 500 m above sea level.

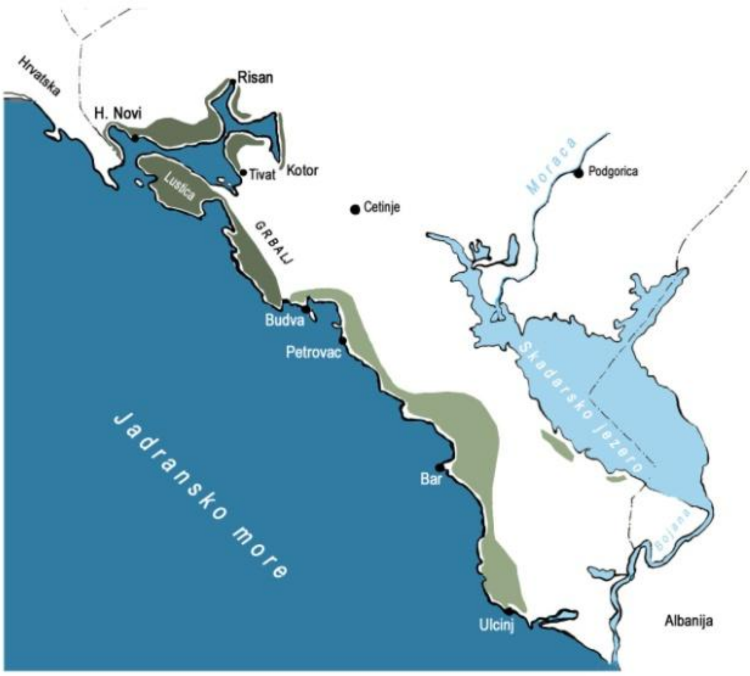

Figure 1. Olive growing sub-areas.

Favourable environmental conditions prevail on the Montenegrin seaside, average temperature is 15.5 °C, with absolute min of -8.5 °C, absolute max of 40.0 °C and average annual rainfall of 1,652 mm, enabled the olive to become the leading plant species in the coastal area and therefore, very important for the country.

In the recent period many challenges appear for olive sector in Montenegro, from the danger of urbanization of olive area as being very attractive for tourism development, to the needs to improve the management in olive growing, increase the production and to improve the olive oil quality. In that sense very important is to use the existing capacities to promote olive production in Montenegro as ecological and to market the products through the tourism.

Olive growing

The olive in Montenegro is spread at around 3200 ha with about 450,000 productive trees, which is 88% of the total number of trees (almost 500,000) (Monstat, Stat. Yearbook, 2012). It is dominantly traditional culture (70%) grown on family farms, which are fragmented to be groves of 0.2-2.0 ha. Bigger plantations exist in the south, around the area of Valdanos with nearly 80,000 trees (Photo 1), which are protected by law, and on Luštica peninsula in the northern coast with 20,000 trees. New olive groves present approximately 10% of the total olive growing and are increasing. Planting of new orchards is the most intensive in area of Ulcinj, then in Boka Kotorska area and recently in the continental part, area of Podgorica. In the last five years around 50,000 olive trees were planted.

Photo 1. Valdanos, area with 80,000 olive trees.

Options for extending olive tree growing area are considerable, especially on the coastal area and the most dynamic in plain areas in the Ulcinj surroundings in the south. The olive growing area of Zeta-Bjelopavlici plain, near Podgorica, with around 5,000 ha is especially interesting, although facing the obstacle of low winter temperatures. However, new trends in agricultural production, awareness on the need of using olive oil in the diet and prestige made family farms in the surroundings of Podgorica interested for olive cultivation. Moreover, the interest of enterprise 'Plantaze' to introduce olive into the production program of wine, table grape and peach, and to use the products through the company's restaurants, guiding other small enthusiasts to be involved in olive sector. Foreign varieties are introduced for growing in this area as being tolerant to the low winter temperatures.

Planting new orchards is permanently supported by the Government, through subsidies that comply with European legislation on benefits in agriculture. However, initiatives to improve olive production come from the producers (olive oil) and through the Association of Olive Growers (AOG), reflecting ownerships – olive orchards and plantations are almost entirely privately owned. The Strategic plan of the AOG is to reach one million of olive trees and to compensate the fund of nearly 200,000 trees destroyed during 20th century.

The coastal as well as the olive area is still exposed to devastation and destruction, influenced by tourism development. On the other hand, old plantations are renewed and functionally used of around 60%. The Strategy of agricultural development (2006-2013) however, considered potential for growing olives in Montenegro many times larger than the existing one.

Olive assortment

The olive assortment is composed of autochthonous varieties, domestic and domesticated, spread in two coastal sub-areas (Figure 1): Bar sub-area – covers municipalities of Ulcinj, Bar and Budva, where the Zutica variety (Photo 2) predominates, with 95-98%; and Boka Kotorska sub-area – includes municipalities of Tivat, Kotor and H. Novi, where beside Zutica, there are other varieties Crnica (15%), Lumbardeska (7%), Sitnica (6%), Sarulja (5%) and others present in around 2% of total. Generally, Zutica variety dominates, approx. 65%. Zutica is appreciated due to its oil quality but also by local use as table variety, prepared on local ways as green and black olives.

During the period of more than 60 years, the different olive collections in Montenegro were established with the aim to investigate the possibility of foreign varieties to be grown in the climatic conditions of the Montenegrin coast and hinterland. However, there are only around 5% of introduced foreign varieties in the new groves on the coastal area, among which are Picholine, Coratina, Leccino. In the area of Podgorica varieties resistant to low temperatures (Leccino, Coratina, Istarska Belica) have been introduced into new plantations and Arbequina variety for high density orchards.

Photo 2. View on the old plantation of cv, Zutica (a) and renewed tree of cv. Zutica (b).

Olive oil production

Olive oil from Bar and general Montenegrin coast was highly valued in the past, and in the first half of the 20th century was exported to European countries and to America. Even today, people are aware of oil from area of Bar, which was served in some European courts. It was, however, the time when the government law committed the obligatory measure for every soldier, government officials, or a man to marry, to plant a certain number of olive trees. As a consequence, the olive sector intensively developed.

The greatest improvement in olive oil production came with establishment of modern olive oil plant ‘Braća Marić’ in 1927 in the southern area of the coast, in Bar. In the second half of 20th century the tradition was continued with ‘Primorka’, state owned factory, which introduced continuous line for olive processing during 80’s.

Development of the olive oil processing technology that has been changed significantly in the last decade contributes to the oil production. Only ten years ago the majority of olive mills were traditional. There are only 11 traditional mills (reducing every year) actively processing olives, and 15 two-phase system plants have been introduced. The processing facilities improvement is followed by improvement of the condition for storing olive oil. Nowadays every olive mill is equipped with tanks of stainless steel of different capacity, providing the conditions for storing the oil after processing. Several producers developed their brands, but the most important are the trade mark 'Barsko zlato' (Bar's gold), ‘Olcinium’, ‘Moric’, etc.

Presently, olive oil production in average is relatively low (below 500 t), making Montenegro importing country for additional 300 t per year. From economical point of view that means from 700 thousand to one million of Euros, of which fund investment only a portion into olive sector, would importantly increase this agricultural sector. This would also take advantage of existing bigger capacities for oil production, of around 2000 t, what was the production in 2012. In this way the import can be significantly reduced by increasing of domestic production and utilization of existing capacities.

There is also a limited production of organic olive oil. In general, the olive production in Montenegro can be considered as organic, with favourable conditions for potential certification and further development. Registered plant for processing olives from organic production in Lustica is incentive to olive growing in this area and wider.

…and consumption

As a consequence of the relatively low production, consumption per capita is low, less than 0.5 l, except in the coastal area, where the diet is based on olive oil usage. Traditional use of olive oil (especially the 'samotok'-free oil’) for medicine is widespread in all parts of Montenegro. With the increase in production it is possible to promote usage of olive oil in the daily diet, not only on the coast but also in the continental part of the country.

The olive is the basis for the coastal rural tourism. The important ways to market olive oil are through the tourism and through organized Mediterranean diet, providing great opportunities and challenge for the olive oil sector.

An obstacle toward increment of the olive oil usage and marketing is the price, which is in the range of 8-15 Euro per liter of oil. Increased production would contribute to the reduction of the oil prize and as a consequence increasing the oil availability to the general population. A good opportunity is to export the olive oil as ‘domestic oil’ to the nearby-region (Serbia, Bosnia and FYROM).

Olive oil quality

The quality of olive oil for farmers is controlled in the laboratory of the Centre for subtropical cultures in Bar, involving determination of the basic quality parameters. The interest to control the free fatty acids and other parameters which show the changes during the olive oil storage increased in the last 5 years. Ministry of Agriculture and Rural Development is supporting basic control of olive oil quality with the aim to upgrade the farmers' knowledge and the quality of production.

To increase the performance and to extend the range of services in olive oil quality control, mentioned laboratory of Biotechnical Faculty in Centre in Bar was recently equipped with GS and HPLC. Besides extending the range of analyses for the market, scientific purpose in characterization of olive oil of local varieties is important as well.

Scientific aspect is very important when it comes to testing the oil quality of individual indigenous varieties from Montenegro (Miranovic, 1994a). Characterization of monovarietal oils started a few years ago, through the bilateral cooperation project with Slovenia (Lazovic *et al*., 2012). However, there is increasing interest in the analysis at this level, especially when it comes to the protection of geographical origin, production monovarietal or specific varietal oils and production from specific micro location or organic production.

The issue of quality control is also significant in terms of import. Since Montenegro imports large quantities of olive oil, quality control of marketed oils, oil from import, control during storage etc., must be provided.

Genetic resources

The great diversity of germplasm in the Mediterranean region characterizes also Montenegrin germplasm, and represents an important source for future breeding. To exploit this resource, more information is needed, including the level and distribution of variability within cultivars, the genetic relationship among cultivars and with wild olives, the markers linked to the main traits under selection, and so on (Rugini *et al*., 2008).

Montenegro is very rich in agricultural genetic resources and olive germplasm as well, considering the small area covered. Montenegro has the oldest exemplars of the old trees in the region and beyond. There are 'Old Olive' in area of Bar (Photo 3), estimated to more than 2,000 years old and protected by law, and the 'Velja maslina – Great olive' in area of Budva, and numerous other exemplars, especially in Bar subarea.

Characterization of local varieties at morphological and molecular level as well as clone selection of the major variety is very important for olive growing improvement. In the early days selection of olive trees for the commercial industry was based mostly on fruit size, shape and only partially on tree performance and yield. Presently, it became evident that choosing the genitors on basis of morphological and performance criteria is often misleading as the genetic information and heredity potential of the different cultivars and the olive species in general are limited (Lavee, 2012).

Photo 3. ‘Stara Maslina’ (Old Olive) at Mirovica, Bar.

Main characterization of the olive germplasm in Montenegro was done by morphological descriptors (Miranovic, 1978, 1994b; Miranovic *et al*., 2008). The next phase was to developed biochemical and molecular analysis by using isozymes and RAPD primarily for initial characterization of Montenegrin varieties (Lazovic *et al*., 2001, 2002). Results showed variability within the major variety Zutica, suggesting the importance of future research.

The evaluation of the genetic variability in a precise manner using molecular biology and technologies that emphasize any genetic changes by means of simple sequence repeat (SSR) (Rugini *et al*., 2008). Thus, recent studies carried out using the SSR technique as a part of PhD thesis, widely involved olive material, including old olive trees, indigenous varieties, clones of cv. Zutica, unknown accessions and oleasters. The work has been performed in collaboration with the University of Koper, Slovenia. Results indicate the variability within the variety Zutica (Lazovic & Adakalic, 2012a) but to a lower extent than on morphological and protein level. Also, the variability within the other local varieties was recorded.

On the other hand the high variability in the total genetic material indicates the need for further and deeper work on this topic (Adakalic *et al*., 2010). More attention has to be paid to individual accessions whose performance proves the value for further expansion in terms of yield, oil content in the fruit or fruit size (Lazovic & Adakalic, 2012b, 2012c).

Plant protection

Plant protection influences very much the olive growing, olive production and olive oil quality. However, the olive protection is conditioned by the terrain structure, traditional olive groves and small properties. In the past the olive protection, performed only against olive fly, was by aerial spraying of all the coastal area, which was forbidden a decade ago. Nowadays, each farmer applies protection on its own plantation/grove. Regular monitoring of the most important pest, olive fly is performed by Plant Protection Dpt. in collaboration with Association of olive growers and Extension service. Research on application of models for spraying prognosis and bio-ecological methods for olive pest protection have been recently conducted (Hrncic *et al*., 2007; Perovic *et al*., 2012).

The frame for olive growing development in Montenegro

Ministry of Agriculture and RD is supporting the olive sector through the frame of national legislative and through harmonization to the EU laws. The main reform document, adopted in 2006, is the 'Montenegro's Agriculture and EU – Food Production and Rural Development Strategy'. It foresees the reform of the Montenegrin agricultural policy through its gradual harmonization with EU Common Agricultural Policy (CAP). National program for food production and rural development (2009-2013) defines support measures to the agricultural sector, with specific focus on rural development. Most relevant measure for further development of olive growing sector is the investment support for restructuring crop plantations, which allows grants up to 50% of the investment. Other schemes also are applied to the sector including support to investment in storage, packing and processing, support to producer organizations, support to improving the quality of agro-food production and products.

Olive farmers are grouped into four Associations of Olive Grower. Their activities are directed in providing: the assistance to the farmers, connection to the Ministry of Agriculture, Extension services, researchers supporting pest control, involved in monitoring in the soil quality control, involvement in projects and programs realization (IPA cross-border), organization of the traditional manifestations 'Maslinijada' and 'Bokeska maslina', etc.

Biotechnical Faculty with its Centre for Subtropical Cultures is very much involved in olive sector through different aspects: realization of scientific projects, education of students in modules of olive growing and oil technology through two study programs, expertise in soil analyses, plant protection, olive growers' education, laboratory for olive oil, etc.

Mutual work on different aspects of olive production, such as: having register in olive growing sector; continuous revitalization of old olive groves; collection and utilization of genetic olive resources; expansion of olive growing area by planting new orchards; olive oil quality control and certification; utilization of olive sector for development of the other sectors (tourism, culture, health); education of farmers and extension service; specialization of experts and technicians; enhance the research and scientific programs in olive sector, will only support the olive sector improvement.

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Session 1
Fundamental research in olive diseases and pests

More information about population genetic structure of *Bactrocera oleae* in the Mediterranean region

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DNA methodologies are contributing significantly to our knowledge of population structures and dynamics, phylogeography and phylogeny of different organisms. Nowadays molecular markers are becoming popular tools for tackling insect pest problems. In this way, the *Inter-Simple Sequence Repeats (ISSR)* technique detects DNA variability in inter-microsatellite loci using specific primers designed from dinucelotide or trinucleotide simple sequence repeats (microsatellite). Several works with ISSR demonstrated the hypervariable nature of these markers. The potential of this method for insect population studies, although scarce, has been proved. The fly *Bactrocera oleae* is a worldwide olive crop pest. Larval feeding and premature fruit drop seriously reduces the fruit oil content and quality. In the Mediterranean basin, where 98% of the world's cultivated olive trees are found, production losses have been estimated at least 15%. Olive tree cultivation accounts for some 3.37% of the total agricultural production of the European Union (EU). Spain, with 2.4×10^6 ha of olive orchards that produce 6.2×10^6 tons of olive, is the foremost olive oil producing country in the world. Scientific knowledge of olive fly is economically relevant for the Mediterranean countries. Despite several interesting works about the population structure of the olive fly in the Mediterranean, still no clear conclusions have been achieved. With the objective of contributing to a better knowledge of the population substructure of *B. oleae* in this region, we are conducting a genetic study using several molecular markers and samples from different Mediterranean countries. The genetic variability of six populations (one from Italy, one from Israel, one from Portugal and three from Spain) assessed with ISSR markers, is reported in this preliminary work. DNA bands ranging from 350 to 1550 base pairs in the 138 analysed flies – 23 specimens/population – were scored. The results show considerable levels of genetic polymorphism ranging from 36% to 60%. However no clear genetic relationship picture among populations is obtained. We hope to achieve a more informative picture of the population structure of this pest when more samples and comparative work with mtDNA markers will be made in the near future.

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Impact of *Resseliella oleisuga* infestation on young olive trees in Tuscan nurseries (Diptera: Cecidomyiidae)

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In Tuscany the heart of production of young olive trees is in the valley of the Pescia river (Pistoia province), with one thousand and two hundred people employed. The annual production from this district is around three million plants, which corresponds to more than 80% of Tuscan production and approximately one third of the Italian production. In recent years, *Resseliella oleisuga* (Targ.-Tozz.) (Diptera: Cecidomyiidae), a pest usually of modest importance for commercial olive crops, showed a considerable increase of its infestations, providing significant concerns among nursery growers of this district. *R. oleisuga* adult presence and oviposition are practically continuous from April to October, and two main flight peaks are usually observed: the first in July and the second in September. The life-cycle and overall larvae behaviour are strongly affected by climatic conditions. During the spring-summer period a part of the population completes its life-cycle (egg to adult) in 35-50 days. Thus, between April and October, three-four overlapping generations can occur. The gall midge female uses any interruption of the bark in twigs with a diameter of 3-15 mm to lay its eggs, but in the nursery the pruning cuts of the lateral branches of the young trees represent by far the most frequent sites of oviposition. As a result of the development of the gregarious larval population in the gallery opened between the bark and the central cylinder, and the associated development of the fungus *Libertella* sp. (Xylariales: Diatrypaceae), the wood of the affected area dries, deteriorates and loses its vascular and static function. One important wound on the stem may be sufficient to set to zero the commercial value of a young plant. Sometimes the overall loss on lots of plants can reach 10-15%. The internal location of the larval population, the favourable microclimate conditions of the nursery, and the succession of several overlapping generations during the year, make it particularly difficult to control this species by means of insecticide treatments. These difficulties are exacerbated by the lack of insecticides authorized for the specific problem. For these reasons we think that in the nursery the most suitable approach to limit the damage caused by the midge infestation is to prevent oviposition properly managing the pruning cuts. Further investigations on the bio-ecology, population dynamics and integrated management of this species are in progress.

Electroantennographic response of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) antenna to olive leaves' essential oils from Portuguese olive cultivars (cvs. Cobrançosa, Madural and Verdeal Transmontana)

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Bactrocera oleae (Rossi) is one of the most important pests of olive crops in the Mediterranean basin. In addition to the damages caused in the olive tree, it affects also table olives production and reduces olive oil quality, inflicting heavy losses to producers. In Trás-os-Montes region (Northeast of Portugal), cvs. Cobrançosa, Madural and Verdeal Transmontana exhibit different susceptibility towards *B. oleae* females' oviposition (lower, medium and higher susceptibility, respectively). In this sense, this work intends to study the composition of the essential oils from leaves of these three cultivars and their effect in the antenna response of males and females of *B. oleae.* Essential oils were extracted by hydrodestillation in a Clenveger-type apparatus, being their composition assessed by gas chromatography-mass spectrometry (GC-MS). Electroantennography (EAG) and GC-electroantennogram detection (GC-EAD) were also carried out. Flies with different age ([0-5]; [5-10] and [10-15] days), were tested at different essential oils concentrations (1 and 10 µg). Leaves' essential oils revealed qualitatively and quantitatively differences among olive cultivars. Damascenone A, nonanal, and caryophyllene were among the most abundant compounds. Antenna responses were dependent on the concentration of essential oil tested, with significantly higher responses recorded in all cultivars at 10 µg. The order of antenna response to essential oils by sexes, age groups and concentration tested was always the following: Cobrançosa > Madural > Verdeal Transmontana. Concerning olive cultivars, higher oviposition susceptibility was associated with lower response in the olive fly antenna, which may support a possible repellent activity, possibly related with essential oils composition. Males reported higher signal values than females in all age groups. We also observed in both sexes that, in general, the younger olive flies presented higher response to leaves' essential oils, with significant differences among groups of [0-5] days and [10-15] days old.

Session 2
Fundamental research in olive diseases and pests (continued)

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Olive fly molecular biology goes –omic

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Abstract: The olive fruit fly, *Bactrocera oleae*, is the major pest of the olive tree. The female fly leaves its eggs in the olive fruit and the resulting larvae destroy the fruit by feeding on its sap. Currently, its control is based on chemical insecticides. In several insect pests, the Sterile Insect Technique (SIT) has been proven to be an effective environmentally friendly alternative. The SIT is based on the mass production and release of sterile insects into field populations. Past efforts to apply the SIT in the olive fly were unsuccessful, mainly due to the low competitiveness of the mass-reared flies. Several years of experience have shown that efficient SIT protocols rely on the availability of fundamental genetic and molecular information and the development of modern transgenic tools. In recent times, molecular and genetic studies in the olive fly have focused on genetic analyses of natural populations, cytogenetics, isolation and characterization of a few genes that control important biological processes, as well as the identification and mapping of several microsatellite loci. Just a few years ago, *B. oleae* was successfully transformed, an achievement that gave new perspective towards the efficient use of the SIT. Lately, this is being coupled with genomics studies and transcriptomics analyses of various important systems (i.e., reproductive and olfactory), as well as efforts in advancing olive fly mass-rearing, that are setting the ground for the application of modern control approaches through the genetic manipulation of the insect.

Key words: olive fly, Tephritidae, genomics, transcriptomics, Sterile Insect Technique

Introduction

The olive fruit fly, *Bactrocera oleae*, is the major pest of the olive tree. It belongs to the Tephritidae family of Diptera, which includes the most important agricultural pests of fruits and vegetables (White & Elson-Harris, 1994). The best studied member of the family, the medfly, *Ceratitis capitata*, attacks over 350 different fruits and vegetables around the world. Instead, the olive fly has a single fruit host: the adult female olive fly leaves its eggs in the olive fruit and the resulting larvae feed on the olive sap, thus destroying the fruit. If the tree is untreated, practically all olives can get infested with the subsequent damage of the olive fruit and the severe impoverishment of the quality of the produced oil. The estimated losses due to the fly reach 5-30% of the global olive production, resulting in economic losses of about 800 million € per year (Daane & Johnson, 2010).

Currently, control of these flies is based on chemical insecticides. Their use, however, not only deteriorates the environment and poses health problems to farmers and consumers, but also leads to insecticide resistance development and the vicious cycle of their increased use. At the moment, European and most other countries in the world are trying to reduce insecticide use. The most promising, environmentally friendly, alternative methods of pest management involve population reduction, mainly relying on the Sterile Insect Technique (SIT), and population replacement.

The SIT is a species-specific insect control approach that relies on the mass rearing, sterilization and field release of large numbers of insects (Knipling, 1955). The competition between released sterile and resident males for mating with wild females leads to the reduction of the reproductive potential and, ultimately, if continued releases of high-quality sterile males in overwhelming numbers over several consecutive generations are performed, a progressive reduction of the population size and eventually the total eradication of the pest population will occur.

