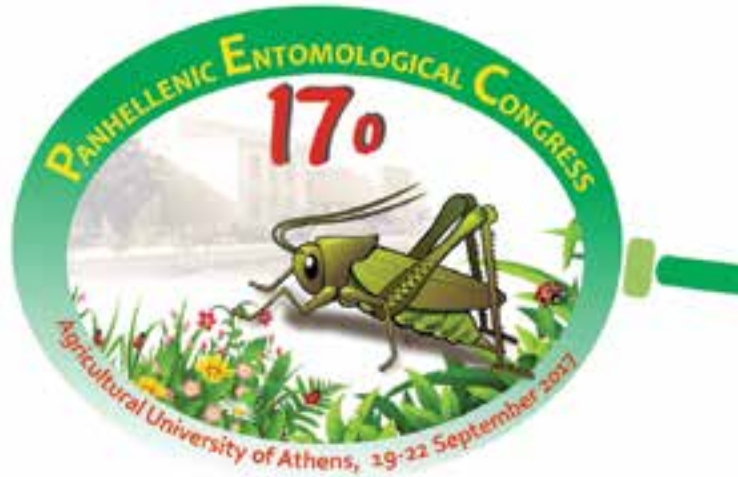




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Αγροτική Ανάπτυξη



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17th PANHELLENIC ENTOMOLOGICAL CONGRESS

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Preface

The aim of the Hellenic Entomological Society is to disseminate the entomological knowledge and the results of the entomological research in Greece. The main tool towards this direction is the organization of the Panhellenic Entomological Congress, in collaboration with the Local Organizing Committee. The forthcoming 17th Congress is going to address efficiently the aims of our society considering that 145 papers are going to be presented, 2 round tables will be organized on ad-hoc topics, invited talks will be given and several parallel activities are going to be performed. Topics on all aspects of Entomology and Acarology will be presented including but not limited to the integrated pest management, stored products pests, medical and veterinary entomology, insect physiology and phenology, insect diversity, evolution and systematics, biological control and other control methods, chemical control and insecticide resistance.

The organizers would like to thank the Scientific Committee, the invited keynote speakers, the members of the round tables and the members of the other committees for their cooperation and valuable contribution to the success of the meeting.

The Organizing Committee would like to acknowledge the Ministry of Rural Development and Food, the Region of Attica, the Geotechnical Chamber of Greece and the Hellenic Agricultural Organization – ‘DEMETER’, that provided their auspice as well as financial support.

We would like thank cordially all the Congress sponsors for their significant financial support.

Finally, sincere thanks are expressed to all those who will honor the 17th Panhellenic Entomological Congress with their participation and particularly the under- and post- graduate students.

We welcome you in the campus of the Agricultural University of Athens, wishing you a constructive and fruitful meeting.

The Organizing Committee
of the 17th Panhellenic Entomological Congress

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Opening lecture

Modern biotechnology against insect pests and disease vectors

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Insects pose tremendous threats to humans in two main areas. Firstly, insect borne diseases, such as malaria and dengue fever, are spread by insect vectors and cause severe global health problems. Secondly, the sustainability of agricultural output, which needs to nearly double in the next 30-40 years to maintain food security and meet predicted population growth, is seriously threatened by insects and mites that devastate crop production. Both the prevention of vector borne disease and the protection of agricultural production are best achieved by controlling the insect population, which largely relies on the use of insecticides. However, the need for non-chemical methods to replace or integrate with insecticides for tackling insect pests is widely acknowledged.

Over the last years, there has been an impressive development of biotechnology - based insect control tools, such as microbial toxins (via transgenic plants or sprayable formats) and more recently dsRNA, “green-chemistry” biopesticides (metabolites, biostimulants etc), *Wolbachia* symbionts and insect transgenic technologies (RIDL, HEG - Gene Drive technologies).

An overview of current advents of modern biotechnology applications against insect pests and disease vectors will be presented, including technical introduction, stage of development and roll out, strengths and weaknesses, regulatory considerations, and operational constraints. Conditions where these alternative vector control methods/tools could offer greatest value will be discussed.



1st Session
Biodiversity, Evolution and Systematics

Invasive species of invertebrates in Greece

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Invasive species pose a significant threat to our environment because they (a) can transform habitats and alter the function of ecosystems and ecosystem mechanisms; (b) multiply exponentially in number or replace native species; and (c) affect human activities, which is a burden on the economy. For example, losses for agriculture, forestry, fishing and other human activities from imported species are estimated at 137 billion dollars a year, only for the US economy.

In this work we present a list of invasive invertebrates recorded in Greece. The list contains information on 301 species belonging to the groups of Nematoda, Myriapoda, Arachnida, Acari, Blattodea, Thysanoptera, Siphonaptera, Hemiptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera.

For each group, we recorded the status, area of installation, place of origin, date of first observation, mode of introduction, impacts, establishment, habitat, frequency of observation and number of specimens.

Establishment of selected flowering plants in a processing tomato field margin to enhance habitats of pollinators and natural enemies

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Conventional agricultural practices degrade the capacity of field margins to offer food and shelter to pollinating insects and natural enemies of pests. In this study, we enhanced the floral biodiversity in the margin of a processing tomato field using Greek biotypes of selected flowering plants (provided by the Greek Gene Bank) and examined their potential to support populations of pollinators and beneficial arthropods compared to native vegetation. Plant species were selected from various families to increase attractiveness for pollinators, excluding potential weeds, alien species or pest hosts. The selected plant species were sown in a strip (70m x 2m) along one side of a 2ha tomato field in Boeotia, Greece, in early December 2014. A similar size area of conventional field margin with native vegetation was used as control. Flowering per species (% cover of surface), numbers of attracted Hymenoptera pollinators [*Apis mellifera* Linnaeus, *Bombus* spp. Latreille (Apidae), mining bees, Megachilidae] based on counts of insect-visits per flower, and presence of beneficial arthropods (suction-sampling) were measured every 15 days for 10 weeks. Sown plant species flowered from April to July and reached a peak in consecutive order: *Lathyrus sativus* L. ≥ *Vicia* sp. (Fabaceae) > *Chrysanthemum coronarium* L. (Asteraceae) ≥ *Coriandrum sativum* L. > *Anethum graveolens* L. (Apiaceae). Control field margins had a limited flowering period and consisted mainly of grasses and small numbers of *Anthemis arvensis* L., *Matricaria chamomila* L., (Asteraceae), *Rapistrum rugosum* L. (Brassicaceae) and *Convolvulus arvensis* L. Convolvulaceae). Flower coverage and number of attracted Hymenoptera pollinators (Apidae, mining bees) were significantly higher in the sown field margin compared to the control at all measurements. In addition, the presence of Hymenoptera parasitoids was more evident in the sown flowering mixture compared to the control. However, the numbers of pollinators on tomato flowers were low regardless of the crop row's distance from the flowering strip. In conclusion, management of field margins by establishing selected plant species can be a good practice to enhance biodiversity in intensively cultivated areas in support of flowering plants, pollinators and beneficial arthropods.

This work was funded by the biodiversity project Operation Pollinator of Syngenta.

Enhancement of arthropod-based ecosystem services in Mediterranean vineyards

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Arthropods, including pollinators, predators and parasitoids play a key role in agroecosystem productivity and environmental quality. Beneficial insects provide valuable ecosystem services, such as pollination and biological control. Vineyards are one of the most important crops in the Mediterranean, with a history dating back thousands of years, and represent an important part of High Nature Value farmland. Vineyards in Cyprus and elsewhere are threatened by both agricultural intensification and land abandonment. In the last 40 years, the area covered by vineyards in Cyprus has declined by 75%, to approximately 7,000 ha.

AgroLIFE was a three-year project that aimed to protect, conserve and promote High Nature Value vineyards. Conservation actions were implemented, including planting of native plants, construction of stonewalls, as well as rock and brush piles. In addition, pheromone mating disruption was used to control the most destructive insect pest of vineyards, the grape berry moth, *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae). Biodiversity monitoring of arthropods was conducted for two consecutive growing seasons to assess the effectiveness of conservation practices. Twelve vineyards were monitored: (i) Four diversified fields, where sustainable agricultural practices and biodiversity conservation measures were applied, (ii) four conventional fields and (iii) four abandoned fields.

Preliminary results suggest that abandoned fields are more diverse and their rich plant biodiversity enhances arthropod diversity. Agricultural intensification has a negative impact on the ecosystem service of biological control. Vineyard margins serve as an important habitat for many plant and arthropod species that are highly important for biodiversity conservation.

An estimation of the quality of urban green; butterflies of the broader region of Thessaloniki as biological indices

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The biodiversity of modern urban areas is affected by severe environmental threats the most important of which is urbanization. This situation combined with the lack of green areas imposes the need to study social, physical and ecological factors that influence rural areas. To this purpose, the study of butterfly communities could exhibit an excellent biological marker of environmental quality, due to their rapid adaptation even to the slightest changes in their habitat. In the present study six parks of the municipal district of Thessaloniki and seven of the municipal district of Pavlos Melas were selected. The aim of this research is to correlate the size, vegetation coverage and pollution levels with the samplings' results. Samplings followed a modified protocol of Pollard line transects lasting 30 minutes each. After ten sampling periods, 15 species were documented in the municipal district of Thessaloniki and 11 in the municipal district of Pavlos Melas. Populations exhibited statistically significant differences, something that is reflected even on biodiversity indices.

Preliminary study on the presence of insects, potential vectors of *Xylella fastidiosa* in olive orchards

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Xylella fastidiosa (Well & Raju) is a xylem-limited bacterium that is exclusively transmitted by insects belonging mainly to the families Cicadellidae, Aphrophoridae and Cercopidae. There is little or no vector specificity for the transmission of *X. fastidiosa*, with the xylem feeding habit to be one of the few requirements for the transmission of this pathogen. At least 38 species of the family Cicadellidae and six species of the families Aphrophoridae and Cercopidae are vectors of *X. fastidiosa*. In Europe species from Aphrophoridae and Cercopidae are the dominant groups of potential *X. fastidiosa* vectors. *Philaenus spumarius* Linnaeus (Hemiptera: Aphrophoridae) has been identified as a vector of *X. fastidiosa* in Italy.

In order to detect the presence of potential vectors of *X. fastidiosa* in Greece, samplings were carried out on olive orchards during 2017. The samplings were performed by sweeping the wild vegetation on the ground and the olive trees, with an entomological net. The overall content of the sweeping net was emptied in a plastic bag which was properly labelled and brought to the laboratory. In the laboratory, insects of the suborder Cicadomorpha were counted and identified. Moreover, direct observations of the ground cover vegetation within the olive orchards and samplings of the nymphal stages of insects belonging to the families Aphrophoridae and Cercopidae, were carried out. Specific sites were sampled only once while others three times, in spring, in summer and fall.

According to the results of the study, insects belonging to the three families (Cicadellidae, Aphrophoridae and Cercopidae) were collected. The number of species was higher in the Cicadellidae family. Moreover, the presence of *P. spumarius* was identified in a large percentage of the areas that were sampled. Generally, our results show that there are several species of potential insect vectors of *X. fastidiosa* in olive orchards in Greece.

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Presence, seasonal appearance and abundance of Auchenorrhyncha associated with olive trees in Greece

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The olive tree (*Olea europaea*) is widely cultivated for the production of both oil and table olives and very significant because of its economic value. Olive and olive oil are the essential components of the Mediterranean diet, with thousands of years of history.

Lately, there is great concern by the European scientific community on the bacterium *Xylella fastidiosa*. This is due to the confirmed infections of the bacterium in olive trees, reported in 2013 in the province of Lecce Region Apulia, Italy, which causes the "Olive Quick Decline Syndrome".

Xylella fastidiosa is exclusively transmitted by Auchenorrhyncha (leafhoppers, sharpshooters and spittlebugs), belonging to the families Cicadellidae, Aphrophoridae and Cercopidae. Moreover, species of Cicadidae and Tibicinidae families (cicadas) can be defined as potential vectors of the bacterium. Although *X. fastidiosa* is a xylem-limited bacterium, some phloem-feeding Auchenorrhyncha were confirmed as vectors of the pathogen in Italy.

A study on the phenology and abundance of Auchenorrhyncha species, and potential presence of *X. fastidiosa* in collected insects, on olive and associated host plants was conducted in Greece. For this reason, surveys over a 1 year period were conducted in from olive groves located in three of the most important olive production regions in Greece: Fthiotida, Attica, and Crete. These surveys were performed with Malaise traps, entomological net and aspirator. Results showed that most of the insects listed as the main potential vectors of *X. fastidiosa* in Europe, are present in olive groves. Furthermore, many insects belonging to the subfamily Deltocephalinae were found, whose role as *X. fastidiosa* vectors should be further studied.