Classically, both male and female insects are released, particularly because the distinction between male and female pupae is an impossible task. Released females, however, although sterile, sting fruits with their ovipositors and compete against wild females over mating with sterile males (Whitten, 1969; Fletcher, 1989). In addition, sterilization is classically achieved by irradiation, a procedure that often renders insects very weak and unfit to compete with the wild males. Such drawbacks and several years of experience have put forward several key requirements for efficient SIT application: intensive rearing of large numbers of insects for mass release, the availability of efficient sex-separation methods, sterilization techniques able to produce large numbers of insects with minimal effects on fitness, effective release methods and efficient marking systems to identify released individuals (for a review see Franz, 2005).

Transgenic technology may enhance operational SIT programmes at three levels: genetic sexing, sterilization and monitoring. One example of the potential role of transgenesis in implementing pest control is given by RIDL (Release of Insects carrying a Dominant Lethal; Alphey & Andreasen, 2002; Alphey *et al.*, 2002), a variant of the conventional SIT, in which both genetic sexing and sterilization are achieved by the same genetic construct (Figure 1).

This population reduction method uses a strain of insects homozygous for a dominant lethal genetic system, so that the sterilization of the released insects is induced not by irradiation but by homozygocity for a dominant lethal gene. Mating with wild individuals results in offspring that are heterozygous for the lethal gene leading to the death of all progeny and hence eventual suppression of the population due to a decrease in its reproductive capacity (Heinrich & Scott, 2000; Thomas *et al.*, 2000).

Efficient repressible RIDL systems were first demonstrated in Drosophila models and in the medfly, using the tetracycline-repressible transactivator (tTA) to control expression of a toxic effector (Thomas *et al.*, 2000; Gong *et al*., 2005).

On the other hand, population replacement aims at introducing a "harmlessness" mechanism to prevent the damaging effect of the pest. This approach requires both the manipulation of the harmlessness system and a method to spread it into a population. In mosquitoes, for example, the RNAi technology has been employed to reduce transmission of dengue in *Aedes aegypti* (Franz *et al*., 2006), artificial peptides (SM1) to inhibit malaria development in *Anopheles stephensi* (Ito *et al.*, 2002) and expression of cecropin to impair malaria development in *Anopheles gambiae* (Kim *et al*., 2004).

Different methods for spreading a gene into a population are currently under investigation, such as *Wolbachia* symbionts, fitness manipulation, meiotic drive systems, transposable elements and others. Population replacement is now under development and several aspects still need to be optimized (Benedict & Robinson, 2003).

At present, the SIT is the most widely applied control method against tephritid fruit flies (Enkerlin, 2005; Klassen & Curtis, 2005). Particularly in the medfly the SIT has proven successful in reducing, controlling and eradicating populations worldwide. The development and implementation of the SIT against this species has been so rapid and effective that it has been validated and practiced on industrial and area-wide scale (Hendrichs, 2000; Klassen & Curtis, 2005).

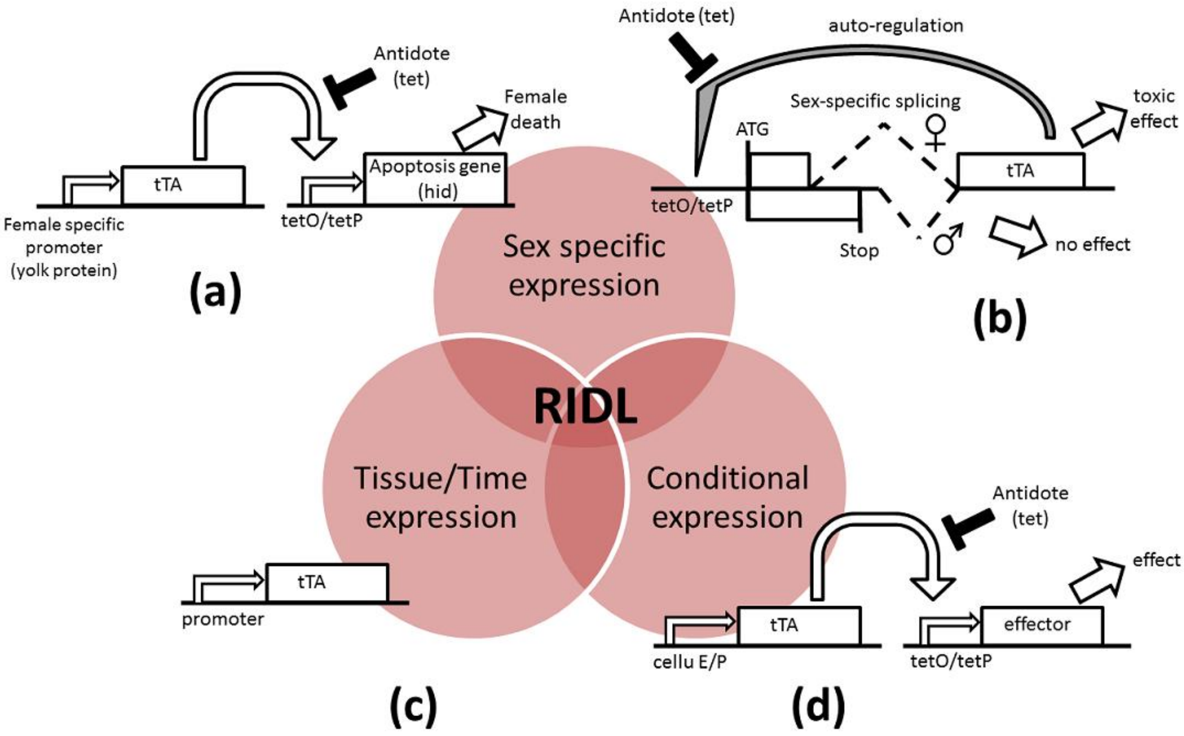

Figure 1.

(a) Original two-component system for female-specific lethality: the tetracycline transcactivator (tTA), under the control of the female-specific yolk protein promoter, binds the tet-response element (tetOperator/tetPromoter) and activates the apoptotic gene *hid* (head involution defective). In the presence of tet, the tTA is inactive. Therefore, supplying the system with tet results in female survival, withdrawal of tet results in female death.
(b) The tTA (lethal at high concentrations for the cell) is placed under the control of the tetracycline operator and promoter (tetO/tetP). In the absence of tet, the tTA activates itself in an auto-amplificatory loop, thus leading tTA to toxic concentrations. An engineered tTA gene, whose coding sequence is interrupted by the insertion of the *Cctra* intron, expresses a full-length protein only in females, because the *Cctra* intron which carries STOP codons is maintained in males but spliced in females. Therefore, in the absence of tet, the concentration of the tTA causes female-specific lethality.
(c) The one-component system of lethality. The RIDL basic principles are applied to obtain larval, pupal and/or adult lethality, irrespective of the sex of embryos.
(d) The two-component system of embryonic lethality is based on the expression of a dominant lethal effector gene (hid^{Ala5}) during the first steps of embryogenesis. In embryos, in absence of tetracycline, tTA, whose expression is under the control of cellu E/P (enhancer–promoters of cellularization genes), binds the Tet-response element and induces the expression of the dominant lethal gene.

(Modified from Labbé *et al*., 2012)

In addition, the medfly was the first non-drosophilid insect to be transformed (Loukeris, *et al*. 1995) and this achievement opened the way to the genetic transformation of many other pest insects that are targets of SIT programmes (Handler & McCombs, 2000; Handler & Harrell, 2001; Handler & O'Brochta, 2005; Koukidou *et al*., 2006; Condon *et al*., 2007a). Different medfly strains are currently available that should increase the efficacy and cost effectiveness of the SIT both at the mass-rearing, releasing and monitoring stages, since they provide: (1) genetic marking for the identification of transformed insects (Zwiebel *et al.*, 1995; Handler *et al.*, 1998; Michel *et al.*, 2001); (2) male-specific fluorescent sorting (Scolari *et al.*, 2008b); (3) sexing for male-only strains (Fu *et al.*, 2007; Condon *et al.*, 2007b); (4) reproductive sterility through embryonic lethality (Gong *et al.*, 2005; Schetelig *et al.*, 2009).

On the contrary, despite decades long efforts to develop an olive fly SIT programme using radiation-sterilised flies (Economopoulos *et al.*, 1977), trials were eventually abandoned due to consistently poor results. This was primarily attributed to low quality of the radiation-sterilised mass-reared flies, high production cost of sufficient numbers of sterilised flies, and assortative mating of released and wild populations (Economopoulos *et al.*, 1977; Zervas & Economopoulos, 1982; Economopoulos, 2001). Indeed, laboratory-reared olive flies were found to mate several hours earlier than wild flies (Zervas & Economopoulos, 1982), presumably due to differential selective pressures in the artificial laboratory-rearing environment. Given the lack of any other available tools at the time, the proposed solution was male-only releases (Economopoulos, 2001).

The molecular biology of the olive fly

The development of classical genetics for the olive fly had been entirely hindered, since several years of efforts did not provide any stable morphological markers (Mavragani-Tsipidou, unpublished; Zacharopoulou, unpublished). Early genetic studies of the species were restricted to comparisons of a few natural populations with laboratory colonies, based on electrophoretic differences of isozymes (Zouros & Krimbas, 1969; Tsakas & Krimbas, 1975; Bush & Kitto, 1979; Tsakas & Zouros, 1980; Loukas *et al.*, 1985; Loukas, 1989; Zouros & Loukas, 1989). Several molecular studies have changed *B. oleae*'s research landscape in recent years. These were mostly directed towards the development of molecular markers for population and genetic analyses, cytogenetics, gene isolation and characterization and, not least, genetic transformation.

Population analyses

The development of several different molecular markers for the species has helped a fairly detailed analysis of population structure and has shed ample light on certain aspects of population dynamics of the olive fly. Historically, the species was distributed mainly in the Mediterranean basin to the Near East and Pakistan, but also in South and East Africa. Initial analyses based on mitochondrial markers identified three genetic groups that corresponded to the three disconnected distribution areas (Pakistan, Mediterranean and Africa) (Nardi *et al.*, 2005). Microsatellite markers were able to further differentiate the Mediterranean populations into three subgroups (Western-Central-Eastern Mediterranean groups) (Augustinos *et al.*, 2002; 2005). Interestingly, the populations analysed showed a gradual decrease in heterozygocity (a measure of variability) in an East-to-West cline, indicating a westward expansion of the species from an unidentified far-Eastern origin that followed the expansion of olive cultivation (Augustinos *et al.*, 2005). A similar pattern of East-to-West expansion was also observed in samples collected from Turkey (Dogaç *et al.*, 2013). A different theory of the species origin was put forward later by Nardi *et al.* (2010), who suggested that most of the evolutionary history of the olive fly predated the domestication of cultivated olives and took place on wild olives. In recent years, the olive fly has also invaded California (Rice 1999; Rice *et al.*, 2003), a phenomenon most likely associated with world trade or tourism. Genetic analyses of the invasion point at an Eastern Mediterranean origin of the flies (Nardi *et al.*, 2005; Zygouridis *et al.*, 2009).

Chromosomes – Genome organization

The cytogenetic data of *B. oleae* is well established. Its mitotic karyotype has a diploid set of 2n = 12 chromosomes consisting of a pair of heteromorphic sex chromosomes (pair 1) and five autosomes (pairs 2-6), while its polytene complement consists of five banded chromosomes (I-V) corresponding to the autosomes of the mitotic complement and a heterochromatic mass representing the sex chromosomes (Mavragani-Tsipidou *et al.*, 1992; Zambetaki *et al.*, 1995; Mavragani-Tsipidou, 2002; Drosopoulou *et al.*, 2012). The correlation between the two chromosome sets is still unclear (Mavragani-Tsipidou *et al.*, 1992). A large number of ESTs (Tsoumani *et al.*, 2011), microsatellites (Augustinos *et al.*, 2008) and other molecular markers (Zambetaki *et al.*, 1999; Drosopoulou *et al.*, 2009) have been mapped on *B. oleae* polytene chromosomes by *in situ* hybridization. However, none of them proved to be informative on mitotic chromosomes. This is due to the fact that polytenization results after several rounds of euchromatin replication without separation of chromatids and, consequently, the sensitivity of signal detection on polytene chromosomes is increased several times, making it possible to map single copy targets on polytene chromosomes, a nearly impossible task for mitotic spreads. However, multiple copy targets, such as the repetitive 18S rRNA genes (Drosopoulou *et al.*, 2012) and/or satellite repeats (Tsoumani *et al.*, 2013) can be localized effectively on both complements and thus provide the correspondence between the two complements.

A preliminary view of the insect's genome complexity was obtained by an accurate estimate of its genome size by quantitative real-time PCR at ~322 Mb (Tsoumani *et al.*, 2012). A closer look at the genome was attempted through the identification, characterization and annotation (by Gene Ontology terms) of about two hundred expressed sequence tags (ESTs), randomly isolated from a cDNA library (Tsoumani *et al.*, 2011). Thirty five of these clones were cytogenetically localized by *in situ* hybridization, highly enriching the entry points to the insect's genome and setting the ground for further genomics analyses of the species.

A few more genes that control important processes have also been independently isolated and characterized, such as genes involved in female germline differentiation and morphogenesis of epidermal cells (Khila *et al.*, 2003), enzyme catalytic mechanisms (Benos *et al.*, 2000), or heat shock proteins (Drosopoulou *et al.*, 2009). Among them, special mention should be made on genes involved in sex-determining cascades and insecticide resistance.

The three major genes of the sex-determination cascade in *B. oleae* [*sex-lethal* (*Sxl*), *doublesex* (*dsx*) and *transformer* (*tra*)] have been isolated and characterized (Lagos *et al.*, 2005; 2007). Their analysis revealed a sex-determination mechanism that follows the pattern of other Tephritids (and dipterans), where splicing of *tra* transcripts is regulated by the master gene *Sex lethal* and *tra* itself regulates splicing of the transcriptional regulator *doublesex* (*dsx*). Indeed, the fundamental role of the *tra* genes was demonstrated by the introduction of *Botra* dsRNA into embryos, which resulted in complete transformation of XX flies into fertile males (Lagos *et al.*, 2007).

Genes and mutations associated with resistance to organophosphate insecticides have also constituted an active area of molecular research in *B. oleae.* For more than 40 years, olive fly populations have been mainly controlled by heavy use of OPs and, inevitably, resistance to OPs has been developed. Two non-synonymous point mutations and one short deletion in the acetylcholinesterase (*ace*) gene have been reported to affect sensitivity to OP insecticides (Vontas *et al.*, 2002; Kakani *et al.*, 2008). The two point mutations, which are found in the active site of AChE, cause steric hindrance of the insecticides (Vontas *et al.*, 2002). The third mutation, instead, is a short deletion of three glutamines (Δ3Q) located at the carboxyl terminal of AChE, which affect the glycophosphatidylinositol (GPI)-anchoring efficiency of the enzyme (Kakani *et al.*, 2008). This mutation indicates a distinct OP-resistance mechanism in which the *Δ*3Q improves GPI anchoring, thus increasing the amount of AChE that reaches the synaptic cleft for its normal role of ACh hydrolysis, allowing the insect to survive higher insecticide dosages (Kakani *et al.*, 2011).

Germline transformation / Genetic manipulation

Germline transformation was achieved in 2006, with the use of a *Minos*-based transposon vector co-injected along with *in vitro* synthesized *Minos* transposase mRNA (Koukidou *et al.*, 2006). The vector carried a self-activating cassette which overexpressed the enhanced green fluorescent protein (EGFP). This initial effort was recently followed by the development of *piggyback*-based conditional female-lethal olive fly strains that provide highly penetrant female specific lethality, dominant fluorescent marking and genetic sterility (Ant *et al.*, 2012). As a proof-of-principle, it was shown that weekly releases of transgenic males into stable populations of caged wild-type olive fly could cause rapid population collapse and eventual eradication. Such developments have renewed the interest of an efficient olive fly SIT based on novel transgenic tools.

A trans-infection with *Wolbachia* has also been achieved in *B. oleae* (Apostolaki *et al.*, 2011). *Wolbachia* is a bacterial endosymbiont of several insect species that has attracted interest because of the wide range of effects on its hosts and the potential applications. *Wolbachia* is known to manipulate host reproduction with several strategies, one of which is cytoplasmic incompatibility (CI), resulting in embryonic mortality in incompatible crosses. While *B. oleae* is not naturally infected with *Wolbachia*, trans-infection was achieved with a cherry fly *Wolbachia* strain. *Wolbachia* was found to induce complete CI in the olive fly, suggesting that symbiont-based approaches can be used as novel environmentally friendly tools for the control of natural olive fruit fly populations.

Mass-rearing

In parallel, efforts are underway (in Israel and Seibersdorf Laboratories, FAO/IAEA) to develop a vigorous and efficient mass-reared olive fly laboratory strain that could be used in such new SIT efforts. As it has been shown in the past, during colonization a strong adaptation to laboratory conditions takes place (Zouros *et al.*, 1982; Loukas *et al.*, 1985; Economopoulos and Loukas, 1986; Zouros and Loukas, 1989). Within 3-5 generations, populations adapt to the new conditions and allele frequencies at particular loci change. Recent microsatellite analysis of the colonization process of the Israeli strain confirmed that substantial changes in allele number and heterozygocity take place between generations F2-F5 and that no more changes were observed after F11 (Zygouridis *et al.*, 2013), thus indicating ways of maintaining the 'wild' vigor of the colonized strain by annual refreshments.

Conclusion

Clearly, modern genomics approaches have a lot to offer in non-model organisms, whose genome is poorly understood and its genetics barely developed. Indeed, the falling costs of Next Generation Sequencing technologies give the opportunity to view the entire genome of the organism and specifically select the loci to study and/or manipulate. Along these lines, a transcriptome analysis of the olive fly was recently performed with 454 pyrosequencing which offers the first large-scale dataset of the insect's transcriptional units (Pavlidi *et al.*, 2013). Such information will undoubtedly facilitate the study and analysis of selected genetic loci and deepen the analysis of the insect's biology.

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Current research approaches for the understanding and control of anthracnose in olives

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The anthracnose pathogen is responsible for epidemics in various horticultural crops around the world, and accounts for $million in crop losses each year in olive-growing countries. In Australia, the disease affects up to 80 percent of olives in susceptible cultivars such as Barnea, Manzanillo, Kalamata and UC13A6. In Portugal, it is very common and has caused losses of up to 100%, particularly in the widely cultivated variety Galega, which is very susceptible. In 2006, significant losses were reported from cultivars such as Arbequina and Picual, previously regarded as moderately resistant, and widely cultivated throughout the Iberian Peninsula. Recently anthracnose disease was reported in Northern California, USA. An olive variety of Italian, Spanish, Greek or Israeli origin will not necessarily behave in the same manner in respect to quality or quantity if grown somewhere else in the world. Anthracnose fungal infection can persist from season to season and its incidence depends on factors including olive variety, environment and the virulence of the pathogen. Warm, rainy, misty and humid conditions or heavy dews have been observed to be associated with severe anthracnose epidemics. It is among the most difficult infections to control and the current practice of applying fungicides to control anthracnose disease has not been successful. Copper-based fungicides are now the main method of disease control but they are not effective in suppressing anthracnose disease in olives under high disease pressure. Disease management in olives is made more difficult by the presence of a number of different species of *Colletotrichum* – *C. acutatum*, *C. gloeosporioides*, *C. clavatum*, *C. theobromicola*, *C. simmondisii* and *C. boninense* – which may infect individually or simultaneously. Colletotrichum is one of the most damaging groups of micro-organisms to plants and utilises a wide variety of infection strategies, both biotrophic (maintaining host viability) to necrotrophic (destruction and consumption of tissue). Olive anthracnose pathogens are capable of epiphytic survival on leaves and brunches and asymptomatic infection can also exist on flowers and young fruits, resulting in subsequent symptom development in mature fruit. Complete coverage of large, tall trees is hard to achieve; spraying is not very efficient and might not be justified or feasible. While control might occur in some situations, anthracnose can return annually and warrant a continued treatment. Especially in rainy years, the application of chemical treatments can be difficult. Preventative measures are therefore the best strategy for control, particularly during the critical phases of flowering and fruit set, commencing with actions to improve plant health and resistance to infection. These should be combined with curative measures for any existing disease, either active or latent.

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EAG response of the olive moth *Prays oleae* to host volatiles from infested and uninfested olive fruits

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Plant volatiles play an important role in the lives of phytophagous insects by guiding them to oviposition, feeding and mating sites. The headspace of olive fruits was studied with regard to volatiles that mediate host-finding or oviposition in the olive moth *Prays oleae* Bern. We studied the volatile profiles from unifested and infested fruits by *P. oleae* through the period of the oviposition by gas chromatography - mass spectrometry (GC-MS) and qualitative and quantitative differences were found among unifested and infested olive fruits. Coupled GC-EAD with *P. oleae* females revealed 4 compounds from fruit headspace that elicited the greatest EAD response. The EAD-active compounds were identified as (*Z*)3-hexenylacetate, (*Z*)-ocimene, citronellol and α-copaene. Electroantennograms (EAGs) were recorded from male and female *P. oleae* in response to individual volatiles identified from olive fruits. Results are discussed in relation to developing a lure for trapping females of *P. oleae*.

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Botryosphaeria dothidea – causal agent of olive fruit rot – pathogen of wounds or not?

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Abstract: The present paper deals with the relationship between olive fruit infections made by the fungus *Botryosphaeria dothidea*, causal agent of olive fruit rot, and the fruit damages made by insects, primarily olive fruit fly, *Bactrocera oleae*. Several authors have studied this relationship, and some have noticed that wounds made by olive fruit fly *Bactrocera oleae* enable the disease occurrence on fruit. *Lasioptera berlesiana* is another insect that influences the development of olive fruit rot disease. On the other hand, there are several statements that the pathogen is not necessarily related to wounds but it can cause the infection by itself. This work reports the results of a study on this matter achieved in Montenegro.

Key words: olive fruit rot, *Botryosphaeria dothidea*, olive fruit fly, *Bactrocera oleae*

Introduction

In Montenegro there is a long-lasting tradition in olive growing. Production of olives could be significantly jeopardized by certain diseases. Olive fruit rot, caused by *Botryosphaeria dothidea*, is a widespread disease frequently observed on olives in Montenegro, in last decade. It causes rotting of olive fruits, diminishing yield and quality of table fruits and olive oil. Damages, therefore, are quite significant, indicating that control measures against the pathogen need to be taken.

Material and methods

Olive fruit with characteristic fruit rot symptoms were collected from several commercial olive plantations along the Adriatic coast of Montenegro in 2005 and 2006. In a laboratory at the Biotechnical Faculty in Podgorica, olives were surface disinfected with 70% ethanol for 2 min, rinsed twice in sterile distilled water, and air dried. Several pieces of symptomatic tissue were cut aseptically, placed on potato dextrose agar medium (PDA), and the plates incubated at 25 °C. Serial transfers were made to obtain pure cultures of *Botryosphaeria dothidea*. In order to define if *B. dothidea* is exclusively the pathogen of wounds in olive fruits or not, two different kinds of experiment were done.

First experiment was done on undamaged, healthy olive fruits. They were inoculated by drops of fungal conidia suspension. For preparing the suspension, conidia were harvested by scraping the sporulating colonies and suspended in sterile distilled water. The concentration was determined using haemacytometer before to reach the appropriate concentration (1×10^6 spores/ml). Part of olive fruits with deposited water drops not containing conidia served as a control.

Second experiment included diseased olive fruits with typical symptoms caused by *B. dothidea* – necrotic, depressed and clearly limited spots, often with visible black pycnidia. A thousand infected fruits from trees of different olive cultivars and different localities along Montenegrin costal area were collected and transferred to plant pathology laboratory where detailed analysis on olive fruit fly or some other insect presence was done.

Results and discussion

Results of pathogenicity test in first experiment were positive in 15% of the cases. It means that in 15 of 100 undamaged fruits inoculated only with conidial suspension drop, without any injuries, necrosis progressively developed leading to total fruit rotting in a few days (Figure 1).

Figure 1. Symptoms on artificially infected olive fruit inoculated by conidial suspension drop of *B. dothidea* (right in both pictures); control fruit (left).

On the contrary, Gigante (1934) could not achieve the infection of uninjured olive fruits by placing drops of distilled water containing spores of the fungus. The same negative results were obtained in experiments performed by Sarejanni & Papaïoannou (1952). However, these authors quote that although sting made by olive fruit fly favorizes fungal development, the fungus does not attack only olive fruits with the sting.

Examination of infected olive fruits with *B. dothidea* presence established three categories in relation to *B. oleae* presence: I category – olive fruits with no stings made by olive fruit fly or any other damages except those made by *B. dothidea*; II category – olive fruits with established presence of *B. oleae* (sterile or fertile stings, empty hallway of the emerged imago, presence of eggs, presence of larvae in different stages or pupae) associated with lesion made by *B. dothidea* (Figure 2) and III category – olive fruits with established presence of *B. oleae* (stings, empty hallway, larvae) but not associated with *B. dothidea* lesion. Based on a sample of thousand of examined fruits, 230 fruits were in I category, 680 fruits in II category and 90 fruits in III category. It could be concluded that there were a significant number of fruits (23% or 32% if III category is included) where no connection with olive fruit fly presence was established. Those results are in accordance with the results achieved in the first experiment, proving that the infection by *B. dothidea* can be realized on totally healthy and not injured fruits. However, high percentage (68%) of fruits where the connection between olive fruit fly and the disease was evident indicates that damages made by *B. oleae* encourage the infection and development of the disease.

Figure 2. Symptoms of the disease on olive fruits in the form of necrotic spots caused by *B. dothidea* originated from pathogen penetration through wounds made by olive fruit fly *Bactrocera oleae.*

Close association between the presence of the disease symptoms caused by *Botryosphaeria dothidea* and the wound created by the olive fly (*Bactrocera oleae*) is reported by Moral *et al.* (2010).

Besides olive fruit fly, in our study there were also larvae of *Lasioptera berlesiana* (parasite of *B. oleae*) found on several examined fruits which also take its role in spread of the disease (Figure 3).

Figure 3. Initial symptoms of the disease appeared as a consequence of *B. dothidea* penetration through injuries made by larvae of *Lasioptera berlesiana.*

Greca & Vrenna (1995) report that, besides *Bactrocera oleae*, the cecidomyiid *Lasioptera berlesiana* plays an important role in the pathogen spreading. According to Goidànich (1990), the cecidomyiid is actually the main carrier of the disease. Sasso & Viggiani (2005) indicate that *L. berlesiana* is widely distributed in Mediterranean countries and it searchs for holes already present in olive fruits to oviposit. Although it is a predator of olive fruit fly, the damages could appear even when only sterile olive fruit fly stings occur, since *L. berlesiana* can transmit the inoculum of the pathogenic fungus (Arambourg, 1986).

According to Kačić *et al.* (1992) olive fruit fly made the most of fruit damages that serve as open door for the development of the pathogen that causes olive fruit rot disease. These authors also mention another insect, olive fruit curculio, *Rhynchites cribripennis* Desbr., which is related to the disease appearance in Croatia. The third insect "responsible" for the disease development, according to these authors, is *Lasioptera berlesiana*.

However, Mateo-Sagasta Azpeitia (1976) denies the claims that the subject fungus is parasite of wounds" and that its development exclusively depends on previous olive fruit fly attack. The author emhasizes that the fungus can act as absolute parasite" by itself, although the disease severity is always more significant on fruits previously damaged in any way. Our study is in accordance with the author's claims.

Considering the obtained results in the study, both in inoculation experiment and in experiment of infected olive fruits in relation to *B. oleae* presence, it can be concluded that damages made by *B. oleae* or any other kind of wounds on fruits encourage the infection and development of the disease caused by *Botryosphaeria dothidea.* However, the damages are not necessary for the infection, indicating that *Botryosphaeria dothidea* – causal agent of olive fruit rot – is not exclusively the pathogen of wounds. Therefore, control measures against *B. dothidea* that causes olive fruit rot in Montenegro should be focused on control against olive fruit fly *Bactrocera oleae,* but also on adequate fungicide applications against the pathogen itself.

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An assessment of peacock olive leaf spot [*Spilocaea oleagina* (Castagne) Hughes] attack in olive growing areas in Croatia during 2011, 2012 and 2013

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Abstract: During 2011, 2012 and 2013 samples of olive leaves were collected from olive growing areas in Croatia to establish an assessment of peacock olive leaf spot – *Spilocaea oleagina*. Collected samples were used to establish latent infection of olive leaves with the fungus. The latent infection is detected using the leaves soaked in 5% sodium hydroxide. Samples were collected from the most common variety Oblica and other varieties – Istarska bjelica, Buza, Carbonaca, Krvavica, Drobnica, Lastovka, Starovjerka, Plominka, Šimjaca, Drobnica and Paska. The results showed differences in susceptibility of cultivars and even within the same locality. Fixed percentages of infections ranged from 0% to 100% infection. Our results indicate that the majority of olive growers have not implemented to date the protection against pathogens of peacock olive leaf spot. The results show the prognostic significance of conducting activities related to monitoring the disease and the need to provide regular forecasts on the protection of olive trees against this disease.

Key words: Peacock olive leaf spot, *Spilocaea oleagina*, quick method for proving latent infection, susceptibility of different olive varieties

Introduction

Croatian Centre for Agriculture, Food and Rural Affairs, Institute for Plant Protection is maintaining measures of monitoring and evaluation of the attack intensity of the peacock olive leaf spot in collaboration with Croatian Agricultural Advisory Service through reporting and forecasting activities (RFA).

Peacock olive leaf spot is a disease caused by the fungus *Spilocaea oleagina*, (Cast.) Hugh. The disease is known around the world where aggravates regular damage (Shabi *et al*., 1994; Civantos, 1999;, Walter *et al*., 2000). It was described in our region called «patula, ospica or šeša» (Vrsalović, 1901; Bakarić, 2004). At that time it was not considered a significant disease. However, gradually it has become the most widespread disease of olives (Cvjetković, 2010). Today it is present in all areas of olive cultivation and is the most common disease in Croatia (Kačić *et al*., 1995; Bjeliš, 2007; Bjeliš, 2009; Buljubašić *et al*., 2011). Intensification of olive growing increases the incidence of the disease (Cvjetković & Vončina, 2011). One of the reasons for the increased severity of the peacock olive leaf spot in our area is that almost over 75% of the olive trees belong to the variety Oblica, which is susceptible variety to the attack of this disease. In the region of Dalmatia's most olive groves are growing conventionally while plantation cultivation is in a small percentage, what is also making difficult the protection against the disease. Symptoms on infected leaves are round spots at the beginning finer, dark oily that, with the development of the disease, become dark brown to black. During the summer, around the spot appears a yellowish colored ring, while in the last stage the spots become whitish due to the separation of the cuticle from the bottom of the epidermis. The reverse does not appear round spots, but with a central vein dark necrotized tissue can be seen.

Elongated spots appear on stalks, while attacked fruits show deformation – in the form of dents. The fungus is present in infected olive groves throughout the year. In terms of favorable temperature (optimum 16 °C to 21 °C) and relative humidity (> 93%), there is a re-infection throughout the growing season (Viruega & Trapero, 1999). Propagation cannot occur if conidiophores exposed to dry air longer than seven days or no humidity, and low or high temperature, however, the fungus continues its development as soon as favorable conditions occur. Studies have shown that the penetration of conidia in the tissue sheet at a temperature of 16 °C takes 48 hours, at 20 °C – 24 h and at 24 °C – 36h (Viruega & Trapero, 1999). Incubation period depends on the environmental conditions and under optimal conditions it lasts 15 days, but can be extended during summer and winter months from 90 to 100 days, while during autumn and spring it lasts from 30 to 50 days. During incubation period, leaves are latently infected and there is no expression of symptoms. After incubation the symptoms develop and the infected leaves produce numerous conidia. New major rainfall creates new infection. During summer, because of the drought and high temperatures, the fungus is stationary, but after a rain it may give new sporulation.

The first (primary) infection occurs in the fall, when conditions of moisture and temperature are favorable for development. Secondary infection is repeated until there are favorable conditions for its development.

The spread of disease is carried out with conidia facilitated by rain and wind. The greatest damage caused by this disease is defoliation, first at the lower canopy branches; it causes total weakening trees and faster decay with a greater number of infections.

Olive varieties showed different susceptibility to disease peacock olive leaf spot. The world's only known resistant cultivar is the Maalot from Israel. Local olive varieties which have proved to be sensitive are: Levantinka, Oblica, Duzica, Drobnica and Buza, while Lastovka, Uljarica and Istria bjelica are less sensitive (Radunić, 2004). According to Bakarić (2004), Uljarica, Mezanica, Sitnica, Grozdulja, Želudarka, Zuzorka, Murgulja, Bjelica, Piculja, Lumbardeška and Crnica proved more resistant than Oblica. On the other side Big Lastovka, Dužica, Jeruzalemka, Kosmača, Paštrica, Drobnica, Levantinka are more sensitive than Oblica. Varieties of Italian origin have shown a higher level of resistance, with varieties Leccino, Pendolino, Ascolana tenera, Sv. Katarina, Cucco, showing less sensitivity than the varieties Frantoio, Coratina, Carolea etc.

A quick method using NaOH or KOH can be used for detection of latent infection (Civantos, 1999). Detection of latent infection gives a true picture of the state of infection in a olive grove, because the symptoms manifest themselves as soon as they achieve favorable climate. This knowledge is important in terms of threshold for protection against the disease.

Our objective was to evaluate the intensity of the attack of peacock olive leaf spot on the most important olive growing regions and notice the importance of timely implementation of protection against the peacock olive leaf spot.

Material and methods

To conduct early detection of the *S. oleagina* infection and detect latent infection, a rapid diagnosis method was used (Zarco & Trapero, 1995). Samplings of the branches 10-15 cm long, from different trees, were taken. Due to higher relative humidity or dew infection in the lower part of the canopy, we took samples from the upper and the lower canopy. From various parts of the canopy 50 leaves were taken in four replications so the sample at each location included 200 leaves. The leaves were treated with a 5% solution of sodium hydroxide. A solution was prepared by heating water (500 ml) at a temperature of about 55 °C to 60 °C in which sodium hydroxide was carefully added, with the use of protective gloves. After 5 to 10 minutes the samples of leaves were put in for about half an hour. After removal from the solution, on the infected leaves at the top of the leaf, there have been one or more dark brown spots, or the outlines which appear darker than the rest of the leaf; this is indicating the fungal infections. The rating is done by counting infected and healthy leaves and infection is expressed as a percentage of infected leaves.

Results and discussion

After treating the leaves with 5% solution of sodium hydroxide on the infected areas on the leaves visible symptoms appeared in the form of a dark blackish round to oval spots on the obverse and reverse of the leaf. "The rapid diagnosis of" patterned leaves obtained by the percentage of the overall infection on 200 leaves. During the three years (2011, 2012 and 2013) the percentage of infected peacock olive leaf spot was established on selected localities in olive-breeding areas.

Tables 1, 2 and 3 show locations, variety and percentages of infection. Most of the results are related to the variety Oblica, which is our most widespread olive variety, except where another variety is mentioned.

Table 1. Rating infection intensity of *S. oleagina* on olive leaves during May and June 2011.

2011		
Area and Site	**Variety**	**% Infection**
Istarska		
Vodnjan 1	Istarska Bjelica	45.5
Vodnjan 2	Buza	44.5
Vodnjan 3	Buza	9
Vodnjan - Krnjaloza	Buza	0
Rovinj - Valalta	Buza	32.5
Motovun, Zamask	Istarska Bjelica	30.5
Motovun, Zamask	Buza	47
Motovun, Zamask	Oblica	10.5
Pula- Valtura	Buza	27.5
Vodnjan	Istarska Bjelica	21.5
Vodnjan	Buza	55.5
Brtonigla	Buza	63.5
Fazana, Valbandon	Carbonaca	88.5
Umag - Sverki	Istarska Bjelica	78.5
Primorsko - Goranska	**Variety**	**% Infection**
Banjol, O. Rab	Oblica	37.5
Supetarska Draga,O. Rab	Oblica	24

Supetarska Draga,O. Rab	Drobnica	6
Supetarska Draga, O. Rab	Istarska Bjelica	0.5
Supetarska Draga, O. Rab	Buza	3
Supetarska Draga. O. Rab	Lastovka	9.5
Mali Losinj,Cunski	Starovjerka	2
Mali Losinj,Cunski	Oblica	21
Mali Losinj.Veli Losinj	Oblica	75.5
Mali Losinj, Veliki Losinj	Starovjerka	13
Papajna, O. Cres	Plominka	5.5
Piskel, O. Cres	Simjaca	53
Piskel, O. Cres	Plominka	23.5
Papajna, O. Cres	Simjaca	59.5
Krk, Malinska	Drobnica	90.5
Krk, Malinska	Plominka	31.5
Krk, Punat	Rosulja	79.5
Krk, Punat	Plominka	50.5
Krk, Krk	Drobnica	72
Krk, Krk	Oblica	80
Krk, Punat	Oblica	88.5
Krk, Punat	Paska	41
Krk, Krk	Plominka	20
Krk, Sv. Juraj	Plominka	11
North Dalmatia		
Biograd 1	Oblica	11
Biograd 2	Oblica	44
Siroke	Oblica	7
Bibinje	Oblica	67
Kali	Oblica	18.5
Preko	Oblica	24.5
Dobro Poljana	Oblica	42
Zadar - Starigrad	Oblica	28.5
Policnik	Oblica	26
Murvica Gornja	Oblica	8
Bozava - Brbinj, Dugi Otok	Oblica	63.5
Pirovac	Oblica	6
Murter	Oblica	39.5
Vodicko Polje	Oblica	77.5
Donje Polje, Sibenik	Oblica	12
Icevo	Oblica	95
Tribunj	Oblica	78.5
Icevo	Krvavica	63.5

Middle Dalmatia		
Primosten Burnji	Oblica	5
Podorljak	Oblica	58.5
Kastel Stari	Oblica	23.5
Selca (O. Brac)	Oblica	97
Mirca (O. Brac)	Oblica	63
Skrip (O. Brac)	Oblica	28
Supetar (Uz More) (O. Brac)	Oblica	52.5
Postira (O. Brac)	Oblica	23
Bobovisca - Milna (O. Brac)	Oblica	26.5
Povlja (O. Brac)	Oblica	67
Starigrad (Ivanje Gomile)	Oblica	37.5
Svirce (Pod Crikvom)	Oblica	93
Hvar (Donci Dolci)	Oblica	79.5
Vrboska (Strane)	Oblica	63
Vrboska (Hreblje)	Oblica	80
Vrboska (Velovi Jalo)	Oblica	76.5
Vrboska (Pelinje)	Oblica	100
Igrane	Oblica	85
Drvenik	Oblica	86
Bacina	Oblica	37
Gradac	Oblica	23
Baska Voda	Oblica	28.5
Marusici	Oblica	29
Solin	Oblica	15
Makarska	Oblica	51.5
Zaostrog	Oblica	87.5
Lorca (O. Vis)	Oblica	12
Zlopolje (O. Vis)	Oblica	57
Podhumlje(O. Vis)	Oblica	13.5
Stasevica	Oblica	28.5
Zagvozd	Oblica	23
South Dalmatia		
Vela Luka (Gudulija)	Oblica	100
Lumbarda (Uvala Racisce)	Oblica	94.5
Konavle - Drvenik (Dobra Voda)	Oblica	53
Konavle -Dubravka (Butkovina)	Oblica	27
Cilipi (Masjesi)	Oblica	88.5
Dubrovnik- Brsecine (Ispod Puta)	Oblica	30.5
Dubrovnik- Mravinjac	Oblica	98
Dubrovnik- Orasac Konjavac	Oblica	21

A total of 96 samplings from different varieties were collected in April 2011 at 74 locations: Istra, Primorsko – Goranska area, North Dalmatia, Middle Dalmatia and South Dalmatia area. Most of the results are related to the variety Oblica, but also other varieties were sampled: in Istria with Oblica, Istarska bjelica, Buža, Carbonaca, in the place Ićevo (North area) variety Krvavica, in the Primorsko - Goranska area Drobnica, Istarska bjelica, Buža, Lastovka, Starovjerka, Plominka, Simjaca, Rosulja and Paška. Complete infestation of 100% showed the analysis of two olive groves, of variety Oblica, one in the Middle Dalmatia area, on the island Hvar, location Vrboska, Pelinje and another in South Dalmatia area in location Vela Luka, Gudulija on the island Korčula. In Istria area the highest percentage of infection (88.5%) was found at the location Fažana, Valbandon, on Carbonaca variety, and the lowest, the only one without infection (0%) in the eco olive grove, variety Buža at Vodnjan - Krnjaloža. In the Primorsko - goranska area - highest rate of infection (90.5%) was found at the location Malinska, island Krk, the variety Drobnica, and the lowest (0.5%) in Supetar draga, island Rab, variety Istarska bjelica. In the North Dalmatia area the highest percentage of infection (78.5%) was found at the location Tribunj variety Oblica, and the lowest (7%) at Siroke also of the variety Oblica. In the Middle Dalmatia area the highest percentage of infection (100%) was found at the location Vrboska - Pelinje on the Hvar island, the variety Oblica, and the lowest (5 %) on the location of Primošten Burni, variety Oblica. In the South Dalmatia area the highest rate of infection (100%) was found at the location of Vela Luka - Gudulija, Korcula island and the lowest (21%) at Dubrovnik, Konjašac - Orašac, variety Oblica. Among the total number of locations sampled, only twelve have got percent of the infection up to 10%. The results show the previously mentioned higher susceptibility of varieties of Oblica, Drobnica and Buža, while variety Istarska bjelica was showing greater resistance than other varieties even within the same location. Very interesting was eco olive grove at Vodnjan where there were no infections. It is considered that 10% of leaves infected is threshold tolerance for protection of olives against peacock olive leaf spot (Bakarić, 2004). At most locations, regardless of the variety registered infections higher than 10% were recorded which means that the protection should be done before our rate of infection. Given that the "quick method" can register latent infection also, the method can be used when decisions have to be made on the implementation of protection (Bjeliš, 2011).

Table 2. Rating infection intensity of *S. oleagina* on olive leaves during May and June 2012.

2012		
Area and Site	**Variety**	**% Infection**
Istria		
Vodnjan,Opg Stipancic, Krnjaloza	Buza	0
Vodnjan, Belci	Buza	2
Vodnjan, Opg Chiavalon	Istarska Bjelica	0
Vodnjan, Pastrovicchio A.	Buza	32.5
Vodnjan, Belci	Istarska Bjelica	0
Vodnjan, Opg Stipancic, Krnjaloza, Eko	Istarska Bjelica	0
Novigrad, Mareda	Istarska Bjelica	26.5
Novigrad, Karpinjan	Frantoio	4.5
Novigrad, Karpinjan	Buza	34.5

Ipsi	Buza	25
Ipsi	Frantoio	25
Salvela	Istarska Bjelica	36
Sv. Lovrec	Istarska Bjelica	1.5
Primorsko - Goranska		
Krk, O. Krk	Oblica	2
Punat, O. Krk	Rosulja	9.5
Malinska, O. Krk	Drobnica	4.5
North Dalmatia		
Petrcane	Oblica	3.5
Kozino	Oblica	47
Bibinje	Oblica	12.5
Bibinje 2	Oblica	3.5
Biograd	Oblica	2.5
Biograd 2	Oblica	10.5
Sv. Filip I Jakov	Oblica	0.5
Sv. Filip I Jakov 2	Oblica	17.5
Turanj	Oblica	7
Sukosan	Oblica	7.5
Drage	Oblica	24
Prosike - Drage	Oblica	22
Sv. Petar	Oblica	7.5
Lukoran, O. Ugljan	Oblica	14
Kali, O. Ugljan	Oblica	3.5
Gornje Selo, O. Ugljan	Oblica	12.5
Dobropoljana, O. Ugljan	Oblica	11.5
Kukljica, O. Ugljan	Oblica	9.5
Ugljan, O. Ugljan	Oblica	8
Zdrelac, O. Ugljan	Oblica	39.5
Preko, O. Ugljan	Oblica	7
Banj, O. Pasman	Oblica	28.5
Pasman, O. Pasman	Oblica	8.5
Nevidane, O. Pasman	Oblica	6
Tkon, O. Pasman	Oblica	4,5
Bozava, O. Dugi Otok	Oblica	0
Murter, O. Murter	Oblica	3.5
Betina, O. Murter	Oblica	5
Tisno, O. Murter	Oblica	1
Zaboric	Oblica	0
Skradinsko Polje	Oblica	68
Icevo	Krvavica	93.5
Icevo 2	Oblica	20.5

Dubravice	Oblica	51
Pirovac	Oblica	2
Pirovac 2	Oblica	7.5
Tribunj	Oblica	11.5
Vodice	Oblica	4.5
Vodice 2	Oblica	7
Middle Dalmatia		
Bilini, Primosten	Oblica	39.5
Draga, Primosten	Oblica	3.5
Primosten Burnji	Oblica	9.5
Primosten Burnji 2	Oblica	4
Primosten	Oblica	3.5
Podorljak	Oblica	18.5
Vrsine	Oblica	16.5
Najevi	Oblica	22
Vinisce	Oblica	6.5
Okrug Gornji, O. Ciovo	Oblica	1
Zedno, O. Ciovo	Oblica	1
Kastel Luksic	Oblica	18
Kastel Stari	Levantinka	10.5
Kastel Stari	Oblica	8.5
Kastel Stari	Ascolana Tenera	8.5
Kastel Stari	Grossa Di Spagna	10
Kastel Stari	St. Catarina	0
Split, Poljud	Oblica	7
Lokva Rogoznica	Oblica	7.5
Marusici	Oblica	5.5
Krvavica	Oblica	21.5
Baska Voda	Oblica	55
Bast Topici	Oblica	3.5
Vruja Tucepi	Oblica	16.5
Zivogosce	Oblica	1
Drvenik	Oblica	7
Postira, O. Brac	Oblica	2
Selca, O. Brac	Oblica	3.5
Postira 2, O. Brac	Oblica	2
Podstrazje, O. Vis	Oblica	0.5
Zlopolje, O. Vis	Oblica	0.5
Dolcic,Hvar, O. Hvar	Oblica	25
Dolcic,Hvar, O. Hvar 2	Levantinka	35
Brojeska, O. Hvar	Oblica	46.5
Pahnisce, O. Hvar	Oblica	34.5
Pelinje, Jelsa, O. Hvar	Oblica	15.5

Vojalo, O. Hvar	Oblica	28.5
Dol.Sv.Ane, O. Hvar	Oblica	2.5
Svirce, Ispod Crikve, O. Hvar	Oblica	41
Ivanje Gomile, Starigrad, O. Hvar	Oblica	27.5
Donce Dolci, O. Hvar	Levantinka	19.5
Gradac	Oblica	5
Bacina	Oblica	9
South Dalmatia		
Glog, Opuzen	Oblica	20.5
Crepina, Opuzen	Oblica	53
Klisevo	Oblica	3.5
Gromaca	Oblica	10
Mravinac	Oblica	4.5
Slano	Oblica	5.5
Slano 2	Oblica	21.5
Molunat	Oblica	48
Orasac	Oblica	11.5
Orasac 2	Oblica	5
Raba	Oblica	2.5
Mali Ston	Oblica	1
Brsecine	Oblica	9.5
Ston	Oblica	2
Klek	Oblica	6.5

During 2012, 113 samples of olive leaves from 91 locations were collected. In the area of Istria the highest percentage of infection (36%) was found at the location Salvela, variety Istarska bjelica, and location without infection (0%) was found at Vodnjan, varieties Buža and Istarska bjelica. The highest percentage of infection (9.5%) was in Primorsko - Goranska area at the location Punat, Krk island, variety Rosulja, and the lowest (2 %) at Krk island, location Krk, variety Oblica. In the North Dalmatia area the highest percentage of infection was found at location Icevo (93.5%), variety Oblica, and two locations with no infection (0%) at the location Božava, Dugi otok island and at the location Žaboric, both with variety Oblica. In the Middle Dalmatia area the highest percentage of infection was found at location Broješka (41%) Hvar island, variety Oblica and the lowest (0%) at the location Kastel Stari, variety St. Catarina. In the South Dalmatia area the highest percentage of infection was at location Crepina (53%), variety Oblica and the lowest (1%) at the location Klek, also variety Oblica.