Population fluctuation of Auchenorrhyncha in citrus orchards with different monitoring methods

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The suborder Auchenorrhyncha (Hemiptera) contains phytophagous species feeding with plant sap. Many species are of economic importance due to their ability to transmit phytopathogenic organisms. In citrus, Auchenorrhyncha are potential vectors of *Spiroplasma citri* Saglio (Mollicutes: Spiroplasmataceae), causing Citrus Stubborn Disease (CSD); and the bacteria *Xylella fastidiosa* Wells *et al.* (Gammaproteobacteria: Xanthomonadaceae), which causes Citrus Variegated Chlorosis (CVC), a disease of great importance. Although both pathogens haven't been reported in Greece, *S. citri* has been recorded in neighboring countries such as Cyprus, Italy, Turkey or Libya. *X. fastidiosa*, while it was considered as a quarantine organism for the European Union, was found in Italy (2013), France (2015), and, lately, in Germany (2016). Potential vectors of this pathogen are insects of the families Cicadellidae, Cercopidae and Aphrophoridae. Their ability to transmit *X. fastidiosa*, combined with the great variety of crops experienced substantial economic losses (olive trees, vineyards and prunus), makes the study of these insects an important tool for a possible future arrival of the bacteria in Greece.

The purpose of the present study was to compare different methods of Auchenorrhyncha population monitoring in citrus orchards.

For this purpose, Malaise traps, as well as yellow sticky traps on the trees, were placed in the citrus orchard of the Agricultural University of Athens. Samples collection from the traps was performed weekly, also carrying out sampling with entomological net in the same orchard. The samples were transported to the Laboratory of Agricultural Zoology and Entomology of A.U.A. to record and identify the insects. The results showed differences between the three sampling methods, concerning trapping and identification of Auchenorrhyncha. Specifically, significantly more nymphs were collected by entomological net compared with the two types of traps, together with other differences, which are listed and discussed in the presentation; emphasizing the apparent inadequacy of yellow sticky traps for the morphometric identification of Auchenorrhyncha, neither at genus, nor subfamily level.

Species and population fluctuation of Auchenorrhyncha (Hemiptera) in citrus orchards

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Auchenorrhyncha are considered as one of the most important insect taxa both in terms of diversity and of agricultural interest. They may reproduce rapidly and become in this prospect harmful for the crops they feed on. Till now more than 26.000 species have been described. Recently, these insects have been considered as very important due to their action as vectors of the bacterium *Xylella fastidiosa*. Although the bacterium has a wide range of hosts, it has gained particular interest in the Mediterranean countries due to the infection of olive trees. The fact that olive and citrus are attacked by the same pathotype of the bacterium, combined with the proximity of citrus orchards to olive groves, makes the study of Auchenorrhyncha fauna on citrus a very useful tool in case of future entry of the bacterium in Greece.

The aim of this work is to study the fauna of Auchenorrhyncha and their population fluctuation on citrus. For this purpose, weekly samplings were carried out in citrus orchards at the Ancient Corinth area, during the period November 2014 - June 2017, using a Malaise trap, with 70% ethyl alcohol as the preservative medium. The collected samples were transported to the Agricultural Zoology and Entomology Laboratory of the Agricultural University of Athens, for identification, which was based mainly on the male genitalia. Detailed information about the results is given in the presentation.

A new insect, drosophila *Zaprionus tuberculatus* (Diptera: Drosophilidae), affects the figs on Crete

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The African fig fly was recorded for the first time in the Europe in Crete in 2008, and again during the summer of 2014 in mature figs. The species identified as *Zaprionus tuberculatus* Malloch (Diptera: Drosophilidae). Since there is no evidence in the literature on this species in Greece and Europe, the aim of this research is to provide basic information on insect identification, stages and damage to figs as a host. In the area where the insect was identified and collected in the summer of 2014, 516 mature figs were sampled from 10/7/2016 to 27/8/2016. In this collection 17.64% (91 figs) had *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) eggs, 5.23% (27 figs) had *Z. tuberculatus* eggs and 0.97% (5 figs) had eggs of both. The main factor determining the laying of females is the thermal requirements, which also greatly affect the viability of the insect. The insects were bred on an artificial diet at a constant temperature of 18, 23, 25 and 30°C, with corresponding relative humidity 60, 50, 50 and 30% and a photoperiod 14D:10L. Four replicates were performed for each different temperature. Of the four different temperatures, the average biological cycle per virgin female and male at 18°C is 34, at 23°C 16,4, at 25°C 13.6 and at 30°C 21 days. At 18°C we have the smallest number of offspring with 148 acres, while at 23°C and 25°C egg production is at the highest yield of 710, 727 acres. The 30°C has limited activity to none at all. Female insects place eggs in laboratory food but do not progress to the larval stage because males are sterile at temperatures above 30°C. The mean adult female life span is in days at 18°C 66.6, at 23°C 40.2, at 25°C 43.6 and at 30°C 56. Average male life span in days at 18°C is 203.2, at 23°C 48.4, at 25°C 57.1 and at 30°C 70.8. These results will help to understand the biology of *Z. tuberculatus*.

Pine processionary moth *Thaumetopoea* sp. (Lepidoptera: Thaumetopoeidae) in Attica – an exciting discovery

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The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Thaumetopoeidae) is one of the most common insect pests of pine forests in Southern Europe and along the Mediterranean basin. Despite the numerous studies, it has recently been proved that the islands of south-eastern Aegean Sea (Crete, Cyprus and islands nearby) and the coasts of Turkey, are being inhabited by the sister species, *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Thaumetopoeidae), whereas in North Africa (Algeria, Tunisia and Libya), a third strongly, genetically diverged mtDNA clade was inferred (East North African clade – ENA clade). Individuals of this clade were detected all over Attika for the first time in 2014, with a single location (Gerakas) being inhabited only by *T. pityocampa*. Analyses carried out, not only verified that this invasion is recent but showed that it is in progress, quite likely originating from Libya as it suggested by the phylogeographic relationship of haplotypes. Further studies should now focus on investigating the interactions with *T. pityocampa* as well as possible differentiations in terms of biology and expansion rate. These results indicate vividly that invasions do not only occur at species level but even at lower taxonomic levels, which are most of the times more difficult to detect; yet their impact might be equally important.

Molecular investigation of the genetic diversity of local honey bee *Apis mellifera* L. (Hymenoptera: Apidae) populations in Greece**L. PAPOUTSIS^{1,*}, M. BOUGA¹, M. TSIKNIA², M. MEIXNER³, P. KRYGER⁴,
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Honey bee *Apis mellifera* L. (Hymenoptera: Apidae) consists of 29 subspecies that can be discriminated using morphometrical and molecular markers. In Greece, according to Ruttner (1988), four subspecies exist: *A. m. adami*, *A. m. macedonica*, *A. m. cecropia*, and *A. m. carnica*. Ruttner also refers to honey bees of the islands of the Aegean Sea as "Aegean race". During the last decades, global apiculture is facing a crisis due among other reasons to the loss of honey bee biodiversity. The aim of this study is to investigate the current situation of the presence of local honey bee populations in Greece using molecular markers, and to compare these data with those obtained from similar studies in the past.

This study is performing in the frame of the European SMARTBEES FP7-KBBE Project 613960 which is a collaborative research European project between 16 partners from Universities, Research Institutions and Companies across Europe and the main goal is to find solutions for the prevention of colony losses. For the purpose of this study, honey bees were collected from different regions of Greece and from other European countries for comparison reasons. Sequencing analysis of mitochondrial DNA (mtDNA) gene segments was performed on these samples. For data processing the packages BioEdit 7.0.9.0, CLUSTALW2, MEGA 4, DnaSp 5.10.00 as well as *vegan* and *physeq* of the statistical platform R are being applied.

The results support the possible presence of local honey bee populations in Greece, like this of *A. m. cecropia* in Central Greece and the presence of different populations in the islands of the Aegean Sea. The results of the study may be useful for the conservation of local honey bee populations in Greece.

Dryocosmus kuriphilus* in Greece – identification of native natural enemies and *Gnomoniopsis castanea**G.I. MEMTSAS¹, G. MELIKA², G.T. TZIROS³,
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Dryocosmus kuriphilus Yasumatsu (Hymenoptera: Cynipidae) is a globally invasive insect pest spreading rapidly in new habitats and causing damage to chestnut trees in many European countries. In Greece, it was first found in Ano Milia (Pieria) in 2014 and since then it has spread in more than ten other areas. The aim of the present study was to identify the native parasitoids that might have responded to the invasion and infested the Asian chestnut gall wasp. For that, galls from seven sites were sampled and dissected, revealing the occurrence of five native hymenopteran species living in the galls of *D. kuriphilus*, whose abundance varied from site to site. Additional analyses, unveiled the occurrence of *Gnomoniopsis castanea*, a fungus causing necrosis of the galls.

A new *Osmoderma* record (Coleoptera: Scarabaeidae) from Peloponnese (Mt Parnon)**P.A. AUDISIO¹, S. SABATELLI¹, G. ANTONINI¹, I. SPARACIO²,
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The Hermit beetles of the genus *Osmoderma* are a group of flower chafers (Scarabaeidae: Cetoniinae: Trichiini). They are quite large scarab beetles (more than 25 mm long) that live as a rule in old hollowed oak trees. The genus is represented in Europe with the species *Osmoderma eremita* Scopoli 1763 (W Europe), *O. cristinae* Sparacio 1994 (Sicily), *O. barnabita* Motschulsky 1845 (E Europe), and *O. lassallei* Baraud and Tausin, 1991 (S Balkans). The taxonomic position of *O. italicum* Sparacio 2000 from southern peninsular Italy is still uncertain (it has been recently interpreted as a subspecies of *O. eremita*). The east European clade comprises *O. barnabita* (from S Finland to NW Greece) and *O. lassallei* (from E Greece to European Turkey). Until now the geographical distribution of the species complex considered that the area on Mt Ossa (Spilea) was a key ecotope separating *O. barnabita* and *O. lassallei* species. In this work we present preliminary data based on a specimen found in a hollowed oak (*Quercus frainetto*) on Mt Parnon, Laconia (S Peloponnese). On the basis of preliminary molecular analyses on this specimen (barcoding using the Cytochrome Oxidase subunit I gene [mtDNA]) with the same methodology used in previous works carried out on other species of the genus, we found that the target specimen is quite apart from *O. lassallei* (ca. 4% of genetic distance). This distance is similar to the interspecific COI genetic divergence estimated between the Sicilian endemic *O. cristinae* and *O. eremita*. Additional fresh material from Peloponnese is needed in order to establish the taxonomic position of these southern Hellenic populations. However, the recent finding offers additional support to the hypothesis that multiple speciation events occurred in forest areas within the Italian and the south Balkan peninsular refugia and Sicily before and during glacial maxima of the Pleistocene. This speciation was followed by fast post-glacial geographical expansion of some species.

Comparison of soil fauna in olive orchard and vineyard on Crete and Rhodes islands (Dodecanese)

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During this study, we compared the soil fauna in vineyard and olive orchard of Rhodes Island and the same cultivations in northern Crete. The study carried on during the spring and the start of summer, by using pitfall traps.

We collected 41 taxa and we worked mainly by using the 18 more abundant ones. The 23 more scarce unified as "Others". Generally the prominent group is the family Formicidae in all four biotopes.

One taxon unifying the two cultivations of Crete is the Crustacean order Isopoda, as there are no extended similarities. Other taxa characterize each combination of island-cultivation, as they are more abundant, checked as statistically significant.

The family Tenebrionidae is more abundant in vineyard of Crete, as also the suborder Blattodea. Another beetles' family Ptinidae (spider beetles) is more abundant in olive grove of Crete.

The vineyard of Rhodes is characterized by big numbers of crickets (Orthoptera). The olive grove of Rhodes is different from the others in numbers of centipedes (Chilopoda), a non – abundant taxon.

The species *Oryzaephilus mercator* (Fauvel) (Coleoptera: Silvanidae), connects different cultivations and islands as it was abundant in olive grove of Crete but it has also a population maximum in vineyard of Rhodes. These two biotopes gave the best stability for the captures of spiders (Araneae).

The vineyards, as more man-influenced cultivations have more extreme values of biodiversity than the olive groves, which they are closer to a natural ecosystem and avoid extreme values, may be more self regulated.

Study of the acarofauna on lichens on Almond, Olive and Pistachio trees

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The aim of this study is to identify the mites on lichens found on the bark of almond olive and pistachio trees. Samples were taken from relevant groves in "Syngrou Estate". Sampling was carried out during February–September 2015. Eight monthly samplings were conducted which constituted 10 samples from each tree, 5 with and 5 without lichens. A total number of 240 samples was examined, 120 with and 120 without lichens. For the extraction of the mites, the Berlese-Tullgren funnel was used. Mites were transferred to microscope slide preparations and were examined under the microscope. A total of 32 mite taxa belonging to 12 families and 4 classes were identified. According to their feeding habits, species are classified as: phytophagous (families Tetranychidae and Tenuipalpidae), predatory (Bdellidae, Phytoseiidae, Cunaxidae, Cheyletidae), fungivorous (order Astigmata) and saprophagous (order Cryptostigmata). Moreover, species with various feeding habits were found, belonging to the families Erythraeidae, Tydeidae, Cryptognathidae, Caligonellidae, Stigmaeidae and Raphignathidae. Using the criteria of Dominance and Frequency the following taxa were found to be most important: Astigmata, *Balaustium* sp., *Bdella* sp., *Biscirus* sp., *Cunaxa capreolus* (Berlese) (Prostigmata: Cunaxidae), *Cryptognathus* sp. Cryptostigmata, *Lorryia woolleyi* (Baker) (Prostigmata: Tydeidae), *Molothrognathus* sp., *Neoapolorryia hellenica* Panou & Emmanouel (Prostigmata: Tydeidae), *Raphignathus* sp., *Triophtydeus triophthalmus* (Oudemans) (Prostigmata: Tydeidae), *Typhlodromus (Anthoseius) foenilis* Oudemans (Mesostigmata: Phytoseiidae). Mites belonging to Prostigmata were dominant with a percentage of 99.30%, on the total of samples. *Balaustium* sp. was found to be constantly present during February and March with high population densities in all three trees species with and without lichens, while was totally absent during summer. Statistic analysis of the results, concerning similarity of the tree-hosts based on all mite species found, pointed out that the habitats with lichens are clearly distinguished from those without. The estimated number of mites in each tree-host as well as in all three hosts is higher than the number found. The mite species that were found in three species of trees are considered to be less than half of what is actually estimated to inhabit the hosts. The less taxa (10) were recorded on olive trees without lichens whereas the most (22) on almond trees with lichens. The number of taxa found on pistachio trees with lichens was also low.

The Entomological Collections in Greece: The need for a common goal

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Since the very first scientific expedition in Greece back in 1832, entomological specimens were collected and deposited to collections abroad. It was not until the founding of the Natural History Museum of the University of Athens that entomological specimens were collected and remained in Greece. Since then, numerous collections have been established. These belong to an array of institutions (i.e. Universities, Research Institutions, Museums) as well as several important collections established by amateur entomologists around Greece. Unfortunately, all of these collections are managed in a fragmented, autonomous way, rarely following well-accepted universal protocols. The lack of scientific and technical staff is evident with numerous collections having severe problems with the curation of their specimens. In addition, the minimal budget issued for curation, specimen exchange and expansion of collections only deteriorates further the specimens deposited in them.

Taking into account the important specimens deposited in some such collections, immediate actions need to be taken to stop further weakening of their status. We thus propose the creation of a National Entomological Collections Network. The first goal is to exchange experiences, information and know-how in order to harmonize and eventually create and utilize common protocols, practices and tools (e.g. databasing software). Furthermore, the NECN will act as a driving force in order to holistically promote the essential role that these collections play in the study of biodiversity; not only for Greece but also regionally and globally. Taking into account the global trend for the protection and potential use of biodiversity (Convention on Biological Diversity and Nagoya Protocol on Access and Benefit Sharing) one can understand that biodiversity collections should be the cornerstone, focal point and ultimate depositories of a nations' natural history wealth. Of course, this initiative can easily expand to other biodiversity depositories, ultimately acting as a much-needed National Natural History Museum.

Monitoring environmental change using nematodes as bioindicators

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Many anthropogenic factors have a negative impact on the quality of soil, air and water leading to detrimental environmental changes. The field of environmental nematology was born by a few colleagues a few decades ago, realizing the potential of nematodes as environmental and ecological indicators and key ecosystem players. Since the pioneering work of Aldo Zullini, Gregor Yeates, Tom Bongers and Howard Ferris, to mention a few, the planet has witnessed environmental change of a high magnitude. This field has considerable applied, economic and societal significance, as scientific awareness grows of the potential consequences of global warming, pollutants and environmental contaminants, and the need to decompose and recycle human, urban, and industrial wastes. In relation to soil health alone, soil degradation costs the EU at least €38 billion annually. These costs are mainly borne by the European society. This presentation will focus on providing an introduction to the area of environmental nematology, from a historical and state of the art perspective, and will emphasize the role of nematodes as environmental indicators especially in complementing environmental monitoring and remediation projects. This role will be further highlighted by work in the authors' laboratory in (1) developing nematodes as sentinels of soil pollution and (2) utilizing nematode communities as indicators of the bioremediating ability of bacterial endophytes.

**Parasitoids of *Physokermes hellenicus* Kozár & Gounari
(Hemiptera: Coccidae) in Greece**

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Genus *Physokermes* includes species that are located in the Holarctic region and attack conifers. The recently described scale *Physokermes hellenicus* is endemic to Greece and attack fir trees of genus *Abies*. It is considered as beneficial species since its honeydew secretions are exploited by honeybees leading to the production of a special honey with certain physiochemical characteristics.

Since there are no data previously recorded on the natural enemies of *P. hellenicus*, an investigation was carried out during 2013 in different Greek mountains.

Among the recorded species, five were identified at species level and three at genus level. The parasitoids *Aprostocetus* sp. (Hymenoptera: Eulophidae), *Eurytoma* sp. (Hymenoptera: Eurytomidae) and *Trichomasthus* sp. (Hymenoptera: Encyrtidae) were recorded for the first time on *P. hellenicus*. The parasitoids *Microterys lunatus* (Dalman) (Hymenoptera: Encyrtidae), *Pseudorhopus testaceus* (Raztaburg) (Hymenoptera: Encyrtidae) and the predator *Anthribus fasciatus* Forster (Coleoptera: Anthribidae) were the most abundant natural enemies of *P. hellenicus* adult female while the parasitoids *Pachyneuron muscarum* (Linnaeus) (Hymenoptera: Pteromalidae), *Aprostocetus* sp. (Hymenoptera: Eulophidae) and *Eurytoma* sp. (Hymenoptera: Eurytomidae) were less abundant. Furthermore, *Metaphycus unicolor* Hoffer (Hymenoptera: Encyrtidae) and *Trichomasthus* sp. (Hymenoptera: Encyrtidae) were found to parasitize *P. hellenicus* male nymphs.

Population outbreaks of *Lymantria dispar* in Greece with effects on goat husbandry

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The last two years (2016 and 2017) the occurrence of severe infestations by *Lymantria dispar* (L.) (Lepidoptera: Erebidæ), gypsy moth, on evergreen pastures, where the yew (*Quercus coccifera* L.) prevails, was recorded in several parts of Greece. The particularly interesting element is the repeatability of the outbreak, the large area and the intensity of infestation. In places, several hundred hectares with yew suffered complete defoliation by the insect's larvae during the months from April to June. Outbreaks have been reported in Larissa, Serres, Preveza, Pieria, Kozani and Drama. Even though, outbreaks do not threaten the viability of yew, as yew leaves re-sprout during June, the impact on goat husbandry is extremely negative since gypsy moth significantly reduces the available food for goats, something that affects goat's milk and meat production. In the current study, emphasis was given to the repeated outbreaks that occurred within the Regional Unit of Drama (Macedonia, Northern Greece), over the years 2016 and 2017. In order to facilitate the monitoring and forecasting of gypsy moth in the area, the affected areas were recorded and mapped by GIS, in order to create a database of the regional occurrence of the species over time and space.

The Entomological Collection of the Goulandris Natural History Museum

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The Entomological Collection of the Goulandris Natural History Museum has a total of 37,231 specimens. The order with most specimens is Lepidoptera with 17,624 specimens, followed by Coleoptera (10,217 specimens), Hymenoptera (4,688 specimens), Diptera (2,426 specimens), and Hemiptera (1,357 specimens). Less specimens exist of other insect orders: Neuroptera 251 specimens, Orthoptera 212 specimens, Odonata 210 specimens, Dictyoptera 40 specimens, Isoptera 17 specimens, Ephemeroptera 15 specimens, Mantodea 14 specimens, Dermaptera 10 specimens, Thysanoptera 10 specimens, Trichoptera 10 specimens, Phasmida 6 specimens, Blatodea 2 specimens, and Isopoda 1 specimen. Also there are 51 specimens of Solifugae, 19 of Scorpiones and 14 of Araneae. The rest 37 specimens are undetermined. There are 17 paratype specimens in the collection.

Sixty-six families of Lepidoptera are represented (785 species), 64 families of Coleoptera (1,158 species), 30 families of Diptera (82 species), 27 families of Hemiptera (123 species), and 22 families of Hymenoptera (149 species). This collection is of great value: a) as there are samples collected in 1889, b) it contains samples from most of Greece and c) allows researchers to perform comparative entomological studies.

The Collection of Coleoptera in the Goulandris Natural History Museum

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The Entomological Collections of the Goulandris Natural History Museum has 10,228 specimens of the order Coleoptera. The following families are represented: Alleculidae (88 specimens), Anobiidae (73 specimens), Anthicidae (118 specimens), Aphodidae (31 specimens), Bostrichidae (34 specimens), Brentidae (1 δείγμα), Buprestidae (271 specimens), Cantharidae (65 specimens), Carabidae (668 specimens), Cerambycidae (612 specimens), Chrysomelidae (577 specimens), Cicindelidae (105 specimens), Ciidae (3 specimens), Cleridae (66 specimens), Coccinellidae (382 specimens), Colydiidae (9 specimens), Cryptophagidae (5 specimens), Curculionidae (744 specimens), Dasytidae (10 specimens), Dermestidae (210 specimens), Dytiscidae (109 specimens), Elateridae (100 specimens), Endomychidae (1 δείγμα), Geotrupidae (36 specimens), Glaphyridae (62 specimens), Gyrinidae (24 specimens), Histeridae (98 specimens), Hydraenidae (43 specimens), Hydrophilidae (37 specimens), Lampyridae (77 specimens), Lathridiidae (9 specimens), Leiodidae (2 specimens), Lucanidae (86 specimens), Lycidae (5 specimens), Meloidae (174 specimens), Melyridae (78 specimens), Monotomidae (2 specimens), Mordellidae (48 specimens), Mycetophagidae (2 specimens), Nanopyidae (15 specimens), Nitidulidae (29 specimens), Ochodaeidae (3 specimens), Oedemeridae (28 specimens), Passalidae (1 δείγμα), Ptinidae (10 specimens), Rhipiphoridae (3 specimens), Scarabaeidae (2,504 specimens), Scaptiidae (21 specimens), Silphidae (22 specimens), Staphylinidae (50 specimens), Sylvanidae (40 specimens), Telephoridae (4 specimens), Tenebrionidae (1,048 specimens), Thorictidae (8 specimens), Trogositidae (2 specimens), and undetermined (1,421 specimens). The aim of this presentation is to notify this collection and its importance for comparison with recent entomological material.

Study of the presence of lepidopteran species of the sub-order Rhopalocera (Lepidoptera : Rhopalocera) in Ossa mountain

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Competition

During the period 2005-2017, lepidopteran species of the sub-order Rhopalocera were collected and studied in the Ossa mountain. A total of 73 species, belonging to 6 families, were identified. Specifically, 3 species of the family Papilionidae, 15 of the family Pieridae, 20 of the family Lycaenidae, 12 of the family Nymphalidae, 11 of the family Satyridae and 12 of the family Hesperidae, were collected. The species biodiversity on various habitat types of Ossa mountain, was also examined. The richest diversity was recorded on mountain meadows and on glades of beech forests (*Fagus* sp.) (61 and 59 species, respectively). It was lower on semi-alpine zone and on oak forests (*Quercus* sp.) (45 and 44 species, respectively). Minimum biodiversity was found on semi-mountainous mixed forests of conifer and deciduous trees (29 species). The distribution of some species such as *Spialia phlomidis* (Herrich-Schäffer), *Muschampia tessellum* (Hübner), *Pyrgus sidae* (Esper) (Lepidoptera: Hesperidae), *Satyrium w-album* (Knoch), *Scolitantides orion* (Pallas) (Lepidoptera: Lycaenidae), *Anthocharis gruneri* Herrich-Schäffer and *Pieris krueperi* Staudinger (Lepidoptera: Pieridae) was very local and limited. Three of those species (*M. tessellum*, *S. orion*, *S. w-album*), are new records for the insect fauna of Mount Ossa.

***Thaumetopoea wilkinsoni* in Crete and nearby regions:
A case of geographic isolation**

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The eastern pine processionary moth, *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Notontidae) is one of the most important pests of pine trees in the southeastern Mediterranean basin (Crete, Cyprus, Israel and Turkish shores). Although the insect is present in Turkish shores and Crete, evidence about its occurrence in islands of the Aegean Sea are missing. Aiming to study and compare Cretan populations and populations of nearby islands and regions, we compared 150 individuals of *T. wilkinsoni* from 13 regions (7 from Crete, 3 from Samos, 1 from Rhodes and 2 from the southwestern Turkey). For every individual, we investigated a sequence of 720 nucleotides from the mitochondrial gene cytochrome oxidase I. Based on the analyses, the populations from Crete were highly differentiated from those of Samos and Rhodes, which seem to be more close to populations from southwestern Turkey. These results further support the hypothesis of the isolation of the insect populations of Crete that is consistent with the geological history of the Aegean Sea and the formation and the last geological isolation of Crete during the Messinian salinity Crisis.