Table 3. Rating infection intensity of *S. oleagina* on olive leaves during May and June 2013.

Area and Site – 2013	**Variety**	**% Infection**
Istria		
Ipsi - Oprtalj	Istarska Bjelica	21.5
Vodnjan - A. Pastrovicchio	Buza	10
P.Z. Vodnjan	Buza	20
Vodnjan I, Opg S.Chiavalon	Istarska Bjelica	26.5
Ipsi	Frantoio	0
Mareda, Novigrad	Mix of Variety	10.5
Primorsko - Goranska		
Krk 1	Drobnica	16
Krk 2	Rosulja	0
Cres 1	Simjaca	3
Cres 2	Plominka	6
Mali Losinj	Plominka	1
North Dalmatia		
Zadar - Bili Brig	Oblica	13.5
Zadar	Oblica	72
Kozino	Oblica	13
Petrcane	Oblica	19.5
Bibinje	Oblica	13
Biograd	Oblica	4
Sukosan	Oblica	1
Sv. Petar	Oblica	8.5
Gornji Policnik	Oblica	5.5
Gornja Murvica	Oblica	13
Islam Latinski	Oblica	3
Smilcic	Oblica	10
Smilicic - Nadin	Oblica	17.5
Zemunik Donji	Oblica	8.5
Vrsi	Oblica	16
Benkovac	Oblica	16.5
Lisane Ostrovicke	Oblica	4
Turanj	Oblica	23
Drage	Oblica	33.5
Pakostane	Oblica	3.5
Prosike	Oblica	13.5
Pirovac	Oblica	13
Tribunj	Oblica	1.5
Zaboric	Oblica	2
Vodice	Oblica	9

Jadrtovac	Oblica	21
Betina, O. Murter	Oblica	23
Tisno	Oblica	35
Jezera, O. Murter	Oblica	2.5
Murter,O. Murter	Oblica	20
Middle Dalmatia		
Draga	Oblica	8.5
Primosten Burnji	Oblica	15.5
Supljak, Primosten	Oblica	7.5
Boraja	Oblica	48
Primosten	Oblica	7
Podorljak	Oblica	36.5
Najevi	Oblica	33.5
Vrsine	Oblica	66.5
Seget Vranjic	Oblica	2
Vinisce	Oblica	3
Dubrava	Oblica	2.5
Trogir, Pantana	Oblica	9
Sv.Kriz, Arbanija, O. Ciovo	Oblica	0
Zedno, O. Ciovo	Oblica	5
Okrug Gornji, O. Ciovo	Oblica	0
Mastrinka, O. Ciovo	Oblica	1.5
Kastel Stafilic	Koroneiki	3.5
Kastel Stafilic	Tosca	17
Kastel Stafilic	Sikitita	9
Kastel Stafilic	Arbosana	26
Kastel Stafilic	Arbequina	54
Kastel Stafilic	Oblica	15
Kastel Luksic	Oblica	28.5
Kastel Stari	St.Agostino	12
Kastel Stari	Ascolana Tenera	12,5
Kastel Stari	Levantinka	0
Kastel Stari	Lastovka	26.5
Povlja, O. Brac	Oblica	19
Supetar, O. Brac	Oblica	31
Vrbanj-Dol, O. Hvar	Oblica	21
Vrbanj	Oblica	39.5
Starigrad, O. Hvar	Oblica	15.5
Sv.Nedilja, O. Hvar	Oblica	18.5
Jelsa, O. Hvar	Oblica	11.5
Pitve, O. Hvar	Oblica	1.5
Mravince	Oblica	7.5
Kucine - Zrnovnica	Oblica	9

Srinjine	Oblica	0.5
Tugare	Oblica	25
Lokva Rogoznica	Oblica	7.5
Krvavica	Oblica	19
Brela	Oblica	13
Zivogosce	Oblica	14
Drvenik	Oblica	18
Podgora - Caklje	Oblica	11.5
Baska Voda	Oblica	4
Tucepi	Oblica	5.5
Podstrana	Lastovka	7.5
Gradac	Oblica	7.5
Podstrazje, O. Vis	Oblica	18
Zlopolje, O. Vis	Oblica	8
Bacina	Oblica	9
South Dalmatia		
Banici	Pendolino	45
Grgurici	Oblica	7
Klek	Oblica	5
Mali Ston	Oblica	9
Ston	Oblica	8.5
Raba	Oblica	9.5
Rogotin	Oblica	35.5
Zaton Doli	Oblica	24.5
Petraca	Oblica	16
Orasac	Oblica	74
Cibaca	Oblica	49.5
Mravinjac	Oblica	19.5
Slano	Oblica	2
Trsteno	Oblica	95
Knezica	Oblica	20
Blato, Sjever, O. Korcula	Drobnica	87.5
Blato, Sjever, O. Korcula	Lastovka	91.5

During 2013, 110 samples of olive leaves from 96 locations were collected. In area of Istria the highest percentage of infection (26.5%) was found at the location Vodnjan, variety Istarska bjelica and the lowest (0%) at the location Ipsi, variety Frantoio. The highest percentage of infection (16%) was in Primorsko - Goranska area at the location Krk, Krk island, variety Drobnica, and the lowest (0%) Krk island, location Krk, variety Rosulja. In the North Dalmatia area the highest percentage of infection was found at location Zadar (72%), variety Oblica, and location with the lowest infection (1%) was at the location Sukošan, variety Oblica. In the Middle Dalmatia area the highest percentage of infection was found at location Vrsine (66.5%), variety Oblica and two location had 0% infection at the location

Kastel Stari, variety Levantinka and St. Križ, Čiovo island, variety Oblica. In the South Dalmatia area the highest percentage of infection was at location Blato (91.5%), variety Lastovka and the lowest (2%) at the location Slano, variety Oblica.

Percentage of peacock olive leaf spot infection primarily depends on favorable climatic conditions in which the fungus infests. Comparing the results obtained we conclude that during the 2011 a large percentage of infection in most of the olive locations had been established as illustrated by the fact that only 12 samples had a percentage of infection less than 10%. In 2012 infection was found in certain locations where there is a problem with this disease due to the poor condition of olive groves, the weak enforcement of protection, irrigation, excessive fertilization etc. 68 localities had less than 10% of infection. During May 2013 on olive growing area *S. oleagina* also made infection in specific localities as in the previous year but at a higher percentage due to favorable climatic conditions; 50 sites had less than 10% infection.

Peacock olive leaf spot disease is the most common disease in our olive groves; its occurrence is expanding and therefore, the primary purpose of monitoring should be the timely recognition of infection, which is achieved by regular monitoring of relative humidity and temperature, rainfall, accompanied with "rapid diagnosis" of leaf infection, which determines the starting of sprayings. It is very important to start protecting as soon as the first sign of infection appears because any delay contributes to the spread of the disease because the fungus is present throughout the year and the production of conidia initiates as soon as favorable conditions appear.

Recently, the disease has become the most common fungal disease of olives. At many locations, regardless of the variety, infections were observed at a frequency exceeding the threshold of tolerance for fungal infections by *Spilocaea oleagina* (Cast.) Hugh. (i.e. 10%), indicating the necessity of introducing monitoring for this disease. The application of the "quick method" has proven to be reliable for the detection of latent infection. The "Quick method" can also be used for spraying decision making against peacock olive leaf spot. The occurrence of the disease is dependent on the sensitivity of the variety. On the variety Oblica very variable percentages of infection ranging from 5% to 100% were recorded, indicating the critical role of climatic factors and the location of olive groves. The "Quick method" can also be used when evaluating the susceptibility of varieties, but apparently such investigation must be set to a single location for uniform microclimate conditions. Monitoring of olive groves with the use of "rapid diagnosis of infection," proved to be the promising basis for a systematic monitoring and evaluation of thresholds for disease control.

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Effect of different food sources on life parameters of the parasitoid *Psyttalia concolor*

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In a conservative biological control strategy, agricultural habitats have an important role in the action of natural enemies. Flowering plants are sources of nectar and pollen, providing sugars, like sucrose, glucose and fructose, that have a beneficial impact on life parameters of parasitoids and consequently in pest biological control. *Psyttalia concolor* (Szépligeti, 1910) is a koinobiont larval-pupal endoparasitoid of *Bactrocera oleae* (Rossi) and its maintenance and action on olive groves should be stimulated. In this context, the objective of the present work was to study the effect of different food sources on the life parameters of *P. concolor*. For this, the following food sources were studied: (1) artificial diet (sucrose, yeast extract 4:1), (2) sucrose, (3) fructose, (4) glucose, (5) honey solution (10% v/v), (6) pollen, (7) honey solution (10% v/v) + pollen, (8) *Dittrichia viscosa* (L.) flowers, (9) extract of *D. viscosa* leaves (1%), (10) *Stevia rebaudiana*, and (11) water. For the bioassays and for each food source, five replicates of newly emerged *P. concolor* were kept in groups of 20 individuals (10 males and 10 females) in cylindrical plastic cages (12 cm of diameter and 5 cm height). All cages were maintained at 25 ± 2 °C, 16 L : 8 D and 75 ± 5% RH (relative humidity) and checked for insect survival on a daily basis. After seven days, parasitisation assays using *Ceratitis capitata* (Wiedemann) larvae as substitution host, were developed offering 30 fully-grown *C. capitata* for one hour. After that, exposed larvae were placed into Petri dishes until pupate. Parasitism ability was measured as the percentage of attacked host (percentage of puparia without medfly emergence) and progeny size (percentage of parasitoids emerged from parasitized medfly puparia). Average longevity was calculated for each different diet. Life expectancy for each different diet was calculated. *P. concolor* longevity was significantly influenced by food source. The highest longevity was obtained by artificial diet (35.23 ± 5.08 days) and sucrose (35.23 ± 4.91 days) followed by fructose (26.17 ± 5.13). Parasitoids feeding on *D. viscosa* (1%) leaves extract presented lowest longevity (1.85 ± 0.21 days). Life expectancy was always high in females in all treatments tested, being the treatments with artificial diet and sucrose where female obtained highest life expectancy, 48.16 ± 5.18 and 48.81 ± 4.67, respectively.

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Biodiversity and pollen feeding habits of syrphids in olive groves and surrounding landscape in northeastern Portugal

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In olive agroecosystems, syrphid larvae potentially prey on *Euphyllura olivina* (Costa), *Euphyllura straminea* Loginova, *Palpita unionalis* Hübner and *Prays oleae* (Bernard). For this, their maintenance in olive groves can contribute for the biological control of these pests. Plants could have a major role once they provide shelters, alternative prey items for larvae and supplementary food for adults, such as pollen and nectar. Thus, the existence of a heterogeneous landscape surrounding olive groves can contribute to the enhancement of the fitness of syrphids and their action as natural control agents. In this context, the aim of the present work was to identify the diversity of syrphids in olive groves and surrounding landscape and to study plant species that constitute pollen sources in a conservative biological control strategy. From August to December 2012, adult syrphids were collected from a total of 45 delta traps (five traps per field), used for *P. oleae* monitoring, and placed in three olive groves and two surrounding fields (a herbaceous and a shrubland) from Mirandela, northeastern Portugal. First, syrphids were identified to species level and then the abdominal contents were extruded onto a glass slide and the pollen grains were stained with fuchsin, counted and identified to pollen type. Flowering plant inventories were also carried out during that period and the abundance for each plant species was registered following the Domin Krajina scale. A total of 254 specimens was collected and ten syrphid species were identified, being *Eupeodes corollae* (Fabricius) the most abundant, representing 64% of the total, followed by *Episyrphus balteatus* (De Geer) with 24%, and *Sphaerophoria scripta* (L.) with 8%. The abundance and richness of syrphids were higher in herbaceous (10 species) than in shrubland fields (six species) and in olive groves (one species – *E. corollae*). After plant inventory, a total of 43 flowering plant species, belonging to 19 families, was identified, being Asteraceae the richest family, followed by Apiaceae. Until now, 35 pollen types were identified in the gut of syrphids. About 70% of the specimens of *E. corollae* have consumed pollen, being the number of grains higher in males than in females. Concerning *E. balteatus*, about 80% of the females and 60% of the males have consumed pollen, being the number of grains higher in females than in males. All the *S. scripta* specimens contained pollen grains in the gut. In all the identified species, the diet varied considerably among specimens, being that they consumed between one and six different pollen types per specimen. The most represented pollen grains found in *E. corollae* and *S. scripta* belonged to Asteraceae and in *E. balteatus* to Brassicaceae.

Evaluation of Degree-day model to predict the adult stage population of *Bactrocera oleae* (Diptera: Tephritidae) in Iran (Tarom-sofla)

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Prediction of olive fruit fly growth and development at field conditions is considered as one of the essential components of its integrated management. Similar to other pests, the IPM of this pest consists of a decision making system for selecting and application of tactics independently or in accordance with management strategies based on the cost/benefit analysis. Eventually, with the lack of such programme (olive fruit fly IPM), the field managers will have no choice but scheduled application of pesticides without taking benefit of pest forecasting and surveillance information. However, the exact timing of pesticide usage may guarantee their effectiveness and also reduction in application intervals. Otherwise, the cost of control is much increased but also levels the natural enemies' population are adversely affected. In spite of differences in climatic conditions of given locations per annum, degree-day (DD) models have been known as very important tools to serve pest management programmes. As an insect, the growth and development of the olive fruit fly is dependent on temperature. Therefore, detection of the pest population by DD is a valuable technique in its population density forecasting. By using McPhail traps and meterological information, a study was carried out to calculate DD through Double Sine method based on flight base thermal threshold of the olive fruit fly during 2008-09. Results revealed that the first fly was entrapped when the DD was 2586 and 2298/8 °C in 2008 and 2009, respectively, in Tarom Sofla (Kallaj).

Session 3
Biological control

The olive fly and bacterial symbiosis: new perspectives for IPM in olive crops

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The olive fly *Bactrocera oleae* shows two types of relationships with bacteria: a close symbiosis with *Candidatus* Erwinia dacicola, a species living in the gut of both the adult and the preimaginal stages of the fly, and a different symbiosis with others bacterial species, which represent the main food source for *B. oleae* adults in the olive ecosystem. Previous researches have shown that treatments carried out with copper-based products were able to break off the bacterial symbiosis, causing high level of mortality in young larvae during field experiments. Here are referred some results dealing with bactericidal compounds in a semi-field trial. Results confirmed symbiotic interruption played by copper products. Concerning the relationships between the olive fly and other bacteria, we previously demonstrated that bacterial filtrates attracted adult olive flies. Here, a lab research dealing with the isolation of volatiles emitted from bacterial filtrates, together with behavioral bioassays performed in wind tunnel and electrophysiological assays are presented. Some active compounds originating from the bacterial activity appear to be worthy of consideration for future research aimed at discovering novel attractants, which are a starting point for the development of future lure and kill techniques in area-wide olive systems.

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Natural enemy complex of *Bactrocera oleae* in organic and conventional olive groves

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Abstract: Around the Mediterranean Basin *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is attacked by a complex of natural enemies. The percentage of parasitism as well as the natural enemy complex of *B. oleae* in organic and conventional olive groves, located in Chania (west Crete) and in Kyparissia (w. Peloponnesus) of Greece, were studied during three successive years from 2010-2012. During the experimental period four parasitoids and one predator attacked olive fruit fly immature stages and eggs, respectively. In Crete, in 2010 the most abundant was the ectoparasitoid *Eupelmus urozonus* followed by the predator *Prolasioptera berlesiana* and the endoparasitoid *Psyttalia concolor*, while the ectoparasitoid *Eurytoma martelli* was less abundant. In 2011 the most abundant were *E. urozonus* and *Pnigalio mediterraneus* followed by *P. concolor* and in 2012 the most abundant was *P. concolor*. In the area of Kyparissia in autumn 2012 the only parasitoid recorded was *E. urozonus*. In general, the parasitization rates in organic as well as in conventional olive groves was significantly fluctuated depending on the year and the locality. Although higher rates of parasitism were recorded in organic olive groves, no significant differences were observed regarding total parasitism rates between organic and conventional olive groves. In spite of the application of poisonous bait sprays against *B. oleae* in the conventional groves, population of its natural enemies showed high densities and it was not significantly different from the population in the organic groves.

Key words: natural enemies, *Bactrocera oleae*, biological control

Introduction

The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is considered the most serious pest of Mediterranean olives (Tzanakakis, 2006). Around the Mediterranean basin immature stages of olive fruit fly are attacked by a complex of natural enemies. It includes the ectoparasitoids *Eupelmus urozonus* Dalman, *Pnigalio mediterraneus* Ferrière & Delucchi, *Eurytoma martelli* Domenichini and *Cyrtoptyx latipes* Rond., the endoparasitoid *Psyttalia concolor* (Szépligeti) and the egg predator *Prolasioptera berlesiana* Paoli (Arambourg & Pralavorio, 1974; Neuenschwander *et al.*, 1983).

Although many studies analyze the impact of natural enemies on olive fruit fly population in conventional olive groves, however it has not been investigated their effect in the organic olive groves.

The objectives of this study were to evaluate the percentage of parasitism as well as the natural enemy complex of the preimaginal stages of *B. oleae* in organic and conventional olive groves in Greece.

Material and methods

The study was conducted in conventional and organic olive groves, located in western Crete (Chania) and in Peloponnesus (Kyparissia) of Greece from 2010 to 2012. The organic olive groves were under the regime of organic farming for more than 10 years. The olive variety was Koroneiki in Chania and in Kyparissia. 'Koroneiki' is the most commonly cultivated variety in Greece, used for olive oil production, and mainly planted at lower elevations.

In the conventional groves, against *B. oleae* were applied bait sprays with pyrethroids and organophosphorus insecticides and protein hydrolyzate from July to end of October. In the organic groves were applied bait sprays with the Naturalyte insect control products spinosad (Success TM 480 SC) or were applied lure-and-kill method using Ecotraps (Vioryl SA). Samples of 500 fruits infested from olive fruit fly were taken from conventional and organic groves at biweekly intervals during the olive season 2010 to 2012. Samplings started when the first infested olive fruits were recorded and continued until harvest. From each sample 350 olives were placed into emergence cages at 25 ± 3 °C until the last olive fruit fly or parasitoid had emerged. Then adult parasitoids were indentified. The rest 150 infested fruits were dissected in the laboratory under binocular. By examination of the samples the rate of parasitism and predation was estimated and the type of infestation classified into several categories:

a. Sterile punctures,
b. Eggs, 1^{st}, 2^{nd} and 3^{rd} instars larvae, pupae (live and dead),
c. Exit holes,
d. Immature stages (egg, larva, pupa) of parasitoids and where practicable, the immature stage of the insect which was parasitized,
e. Living midge larvae and
f. Fruits with *Botryosphaeria dothidea* infestation.

The percentage of ectoparasitism was calculated as the ratio of the number of parasitized larvae and pupae by ectoparasitoids to the total number of all host stages (van Driesche, 1983).

In order to measure the percentage of endoparasitism, living third instars larvae and pupa of olive fruit fly from the dissected olives were placed into petri dishes until adults of *P. concolor* or *B. oleae* have emerged. The percentage of endoparasitism was calculated as the ratio of the number of parasitized larvae and pupae by the endoparasitoid to the total number of all host stages. The midge *P. berleiana* predatory action was calculated as the ratio of the number of fruits with the living larvae of *P. berlesiana* and fruits with the disease *Botryosphaeria dothidea* spots to the the total number of all host stages.

In the area of Kyparissia 19 samplings were conducted from 30 September to 29 November 2013 (on 30/9 samples were collected from 2 groves, on 28/10 from 5, on 11/11 from 6, on 26/11 from 4 and finally on 23/11 from 2 groves). At each sampling at least one organic olive grove had been included. The methods used were similar to those reported for the samplings conducted in Chania.

The data were analyzed using one-way analysis of variance (ANOVA), with the factor being cultivation method (organic, conventional). Data were arcsine transformed before the analysis. When significant variation was noted, the differences were identified by the LSD Student's t-test at $P < 0.05$. Analyses were conducted using the statistical package JMP 7.0.1 (SAS Institute, 2007).

Results and discussion

The natural enemy complex of preimaginal stages of *B. oleae* included the parasitoids *E. urozonus*, *P. concolor*, *P. mediterraneus*, *E. matrelii* and the predator *P. berlesiana*. The natural enemy complex of olive fruit fly obtained from organic and conventional olive groves from 2010 to 2012 in Western Crete (Chania) is presented in Table 1. In 2010 and 2011 the most abundant was the *E. urozonus* while in 2012 it was the *P. concolor* (Table 1).