Recording of insects and other arthropods on fiber hemp cultivation

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Cannabis sativa (Rosales: Cannabaceae) was established at the Experimental Farm of the Technological Educational Institute of Thessaly (T.E.I.; Larissa plain) in 2017. The presence of insects and other arthropods was recorded during crop development. Arthropod collection was conducted i) with visual inspection and arthropod individuals collection with aspirator, ii) with direct observation of plant and soil samples (leaves, flowers, shoots) in the plant protection laboratory, iii) with the modified Berlese-Tullgren method to extract arthropods from plant and soil samples. The presence of 17 arthropod taxa, mainly insects, was verified. As far as phytophagous species are concerned, the presence of polyphagous species such as the cotton aphid *Aphis gossypii* Glover, the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), two Mirid bugs *Lygus pratensis* L. and *Closterotomus (Calocoris) norvegicus* (Gmelin) (Hemiptera: Miridae), the Western Flower Thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), a Psyllid bug *Psylla* sp. (Hemiptera: Psyllidae), the southern green stink bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and the two-spotted spider mite *Tetranychus urticae* Koch (Prostigmata: Tetranychidae), was more intense. The presence of soil insects such as wireworms *Agriotes* sp. (Coleoptera: Elateridae), black cutworms *Agrotis* sp. (Lepidoptera: Noctuidae), fungus gnats *Bradysia* sp. (Diptera: Sciaridae) and springtails (Collembola: Entomobryidae), was also recorded. Predatory insects like the seven-spot ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), a banded thrip *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae), a minute pirate bug *Orius vicinus* Ribaut (Hemiptera: Anthocoridae) and a mirid *Atractotomus* sp. (Hemiptera: Miridae) were also recorded frequently. Species presence was analyzed with the criteria of dominance and frequency. Despite the intense presence of many phytophagous species, no negative impact on crop development and yield was recorded, verifying that the fiber hemp seems to be a crop of low or zero plant protection treatments.

Revision of genus *Xylotrechus* and its impact in Greece**E.I. LEIVADARA¹, E. RODITAKIS^{2,*}, K.B. SIMOGLOU³, I. LEIVADARAS⁴,
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Even though the cerambycid genus *Xylotrechus* (Coleoptera: Cerambycidae) is represented in Greece with four species, no particular damages have been reported thus far. It is therefore the first time that *Xylotrechus stebbingi* Gahan and *X. rusticus* (Linnaeus) have caused damages in Drama and Crete respectively. In particular, wood boring larvae have been found in olive and lemon trees in March 2014 (Houdetsi, Heraklion, Crete) causing extensive damages in various orchards. The species was then identified employing morphological features as *X. rusticus* and thus it is the first time that this species is reported as pest. On the other hand, morphological identification coupled with molecular DNA barcoding verified that *X. stebbingi* occurred in logged poplar trees (Drama), reducing significantly the value of the infested wood. Finally, quite recently (2016-17), an exotic *Xylotrechus* species has been found in mulberries planted in Heraklion (Crete) – this species has been identified as *X. chinensis* (Chevrolat), and further phylogeographic analysis will focus on understanding the route that this invasive species has followed.

The authors would like to thank the agronomists of the Regional Center for Plant Protection and Quality Control of Heraklion / Hellenic Ministry of Agriculture and Food, for the detection of the *X. stebbingi* and *X. rusticus* damages.

First report of *Gymnoscelis rufifasciata* as a pest of loquat, *Eriobotrya japonica*

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Infected branches of loquat (*Eriobotrya japonica*) were collected in December 2016 from the region of Agios Nikolaos, by the Department of Quality and Phytosanitary Inspections of Lasithi. The loquat branches were carefully examined and larvae of unknown lepidoptera pests were detected actively feeding in numerous mines below the tree bark. Infected trees exhibited restricted development, drying branches and twigs, and reduced fruiting. The species was identified using morphological taxonomic characters as *Gymnoscelis rufifasciata* Haworth (Lepidoptera: Geometridae), also known as Olive pug moth. DNA barcoding confirmed the species identification. Previous records of *G. rufifasciata* exist in the Greek territory. Olive pug moth is extremely polyphagous but generally it is considered a minor pest of agricultural crops. Its main feeding activity is observed on plant flora and leaves. Damages have been reported on crops of the *Citrus* and *Olea* genera, but overall, very few such cases have been recorded to date. Thus, the type of damages reported (twig borer), the symptoms described and the plant species infected (loquat) in this study, have not been previously described in the literature. In March 2017, *G. rufifasciata* infestations were recorded again on loquat in Heraklion, Crete. Based on these findings *G. rufifasciata* could be considered as potential novel pest of loquat in Greece.

First record of *Antigastra catalaunalis* on sesame in Greece**K.B. SIMOGLOU^{1,*}, A.I. ANASTASIADIS², J. BAIXERAS³
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In August 2016, infestations of sesame crops by *Antigastra catalaunalis* (Duponchel) (Lepidoptera: Crambidae) were observed in rural areas of the Regional Unit of Drama, Northern Greece. The symptoms recorded were the result of infestation of the foliage, shoots and capsules by the larvae of the insect. The feeding damage of 1st instars was observed as superficial erosions on the leaves. Later instars were observed causing leaf twisting by binding their edges with a silky web, boring galleries in the shoots and entering the capsules where they fed on the developing seeds. The degree of infestation was particularly high and almost total defoliation of most plants of the sesame crops was recorded.

Antigastra catalaunalis is a species of tropical origins, widespread in the Mediterranean region. It is considered a major pest of sesame in Asian and African countries. In India, it completes as many as 14 generations per year. The prevalence of high temperatures (average daily temperature of 27°C) and low rainfall (< 55 mm), during the flowering and fruit development stages of the sesame, favor the development of this species.

In the present study, a detailed recording and presentation of symptoms caused by the pest in sesame crops is made, mainly to aid stakeholders in the timely detection of the species' presence in order to protect their crops from this pest in the future.

Re-examination of the taxonomic status of *Anopheles hyrcanus* and *Anopheles pseudopictus* using a multilocus genetic approach

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Competition

Mosquitoes-members of the *Anopheles hyrcanus* (Pallas) group (Diptera: Culicidae) are believed to be important vectors of malaria pathogens. More than 25 different sibling species are included in the group worldwide, several of which are morphologically indistinguishable. Two members of the Hyrcanus group have been recorded to inhabit Southern Europe, namely *An. hyrcanus* and *Anopheles pseudopictus* Grassi, the taxonomic status of which remains unclear. Recently published studies and identification keys refer to these 2 taxa either as separated or synonymous species. The only morphological difference, between these two taxa, is a chromatic variation in tarsomere IV of hind leg. Further, significant genetic similarity between specimens of the two taxa originating from France, has been revealed, indicating greater differentiation by distance than inter-taxa genetic differentiation. The main objective of the present study was to evaluate the systematic status of *An. hyrcanus* and *An. pseudopictus* taxa. To achieve this goal, we examined their genetic relationships using a multilocus, mitochondrial and nuclear approach in specimens from a wider geographical area. Mosquito samples from various locations of Greece were used in the analysis. Haplotypes obtained from the GenBank database, originating from other European and Asian countries, were also included in the analysis. Our results revealed absence of genetic variability between *An. hyrcanus* and *An. pseudopictus* samples from both Greece and Austria and low genetic differentiation from Chinese *An. hyrcanus*, which was clearly attributed to geographic distance. Altogether we here suggest that *An. hyrcanus* and *An. pseudopictus* may belong to a single species.

**Study of the Vespidae family species in the area of Plaka
in the prefecture of Lasithi and evaluation
of the efficiency of food and pheromone attractants**

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Competition

In this work three odor attractants were evaluated towards wasp species. The experiment took place in Plaka, Lasithi Prefecture, Crete with a three month duration (summer 2016) and 6-day sampling intervals. Two food attractants were used, orange juice and beer, while also their combination with a synthetic pheromone targeting wasps (Csalomon® Hungary).

Plastic bottles were used as traps and filled with 200 mL of each one of the food attractants or 300 mL of the orange juice and beer mixture (1:1) combined with a pheromone dispenser. The traps were placed in an apiary to evaluate their efficiency as a tool to suppress wasp population.

The insects found in the traps were Hymenoptera, Diptera, Lepidoptera and Neuroptera, with Diptera being the most abundant. Insects captured were higher in numbers at the beginning of the experiment. Comparing the traps containing orange juice and beer showed no differences, but the trap employed with pheromone captured the highest numbers in total as well as Hymenoptera species.

Food traps captured mainly Diptera and Lepidoptera, while the combined one Hymenoptera. This was expected since the pheromone targets wasps. Almost three quarters of the total captures of Hymenoptera were found in the combined trap. Total numbers of Diptera were equally divided to the three traps and it can be assumed that they randomly occur in traps. The combined trap attracted more Lepidoptera than the orange juice.

Regarding members of the Vespidae family, the following species were present in absolute numbers and in descending order: *Vespula germanica* (Fabricius), *Polistes dominulus* (Christ), *Vespa orientalis* Linnaeus and *Polistes gallicus* Linnaeus (Hymenoptera: Vespidae). The combined trap captured almost 60% of wasps. On the other hand it also trapped high numbers of honeybees, the numbers of which was quite lower in food traps.

Biodiversity of Auchenorrhyncha in alfalfa crops

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Competition

Auchenorrhyncha (Hemiptera: Homoptera) consists of insects behaving as plant pests, which can create severe damage to their hosts, either as phloem and xylem feeders, or as vectors of plant pathogens. They are divided in two superfamilies, Cicadoidea and Fulgoroidea. Cicadoidea includes five families: Cercopidae, Cicadellidae, Cicadidae, Aphrophoridae and Aetalionidae (Membracidae). Fulgoroidea includes twenty families, from which most known are Cixiidae, Delphacidae, Issidae and Flatidae. The scope of the present study is to study the biodiversity of the Auchenorrhyncha in alfalfa crops and to follow their population fluctuation, as well. For this reason, Malaise traps were installed in alfalfa crops. Samples were taken from alfalfa fields in Copais and from Mantoudi. In alfalfa crops insects were collected by Malaise traps, sweeping nets and glass aspirators were they directly stored in 70% ethyl alcohol. Collected material was transferred to the Laboratory of Agricultural University of Athens for classification. After sex separation, genitalia were prepared for observation in binocular microscope, by maceration in 5% natrium- hydroxide (NaOH) and 10% potassium- hydroxide (KOH). Results showed that 2,856 Auchenorrhyncha individuals were collected in Copais (2 traps), belong to 71 species and that 1,830 Auchenorrhyncha individuals were collected in Mantoudi (1 trap) belong to 51 species. Results in Copais show high population density of *Zyginidia pullula* Boheman, 1845, *Empoasca pteridis* Dohlborn, 1850, *Empoasca decipiens* Paoli, 1930. Some of the other collected species are *Anaceratagallia frisia* Wagner, 1939, *Anaceratagallia ribauti* Ossiannilsson, 1938, *Agallia leavis* Ribaut, 1935 and *Cercopis sanguinolenta* Scopoli, 1763. Results in Mantoudi showed high population density of *Zyginidia pullula* Boheman, 1845, *Asymmetra scadecedens* Paoli, 1932 etc. Some of the other species are *Balclutha rhenana* Wagner, 1939, *Anaceratagallia frisia* Wagner, 1939, *Philaenus spumarius* Linnaeus, 1758, and *Neophilaenus campestris* Fallen, 1805.

Classification and population variability of Auchenorrhyncha in *Capsicum annum* cultivation

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Competition

Insects of the suborder Auchenorrhyncha (Hemiptera) are, especially the recent years, of create importance. In particular, this suborder includes mainly small-sized insects, in a variety of shape and coloration, which can be found in a number of cultivated and wild plants. It is worth noting that Auchenorrhyncha can be proved very damaging, either by sucking sap, or by transmitting phytopathogenic microorganisms. Specifically, certain species of this suborder are vectors of *Xylella fastidiosa*, a bacterium, which, while making its first appearance in the 1880s, seems to become of great interest lately. For this reason, it was considered necessary to record the species of Auchenorrhyncha and their population variation found in various crops. The scope of this study is the classification and the population estimation of Auchenorrhyncha in *Capsicum annum*.

For this reason, Malaise traps were placed in *C. annum* cultivation, in the region of Kiparissia Messinia. Insects were collected from the trap containers, which have 70% ethyl alcohol in the batch, on a weekly basis. At the same time, sampling was carried out in the surrounding wild vegetation with entomological sweep net and aspirator. Collected samples were transferred to the Laboratory of Agricultural Zoology and Entomology of the A.U.A., where they were placed in vials with 70% ethyl alcohol. Classification of Auchenorrhyncha was accomplished by genitalia identification. Preliminary results showed a high population density of the genera *Zyginidia*, *Euscelis* and *Anoplotettix*. Details about the species and their population abundance are given in the presentation.

***Ceroplastes japonicus* (Gray) (Hemiptera: Coccidae)
on *Laurus nobilis* and *Diospyros kaki*: first record for Greece**

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Following several years of insect pest surveys on different host plants, important soft scale insect (Hemiptera: Coccidae) infestations were observed on *Laurus nobilis* L. (Lauraceae) and *Diospyros kaki* Thunb. (Ebanaceae) in Northern Greece. Initial investigations showed the presence of the *Ceroplastes* Gray genus responsible for this damage. Specimens of *Ceroplastes* were collected in regions (northern Greece) of Thessaloniki and Kavala by S. Papadopoulou and Naousa by A. Manganaris and later in other regions as well, in the years 2012-2016. The infested plants and adults of the attacking scale insect were collected and brought to the insect laboratory of the Alexander Technological Educational Institute of Thessaloniki, for identification. The specimens were identified based on female morphological characteristics. The results showed that it is the species *Ceroplastes japonicus* (Gray) (Hemiptera: Coccidae). The scale insect fauna of the whole Greek territory includes 207 species of which 35 species belong to the Coccidae family. *Ceroplastes* species that were recorded in the Greek mainland are namely; *C. rusci* (Linnaeus), *C. sinensis* (Del Guercio), *C. floridensis* (Comstock) and *C. cirripediformis* (Comstock, 1881).

C. japonicus is a new insect species in Greece, observed for the first time on *L. nobilis* and *D. kaki*. This species has not been, until this work, reported in Greece to attack any plant. Part of the research has been funded by the Research committee of the Alexander Technological Educational Institute of Thessaloniki.

The ant fauna of Cyprus

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The aim of this paper is to create a catalog for the Cypriot ant fauna, as well as its' biogeographical analysis. The ants studied mainly came from pitfall traps in different habitats, mainly in the areas of Lemesos and Pafos.

For Lemesos area, 41 different species belonging to 14 genera and 3 subfamilies were identified. *Camponotus sanctus* Forel was first in number, followed by *Pheidole pallidula* (Nylander), *Crematogaster sordidula* (Nylander), *Tapinoma simrothi* Krausse and *Camponotus ceconii* Emery. Greater species diversity was recorded in phrygana and then the pine forest, while on the other hand the least diversity was recorded in the dunes habitat. For Pafos area, the results of our survey led to the recording of 34 species belonging to 14 genera and 3 subfamilies. The most common species were *Monomorium bicolor* Emery, and with a slight difference in the number of appearances, followed by *Lepisiota frauenfeldi* (Mayr), *P. pallidula*, *C. sanctus*, *Monomorium dentigerum* (Roger) and *Cataglyphis aenescens* (Nylander). A greater number of species was recorded in the areas of the phrygana, while the least number of species was observed in the pine forest.

Finally, with this study, the number of species (and subspecies) in Cyprus is increasing from 61 to 77, with possibly two new species to science. It is expected that many more species will be found as well as endemics since there are many areas of the island that have not been extensively or even at all censused. Also, due to the limited research that has been carried out for the Cypriot ant fauna in the past, there were no available keys, and those used were for Greece and surrounding countries. Therefore, there is still a lot of data that can be researched in the future so as to give us a more complete view of the Cypriot ant fauna.

Mitochondrial DNA sequence diversity of *Drosophila suzukii* in Greece

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Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae), originally from Southeast Asia, is rapidly spreading across the world, causing serious losses, especially to the soft fruit production. Unlike most other drosophilids, which are primarily attracted to rotten or fermenting fruit and are therefore not considered pests, *D. suzukii* females bear a serrated ovipositor, which allows them to pierce the skin of healthy, ripe fruits to lay their eggs, thus causing significant crop damage.

D. suzukii was first reported in Greece in the northwest region of Ioannina, in 2013, through the national surveys programme. Since then, it has been trapped in numerous locations across the country. Samples collected during the surveys programme, during 2014-2016, were used to assess the genetic diversity and population structure of the Greek populations of *D. suzukii*. As an initial approach, the analysis was based on mitochondrial DNA, namely the sequence of a fragment of the cytochrome oxidase subunit 1 (COI) gene.

A 850 bp COI gene fragment was sequenced in 282 individuals from 18 locations of Greece, and a total of 5 haplotypes were defined. Two of them were the most common and included 68 and 29% of the sample, while the remaining three were found in only 4, 2 and 1, respectively, of the individuals analysed. No patterns of population structure were detected, as both of the most common haplotypes were reported from most locations. Haplotype diversity (Hd) was estimated at 0,45. The two main haplotypes were identical to sequences from samples collected in Europe (Italy, Spain, Portugal), two others had been widely reported from America and Asia, while one did not match any published *D. suzukii* COI sequence.

In general, our results are consistent with a recent, local invasion and indicate relatively low genetic diversity in the *D. suzukii* population in Greece.

Insects of Salamis island

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Competition

The present work entitled “Insects of Salamis island” describes an attempt to record the insect fauna of Salamis island. It is worth noting that this recording is not based on any scientific method, and is purely amateur.

The foundation of this work is a photographic collection, which contains predominantly adult, living insects, in their natural environment. This photographic collection belongs entirely to the author and is a result of systematic insect search for the last two years, while the taking of the photos has not taken place in selected places, but along the entire island.

It is important to note that this work is not the final list of Salamis insects, but a portion of them, which also justifies the form of the title. In the work, reference is made to the insects and their number by order, accompanied by photographs, which, to a certain extent, constitute evidence of their existence on the island of Salamis.

The most important thing is to clarify the purpose of this work. The aim is to highlight the richness of the insect fauna of Greece through an amateur record on the more urbanized island of the country¹, and the emergence of lesser known Orders and Families of insects by the macrophotography method.

Finally I would like to give particular thanks to all the people who helped me in the partial or full identification of insects, but also to the patience of my colleagues and friends who accompanied me in several phases of this recording.

¹41.8% of the area of Salamis consists of roads, buildings, etc. Source: www.kathimerini.gr

**Auchenorrhyncha (Hemiptera) – potential vectors
of the *Xylella fastidiosa* in neighboring olive groves and vineyards:
Species and population variability**

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Competition

The bacterium *Xylella fastidiosa* has been a major concern in the scientific community in Europe. This is due to the confirmed infection of olive trees by this bacterium in the Apulia region of southern Italy, in October 2013. The disease, which is caused, by this bacterium called “Olive Quick Decline Syndrome”. Initially, affected trees show wilt and desiccation in some shoots, which gradually spreads over their entire stem, resulting in the collapse and death of the tree. Also, this bacterium causes the Pierce disease in the vineyard. This disease first appeared in California in 1887 and has since destroyed a large number of stumps across the American continent. The main symptoms are severe cachexia, death of the leaf region, delayed development of the eyes, dwarfism of the trees, drying of the grapes and finally the death of the stumps.

Xylella fastidiosa has four sub-species: *-pauca*, *-multiplex*, *-fastidiosa* and *-sandyi*, which infect different plant species. In the case of olive tree, the disease is due to the *Xylella fastidiosa* subsp. *pauca*, while the vineyard is *X. fastidiosa* subsp. *fastidiosa*. This bacterium is transmitted by sucking insects of the suborder Auchenorrhyncha. These insects are very small in size and they cause direct (suck plant sap) and indirect (vectors of pathogenic microorganisms) damage to the plants from which they are fed.

This study attempts to compare the species of Auchenorrhyncha which are considered as potential carriers of *X. fastidiosa* and their population density in neighboring olive groves and vineyards. For the purpose of this study, samplings were conducted from olive grove in Messinia. The samplings were performed weekly with Malaise traps with ethyl alcohol 70% as preservation medium. Then, the collected samples were transferred to the Laboratory where the systematic classification of the leafhoppers took place through the removal and cleansing of the male genitalia. The results showed the presence of Auchenorrhyncha from Aphrophoridae, Cicadellidae and Issidae families which are potential carriers of the bacterium. Detailed information about the species, the population density and the differences between the two cultivations are given in the presentation.

Species and population variance of Auchenorrhyncha in vineyards

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Competition

Auchenorrhyncha (Hemiptera: Homoptera) are one of the most important groups of insects, both in terms of diversity and geography. Usually, are small to very small, rarely of large size. Most of them affect the crops. So far, are described more than 26,000 species, 9,000 are belong to the Fulgoroidea and 17,000 to Cicadoidea.

After observing the yellowing of the foliage of leaves and twists, it was considered advisable to investigate this situation.

For this purpose, samples were carried out from the vineyard of table grapes (thompson seedless) in Stimaga Korinthias. A Malaise-type trap was used. For the identification, the samples were transferred to the laboratory of the University. Also, samples from the vineyards of the university were taken.

The systematic classification of the samples was performed after the notice of genitalia. Results showed a high population of the genus *Empoasca*.

**The distribution of the invasive pest, rice water weevil
Lissorhoptrus oryzophilus Kuschel (Coleoptera: Curculionidae),
is expanding in Europe: First record in the Balkans, confirmed by
CO1 DNA barcoding**

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Competition

The rice water weevil *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is a semi-aquatic beetle that is considered one of the most important rice pests globally. *L. oryzophilus* is also described as a highly invasive species on account of its spreading capability as well as its diverse feeding behavior (wide range of wild grasses). The native populations of *L. oryzophilus* originate from the USA, however it has invaded several productive rice-growing areas throughout the world, including Japan, China, India and Korea, while recently it was reported for the first time as an invasive pest in Italy. During July and August 2016, bi-weekly sampling surveys were carried out in the rice field region of western Thessaloniki, where totally 30 *L. oryzophilus* specimens were collected throughout the survey period. Morphological identification was verified by DNA barcoding, and the first COI sequence of *L. oryzophilus* from Greece was deposited in the GenBank database (accession number KY212031). To the best of our knowledge, this is the first report of *L. oryzophilus* in the Balkans, comprising a serious challenge for rice production that deserves special attention. Considering the highly invasive nature of this species, it is possible that it has spread to neighboring rice wetlands of the Southern Balkans, and additional field investigations are essential to accurately determine its geographic distribution and to help contain its spread.

The entomofauna on the cones of *Picea abies* in a manage Rhodope mountain forest

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In Northeastern Greece, especially in the managed forest of Elatia (41° 29' N, 24° 18' E) on Rhodope mountain, a total of 10,000 cones (4,432 cones in 2007 and 5,532 cones in 2017) were sampled in order to study the entomofauna of *Picea abies* L.

In the first sampling, in 2007 the following species of insects were identified attacking and or parasitizing the cones of *Picea abies*: *Cydia strobilella* (Linnaeus) (Tortricidae: Lepidoptera), *Dioryctria abietella* (Denis and Schifferrmüller) (Lepidoptera: Pyralidae), *Ernobius abietis* (Fabricius) (Coleoptera: Anobiidae), *Megastigmus strobilobius* Ratzeburg (Hymenoptera: Torymidae), *Gastrodes abietum* Bergroth (Hemiptera: Rhyparochromidae) and *Raphidia notata* (Fabricius) (Raphidioptera: Raphidiidae).

Ten years later a second sampling was carried out.

According to this the same species of insects were recorded, however the populations of most of the species, especially of the two seed cone insects *D. abietella* and *M. strobilobius*, were extremely high. Did climatic change and the following stress to the plant physiology cause this increment? Therefore, additional research in the forest is necessary in order to determine the factors affecting the increase of the population of those species that attack or parasitize spruce cones.



2nd Session
Biology and Ecology

Temporal and spatial distribution of *Helicoverpa armigera* in Fthiotida (Central Greece)

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Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is one of the most important insect pests of cotton. In the present study, the temporal and spatial distribution of *H. armigera* was studied in the area of Moschochori (Fthiotida, Central Greece). For this purpose, a network of pheromone funnel traps was established in selected cotton fields. Three types of pheromone traps (funnel traps with black-and-white stripes, green funnel traps and white-yellow funnel traps) were tested. The captures of male adults of *H. armigera* in the pheromone traps were recorded weekly from July to September 2015. The results of the current study showed that the black-and-white traps captured significantly more male adults of *H. armigera* in contrast to the other two types of trap. Furthermore, the results revealed extensive overlapping of *H. armigera* generations. Finally, the current study is one of the few attempts to study the spatial and temporal distribution of one of the main insect pests of cotton in the area of Fthiotida and may be used for the development and implementation of effective control strategies of *H. armigera*.

Effect of the type and quantity of feed on biological parameters of *Hermetia illucens* (L.) (Diptera: Stratiomyidae)

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Nowadays, there is an extensive discussion on the issue of environmental protection, the reduction of organic waste and the pollution management they cause. In order to manage this issue, Conversion of Organic Refuse by Saprophages (CORS) technology promises solution, with the non-pest insect *Hermetia illucens* (Diptera: Stratiomyidae) (Black Soldier Fly) being the basic tool. Larvae of this species are voracious feeders which consume a wide range of organic materials. Thus, they can be used in simple mechanical systems to reduce organic waste, but also as a nutritional factor for animal livestock, in the context of recycling of organic matter. In this study we investigated the effect of two combinations of nutrient substrates on biological parameters of *H. illucens*. For this purpose, poultry feed and urban bio-waste (catering organic waste) were used as nutritional substrates in two rates, 150 and 250 mg/larva. For each treatment, immature development, survivorship and larval body weight, as well as adult longevity and sex ratio were recorded. Results showed significant differences on the biological parameters of *H. illucens* between the different treatments. In total, significantly shorter immature development was recorded in poultry feed treatment compared with the catering waste. Moreover, immature development time in the 250mg/larva/day treatment was significantly shorter compared with the 150mg/larva/day treatment. Detailed results and discussion are given in the presentation.

Effect of the olive fruit size and volatiles on oviposition preference and egg laying in the olive fruit fly

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Contact and volatile stimuli linked with the olive fruit may affect the behavior and physiology of *Bactrocera oleae* Rossi (Diptera: Tephritidae). We studied whether the size and volatiles of the olive fruit affect oviposition preference and egg laying of *B. oleae* females. Mated adult females were allowed to lay eggs on small and large size olive fruits of the same variety (Chalikidikis) covered with a thin membrane (Parafilm M[®]) with or without small holes. We scored the number of females that visited the differently treated olive fruits and the number of eggs laid on the fruits for a period of 4 hours. It was found that the size of the olive fruits and volatiles emitted through them affect the oviposition preference and the number of eggs laid.