Table 1. Relative abundance of *B. oleae* natural enemies recorded on Koroneiki fruits of organic and conventional groves during the whole sampling period, as determined from rearing cages, Western Crete, 2010-2012.

	Eupelmus urozonus %	*Pnigalio mediterraneus* %	*Eurytoma martelli* %	*Psyttalia concolor* %	*Lasioptera berlesiana* %
2010					
Organic grove	44.1	7.8	3.4	17.9	26.8
Conventional grove	53.9	9.5	0.0	10.6	25.9
2011					
Organic grove	32.4	52.6	0.0	10.6	4.2
Conventional grove	52.6	25.8	0.0	19.8	1.7
2012					
Organic grove	22.7	7.6	0.0	63.6	6.1
Conventional grove	13.5	5.8	0.0	80.8	0.0

E. urozonus and *P. mediterraneus* were recorded throughout the sampling period during the three successive years of the study in all olive groves (organic and conventional). *E. urozonus* becomes abundant during September-October and *P. mediterraneus* at the end of summer beginning of September. *P. concolor* was the most abundant parasitoid of *B. oleae* in 2012. The first individuals appeared by the end of summer. From then its population gradually increased until the end of the sampling period. These findings are in agreement with the results of previous studies which described these species as the most abundant (Pappas *et al.*, 1977; Neuenschwander *et al*., 1983). *E. martelli* was found in very low numbers only at the end of August in 2010. The egg predator *P. berlesiana* was found also in low numbers throughout the sampling period in 2011 and 2012, however in 2010 higher number of individuals were found (Figure 1).

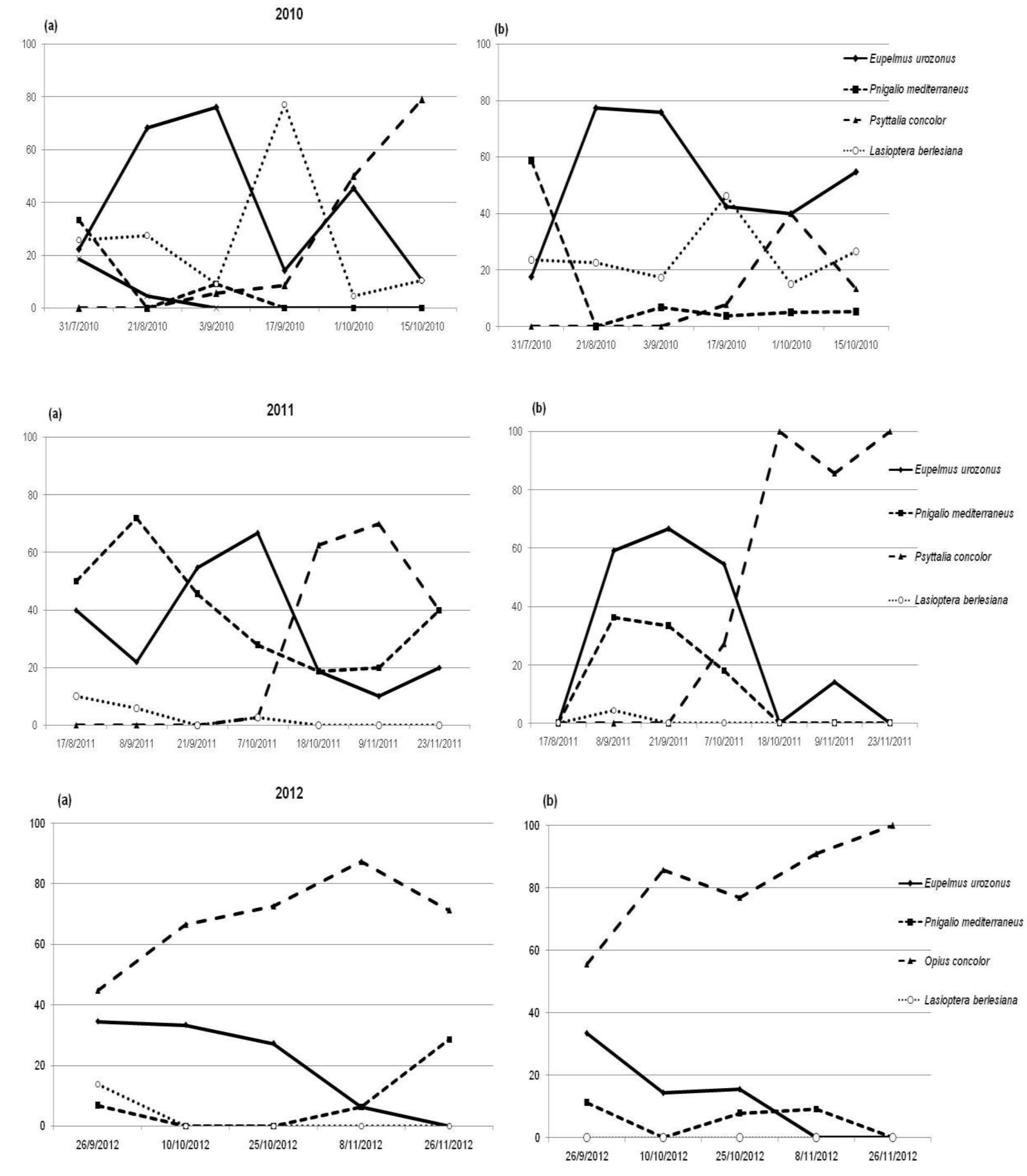

Figure 1. Relative abundance of olive fruit fly parasitoids obtained from infested olive fruits in organic (a) and conventional (b) olive groves, as determined from rearing cages, Western Crete (Chania), 2010-2012.

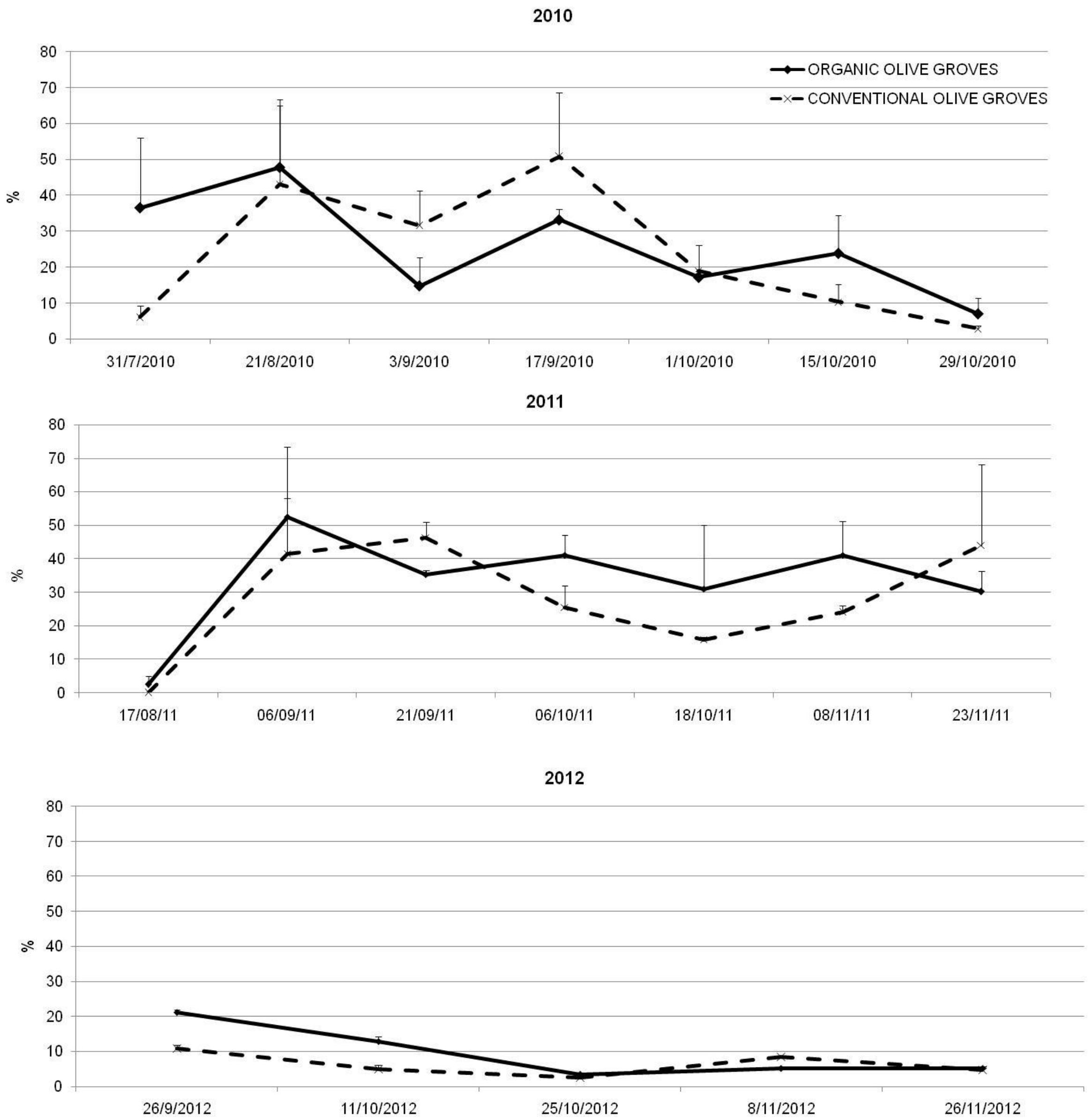

Figure 2. Percentage of ectoparasitism of *Bactrocera oleae* (x + s.e.), from dissections of olives, from organic and conventional olive groves in Western Crete (Chania), 2010-2012.

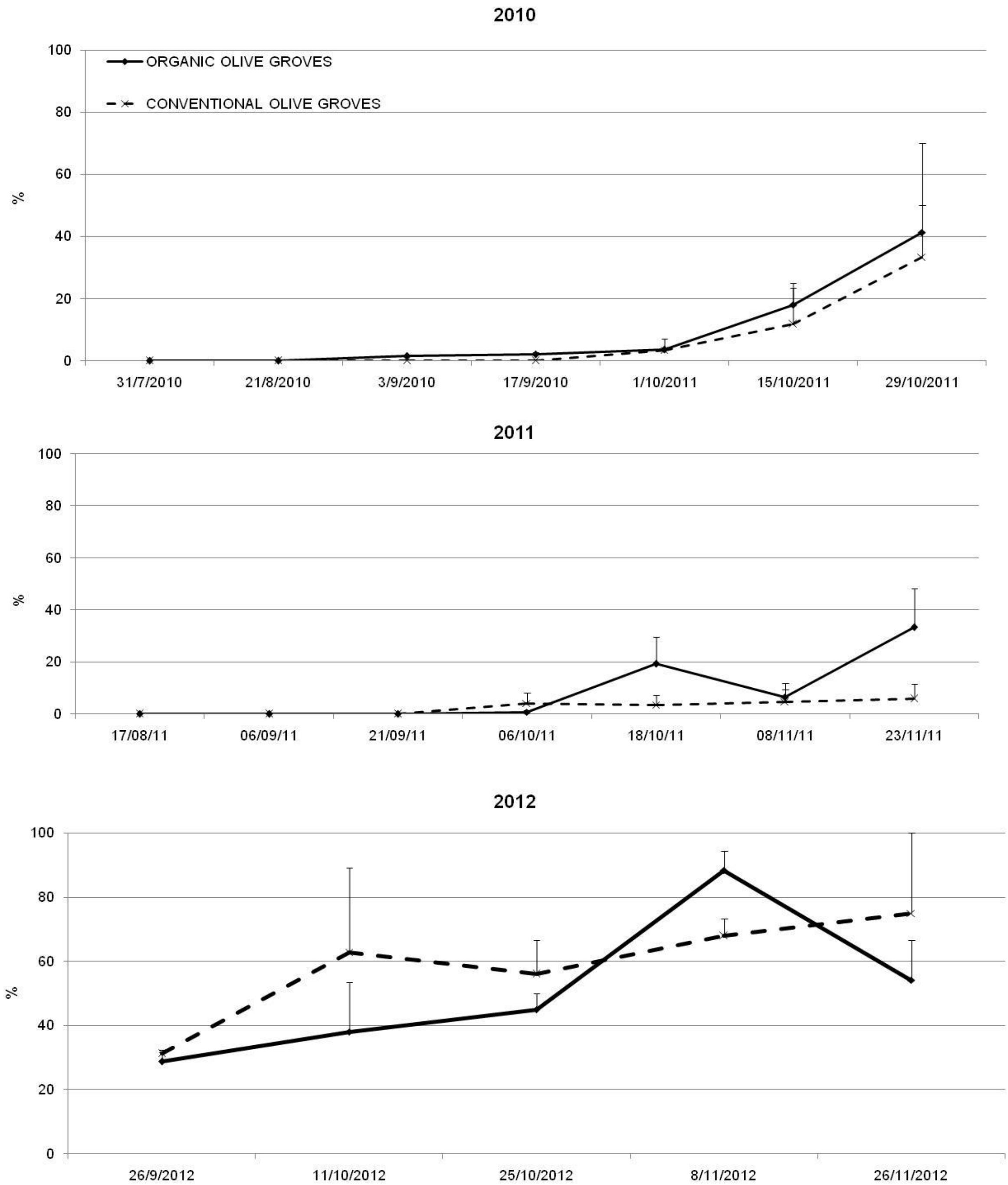

Figure 3. Percentage of endoparasitism of *Bactrocera oleae* (x + s.e.) from dissections of olives, from organic and conventional olive groves in Western Crete (Chania), 2010-2012.

The percentage of parasitism of *B. oleae* due to ectoparasitoids, endoparasitoid and predator action on olive fruits from organic and conventional olive groves in Chania during the three successive years of the study is presented in Figures 2, 3 and 4, respectively.

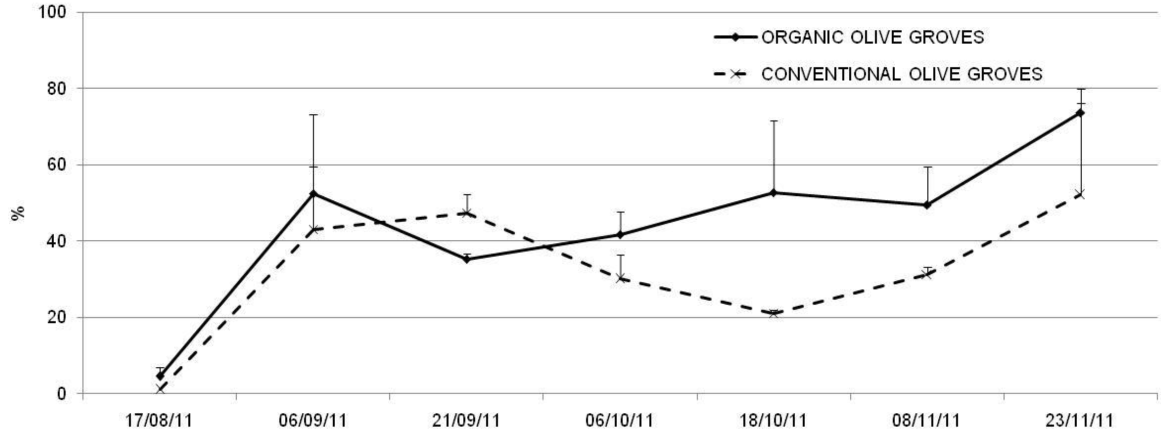

Figure 4. Percentage of predation of *Bactrocera oleae* eggs (x + s.e.) due to *P. berlesiana* action, from dissections of olives, from organic and conventional olive groves in Western Crete (Chania) in 2011.

The parasitization rates in organic as well as in conventional olive groves was significantly fluctuated depending on the year and the locality. The ectoparasitization rates were peaked in September and reached 52.5% in 2011 in Chania. Parasitization rate by *P. concolor* was highest at November and reached 88.4% in 2012 (Figures 2, 3).

In Chania, during the entire sampling period, the highest percentage of parasitism (ectoparasitism, endoparasitism) of *B. oleae* and of predation by *L. berlesiana* was found in organic olive groves compared to conventional, however the differences were not statistically significant.

The observations made in Chania indicate that the percentage of natural enemy action reach the highest levels during November (73.8%) (Figure 5) nevertheless natural enemies are not able to maintain the population of olive fly below the economic threshold. Similar trend has been observed by Neuenschwander *et al.* (1986).

It is concluded that although the conventional olive groves considered in this study were subjected to 4-5 bait sprays against *B. oleae*, no significant differences were observed between conventional and organic olive groves. This is may be because the used hydrolyzate proteins in bait sprays do not attract hymenopterous parasitoids (Samish, 1973).

In the samplings from the area of Kyparissia, parasitism was recorded in 5 out of 19 samples. The only parasitoid emerged was *E. urozonus*. The percentage of parasitism in those 5 samples was in average 6.5%, ranging from 14 to 2%.

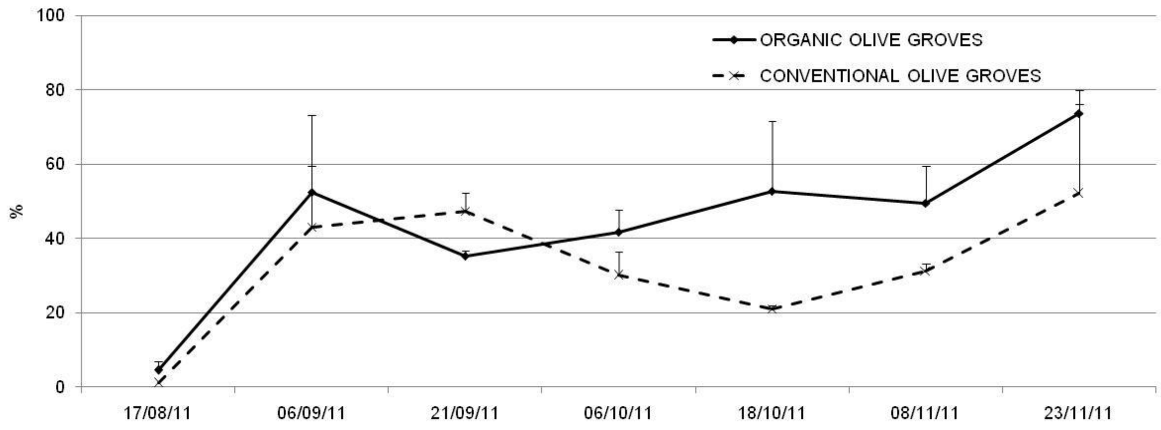

Figure 5. Percentage of parasitism and predation of *Bactrocera oleae* (x + s.e.) on olive fruits from organic and conventional olive groves in Chania in 2011.

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Entomopathogenic fungi associated to olive pests: isolation, characterization and selection for biological control

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Entomopathogenic fungi (EF) are one of the most promising alternatives to chemical pesticides, and, although a bulk of publications is available, knowledge about the interaction of EF and olive pests is scarce. Hence, the goals of this work were i) to identify naturally occurring entomopathogenic fungal species associated to olive pests; ii) to appraise factors affecting abundance and diversity of EF; iii) and to evaluate the pathogenicity of EF isolates. For this, EF were isolated from mycosed larvae and pupae of *Prays oleae* and identified by sequencing of the internal transcribed spacer region. A total of 8 EF species was identified, being *Beauveria bassiana* the most abundant. Differences on EF community associated to each generation of *P. oleae* were detected. The species *B. bassiana*, *Paecilomyces formosa* and *Cladosporium oxysporum* occurred almost exclusively in the phyllophagous, carpophagous and antophagous generations, respectively. In an attempt to elucidate the mechanisms responsible for this variation, the influence of olive plant organ and type of compound produced on fungal behaviour were assessed. *B. bassiana* development was inhibited by diffusible compounds released from olives, and stimulated by volatile compounds of both leaves and flower clusters. By contrast, the influence of plant organs on *P. formosa* development was more subtle. The effect of different soil management systems (tilled and not tilled) on the EF community in olive orchards was also evaluated. Olive groves under no-till system showed higher occurrence, diversity and abundance of EF than tilled system. Although these differences were found to be not significant, the highest number of exclusively species found in non-tilled orchards indicated that vegetation cover may act as a reservoir for fungal species. The virulence of *B. bassiana* isolates was also tested against fourth instar larvae of the lepidopteran *Cydia splendana*. Concentration-dependent mortalities were observed as well as noticeable variation of virulence between isolates. Rapid mortality (< 5 days) and low LC_{50} were observed, showing high pathogenicity of the isolates. The combined data shows that EF are naturally present in olive orchards, and provide new insights about the population dynamics of these fungi and of their relation with agricultural factors.

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The parasitoid complex associated with the olive fly, *Bactrocera oleae*, in Southern Portugal (Algarve)

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Abstract: The olive fly, *Bactrocera oleae* (Gmelin), is the most destructive pest of the olive trees in the Mediterranean Basin. Since 2004 several studies have been carried out in order to identify the beneficial insect species of the Hymenoptera order that usually inhabiting the olive ecosystem in Algarve. For that purpose samples of infested olive fruits were collected during the autumn season from several olive trees at Loulé and S. Brás de Alportel regions. The collected fruits were placed in laboratory conditions (temp.: 23 ± 2 °C; 60 ± 5% relative humidity and 12 light: 12 dark photoperiod cycle) until larvae completed their development and left the fruit to pupate. After that they were collected and placed into a small glass tubes with a honey drop until the emergence of the adults in the same laboratory conditions. Adults of the parasitoids were characterized and identified. The obtained results showed that the most abundant Hymenoptera species of the parasitoid complex associated with the olive fly in Algarve are *Psytallia (Opius) concolor* (Hym.: Braconidae), *Pnigalio mediterraneus* (Hym.: Eulophidae) and *Cytoptyx latipes* (Hym.: Pteromalidae).

Key words: *Bactrocera oleae, Opius concolor*, *Pnigalio mediterraneus*, *Cytoptyx latipes*, parasitoids

Introduction

The olive tree, *Olea europaea*, was a native from Asia Minor and spread to the Mediterranean basin 6000 years ago (Costa Lobo, 2012). It is a major crop in the Mediterranean basin and its cultivation goes back to ancient times. It was being grown on Crete by 3000 BC and the Phoenicians spread the olive to the Mediterranean shores of Africa and Southern Europe. The olive culture was spread to the early Greeks then Romans. As the Romans extended their domain they brought the olive with them (Costa Lobo, 2012).

With the discovery of America olive farming spread beyond its Mediterranean confines. The first olive trees were carried from Seville to the West Indies and later to the American Continent. By 1560 olive groves were being cultivated in Mexico, then later in Peru, California, Chile and Argentina (Gonçalves *et al.*, 2010). In more modern times the olive tree has continued to spread outside the Mediterranean and today is farmed in places as far removed from its origins as southern Africa, Australia, Japan and China (Costa Lobo, 2012). Spain is the principal European olive producer, followed by Greece, Italy and Portugal.