Effects of host plant and temperature on the development and survival of *Closterotomus trivialis* (Hemiptera: Miridae)

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The phytophagous plant bug *Closterotomus trivialis* (Costa) (Hemiptera: Miridae) is widely distributed in the Mediterranean region. It is a polyphagous species commonly recorded on olive (*Olea europaea* L.), citrus, as well as on wild growing plants such as *Urtica* sp., *Parietaria officinalis* L., *Mercurialis annua* L., and *Sinapis alba* L. Survival and developmental duration of *C. trivialis* nymphs on the olive cultivar 'Koroneiki', 'Washington Navel' sweet oranges, as well as on *M. annua*, *U. urens*, *P. officinalis* and *S. alba* were studied under four constant temperatures 15, 20, 24 and 27°C with 60±5% RH and 14L:10D h photoperiod. Results demonstrated that *C. trivialis* can successfully complete its development on all tested host plants, except *U. urens* at 15 and 20°C, on olive at 24 and 27°C and sweet orange at 27°C. The effect of the host plant and much more the effect of temperature were significant during the nymphal development of *C. trivialis*. The assessment of the nymphal development in various host plants and temperatures is particularly important for understanding the insect biology and provides useful information to optimize the management strategy under integrated pest management system.

Adjustment of frequency and daily pattern of male sexual signaling in response to different photoperiods

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Photoperiodic stimuli drive several biological phenomena in insect species ranging from dormancy responses to circadian adjustments of reproductive behaviours including sexual signaling. We used the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), a polyphagous invasive species, to test plastic responses in expression of male sexual signaling in response to photoperiodic and dietary stress. Males (from a laboratory strain) that were fed on either yeast and sugar (protein rich) and sugar only (protein stressed) were exposed to different photoperiod regimes under constant temperature and humidity conditions, in walk-in chambers. Soon after emergence, males were exposed for 5 days to constant light (L24:D0) conditions before being (a) transferred directly to L8:D16 conditions, (b) first taken to L14:D10 for 5 days and subsequently to L8:D16 and (c) kept continuously to L24:D0. Males that were exposed continuously to L14:D10 served as control. The frequency of sexual signaling was recorded at hourly intervals from 07:00 until 20:00h. Diet context significantly affected the frequency of sexual signaling regardless of the photoperiod regime. Photoperiod shifts increased sexual signaling rates on sugar-fed males and decreased them on males fed yeast and sugar. Sexual signaling was evenly distributed at the L24:D0. Substantial differences were recorded in the daily patterns of sexual signaling between L14:D10 and L8:D16. The interaction between photoperiodic and food stresses seems to regulate both quantitative and qualitative aspects of male sexual signaling.

Effects of food deprivation on the sexual behavior of Mediterranean fruit fly

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We tested if food deprivation affects the sexual behavior of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Specifically, we monitored the frequency of male sexual signaling in two different deprivation periods (12 and 24 hours) compared to males that had access to food. Calling was monitored for 10 consecutive days, using males of 8, 13, 18 and 23 days of age. The results showed that the effects of food deprivation vary with its duration. A 12-hour lack of food had a positive impact on sexual calling whereas a 24-hour had a negative effect. The effects seem to vary in relation to the age of males. In an additional experiment, food shortage did not seem to evoke any significant effect on male sexual competitiveness.

Efforts to identify the major sex pheromone component of the mushroom sciarid fly *Lycoriella ingenua***S.S. ANDREADIS^{1,*}, K.R. CLOONAN¹, A.J. MYRICK¹,
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Lycoriella ingenua (Dufour) (Diptera: Sciaridae) is a serious pest of the white button mushroom, *Agaricus bisporus*, worldwide. Previous reports concerning the sex pheromone of *L. ingenua* erroneously identified n-heptadecane (C₁₇) as the major component as well as several additional saturated n-hydrocarbons n-C₁₅, C₁₆, C₁₈, C₂₀ as active attractants. Recently, we reinvestigated the sex pheromone of this species by confirming that virgin females were attractive to males in a Y-tube bioassay and by collection of extracts from virgin females. Extracts were analyzed by gas chromatography coupled to electroantennographic detection, and by the less widely-used technique of gas chromatography coupled to a behavioral bioassay to detect compounds causing wing fanning and copulatory abdomen curling in males. As a result a single behaviorally-active pheromone component was isolated and characterized by gas chromatography coupled to mass spectrometry. This component is proposed to be a sesquiterpene alcohol having analytical characteristics that closely matched those of reference germacradienols. At present our task is to chemically confirm which of the several possible enantiomers is responsible for the particular sex pheromone activity. Once this compound is finished being fully chemically characterized, it will have potential utility for monitoring populations of *L. ingenua* and reducing their density and damage to the mushroom crop.

The variance to mean ratio of the Poisson distribution as indicator of the probing prefeeding behavior of the aphid *Myzus persicae*

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In this work, we examine the prefeeding probing behavior of the aphid *Myzus persicae*, focusing on the probability distribution of probes. Treatments were starved and unstarved aphids and aphid activity was monitored individually into two successive zucchini plants or three min after aphids had been placed on the leaf surface.

Our results show that aphid probes almost always fit a Poisson distribution although with different rate λ , depending on the treatment. The Poisson distribution describes random events when the variance to mean ratio (VMR) equals one, but suggests dependence among the events in different cases. In our experiments, VMR values of unstarved ($n=145$) or starved ($n=245$) aphids in the first host were 1.05 and 0.56, respectively, whereas those in the second host were 1.27 and 0.84, respectively.

According to the results, the probing distribution of unstarved aphids in the first host tends to be random, suggesting independence among the events, whereas, it shows overdispersion in the second host, suggesting dependence among the events and heterogeneity. In contrast, the probing distribution of starved aphids in the first host shows always underdispersion that is preserved in the second host although with alleviating intensity, suggesting dependence among events and homogeneity.

In conclusion, our work shows that the probes of aphids in a three-min interval follow a Poisson distribution, the random variable VMR could be used to test randomness of the aphid probing behavior and that underdispersion may constitute a characteristic trait of starved aphids important for host colonization and/or non-persistent transmission of plant viruses.

Recording the mating behaviour of macedonica (*A. m. macedonica*) and cecropia (*A. m. cecropia*) honey bee queens**F. HATJINA^{1,*}, L. CHARISTOS¹, L. PAPOUTSIS² AND M. BOUGA²**¹*Division of Apiculture, Institute of Animal Science, H.A.O. "Demeter", Nea Moudania, Greece*²*Laboratory of Agricultural Zoology and Entomology, Faculty of Crop Science, Agricultural University of Athens, Greece***e-mail: fhatjina@gmail.com*

In the framework of a project funded by the EU the Division of Apiculture of H.A.O. "Demeter" implemented an innovative method for the controlled and at the same time natural fertilization of honey bee queens. "The Virgin Queen Train"(TVQ) as it is called, is an innovative method that involves the existence of a cabin in which the virgin queens are kept at 14-15°C in the dark and their small mating colonies are on rails like a train. In the afternoon and when all available free flying drones have returned to their hive, the selected drones are released and the virgin queens are pulled out of the cold cabin and placed in specific positions. The queen's mating flights starting time, the duration of the flights and the frequency of flights were recorded daily for both, natural queens and queens from TVQ. At the same time, the ambient temperature (in shade) was also recorded. Measurements were made in two consecutive years, 2016 and 2017, for the macedonica and cecropia queens respectively. The macedonica queens in May 2016 started the mating flights around 15:15 and returned at 16:30 to 17:00. In June, there was an average shift of at least 1 hour later on departure and return, and flight time was also prolonged by 15 minutes (from 60 minutes to approximately 75 minutes). The queens at the TVQ always departed at least 30 minutes after the mating colonies were transported to the open area, possibly allowing a relative temperature and homeostasis to be restored first in the hive. During May 2016, it was observed that most TVQ queens departed around 18:15 and returned around 19:15 (flight time of about 60 minutes), while in June the departure occurred a little later, around 18:30, and the return between 19:00 and 19:30. With the temperature rising from May to June, queens departed for the mating flight later in the day. The behavior of cecropia queens was somehow different in 2017, when the open area queens started the mating flights after 16:30 in the afternoon. During the same days, queens from the TVQ were leaving their hive after 19:00 in the afternoon. The ambient temperatures recorded for June 2017 were much higher than June and July of 2016. The above work is the first recording of the mating behavior of the Greek queens, in the Greek territory. The results will be very useful for determining the day intervals during which a controlled mating can occur.

Study of the effect of glass McPhail trap density on monitoring of *Bactrocera oleae* population (Diptera: Tephritidae)

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The average number of olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) adults per trap and week, the sex ratio and the fertility rate of females are among the most important criteria for the decision to carry out ground bait sprays in a region. The timing of bait spray applications is based on a weekly monitoring system of populations of the pest with a net of glass type McPhail traps, in a ratio of 1 trap per 1,000 olive trees. However, in the last four years, the ratio of traps fell to 1 trap per 2,000 trees, approximately. The aim of the study was to investigate the effect of the trap density in the net on monitoring of *B. oleae* population. The study was conducted for three consecutive years (2014-2016) in the semi-mountainous region of Syrili, Chania, Crete in about 30,000 olive trees of Koroneiki variety. For this purpose two different trap networks were installed: 1 trap per 1,000 olive trees and 1 trap per 2,000 olive trees. McPhail traps were renewed with new bait solutions once per week. The captured adult olive flies were counted, and the sex ratio and the fertility of the females were determined in the lab. Throughout the study period, the average number of captured adult olive flies per trap and week, the sex ratio and the fertility rate of females in the net of 1 trap / 1,000 tree density did not differ significantly with the net of 1 trap / 2,000 trees density.

Use of GIS and spatial analysis to control insect infestations in permanent plantations: Pilot implementation to the olive fruit fly control program in Samos

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Competition

The cultivation of olive groves in Greece is a very important economic activity, with cultural and social significance and environmental benefits, especially in low-input farming systems that are still found in many parts of Greece. Their most important enemy is the olive fruit fly, *Bactrocera oleae* Rossi (Diptera: Tephritidae), with recorded losses of many millions of euros per year. It has been recently observed that olive fruit fly control programs (run collectively for major olive zones) are more dependent on available funds and organization, rather than landscape ecology of the insect, relief and land cover. Also, extensive abandonment of olive cultivation creates outbreaks and sometimes renders the programs ineffective.

In this paper, we present some exploratory findings from the analysis of the olive fruit fly control program on the island of Samos using GIS and spatial analysis. We use the results of the trap network of the program for the 2016 period to approach spatial and temporal changes in the insect population relative to geomorphology and spraying interventions. The trap data from 12 experimental plots (1Km²) was also used, two of which were placed in areas not covered by the program. In these plots, temperature and relative humidity inside the tree canopy were measured for 8 weeks. Land cover was also recorded from photo interpretation with empirical verification of the categories in the field. The findings show seasonal changes in the insect population spatially, as well as significant differences between areas inside and outside the control program. On the basis of these preliminary findings and extra data from repetitions of the experiment, the design of an estimation model with a risk map that can be used in designing more effective future programs is suggested, which will be based on actual spatio-temporal changes of the insect population with meteorological data and olive fruit availability.

Induction of prolonged dormancy via exposing *Rhagoletis cerasi* (Diptera: Tephritidae) pupae in various combinations of periods in high and low temperatures

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Extended exposure of *Rhagoletis cerasi* pupae to low temperature increases the percentage of individuals entering prolonged diapause. However, whether the duration of exposure to high temperature, immediately after pupation, affects diapause termination of *R. cerasi* remains unknown. In the present study we tested the effect of different time intervals of exposure to high temperature right after pupation on obligatory diapause termination and on the induction of facultative prolonged dormancy of *R. cerasi* pupae. To this end, we used 5,000 pupae of a highland population that were exposed after pupation, in groups of 100 individuals, to three high temperatures (15, 20 or 25°C) for 12 different periods (0–12 months). Subsequently, pupae were subjected to low temperature (4°C) for two different periods (5 or 8 months). Finally, pupae of each treatment were transferred to 25°C, placed in Plexiglas cages with food and water where the number of emerging males and females and the number of dead pupae was recorded daily. The results showed that individuals that remained in 4°C for 8 months entered prolonged diapause in higher percentage than those kept at 4°C for 5 months, regardless of the previous period of exposure to high temperature. The percentage of pupae that entered prolonged diapause increased progressively as the period of exposure to high temperature was longer. In detail, pupae exposure for 12 months to 15 and 25°C, before being transferred to low temperature for 5 months, lead 40 and 70% of pupae to prolonged diapause, respectively. The combined exposure of pupae to 25°C for more than 8 months and to 4°C for 8 months induced prolonged dormancy to more than 65% of individuals. Pupae that remained in diapause exhibited low mortality and regardless of the duration of the second period in low temperature (6–8 months) yielded adults in percentages of 40–80%. The ecological impact of induction of *R. cerasi* pupae in prolonged diapause is commented and a possible practical implication of the phenomenon is discussed.

Data on ecology of the scale insect *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae)**G.J. STATHAS* AND P.J. SKOURAS**

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Phenology and natural enemies of the scale insect *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), on citrus, in Attica, during the years 2015-2017, were studied.