The fruits, commonly called olives can be used as condiments or for oil production. The olives destined to be used as condiments are picked in autumn and left to soak in salt water. In Algarve, the olives to be used for oil production are mostly harvested in November and December. In southern Portugal (Algarve region) the olive tree is affected by some pests, although it has fewer problems than most fruit trees (Gonçalves *et al.*, 2010; Gonçalves & Andrade, 2012). In this region the most important insect pest of the olive crop is the fruit fly *Bactrocera oleae*. The olive fruit fly causes quantitative and qualitative damages on olive production. Quantitative damages are caused by larvae of the two last stages (second and third larvae stages), by the removal of the significant proportion of the pulp which causes a reduction in the yield of olives and also part of the production is lost due to premature falling of the infested fruits. The qualitative damage is the significant deterioration of the oil quality extracted from olives with a high percentage of attacks by larvae of the last stage. The oil obtained from infected olives has a high acidity level and a consequent lower shelf life. Secondarily, qualitative impairments of varying severity derive from the olive fruit fly attacks due to the arrival of fungus through the holes resulting from the larvae hatchings.

The main goal of this study, which took place in the period between 2004 and 2012, was to identify the beneficial insect species of Hymenoptera usually inhabiting the olive ecosystem in Algarve region.

Material and methods

Samples of infested olive fruits were weekly collected during the autumn season from several olive trees at Loulé and S. Brás de Alportel regions. The samples were composed by five hundred infested olive fruits collected from ten olive trees, in each region. The collected fruits were placed in dark boxes in laboratory conditions (temp.: 23 ± 2 °C; 60 ± 5% relative humidity and 12 light: 12 dark photoperiod cycle) until larvae completed their development and left the fruit to pupate. After that, the larvae were collected and placed into small glass tubes with a honey drop until the emergence of the adults in the same laboratory conditions. The emerged insects were placed in 70% alcohol and the parasitoids were characterized and identified in families, genera and species according to their morphological characteristics. The results were statistically analyzed by Student T-test ($P < 0.05$).

Results and discussion

The hymenoptera parasitoids emerged from infested olive fruits, during the autumn season from several olive trees at Loulé and S.Brás de Alportel regions, in the period between 2004 and 2012, are indicated in the Figures 1 and 2, respectively. In both regions the identified parasitoids belonged to the Braconidae, Eulophidae and Pteromalidae families. In both regions the Braconidae family was the most abundant, followed by the Eulophidae and Pteromalidae families, respectively. In Loulé region the relative importance of the emerged parasitoids families are 51%, 32% and 17%, for Braconidae, Eulophidae and Pteromalidae, respectively, whereas in S. Brás de Alportel region for the same families the emerged parasitoids were 50%, 32% and 18%, respectively. Based on their morphological characteristics, the emerged parasitoids from the olives infested by the fruit fly *Bactrocera oleae* during all the period of observation belong to the following genera and species: *Cytoptyx latipes*, *Psytallia concolor* and *Pnigalio mediterraneus* (Table 1). Significant differences ($P < 0.05$) were not found in the total number of parasitoids between the two studied regions (Loulé vs. S. Brás de Alportel) ($P = 0.1127$).

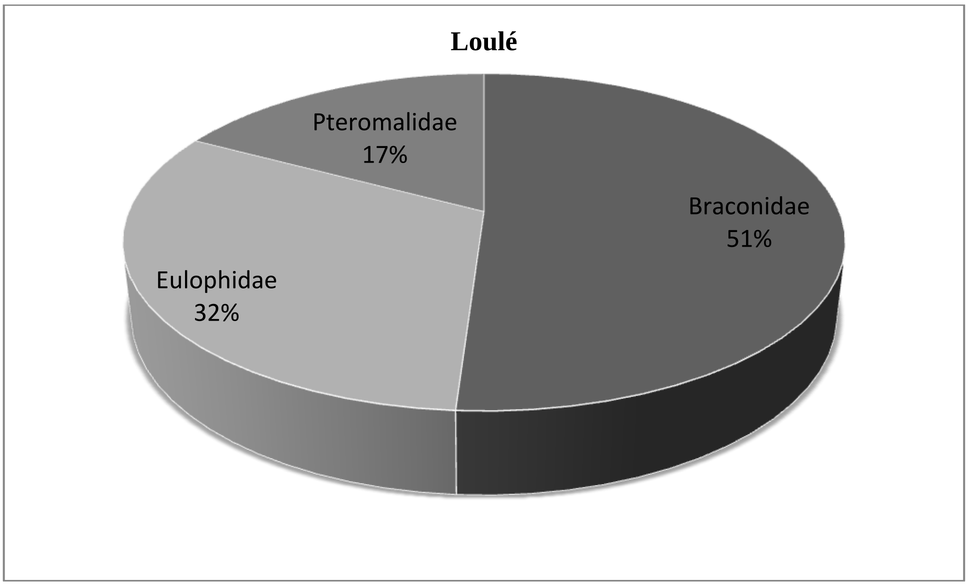

Figure 1. Relative importance of the parasitoid families emerged from the olive fruits infested by the olive fly collected from olive trees in Loulé region, from 2004 to 2012.

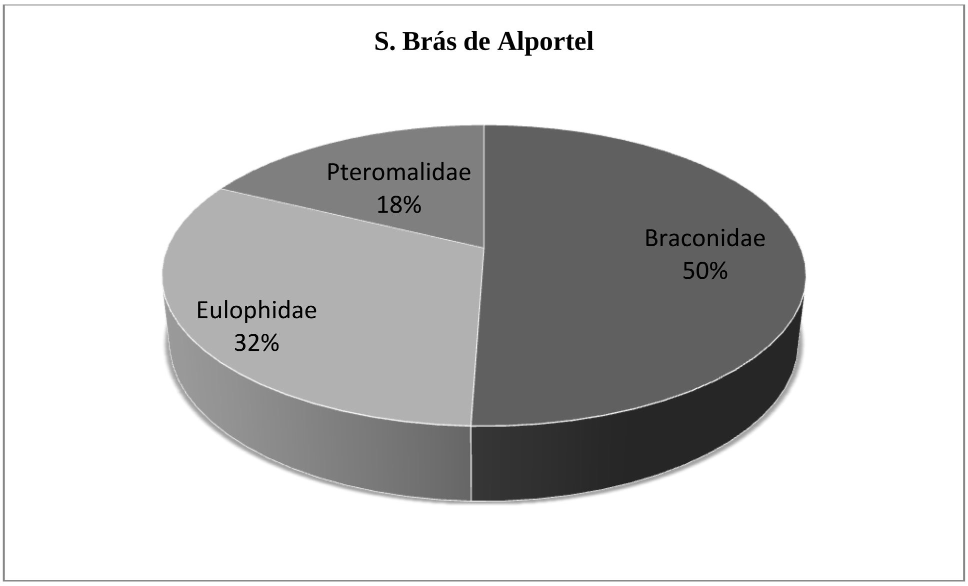

Figure 2. Relative importance of the parasitoid families emerged from the olive fruits infested by the olive fly collected from olive trees in S. Brás de Alportel region, from 2004 to 2012.

Table 1. Hymenoptera parasitoid species of the olive fly *Bactrocera oleae* emerged from infested olive fruits, during the autumn season from several olive trees at Loulé and S. Brás de Alportel regions, from 2004 to 2012 (500 fruits weekly sampled from 10 olive trees, in each region).

Region	**Loulé**			**S. Brás de Alportel**		
Year	*Psytallia concolor*	*Pnigalio mediterraneus*	*Cytoptyx latipes*	*Psytallia concolor*	*Pnigalio mediterraneus*	*Cytoptyx latipes*
2004	10	8	2	11	7	3
2005	8	4	4	9	5	3
2006	9	5	3	8	5	4
2007	10	6	4	17	6	4
2008	11	8	3	9	7	3
2009	13	9	4	10	8	4
2010	10	7	5	11	6	3
2011	14	8	4	9	7	5
2012	13	6	4	10	8	4
Total	**98**	**61**	**33**	**94**	**59**	**33**

With this study we verified that the natural enemies of the olive fruit fly with a significant role are mostly parasitoids of the Hymenoptera order. In conclusion, the most abundant species of the olive fruit fly parasitoid complex in Southern Portugal is the larvae endoparasite *Psytallia concolor* followed by and the larvae ectoparasite *Pnigalio mediterraneus* and *Cytoptyx latipes*.

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Initiation for the study of moss as a cover crop in Mediterranean olive groves

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Abstract: In Cabra, Spain, we selected an olive grove with a dense presence of moss as a cover crop (85% surface area) in order to study the influence of its presence on the growth of weeds, as well as its tolerance to common herbicides and chemical substances used in olive crops. We evaluated the installation of 6 different weed species (*Brachypodium distachyon, Capsella bursa-pastoris, Papaver rhoeas, Sagina apetala, Sinapis alba* and *Stellaria media*) in pots kept in a semi-protected environment for non-vegetation soil facing to soil covered with moss. The emergence that was measured over 10 weeks showed significant differences between species, which were established themselves quicker and in greater abundance in the absence of moss. The evaluation was held based on the analysis of variance and Tukey test at $P < 0.05$. Over a period of six weeks we also evaluated the tolerance/susceptibility to 19 different herbicides authorized in olive groves in Spain and Israel and to chemical substances such as fertilizer of ammonium sulfate and fungicide containing copper oxychlorid. Significant differences were found in the moss's reaction to the distinct treatments in both the time of reaction and recovery. On the one hand the moss was very susceptible to fertilizer but on the other hand tolerated fungicide. The first experiment was based on a factorial design (species x soil/moss) with 4 repetitions and 100 seeds per pot (48 samples) and a randomized design with 5 repetitions (125 samples) in the second experiment. The presence of moss in the soil of the olive grove, and its reduction or delay in the installation of weeds, demonstrates a non-chemical control method which could be useful in the integrated management of weeds. Its tolerance to several herbicides that are commonly used in olive groves, encourage its presence and its advantage as a good cover crop which might reduce over time the need for the application of herbicides.

Key words: Weed, emergence, herbicides, biological control, chemical substance, integrated pest management

Introduction

Weed control in olive groves faces several environmental difficulties. Saavera & Shepherd (2002) indicated that as a crop adapted to Mediterranean climate, the weed flora is highly diversed. Traditional control consists on the aplication of herbicides and soil tillage. These techniques lead to water contamination in some cases, while in others to soil erosion (Pujadas-Salva, 1986).

In non-tilled groves that are treated with herbicides, it was observed a dominant bryophyte flora that could reach 68% soil cover in Andalusian groves (Rams *et al.*, 2011). Moreover, Brown (1967) shows that moss carpet reduces the instalation of vascular species, and Miller & Miller (1979) indicate that in the case of *Sphagnum* moss, when it is mixed within the soil surface, it prevents the growth of weeds. The poor seedling height of weeds when moss is present as a cover crop might be either because of the fact that the seeds can´t reach enough soil water or because of their lack of light to germinate.

The effect of herbicides on the ecology and growth of mosses in agriculture is poorly documented (Stjernquist, 1981; Brown, 1992; Söderström *et al*., 1992; Rowntree *et al*., 2001), although Porley (2001) considers it an issue of great importance. Kershaw *et al*. (1994) claim that on the one hand the use of herbicides favors the growth of mosses by the lack of competition with vascular plants, but on the other hand, ecosystem studies showed that herbicides drastically reduce the diversity of mosses and lichens (Bell & Newmaster, 1988). Different species of mosses differ from each other in their tolerance or susceptibility to herbicides (Horrill *et al*., 1978). However, some researchers argue whether bryophytes and lichens can be recovered after being treated with herbicides (Stjernquist, 1981; Brown, 1992). In other tests herbicide application showed no significant effect. Moreover in some cases they even became manifest resistance (Bond, 1976; Atkinson *et al*., 1980; Ougham, 1983; Skender, 1983; Rusinska & Balcerkiewicz, 1987; Anon, 1989).

In the groves studied in the context of this work it is very common to use herbicides to control weeds. However, moss is found relatively frequently. Still, it is not known which active materials are harmless or harmful to moss´s species or their degree of tolerance to a maximum level in this crop.

Mosses usually have low N:C ratio, indicating a scarce mineral nutrient requirement (Estébanez-Pérez *et al*., 2011). Some authors have measured a range of $10\text{-}50^{-3}$ g/kg of N in dry matter (Epstein, 1965; Larcher, 1983), compared with the olive, where in a concentration lower than 1.4% N in leaf is considered deficit (Beutel *et al*., 1983). In mosses´s fertilization it is common to use dilutions of the means employed to 1:10 respect to vascular plants, since a high concentration of nutrients has a toxic effect on them. It appears that the bryophytes play an important role as substrates for N fixation in many nutrient-poor habitats, making them an essential component in these ecosystems. Moreover, bryophytes quickly absorb heavy metals without the regulation characteristic of the absorption of nutrients (Glime, 2007). Its ability to sequester heavy metals without damage makes them a good biomonitors.

On the one hand, in olive groves nitrogen is usually the most commonly used mineral element in the fertilization programs (Fernández-Escobar, 2008). In integrated production programs (BOJA no. 117, Sevilla, 2010), it is recommended not to exceed the dose of 15 kg N per ton of olives produced, representing amounts around the 60-70 kg/ha of nitrogen to much of Andalusian olive groves.

On the other hand, the olive tree in Mediterranean conditions, even in the most drought areas, suffered aerial attacks of fungi. The most important among them is *Fusicladium oleagineum* (Cast.) Hughes (Trapero *et al*., 2009), which is treated generally with fungicides contained cation´s metals, preferably copper oxychloride.

This paper therefore seeks to evaluate the tolerance or susceptibility of mosses against the most common applications of chemical substances; herbicides, fertilizers and fungicides used in the olive grove and to determine inference of olive moss crust developed, compared to bare soil on emergence and installation of weeds in order to find out whether moss crust could have a beneficial effect in the biological control of weeds.

Material and methods

During June, 2012, 6 species of weeds; *Sagina apetala* Ard., *Stellaria media* L., *Capsella bursa-pastoris* (L.) Medicus, *Sinapis alba* L., *Brachypodium distachyon* (L.) Beauv. and *Papaver rhoeas* L. were collected in the province of Cordoba and stored under laboratory conditions. These species were chosen for their autumnal germination, their different seed size and belongs to different botanic families.

In November 2012 we collected soil samples with and without moss crust, using an iron mould of 10×10 cm size from an olive grove in Cabra (37°30´55"N 4°24´29"W). Then we planted moss´s carpet and bare soil in pots and located them in humid conditions. On every pot we seeded 6 weed species, individually, at 100 seeds/pot. The experimental design was factorial randomized (6 species × 3 locations × 2 treatments, with or without moss) with 4 repetitions. We assessed the percentage of plants installed over the sown seeds weekly, from the third week until the 10^{th} week. We then established that the installation had occurred when the seedling had the first true leaf visible. Seedlings have also been removed on each count. In the case of *S. apetala*, due to its small size, it was difficult to assess the emergency at the 4^{th} and 5^{th} weeks without magnifying glasses; hence it led to a major emergency data for accumulation in the 6^{th} week. Emergencies were expressed in percentages and ANOVA was performed for the data transformed by arcsin $\sqrt{x/100}$ expression and using the program STATISTIX 9.0. The average separation was performed using Tukey test ($P < 0.05$).

In continuation, on early February, on samples of mosses from the same olive grove, 19 herbicides were applied at the maximum authorized doses in Spain as well as in Israel (Table 1), with a OMPAK 10 pulverizer provided with a homogeneous distribution dripper 8001 E Teejet which moves with a moving chain, pulverizing the surface on 50 cm height in 2 bar pressure and volume of 250 l/ha. In the case of glyphosate, further treatment was applied to fifty percent of the maximum permissible dose due to the fact that usually it is implicated in lower doses. The design was factorial (herbicide x location) with 5 repetitions.

Following the same procedure, ammonium sulfate (21.5% N) and copper oxychloride (38% Cu) were applied. The ammonium sulfate dose was calculated at 900 kg/ha with 2 application procedures in solid and liquid, simulating the entire product is applied at a dose of 300 kg/ha of olive groves (64.5 kg N/ha), concentrated under the cup, which occupies 33% of the surface, thus resulting in a dosage of 900 kg/ha actually applied. The solid treatment of N was realized by distributing it manually over the samples (0.9 g/pot of 100 cm^2).

The maximum permissible dose of copper oxychloride, Sanagricola 38 Flow (pc) is 6 kg/ha. The dose used in this experiment was calculated by estimating the area occupied by the foliage of the olive is 25%, thus resulting in a concentration of 24 kg/ha and simulating 2 levels of washing the product from the tree (10% and 50%) on the moss cover (resulting in a dose of respectively 2.4 and 12 kg/ha actually treated). The application was realized by the pulverizer. The experimental design was factorial (treatment x location) with 5 repetitions.

After application, we left all the pots without watering for 24 hours in order to avoid washing products and promote absorption. The evaluations were realized by 4 reviewers every 7 days from the third week estimating the damage percentage as a phytotoxicity compared to the control, following EWRS scale (European Weed Research Council) of 0-100 (0 = no damage, 100 = mortality). Symptoms to assess the phytotoxicity observed were: colour change to brown-yellow, drying, leaf necrosis and lack of vegetative growth. ANOVA was realized for the transformed data to $\sqrt{x/100}$ arc, using the 9.0 STATISTIX program. Average separation was realized using Tukey test ($P < 0.05$).

Table 1. Active materials of herbicides and their commercial product name applied in this experiment.

Treatment	**Active materials**	**Commercial product**
T1	Flazasulfurón	Terafit
T2	Fluometuron	Cottolinz
T3	Terbutilazina	Cilotor
T4	Diflufenicán	Mamut
T5	Flumioxacina	Pledge
T6	Oxiflurofén	Goal Supreme
T7	Glifosato	Pitón
T8	Glifosato ½ dosis	Pitón
T9	Fluroxipir	Starane
T10	Quizalofop-p-etil	Nervure
T11	Amitrol	Etizol
T12	MCPA	U-46 DMA fluid
T13	Tribenurón metil	Granstar
T14	Diquat	Reglon
T15	Glufosinato	Finale
T16	Piraflufén	Kabuki
T17	Simazina	Simanex
T18	Diurón	Diurex
T19	Imazapir	Arsenal
T20	Isoxafutol	Balance

Results and discussion

In all weed species studied, the cumulative emergence rate was significantly lower in soil covered with moss than in those with bare soil, except for *Brachypodium distachyon*, that showed significant differences only until the 7th week. In this species the emergence was high and quite similar both with soil covered with moss as well as with bare soil (Figure 1). The decrease of emergence in the presence of moss was especially important in the cases of *Sinapis alba* and *Stellaria media*. Nevertheless, in the cases of *Papaver rhoeas* and *Capsella bursa-pastoris* the percentage of emergence in the presence of moss decreased almost 0% (Table 2; Figure 2).

Another interesting fact is that there was no effect of seed size on the rate of emergence and plant seedlings. However, within seed species of high weight emergence rate varied (*B. distachyon* and *S. alba*). Emergence rate also varied when comparing underweight seeds species (C. *bursa-pastoris* and *P. rhoeas*).

Moss´s carpets absorb water quickly, which causes retention of moisture for seed germination. Furthermore, frequent cycles of dehydration and drying of the surface layers of soil make it difficult for the seedlings and the instalation of emergence in the substrate. During germination the seeds are exposed to environmental conditions that might harm their emergence and instalation such as wind, or solar radiation, consequently they become more vulnerable to desiccation, and thus may lose their ability to germinate. Furthermore, it might be attacked by microorganisms or eaten by insects or birds. This can reduce the seed bank of weeds in the ground, and thus reduce the need to use lethal treatment in the control of weed population.

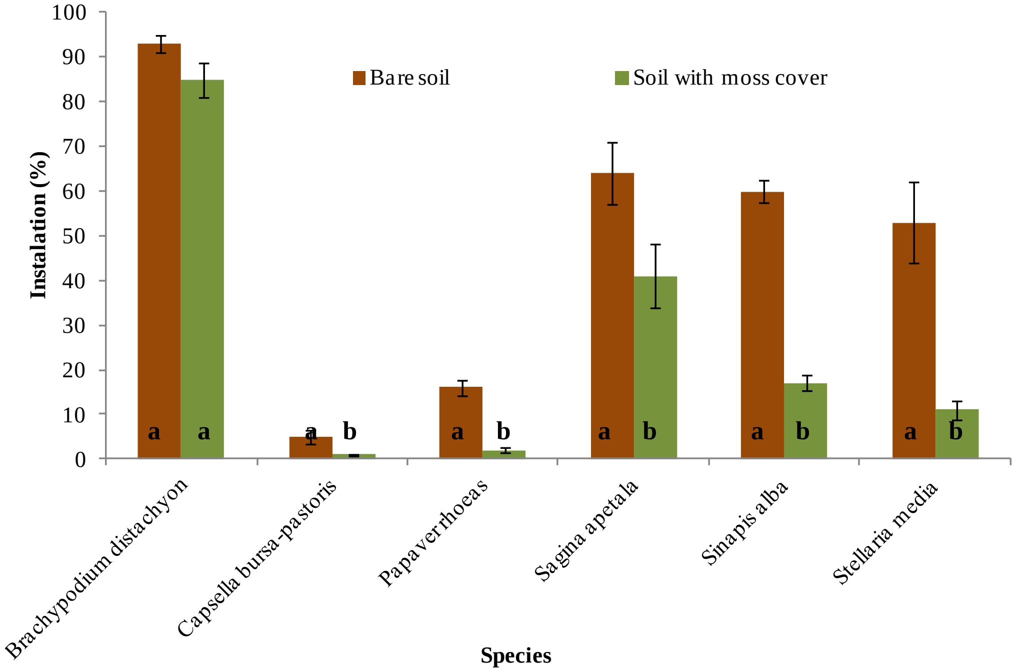

Figure 1. Percentage of accumulate emergences 70 days since seeding (6 February 2013). Vertical bars represent standard error. Letters indicate differences in type of soil between each species (P ≤ 0.05, Tukey test).

Between the 19 active materials applied on the moss species *Didymodon vinealis* (Brid.) R. H. Zander that dominants the moss´s flora in Cabra, 8 herbicides have proved to be safe or produced a minor level of phytotoxicity (0-20% damage): diquat, fluroxypyr, glufosinate, imazapyr, isoxaphlutol, MCPA, Quizalofop-p-ethyl and simazine. In contrast, 3 of herbicides: diuron, flummioxacine and flazasulphuron were lethal (Figure 3).

At 21 DTA (days after treatment), 8 herbicides quickly produced phytotoxicity higher than 70%. At 42 DTA, mosses treated with 2 of these herbicides (glyphosate and pyraflufen) showed signs of recovery characterized by new growth and greening of their aerial parts (Figure 3). Mosses treated with fluometuron and terbuthylazine, which produced initial damage between 30 and 40%, also manifested a substantial recovery.