Coccus pseudomagnoliarum infests the shoots and the leaves provoking damage by sucking the juice and creating sooty molds developed on the honeydew excretions of the scale. It is viviparous and parthenogenetic species. It overwinters as 1st instar nymph, until the beginnings of April, when they appear 2nd instar nymphs and later they develop to mature female adults. During the first half of May, crawlers appeared, until the middle of June. From the middle of June, until next April, the population of the scale is consisted of 1st instar nymphs, settled on shoots and leaves, preferring the upper surface, along of the main ribs.

A parasitoid species of genus *Coccophagous*, recorded as the most important natural enemy of the scale. The percentage of parasitism about the end of May, reached up to 35%.

Presence, description and developmental characteristics of the newly emerging quarantine pest *Aleurocanthus spiniferus* in Corfu

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Competition

The black spiny whitefly, *Aleurocanthus spiniferus* (Quaintance) (Hemiptera: Aleyrodidae), was reported for the first time, in Corfu, in the area Alepou, on July 14, 2016. *Aleurocanthus spiniferus* is native to Southeast Asia that was detected in Europe in 2008 on a citrus orchard in Italy. Adults are approximately the same size as *Aleurothrixus floccosus*, about 1.4–3 mm in length, with the females slightly larger than the males. Wings are charcoal-grey in color with characteristic light spots, while eyes and abdomen are reddish-orange. Eggs are elongated, oval or kidney-shaped, 0.2 x 0.1 mm in size and deposited in a spiral path on the lower surface of the leaves. Initially they are yellowish, however soon they become dark brown and finally black as the embryo develops. Young larvae are black, flattened, with 6 thoracic legs and 2 long and several shorter, radiating spiny marginal filaments.

The life cycle of this species, in the particular area, lasts typically 2 months and has 5 overlapping generations per year. Primarily, it damages host plants by sucking the sap and leading to a general weakening but it also causes indirect damage by producing honeydew and subsequently promoting the growth of sooty mold. Dispersal over long distances takes place through the transportation of infected seedlings or infected plant parts at which eggs or immature stages of the species are present. Besides citrus, in humid areas other hosts include grapes, pear, apricot, peach, lotus, avocado, pomegranate, quince and ornamental plants. *Aleurocanthus spiniferus* is a quarantine pest and special handling is required to avoid possible new cases in other areas in Greece.

Population variation and control of *Rhagoletis cerasi* in two regions with different altitude of Naoussa

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Competition

The insect *Rhagoletis cerasi* (L.) (Diptera: Tephritidae) is the most damaging insect for cherry orchards. The population variation has been studied in two study areas with different altitudes: in the area "Blana" at 400m and in the area Taxiarches at 600m. The varieties used in both estates were Lapins, Sunburst and Ferrovia. Four pheromone traps were placed on each farm (one in each variety and one in the untreated) on 05/05/2016 to monitor the flight and fluctuation of the insect population. Plant protection treatments and fertilization in the cherry orchards were exactly the same until the stage of fruit change color of each variety where the interventions were performed in a fully randomized four-repeat pattern with Profil (acetamiprid) 250 g/1,000 liters per hectare, Ikarus (Deltamethrin) 500cc per hectare, Deltathrin (deltamethrin) 500cc per hectare and combination of the first two in one spray. Estimation of the cherry damage by *R. cerasi* occurred one day prior to the harvest of each variety.

From the captures of the pheromone traps it was apparent that in the highest altitude orchard the populations were smaller in all three varieties. The maximum of arrests was observed at 600m on 10/06/2016 while at 400m on 14/06/2016. The best results in protection of insect attack were given by the combination Profil and Ikarus in both areas. Infestations were greater in the orchard with the smallest altitude in all single-use insecticides.

Intraguild predation between endemic and exotic species of phytoseiid predatory mites

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The family Phytoseiidae includes species of predatory mites that are used with great success in biological control of several insect and mite pests. In the present study, we studied intraguild predation among the exotic predatory mites *Amblydromalus limonicus* and *Transeius montdorensis* and three endemic species (*Euseius stipulatus*, *E. finlandicus* and *Iphiseius degenerans*) for the European fauna. The bioassays were conducted in the absence of prey with or without pollen (*Typha angustifolia*) provisioning as alternative food source. Parameters evaluated were aggressive behavior (aggressiveness), intraguild prey preference and predation rate against larvae of the same (cannibalism) or the competitive species, as well as ability to complete juvenile development and reproduce in the presence of prey. We found that in the absence of alternative food source exotic species were superior intraguild predators compared to indigenous *Euseius* species. Pollen provisioning resulted in the reduction in the intensity of intraguild predation; however, this effect was asymmetrically expressed among the interacting phytoseiid species, with a stronger decline recorded in intraguild predation by the endemic *Euseius* species. Our results highlight the need for further evaluating ecological risks associated with the release of exotic phytoseiids in biological control.

On the viability of the steady-state satiation equation

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Mathematical models allow the description of ecological processes and can thereby synthesize the existing knowledge of a system that has been studied or is under study. In this direction, modelling predator-prey interactions is a central goal for ecologists. Functional response models are, in turn, central to predator-prey models, describing the relationship between prey density and per capita prey consumption rate by a predator. Holling's modelling seminal approach, commonly known as the disc equation, has been the base upon which much of modern functional response theory has been developed, becoming the "null" functional response model. While examining the foraging cycle of individuals, Holling used two parameters in order to develop an improved explanation of their feeding behavior: the attack rate, i.e. the predation ability in low prey densities and the handling time, i.e. the time a predator spends pursuing, subduing, and eating its prey. Holling did not include digestion time in the disc equation. Although some authors have later extended the interpretation of "handling time" by also including digestion time, this violates the key assumption of the disc equation that the processes searching for and handling prey are mutually exclusive, as a predator *can* search for prey while digesting its last meal. Ecologists often utilize Holling's approach in order to illustrate co evolutionary associations and improve functional predictive power in predator-prey dynamics. However, the mechanistic basis of the disc equation has been challenged by the steady-state satiation (SSS) as the authors presented a detailed examination of the fundamental process of predators' feeding behavior. The application of the SSS equation is underutilized so far in the ecological literature, probably due to its complexity relative to the simpler disc equation.

In this study we evaluated the viability of the SSS equation, using functional response data of the aphidophagous ladybird beetle *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae), feeding on the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae). The model parameters were assessed through a customized Markov Chain Monte Carlo algorithm, suitable for ordinary differential equation models. The results highlighted the viability of the SSS equation. Its use, compared to the Holling model, can lead to a more reliable estimation of the handling time, as it is characterized by a clear discrimination of predators' handling and digestion time.

Biological parameters of *Lucilia sericata* (Diptera: Calliphoridae) on various substrates

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Competition

Skin ulcers are a major health problem, which is constantly increasing, in proportion to life expectancy. Bigger problems of ulceration are faced by people in poor and developing countries. Complications in chronic ulcers often include mutilation, poor quality of life and they can even lead to death. Cataracts and diabetic ulcers are more prevalent. Despite the extensive research, the results about the healing ability of pharmaceutical and parapharmaceutical formulations remain poor. Remarkable results in the treatment of chronic ulcers have been achieved by the use of larvae of the *Lucilia sericata* (Diptera: Calliphoridae). The above therapeutic method has been approved by United States Agency for Drugs and Drug Administration (FDA) and by the relevant authorities of several European countries such as Germany, the United Kingdom, Austria, Switzerland etc. Although research for the use of *L. sericata* on the treatment of ulcers has progressed a lot, the knowledge about the biology of the insect remains deficient.

In this study the biological parameters of *L. sericata* were studied on various substrates. For this purpose, experiments were performed under laboratory conditions (temperature $27\pm 2^{\circ}\text{C}$, relative humidity $65\pm 5\%$ and photoperiod 18:6 (L:D)). The substrates which were used for the breeding of the larvae were raw beef liver, ground fish feed (dry and wet) and ground soybean meal (dry and wet). For the study of the incomplete development were used petri dishes, in which larvae of the first instar were placed and their growth was observed on a daily basis. Three (3) replicates of ten (10) plates were made for every substrate. For the study of spawning and longevity, insects grown in fresh bovine liver and ground fresh fish feed were used. In total were observed forty four (44) pair of adults of which the larvae of the twenty one (21) pairs had been fed with raw beef liver and the larvae of the remaining twenty three (23) pairs of adults had been fed with ground wet fish feed. These were individually placed in plastic utensils containing saturated cotton honey, dissolved in water and fresh beef, as an insect spawning site. The counting of oviposition results was done every day. However, the rate of egg production was low because of the difficulty to succeed sexual maturity of both sexes and coupling phase at the same time. The larvae developed in dry fish feed, dry soybean meal and ground wet soybean meal did not survive. The spawning rate was greatest in pairs that had been bred with ground fresh fish feed. Extensive results and conclusions are given in the presentation.

Effect of abiotic factors on population dynamics of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) in the field

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The bio-ecology of the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) has been studied for long in the lab and in the field. In this paper, we examine the cumulative effect of meteorological factors (temperature and humidity) in the olive fruit fly population and the availability and state of olives in the field. Data come from monitoring populations for three years (2014-2016) in Koroneiki variety olive trees, 40 years old approximately, in the Nerokouros area in Chania. Each year three categories of olive tree production were used: (i) "low" fruit production (25-30%), (ii) "middle" fruit production (40-50%) and (iii) "full" fruit production (70-100%). Equal numbers of two different types of traps were used (glass McPhail trap, with 2% Ammonium sulfate water solution and yellow sticky traps with pheromone and ammonium bicarbonate). Adults in the traps, female ratio and fertility of females were conducted every 7 days from July to November. In the same period, from each selected tree the understory fruits were collected and randomly from tree canopy and examined with the use of stereoscope to determine damage rates. Hourly meteorological data were gathered from the whole cultivation period (temperature, relative humidity, wind speed and rainfall). Meteorological data were converted from hourly measurements to 7 day sums for six temperature thresholds (from $\geq 30^{\circ}\text{C}$ to $\leq 35^{\circ}\text{C}$) and six relative humidity thresholds (from $< 50\%$ up to $> 70\%$ per 5%). The dependent variables (total number of adults in the trap, female fertility rate and damage percentage) statistical significance was tested for all possible combinations of temperature and humidity thresholds. Results indicate the importance of combining temperature and relative humidity to explain the changes of the values of the dependent variables and highlight the cumulative statistically significant thresholds. The study was partly financed by Hellenic Ministry of Rural Development & Food, in the frame of the program "Comparative experimental studies for the olive fruit fly control".

Study of the bio-ecological parameters of the predacious mite *Typhlodromus (Anthoseius) recki* Weinstein (Acari: Phytoseiidae)

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Competition

Typhlodromus (Anthoseius) recki Weinstein is a predatory mite belonging to the subfamily Typhlodrominae, of Phytoseiidae family. Many species of the Phytoseiidae family are successfully used in biological pest control especially in greenhouse crops. *T. recki* is widely distributed in Greece, and has been recorded mainly in arboricultural, floricultural and horticultural cultivations. However, its biological and ecological parameters have never been studied. The present study deals with this species bio-ecology, in order to evaluate its significance as a biocontrol agent. Survival, development, fecundity, longevity and life table parameters of *T. recki* were explored, under constant laboratory conditions (25±1°C, 65±10% RH, 16L:8D photoperiod). Pollen from *Typha* sp. and the herbivorous mite *Tetranychus urticae* Koch were used as tested food. The survival of immature stages, in two different foods tested, was very high. Development time from egg to adult varied from 7.97±0.16 to 7.77±0.14 days for females and from 7.75±0.28 to 8.02±0.23 days for males. Female longevity was estimated to 29.67±3.3 days, when the food was *Typha* pollen and 39.89±2.42 days when the food was *T. urticae*. Fecundity varied from 15.5±2.65 to 27.08±1.99 eggs per female. The highest intrinsic rate of increase (r_m) of mites was estimated for *T. urticae* (0.134159 days⁻¹) while the lowest for *Typha* sp. pollen (0.119657 days⁻¹). The predatory mite was successfully developed in both of the tested foods. In case of *T. urticae*, longevity and fecundity of the predator was higher.

Studies on the cannibalistic behaviour of equal-aged nymphs of the omnivore *Dicyphus errans* (Hemiptera: Miridae)

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Negative intraspecific interactions, especially cannibalism are usually density-dependent and become more frequent when food resources are scarce. The existence and the intensity of cannibalistic behavior were searched among nymphs of the zoophytophagous mirid predator *Dicyphus errans* Wolff (Hemiptera: Miridae) during their developmental period. Newly emerged nymphs (<24h old) were grouped at several densities on a tomato leaflet in a dish. Depending on the treatment, nymphs were used in cohorts of 2, 4, 8 and 16 individuals, with or without prey (*Ephestia* sp. eggs and *Artemia* sp. cysts). As controls nymphs were used individually in the dish. All experiments were conducted at 25±1°C, 65±5% RH and 16L:8D photoperiod. The number of alive, consumed and injured nymphs was monitored daily till death or adult emergence and sex and weight of adults were determined. In each treatment 15 replicates were used. Cannibalism was observed in all densities when prey was not available and no nymph succeeded to complete development. The survival was higher when nymphs were placed individually or in pairs. Most of the nymphs succeeded to complete development under prey presence at the densities of 8 and 16 nymphs per dish. The developmental period was significantly extended in the treatment of 8 nymphs. The female and male weight was similar among the treatments but significantly lower than the control (single nymph). *D. errans* seems to exhibit cannibalism as an adaptive survival strategy when prey is scarce. This behaviour may have important consequences in the population size and structure of this predator and should be further studied.