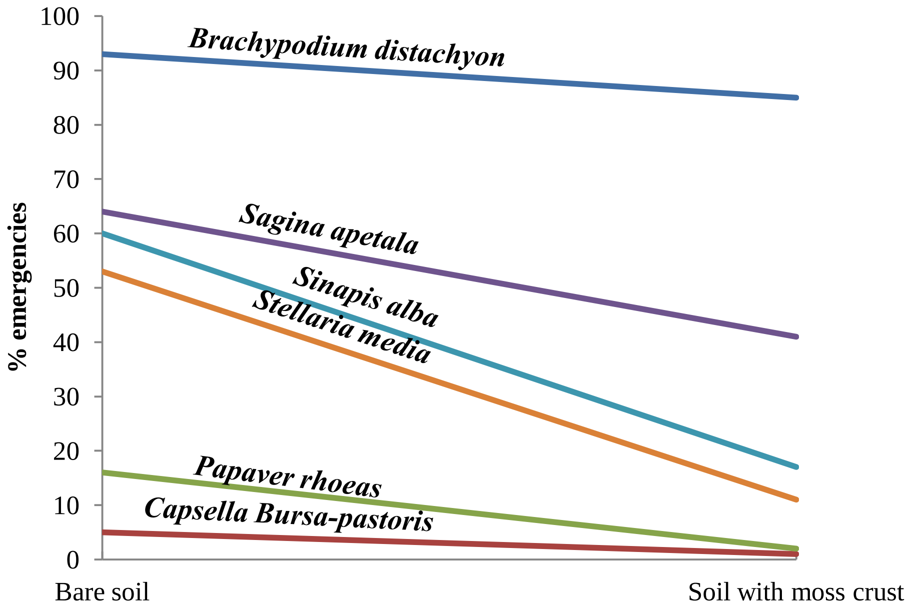

Figure 2. Interaction between species in the percentage of emergence between bare soil and soil with moss carpet at 70 days after seeding.

Table 2. Reduction of weed emergence (%) in soil covered with moss compared to bare soil.

Species	(%)
B. distachyon	9
C. bursa pastoris	80
P. rhoeas	88
S. apetala	36
S. alba	72
S. media	79

It could not observed any relationship between the level of phytotoxicity on moss and the form of control of the active material. Thus, moss manifested susceptibility or tolereance to herbicides without connection to be of pre- or post-emergence, as well as for the type of herbicides. Hence, fluometuron caused minor effect on moss growth while diuron was highly phytotoxic. Also terbuthylazine cuased high damage while simazine not (Figure 3). The treatments with the fungicide copper oxychloride did not cause any damage to mosses in any case. Instead the fertilizer ammonium sulfate, both as a solid powder as pulverized, produced a quick and enormous phytotoxicity on the moss.

Based on this study, moss manifests tolerance to some of the herbicides that are in common use in the control of weeds in olive groves. Its use may not affect the growth and abundance of moss, which may reduce the emeregence and can facilate the control of weeds. Saavedra and Pastor (2002) noted future intention toward an important decrease of the number of active substances applied, as well as their licensed doses. The European Union has regulated the use of pesticides, among others, through the Directive 414/91, which led to a significant reduction in the number of authorized active substances. In Spain, the transposition of the rule has been made by Royal Decree 1311/2012, of September 14, which establishes the framework for action to achieve the sustainable use of plant protection products. One of the key points of this Royal Decree is compulsory from 2014, to follow the general principles of integrated pest management on farms, except those regarded as low use of pesticides. It is very common to find moss cover as dominant species in olive groves, where herbicides have been used frequently, constituting a flora of bryophytes that shifts almost entirely the vascular flora. It might be possible to keep an olive grove free of weeds with few applications of herbicides, or even without any, if there is a dense presence of moss carpets. According to the results of this study, a herbicide may be replaced by other of the same family of mode of action, in order to prevent damage to the muscinal cover. Similarly, foliar application of nitrogen is recommended compared to localized solid application, in order to preserve moss carpet and reduce the direct contact between the fertilization and moss. Following the new policy of sustainable use of pesticides, proper handling of herbicides, considering its phytotoxicity mosses, will be advantageous.

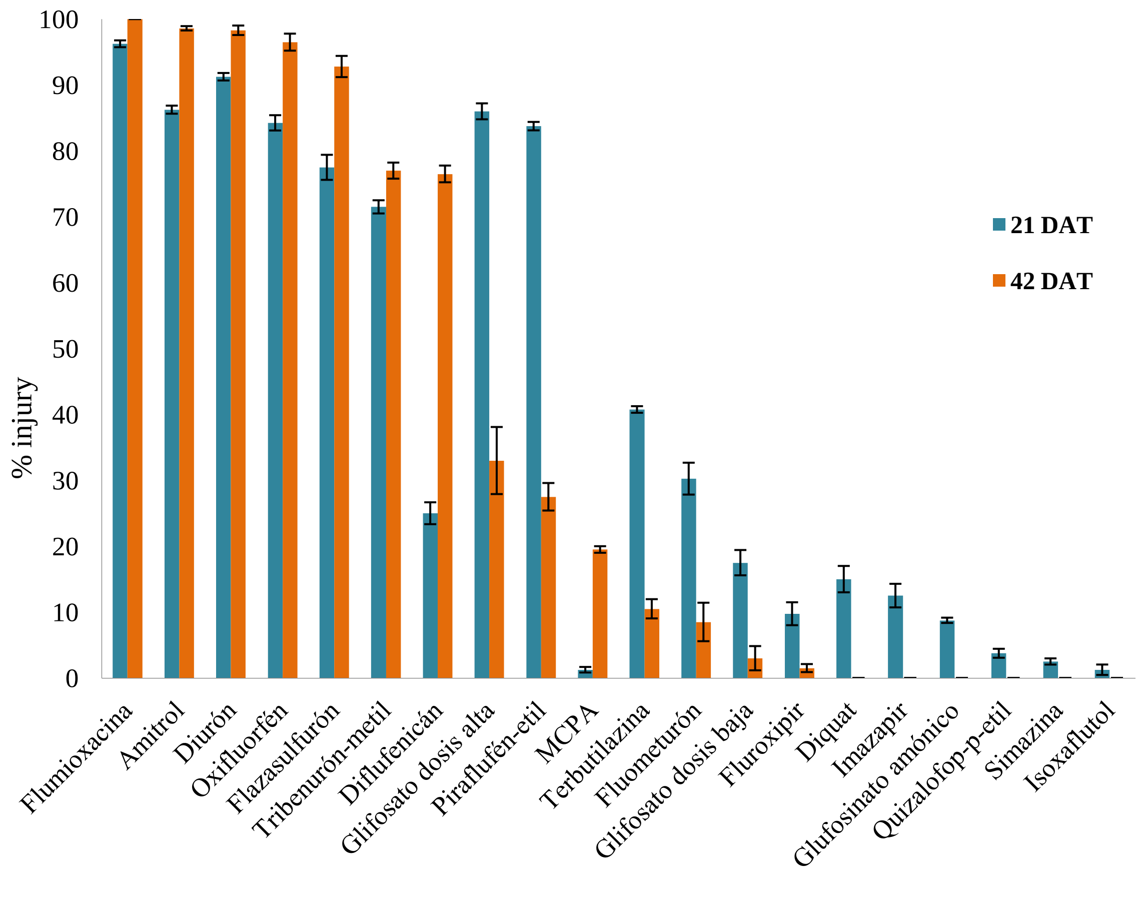

Figure 3. Phytotoxicity (%) produced at 21 and 42 days after application (DAT). Vertical bars represent standard error.

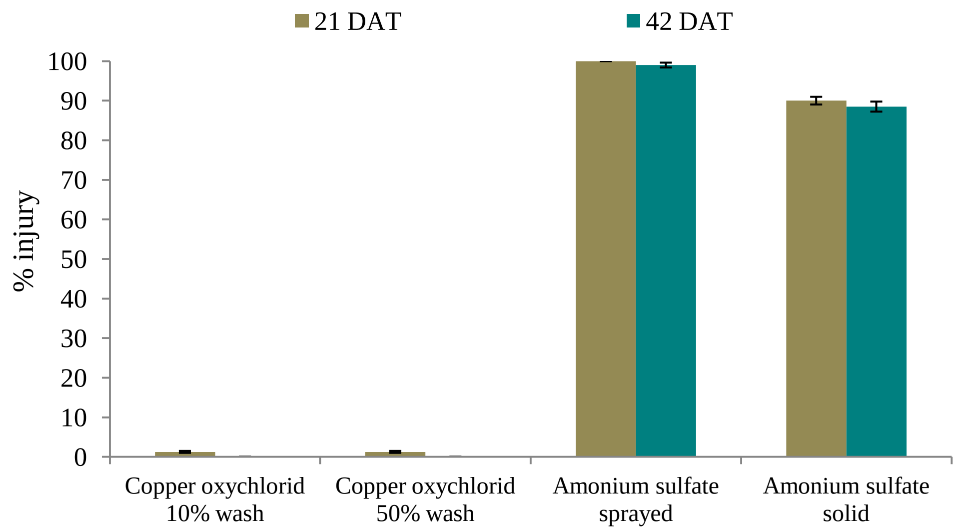

Figure 4. Phytotoxicity (%) caused by copper oxychloride and ammonium sulphate at 21 days after application (DAT). Vertical bars represent standard error.

The present study demonstrates that the presence of moss carpet can be considered as a biological control agent of other plant species. Moreover, it might be useful in the context of integrated production. Thus it could replace or reduce the use of herbicides. This approach is new in the sense that up to now the only biological control is realized as a control between natural enemies, particularly among insects or microorganisms.

According to Sheppard *et al.* (2006), the excessive use of agrochemicals and intensive management of soil in Europe, compared to other less populated regions, have caused serious environmental problems. This situation is changing with the new EU targets to reduce the use of herbicides through sustainable production methods (Directive 2009/128/EC on the sustainable use of pesticides). The potential use of moss as a cover crop in olive groves has not been studied up to now.

In conclusion, given the economic importance of the olive grove in our Mediterranean region, promoting the presence of moss as a cover crop in olive groves, is shown in this paper as a useful method of population control in the integrated management of weeds.

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Is the fungal endophyte community of olive tree active in biocontrol?

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Several reports indicated that endophytes protect their host against abiotic and biotic stress, including pathogens. However, up to date studies on endophytes of olive tree are still lacking and their involvement on plant disease resistance was never studied. Thus, the objectives of this work were (i) to assess the diversity and distribution pattern of fungal endophytes in two olive tree cultivars with different susceptibilities to olive anthracnose and verticillium wilt (cv. Cobrançosa and cv. Galega); and (ii) to disclose potential endophytes able to promote resistance to both diseases and select the most efficient biocontrol agent (BCA). The endophytes were isolated from roots, leaves and branches of healthy olive trees and identified by rDNA sequencing. The *in vitro* effect of the isolates obtained against *Colletotrichum acutatum* and *Verticillium dahliae*, the causal agents of anthracnose and verticillium wilt, respectively, was analyzed by the dual-culture method in PDA medium. A total of 1106 isolates belonging to 44 fungal species and 29 genera were isolated from the 5775 segments examined. The genera *Penicillium* had the greatest species diversity. Among the species isolated, *Paecilomyces lilacinus*, *Phomopsis columnaris* and *Fusarium oxysporum* were the most frequently isolated. The frequency of endophytic colonization was greater on roots (53-57%) followed by leaves and branches (1.5% each). The colonization frequency of endophytes was higher in cv. Galega (23%) than in cv. Cobrançosa (19%). Among the fungi isolated from cv. Cobrançosa, *P. lilacinus*, *Hypocrea lixii* and *Penicillium roseopurpureum*, significantly inhibited growth, sporulation and spore germination of *C. acutatum*. By contrast, only one species (*Trichoderma gamsii*) isolated from cv. Galega was inhibitory to *C. acutatum*. The highest inhibitory effect on mycelia growth of *V. dahliae* was displayed by *F. oxysporum* and *T. gamsii* isolated from cv. Galega. The results could be very useful in the elucidation of the mechanism of enhanced disease resistance in olive tree cultivars and in BCA finding.

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Effect of naturally occurring plants and *Saissetia oleae* (Oliv.) and *Euphyllura olivina* (Costa) honeydews on *Elasmus flavelatus* (Fonscolombe) longevity

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The olive moth (*Prays oleae* Bernard) is the most important pest of olive trees in the North of Portugal. Additionally, naturally occurring plants and insect honeydews constitute a potential food for beneficial arthropods in olive agroecosystems. *Elasmus flabellatus* (Fonscolombe) is a *P. oleae* parasitoid referred to as playing a sparse action on the olive moth mortality and undesirable because of its hyperparasitic behaviour. Nevertheless, in some olive orchards from Trás-os-Montes *E. flabellatus* was found to be responsible for 38% of the parasitism of the olive moth anthophagous generation. In this context, the objective of this work was to study the *E. flabellatus* females longevity under laboratory conditions in the presence of *Saissetia oleae* (Oliv.) and *Euphyllura olivina* (Costa) honeydews and some naturally occurring plants in the olive orchard during the anthophagous generation of the olive moth, namely, *Andryala integrifolia* L., *Anthemis arvensis* L., *Centaurium erythraea* Rafn subsp. *majus* (Hoffmanns. & Link) Laínz, *Chrysanthemum segetum* L., *Coleostephus myconis* (L.) Rchb.f., *Crepis capillaris* (L.) Wallr., *Daucus carota* L. subsp. *carota*, *Echium plantagineum* L., *Hypericum perforatum* L., *Jasione montana* L., *Malva sylvestris* L., *Spergularia purpurea* (Pers.) G. Don and *Tolpis barbata* (L.) Gaertn. *E. flabellatus* females were obtained from larvae of *P. oleae* anthophagous generation. For this, following its collection, each *P. oleae* larva was introduced in a tube (1.7 cm diameter and 12 cm height) until adult emergence. *E. flabellatus* females emerged from *P. oleae* larvae were transferred into tubes (2.7 cm diameter and 12 cm height) provided with water as one of the treatments. Negative and positive controls were carried out. Mortality of *E. flavellatus* was recorded at 24 h intervals. In the presence of plants, the average longevity of *E. flabellatus* females varied between 3 and 10 days while in the presence of honeydews it varied between 13.5 and 28 days. Longevity in the presence of *D. carota*, *M. sylvestris* and honeydews was significantly higher than longevity in the negative control. Additionally, in the presence of honeydews longevity was not significantly different than longevity in the positive control. These results suggest that *M. sylvestris, D. carota* and both *S. oleae* and *E. olivina* honeydews could benefit the longevity of *E. flabellatus* females.

Diversity patterns of Carabidae across a gradient of farming practices in olive groves from Trás-os-Montes (Northeastern Portugal)

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Olive grove is an important land-use type in the Mediterranean region, with great ecological value because of the complex composition of its biocenosis and, consequently, diverse food webs established among organisms. This work is part of a broader project aiming to develop biological indicators for assessing the quality and sustainability of the olive grove. Suitable indicators can help detect changes in agricultural areas and the subsequent provision of ecosystem services over time, namely pest control and decomposition of organic matter. Carabids were one of the taxa studied in this project due to their diversity and function in agroecosystems as well as their susceptibility to farming practices, such as tillage and pesticide application. Among the coleopteran community, edaphic carabids have been considered important predators of the olive fruit fly, *Bactrocera oleae* which may be important for reducing populations of hibernating pupae and newly emerged adults. The objective of this work was to study the diversity patterns of Carabidae community across a gradient of farming practices in olive groves from Trás-os-Montes. In 2011, carabid specimens were sampled in May in eight olive groves located near Mirandela. These fields were grown under different types of soil management: (i) tilled soil – 2 groves; (ii) spontaneous vegetation controlled with herbicides sprayed on the plantation row – 2 groves; (iii) soil covered with leguminous plants – 2 groves; and (iv) soil covered with spontaneous vegetation (wildflowers) – 2 groves. In each grove, 16 pitfall traps were installed 50 m apart and arranged alternately with four traps placed along two rows and the other traps placed between the rows of trees. Pitfalls were left in the field for seven nights and carabids were sorted in the laboratory and identified to species level. A total of 897 specimens, belonging to 33 species, were collected in the eight olive groves studied. The most abundant species were *Nebria (Nebria) salina* Fairmaire & Laboulbene, 1854, *Calathus* (*Neocalathus*) *mollis* (Marsham, 1802) and *Pterostichus* (*Steropus*) *ebenus* (Quensel, 1806) with relative abundances of 51.5%, 12.7% and 11.0%, respectively. Higher number of individuals was found in groves covered by spontaneous vegetation (315), followed by leguminous plants (235), application of herbicide (190) and tilled (143). Considering species richness, groves covered with leguminous plants reached higher number (18), followed by spontaneous vegetation (17), application of herbicide (15) and tilled (11). The number of carabids was higher in the plantation row than between the rows in tilled groves, groves with application of herbicides and with spontaneous vegetation. An opposite pattern was found in olive groves covered with leguminous. These results seem to indicate a negative effect of tillage and herbicides on carabid community.

Isolation of entomopathogenic nematodes from soil of olive orchards and their evaluation in biological control of the olive scale *Parlatoria oleae* Clove. (Homoptera: Diaspididae), in Al jouf region, Saudi Arabia

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Soil samples were collected from olive orchards at Al Jouf region, Saudi Arabia to isolate and identify entomopathogenic nematodes (EPNs). EPNs extracted by greater wax moth, *Galleria melonella* L. larvae baiting technique. In two samples *Heterorhabditis* spp. (Rhabditida: Heterorhabditidae) and in a single sample *Steinernema* spp. (Rhabditida: Steinernematidae) were identified. The soil acidity (pH) in the samples ranged between 7.6-7.9. Most isolates were found in olive orchards where drip irrigation was used. This finding indicates the role of this type of irrigation in the distribution of entomopathogenic nematodes, most likely due to appropriate soil moisture ventilation and the ease of nematodes spread. The pathogenicity of these nematode isolates was tested on the olive scale, *Parlatoria oleae*, under laboratory conditions. The aim of this work was to determine if the tested nematodes could reach and kill the pest nymph inside the scale. Water suspensions of different nematodes were prepared and sprayed on the infested leaves and branches. Doses of 100, 200, 400, 800, 1,200 infective juveniles (IJs) nematodes per/ml. were applied; leaf disks and branches were sprayed with 1 ml (0.5 ml/side) of different concentrations of a nematode suspension. A handheld aerosol sprayer was used to apply the spray. Sprayed leaves were left for several minutes to avoid water condensation. Doses used showed a highly positive response. The nymphal mortality was more than that of the adults when Oleyl-polypeptide (wax remover) was added in the nematodes solution. *Heterorhabditis* spp. caused the highest mortality (76%) at the dose of 1,200 Ijs/ml. The mortality rates increased with the increase of nematode concentration.

Session 4
Chemical control

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Bait sprays: Area wide control program against *Bactrocera oleae* in Greece

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The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most serious pest of olives in Greece (Tzanakakis, 2006). It infests olive fruit causing quantitative and qualitative damage (Neuenschwander & Michelakis, 1981). Potential crop losses that can be caused by this pest, in periods and locations with conditions favoring high population densities and where no control measures are taken, are quoted by various authors to be as high as 80% of production with an average range of about 40-50%. However, economic losses due to this pest have been estimated to reach up to 15% of the olive crop, in spite of the fact that, pesticide treatments are applied every year to control the olive fly population (Haniotakis, 2005).

In Greece the application of bait sprays against *B. oleae* started in 1913. In 1937 was organized and started the application of the national program "Collective control against olive fruit fly" in all olive growing areas of Greece. In the mid-tenth of 1970, parallel with the ground bait sprays, began the implementation of the aerial bait sprays. However, in 1996 air applications of bait sprayings against *B. oleae* have been abolished in Greece due to their strong adverse side effects (Ziogas, 1996).

The "Collective control program against olive fruit fly" is funded annually from the Hellenic Ministry of Rural Development and Food and supervised from the Directorates of Agriculture and Veterinary of the 36 olive growing Regional Units (Prefectures) of Greece. The program is implemented annually to a total of about 96.6 million olive trees.

Conditions for the application of the program:

a. Should be requested the accession of the olive growing area to the program by the competent authority (i.e. the boards of municipalities)
b. Fruition percentage should be higher than 25% and 20% of full production for oil extraction and table olive trees respectively, since in regions with high population and fruition rate lower than this percentage the method is not effective.
c. Each farmer which is included in the program should pay the relevant levy (i.e. 2% on the value of oil produced and is calculated at the lowest value (intervention value)).

Bait spray is a preventive control method since the bait attracts and kills adults of both sexes before the females oviposit in the olive fruit. Bait solution consists of a food attractant (protein hydrolyzate) and a registered insecticide.

Requirements for high efficacy of this method are:

a. Spray early in the morning, because the insect is more active early the morning searching for food.
b. Should spray every 2^{nd} or 3^{rd} tree or row of the grove, depending on the planting density.

c. The baits should be applied by high-pressure sprayers on tractors or by equipments which provide distribution of the product on a small part of the foliage of the sprayed tree "spot spraying" at quantity not more than 300 ml/tree.
d. Directional spray inside the tree canopy for protecting the bait from the effect of sun radiation for achieving prolonged residual activity of the bait.
e. The pressure in the spraying means should be regulated properly to ensure that the spraying nozzle should be as large as it can be (droplet size diameter > 5mm).
f. Should not be left unsprayed olive groves and area-wide application is needed due to high mobility of the adult flies.
g. Suitable environmental conditions. The spraying should be stopped if the temperature is higher than 28^{o}C, the wind speed is higher than 5 Bf and when it rains or if it will rain.
h. The duration of each spraying in a region should be as short as possible (not more than 8 days).
i. Prolonged residual activity of the baits since the residual activity of the currently used baits ranges from 3-7 which is not enough to cover the continuous emergence of adults, due to the overlapping generations of this pest or the immigration of adult flies into the protected area.
j. Effective insecticides. The used product should be applied at the dosage specified for the intended use.

The timing of applications is based on a weekly monitoring system of populations of the pest with a net of McPhail traps. In addition to population of olive fly monitoring fruit sampling is also used as it provides direct information about the extent of damage caused by *B. oleae* as well as indirect information about its population size and composition.

In Greece, 2-5 bait sprays are usually applied annually depending on the local conditions. The annual cost is about 30 million € from which the 80% comes from the National Budget and the 20% from the EU in the frame of the project "Improving quality of olive oil". The economic benefit calculated from the following equation is about 550 million €:

Economic benefit = Anticipated damage without control measures – Actual total loss - Total cost (labor cost + insecticides +baits + overheads).

When properly and timely applied, the method is very effective and strongly recommended against olive fruit fly in IOBC Guidelines for the Integrated Production of Olives (IOBC-WPRS, 2012). Advantages of the bait spraying are:

a. Considerably less amount of active substance/ha (0.10 of the cover spraying).
b. Lower cost of application/spraying compared to other methods (cover sprays, mass trapping etc).
c. The damage to the fauna of beneficial insects is greatly reduced (since the baits do not attract hymenoptera parasitoids).
d. There is no risk for bees, birds and the environment.
e. There is no risk of detection of residues in olives if properly applied.

For the improvement of the method, extensive research has been carried out in the last 20 years, which has focused mainly on:

a. Studying the effectiveness of various insecticides against *Bactrocera oleae*;
b. Improving the bait effectiveness and extensive of its life;
c. Reduction of pesticide use;
d. Improving spraying techniques;

e. Studying the development of insecticide resistance in the olive fruit fly and risk analysis for development of resistance of the olive fly;
f. Studying the effect of bait sprays on arthropods fauna of the olive grove.

Research is ongoing to further improve effectiveness of this method. The effort is concentrated on the improvement of the method through the improvement of the bait effectiveness and extensive of its life, the use of effective and more selective insecticides, the use of GPS-GIS technology, the development of computer models that predict population dynamics etc.

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Influence of age, sex, and stimulus concentration on the electroantennographic response of *Bactrocera oleae* (Rossi) adults to *Olea europaea* volatiles, chemical repellents and sex pheromone

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Plant volatiles play a key role in host plant location of phytophagous insects. Knowledge concerning the electrophysiological response of *Bactrocera oleae* (Rossi) to different chemical compounds and host plant is scarce. Electroantennographic (EAG) analysis was used as a method to screen host plant volatiles for potential semiochemical-associated behavioral responses. In this sense, in the present work five compounds from leaves and/or green olive fruits were tested: (*E*)-2-hexenal, α-pinene, farnesene, xylene and nonanal. Those EAG dose–response studies were compared with results from known *B. oleae* semiochemicals: (*Z*)-9-tricosene and Spiroketal. Males and females of *B. oleae* were tested at different physiological stages: 0-5, 5-10 and 10-15 days old. Chemicals were all tested at the concentrations of 1, 10 and 100 µg. It was observed that the response obtained was directly dependent with the concentration tested. An effect of age in the response of both sexes was also observed, being reported higher signals in the age group of 5-10 days old, after that the signal decreases in the majority of the compounds tested. Generally, males reported higher signals to the stimulus received by their antenna. Among *Olea europaea* volatiles, (E)-2-hexenal and nonanal elicited the largest EAG responses. (E)-2-hexenal is known as a oviposit repellent from wounded olives and its response on females generally increases with their age. α-Pinene and xylene, reported as oviposition stimulants, showed weaker response in both sexes, even with higher concentrations and during aging process, the same observations were recorded with farnesene. Spiroketal elicited higher response on males at 5-10 days old at 100µg, which coincides with the sex maturity of females. (*Z*)-9-tricosene, reported values below or similar to those presented by hexane. No effect was observed in the sex, stimulus concentration, or flies age with this chemical.

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Influence of copper oxide and mancozeb to mycelia growth of *Botryosphaeria dothidea* – causal agent of olive fruit rot *in vitro*

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Olive fruit rot, caused by *Botryosphaeria dothidea*, is a disease which causes very significant damages in olive orchards on Montenegrin seacoast in the last decade. Symptoms manifest on fruits as depressed brown necrotic spots that lead to fruit rotting. Thus, the pathogen influences significantly the quantity and quality of olive yield. Since in Montenegro there is no developed strategy for the control of this disease, two active ingredients were evaluated for their potential in this respect. The *in vitro* fungicide testing against the pathogenic fungus included two contact fungicides Penncozeb WP and Nordox 75WG, containing active ingredients mancozeb and copper oxide, respectively, in the same amount (750 g/kg) each. In laboratory conditions both fungicides in five different concentrations were incorporated in potato-dextrose agar (PDA) in Petri dishes. The Penncozeb WP was tested in the concentrations 0.0313, 0.0625, 0.125, 0.25 and 0.5 (%) and the Nordox 75WP in 0.125, 0.25, 0.5, 1.0 and 2.0 (%). Petri dishes with PDA without fungicides incorporated served as a control. Fragments of the fungal colonies gained in culture were placed in the centre of the medium poured in all Petri dishes and incubated at 25 °C. After three days comparation in mycelia inhibition was measured. Data about fungicides' effects on fungal mycelium growth were processed by Duncan's Multiple Range Test, ANOVA. The comparison of fungicides in three common concentrations (0.125, 0.25, 0.5) revealed significantly higher efficacy of mancozeb, causing 92% mycelium inhibition at 0.125% concentration and 100% mycelium inhibition at 0.25% concentration. In the same concentrations, respectively, copper oxide revealed only 8.9% and 17.4% mycelium growth inhibition. These two fungicide concentrations are generally recommended for field protection and the results clearly demonstrate that mancozeb is an appropriate fungicide for preventive management of *Botrysphaeria dothidea* in olives. The copper oxide revealed higher mycelium inhibition in concentrations from 0.5-2%, however these concentrations and dosages are not consistent with environmental standards due to copper soil accumulation and negative impact to some organisms. Beside these, applications of copper fungicides applied in higher concentrations could lead to copper residues in olive fruits and oil, which is especially important in organic production. Additional field efficacy testing and *in vitro* assays in order to determine the inhibition of *B. dothidea* spore germination should be performed in near future.

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Insecticide resistance in the olive fly

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For the last 40 years, the management of the most destructive olive pest, the olive fruit fly *Bactrocera oleae*, has been based on the use of organophosphate insecticides. More recently, pyrethroids and the naturaly tespinosad have been added in the arsenal against it. However, the extensive use of any insecticide inevitably leads to the development of resistance. More than 60-fold resistance ratios to organophosphates (OP) have been observed in olive fly populations in Crete. Acetylcholinesterase (AChE) is the principal target of OPs and, consequently, its gene is the most likely locus where resistance mutations appear. In fact, two point mutations in the catalytic site of the enzyme have been implicated in resistance development. In addition, a small deletion in the carboxyl terminal of AChE has indicated an entirely novel mechanism of OP resistance. Furthermore, over 50-fold resistance to alpha-cypermethrin has also been documented, even though no resistance-associated mutations have been identified thus far. Finally, incipient spinosad resistance has been demonstrated in flies caught in California, where the drug is the only insecticide used for the control of the fly. In *Drosophila*, the α6 subunit of the nicotine acetylcholine receptor (nAChR) has been implicated in spinosad resistance. On the contrary, the α6 subunit of the nAChR does not seem to be spinosad's target in *Musca domestica*. Therefore, while the investigation of the particular locus in the olive fly may indicate its involvement, a conclusive picture of the spinosad resistance mechanism requires a whole transcriptome and/or proteome analysis.

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Influence of treatment period on the olive leaf spot with copper fungicide on the quality of olive oil

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Fungus *Spilocaea oleaginea* Cast. causes olive leaf spot disease. Today it is present in the entire world, in all areas of olive cultivation. Experts consider it one of the most widespread and dangerous fungal olive disease. In fact, it is a very serious problem in almost all Croatian olive-growing areas. The pathogen affects the fertility of infected trees, and its successive occurrences, from year to year, causing degradation of the entire olive trees, especially the younger ones. This disease can be controlled by copper fungicides. For a long time it has been considered that olive leaf spot infections can be prevented with only two treatments by copper formulations (September-October and March-April); however, for effective protection several treatments are needed annually. Concentrations of applied preparations must be strictly controlled for possible copper residues in olive fruit and olive oil, which are limited by law regulation (up to 0.4 mg Cu/kg). In years with very warm and rainy autumns, a copper fungicide treatment in the fall is certainly not sufficient. It is necessary to perform at least three treatments. The experiment was conducted in 2009, 2010 and 2011 in an olive grove in Kastel Stari, to determine whether several successive copper fungicide treatment, in the fall, cause accumulation of unallowed Cu concentrations in olive oil. In three years research, the olive trees were treated 3 times in the autumn every 15 days with a copper fungicide (copper oxychloride). After the harvest, the olive samples were processed in a laboratory mill. Chemical and sensory analysis of obtained oils was performed and Cu concentrations in oils were determined by atomic absorption. The results showed that chemical and sensorial characteristics of examined oils were not influenced by the several consecutive treatments during autumn. From obtained results it's also evident that concentrations of copper were lower than the law regulation limit in any of the analyzed olive oil samples (0.13 to 0.26 mg Cu/kg).

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Optimization of insecticide application timing in the control of olive fruit fly *Bactrocera oleae* Gmel.

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Abstract: The objective of this paper was to optimize timing of insecticide application using two infestation forecasting models in conditions of Montenegro seaside. Both models define an index of the gravity of infestation (Z), whose trend is correlated with the development of the infestation. Results show that the model based on monitoring of *B. oleae* flight dynamics using yellow sticky traps in conditions of Montenegro seaside is not acceptable. According to this model protection period begins in the second half of September when the infestation is far above economic control threshold. The second forecasting model, based on flight dynamics monitoring by pheromone traps is useful in determination of optimal timing for control of this pest. According to this model the period in which olive should be protected from the olive fly is from the middle of August to the middle of October.

Key words: *Bactrocera oleae,* infestation, insecticide application timing, forecasting model

Introduction

The most important Montenegrin olive variety Žutica is very sensitive to the olive fly attack. Number of generations per year varies, usually, two to three, folding over each other. In some years, especially favourable for the development of the olive fly, the damage may significantly affect the production. Therefore, the protection measures are highly important in preserving the yield and quality of products. The measures are primarily directed to suppression of adults. Thought, it is important to determine the moment of application of insecticides and the other products.

In order to determine the optimal timing of insecticides application, a large number of researchers have been working on establishing a relationship between olive fly flight dynamics and fruit infestation and infestation forecasting models (Ricci *et al*., 1979; Pucci *et al*., 1990; Pucci, 1993; Raspi, 1999; Gilioli & Cossu, 2002; Lo Duca *et al*., 2005). Literature data indicate that the use of appropriate forecasting model of infestation can successfully determine the time of application of insecticides and provide satisfactory protection of olives. The objective of this investigation was to determine the applicability of two models in conditions of Montenegro Coastal area. Both models define an index of the gravity of infestation (Z), whose trend is correlated with the development of the infestation.

The first model – **"female forecasting model"**, based on the capture of females with yellow sticky traps - was completed in the olive area of Northern Lazio and then successfully applied in different olive areas in Italy and abroad (Croatia) (Pucci, 1993).

The second model – **"male forecasting model"**, based on the capture of males with pheromone traps - was also completed in Central Italy (Lo Duca *et al.,* 2003).

Material and methods

The investigation was conducted in an experimental olive grove, of variety Žutica, at the Center for subtropical cultures in Bar, during a two-year period (2005 - 2006).

Olive-fly population was monitored using yellow sticky traps and pheromone traps, in the period from the beginning of July to the end of October. In both years, 8 plants were chosen from the end of June. A pheromone trap (Dacotrap) was placed on 4 plants at medium height of the canopy. The pheromone dispensers were replaced every four weeks. At the other 4 plants yellow sticky traps were placed at medium height and south side of the canopy. The numbers of individual olive flies captured were counted weekly.

At the same time the olive sampling was performed by randomly picking 40 drupes per plant from the canopy of the same tree chosen for flies trapping. For quantification of infestation, the number of eggs, 1^{st}, 2^{nd} and 3^{rd} instars larvae, pupae, empty cocoons and abandoned galleries were counted in each olive fruit.

During the investigation period, meteorological parameters as temperature and rainfall were recorded.

"Female forecasting model" defines index of gravity of infestation Z based on the average number of females on yellow sticky traps and mean daily temperature, and treatment is recommended when the $Z > 0.1$.

The index of the gravity of infestation Z which was calculated by the formula:

$$\mathbf{Z = 0.039(Fm-9.7) - 0.187(Tm-22.1)}$$

where:

Fm is the average number of females weekly captured by means of yellow sticky traps,

Tm is the average of the seven daily mean temperatures recorded in the same capture week.

"Male forecasting model" defines index Z based on the average number of males caught on pheromone traps and mean daily temperature, and treatment is recommended when $Z > -1$.

The index of the gravity of infestation Z which was calculated by the formula:

$$\mathbf{Z = 0.027Mm - 0.399Tm + 8.71}$$

where:

Mm is the average number of males weekly captured by means of sex-pheromone traps,

Tm is the average of the seven daily mean temperatures recorded in the same capture week.

Results and discussion

According to the "female forecasting model", in both years of investigation, index Z, was above the threshold value in the third decade of September (Figure 1 and Figure 2). At that time, the level of infestation was high, 38.7% in 2005 and over 70% in 2006 (Figure 3). The index Z was above the threshold by the end of October.

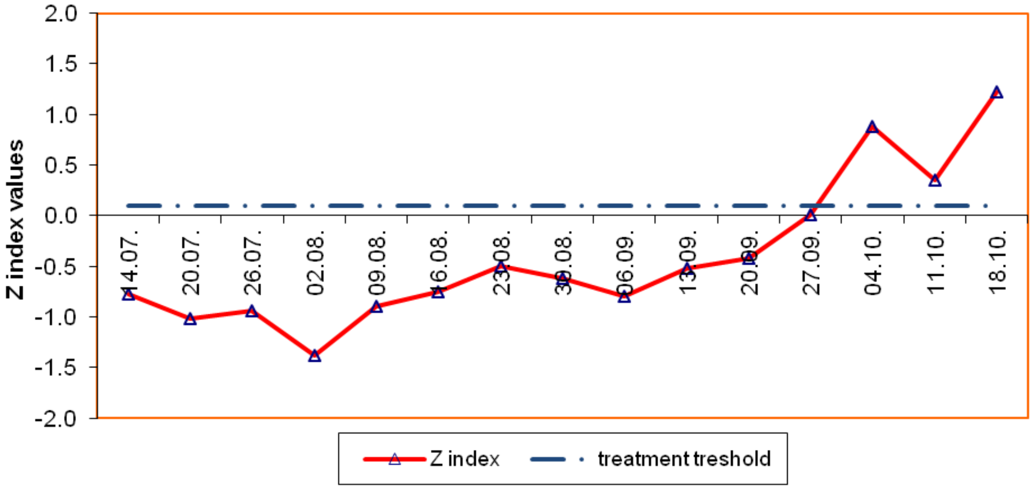

Figure 1. Trend of Z index value in 2005 ("female forecasting model").

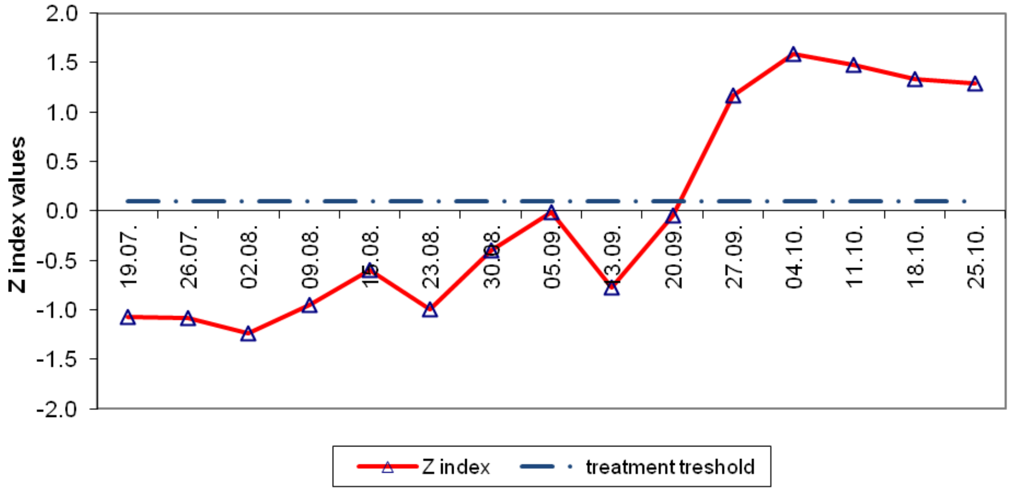

Figure 2. Trend of Z index value in 2006 ("female forecasting model").

The above mentioned suggests that this model is not applicable for the conditions of the Montenegrin Coast, and need to be improved.

According to the "male forecasting model", in the first year of investigation the index Z reached the threshold value on August 16th, when the infestation was 1.25% (Figure 4). From mid-August until the end of October index Z was above the threshold and this is optimal timing for control measure. In this period three maximum of index Z value were observed, followed by rapid increase of infestation in the period from one to two weeks.

The first maximum was observed on August 30th at infestation of 7.5% (Figure 3). In the next two weeks the infestation reached 20%.

The second maximum was on September 12th at infestation of 20%. Two weeks later infestation reached 38.7%.

The third maximum was on October 4th at infestation of 54.4%. The level of infestation rapidly increased, and two weeks later, on October 18th was 78.7%.

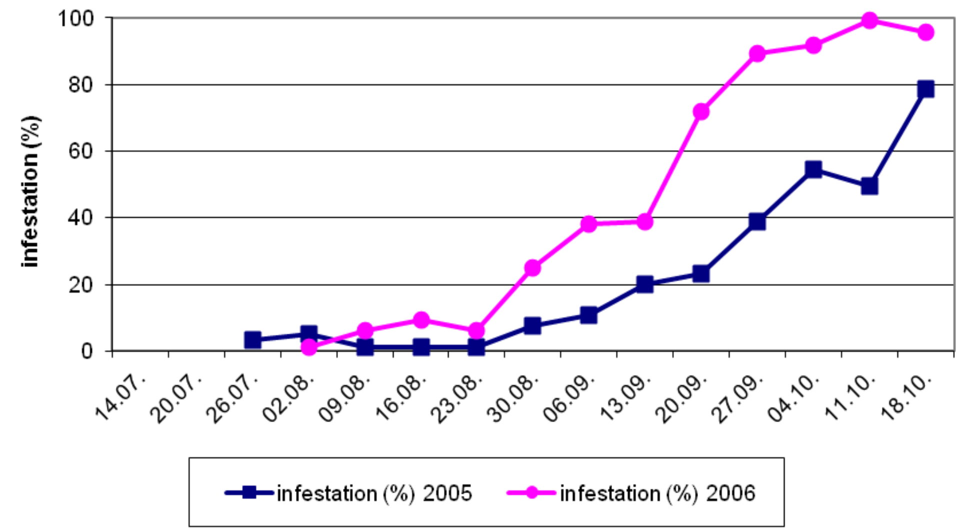

Figure 3. Fruit infestation (%) in 2005 and 2006.

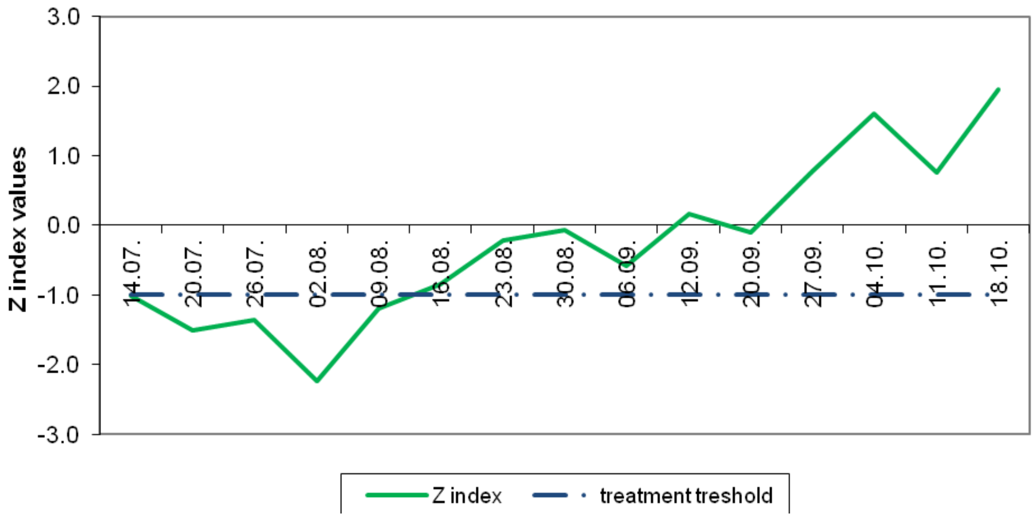

Figure 4. Trend of Z index value in 2005 ("male forecasting model").

In 2006 index Z reached the threshold value on August 9th (Figure 5), one week earlier than in 2005. At that time, the level of infestation was 6.3%. The index Z was above the threshold by the end of October and this is optimal timing for control measure. In that period three peaks of index Z value were observed, followed by rapid increase in infestation within a period from one to two weeks.

The first maximum was on August 15th at infestation of 9.4% (Figure 5), then the index value and infestation decreases. Two weeks later the infestation rapidly increased (6.3-25%).

The second maximum was on September 5th at infestation of 38.1%, and remained at the same level the following week, and then increased to over 70%.

The third maximum was on September 20th at infestation of 71.9%. The level of infestation in the following week increased to 89.4%, and continued to be over 90%.

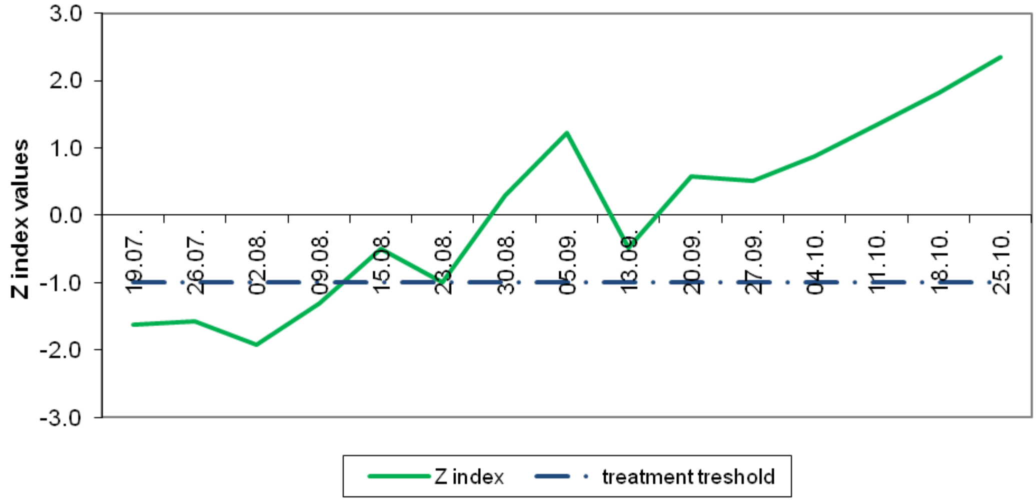

Figure 5. Trend of Z index value in 2006 ("male forecasting model").

In both years of investigation, each maximum of index Z was followed by rapid increase of infestation in the following one to two weeks. This suggests that "male forecasting model" can be used to predict the olive fly infestation under the conditions of Montenegro coastal area.

Conclusion

Presented results show that the model based on monitoring of *B. oleae* flight dynamics using yellow sticky traps in conditions of Montenegro coastal area is not acceptable. According to this model the period of protection should start in the third decade of September, when, however, the infestation is far above economic control threshold.

The second forecasting model, based on flight dynamics monitoring by pheromone traps is useful in determination of optimal timing for control of this pest. According to this model the period in which olive should be protected from the olive fly is from the middle of August to the middle of October. The maximum of index Z indicates an increase of infestations one to two weeks in advance.

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