Effect of temperature and storage time on soil samples contaminated with root-knot nematodes (*Meloidogyne* spp.)

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Competition

Root-knot nematodes (*Meloidogyne* spp.) cause serious problems to a large number of economically important greenhouse crops in Greece. Many producers take samples and send them for analysis so that the problem is confirmed by experts and they will be advised to employ certain control measures to solve the problem.

In this study we examined the effect of temperature and storage period on infested with root-knot nematodes soil samples. Experiments were carried out using four different biological stages of root-knot nematodes (second stage larvae, undifferentiated eggs, differentiated eggs and egg masses). The temperatures tested were: 4, 8, 13, 22 and 30°C. At these temperatures soil samples remained stored for 1, 10 and 20 days.

Both factors (temperature-time) that were tested seem to have a significant impact on survival of nematodes in the soil samples, differently at each stage of the life cycle. Thus, knowing the main stage of the biological cycle of the nematodes in a soil sample to be examined, it is possible to choose the most suitable storage temperature and to be able to examine the sample in a timely manner, by isolating the highest number of nematodes present in the soil sample.

Relative humidity effects on the performance of the European *Chrysoperla carnea* (Neuroptera: Chrysopidae) species complex**K. ATHANASIADIS¹, M.L. PAPPAS¹, V. MORAITIS¹, A. PEKAS²,
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Lacewings are natural enemies with great potential in biological control. The Holarctic *carnea*-group of the genus *Chrysoperla* includes at least 16 cryptic species, distinguishable by their species-specific substrate-borne courtship songs. Whereas five of these cryptic species are endemic in Europe in overlapping geographical distributions, only one species, namely '*C. carnea*' is currently marketed for biological pest control. Ecological differences such as habitat associations documented for some of the European cryptic species may also imply existing diversity in their biology, such as responses to relative humidity. We conducted a series of experiments to reveal species-specific differences among the five European cryptic species (*C. agilis*, *C. carnea sensu stricto*, *C. lucasina*, *C. mediterranea*, *C. pallida*) in their responses to two relative humidity levels (11% and 75% RH) and 25°C. We found that juvenile development and survival were significantly affected by relative humidity with dry conditions (11% RH) resulting in a significant reduction in total developmental time and juvenile survival rate as well as in egg production. In addition, significant differences were recorded among the five species at the two relative humidity levels, with *C. agilis* and *C. carnea* developing faster than the other species at the low relative humidity. Our results show that the different cryptic species are differentially adapted to low relative humidity. This information could find application in the selection of cryptic species for biological pest control in arid conditions.

Invited Speaker

Old and new challenges related to vector and vector-borne diseases in Europe

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Vector-borne diseases are a specific group of infections that represent a (re-) emerging threat to Europe requiring particular attention. Their eco-epidemiology and control are challenging due to their complex transmission systems driven by the interaction between the pathogen, the hosts, the vector, the environmental and social context. Moreover vector-borne diseases tend to globalize by the increasing mobility and travel resulted in the fast and worldwide spread of vectors and the pathogens they transmit. Autochthonous transmission of vector-borne disease likely occurs in environments previously free of these diseases. This presentation will provide an overview of a number of important vectors and vector-borne diseases of specific concern for Europe.



3rd Session
Biological Control and other Management Methods

Uptake of double-stranded RNA by plant insects and mites from tomato upon its exogenous application on tomato leaves

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Double-stranded RNA (dsRNA) acts as the elicitor molecule of the RNA silencing (RNA interference, RNAi), the endogenous and evolutionary conserved surveillance system present in all eukaryotes. DsRNAs and their subsequent degradation products, namely the small interfering RNAs (siRNAs), act in a sequence-specific manner to control gene expression. Exogenous application of dsRNAs onto plants elicits resistance against plant viruses. In the present work, exogenously applied dsRNA molecules, deriving from *Zucchini yellow mosaic virus* (ZYMV) HC-Pro region, onto tomato plants were detected in aphids (*Myzus persicae*), whiteflies (*Trialeurodes vaporariorum*) and mites (*Tetranychus urticae*) that were fed on treated as well as systemic tomato leaves. Furthermore, four siRNAs, deriving from the dsRNA applied, were detected in tomato and the herbivorous pests fed on treated tomato plants. More specifically, dsRNA was detected in the three herbivorous pests at 3 and 10 dpi in dsRNA-treated leaves and at 14 dpi in systemic leaves. In addition, using stem-loop RT-PCR, siRNAs were detected at 3 and 10 dpi in aphids and mites. Surprisingly, in whiteflies carrying the applied dsRNA, siRNAs were not molecularly detected. Our results showed that upon exogenous application of dsRNAs, they move rapidly in tomato and were uptaken by herbivorous pests fed on tomato. As a result, this non-transgenic method has the potential to be used to control important crop pests via RNA silencing of vital genes of the respective pests. It should be noted that dsRNA-mediated silencing of vital genes of pests has been exploited in transgenic plants with positive outcome against aphids, whiteflies and mites.

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Developing new methods of application of entomopathogenic nematodes in combination with attractants for control of the cabbage fly *Delia radicum*

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Cabbage root fly *Delia radicum* L. (Diptera: Anthomyiidae) is an important pest of brassica crops. Our current approaches are to first screen and identify attractants for cabbage root fly larvae and then incorporate them in lure and kill formulations in combination with entomopathogenic nematodes (EPN). First, we found that *Steinernema feltiae* (Rhabditida: Steinernematidae) is the most efficacious species against fly larvae feeding on radishes. Then we found that cabbage root fly larvae are strongly attracted to dimethyl disulphide (DMDS), which is emitted by root fly-infested roots of Brassica plants. Also, other attractants from brassica plants are currently being identified. Current efforts are focused in incorporating these compounds in polymer beads bearing entomopathogenic nematodes with the aim to lure the larvae and deviate them from the plant to the capsule. Several other parameters on using these potential attractants in beads in combination with EPN will be discussed as well their application on soil for control of the root pests.

Positive effects of pollen provisioning on *Amblydromalus limonicus* following flonicamid application

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Flonicamid (Teppeki 50WG) is an insecticide with moderate toxicity against phytoseiid mites. In the present study we evaluated the effect of pollen provisioning as supplementary food source following spray application of flonicamid on the subsequent survival, reproduction and prey consumption (thrips larvae of *Frankliniella occidentalis*) of the predatory mite *Amblydromalus limonicus*. For the experiments, two application frequencies of *Typha angustifolia* pollen were used: a) pollen was provided to the mites once only, within half an hour following flonicamid application and, b) the same amount of fresh pollen was gradually provided to the mites every 48 hours. We found that pollen provisioning resulted in increased prey consumption by *A. limonicus* irrespectively of its application frequency. Frequent provisioning of fresh pollen had a positive effect on juvenile survival of the predatory mite; however, mixed diet (thrips and pollen) provided on flonicamid-treated arenas did not affect *A. limonicus* oviposition and adult survival. The results of our study highlight the role of pollen provisioning in mitigating the negative side-effects of pesticide residues on phytoseiid predatory mites. Ongoing experiments aim to analyse the behaviour of *A. limonicus* on flonicamid fresh spray residues in the presence of fresh pollen.

Sugar provisioning disrupts the ant-hemipteran mutualism and improves biological control

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The role of ants (Hymenoptera: Formicidae) regarding plant protection is controversial. Ants can act as predators; therefore, their role can be positive. However, due to their protecting behavior towards honeydew producers, ants can interfere with the activity of natural enemies and often are becoming important indirect pests. Therefore, ant-exclusion is often employed to reduce the risk of population outbreaks of honeydew producing pests. By excluding ants however, we also lose their positive services as biocontrol agents.

Herein, we tested the provisioning of artificial sugar supplements to disrupt the ant-pest interaction and eventually improve the control of aphids (Hemiptera: Aphididae) and mealybugs (Hemiptera: Pseudococcidae) in citrus and vineyards in Spain. We assessed how the provisioning of artificial sugar sources impacted ant activity, the association between the ants and the aphids or the mealybugs and the pest populations.

Our results showed that the provisioning of sugars altered ant activity reduced the ant tending incidence and the colony size of the honeydew producers and significantly increased the probability of natural enemies being associated with the honeydew producers. We conclude that the provisioning of artificial sugar sources has practical potential as a strategy for ant management enhancing the biological control of honeydew producing pests.

Efficiency of alternative control methods for olive fruit fly in Crete**E. ALISSANDRAKIS^{1,*}, K. KALAITZAKI² AND N. BOUNAKIS²**¹Laboratory of Entomology and Pesticide Science, Department of Agriculture, T.E.I. of Crete, Greece²Proactive S.A.–Business consultants, Heraklion Crete, Greece

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Olive fruit fly, *Bactrocera oleae*, Gmelin (Diptera: Tephritidae) is by far the most economically important pest of olives. In the present study, two alternative methods to pesticide spraying were applied alone or in combination, namely kaolin spraying (Surround WP Crop Protectant) and attract and killing (Vio-Trap). The whole study was part of the Action B.III.1, practical demonstrations of alternatives to chemical products for combating olive fly, of the regulation (EU) No 611/2014, regarding the support programmes for the olive-oil and table-olives sector.

The applications were performed in 5 areas, Archanes, Panagia, Sykologos and Harakas of Heraklion prefecture and Achlades of Rethymnon prefecture and in more than 45 hectares, counting around 10,000 trees. Vio-Trap traps were applied before the hardening of the fruit pit when applied as a sole method and right after kaolin spraying when both methods were combined. Kaolin was applied after the pit hardening and before the full size development of the fruit. Supplementary applications were performed in September when necessary.

The effectiveness was estimated by measuring adult population dynamics and fruit damage. In total, 42,165 insects were counted, of which 27,721 were olive flies. Additionally, 23,768 olive fruits were investigated. Among experimental plots, great variability was observed regarding olive fly parameters, such as insects per trap per day, sex ratio, fecundity and parasitism.

All methods showed good efficacy, with average living infestation being in the rate of 2%, reduced by 57.9% compared with plots where bait sprays were applied through the national programme for olive fly control (Dakoktonia). No statistical differences were found among the three applications. In the last fruit sampling, infestation levels were 5%, 3.2% and 3.7% for Vio-Trap, Kaolin and combination respectively. In general, all three applications showed good results, with no statistical differences amongst them.

The use of terpenes for the control of *Meloidogyne javanica*

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the most economically important pests to a number of greenhouse crops in Greece. Since the withdrawal of methyl bromide, and in the absence of any replacement substance, the continuous use of chemical nematicides has raised environmental and health issues. Currently, there are only few nematicides left in use, and their repeated applications interfere with the mechanisms of enhanced degradation in soil, expressed in field as inconsistent efficacy. Therefore, there is an urgent need for development of alternative nematode control methods, such as plant-natural products of secondary metabolism. Among these are terpenes, found as active constituents in several plant essential oils which have been shown to have a broad range of biological activities. It has been reported that they have shown antimicrobial, antioxidant, antifungal, insecticidal and nematicidal activities. In this study, we have evaluated the nematicidal activity of three terpenes: carvacrol, geraniol and eugenol. Specifically, the nematicidal activity of terpenes has been tested in paralysis trials against second stage juveniles (J2s), egg hatch arrest and egg-differentiation inhibition bioassays as well as in pot experiments after incorporation of terpenes in nematode infested soil at concentrations of 62.5 to 1,000 ppm. Also this study aimed to assess the sublethal doses (ED_{50}) of terpenes on nematodes infecting tomato plants. It has been observed that the nematicidal activity could be correlated with the level of doses and the duration of immersion and incorporation of terpenes. Biopesticides of plant origin are a promising tool in plant protection and their use has shown an effect on soil microbial communities.

The use of pheromone traps in monitoring and management of wasps in apiaries

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Beekeeping is an important sector of the rural economy with multiple positive impacts on employment, family income and plant pollination. Wasps (Hymenoptera: Vespidae) cause great damage to apiaries. The present study investigated the use of pheromone traps for the control of wasps in apiaries in an experiment which lasted from July 2015 to the end of 2016 (2 seasons). The pheromone trap used was of VARL type, which consists of a synthetic attractant (pheromone) and of a container with natural bait fluid. The liquid bait was a mixture of beer (5% alcohol) and commercial fruit juice at 1:1 ratio. The amount of liquid bait was 0.3 L per trap. Two traps were placed at a height of 165 cm from the ground, in each of the two apiaries of the Agricultural University of Athens. The traps were fully exposed to the sun and at a sufficient distance between them. The pheromone lure was renewed once a month and the attractive bait once a week. Trapped wasps and other insects were collected every week. The results revealed that the collected wasp species belonged to all the three harmful for the apiculture wasp genera (*Vespa*, *Vespula* and *Polistes*) and specimens belonged to all castes (queens, workers, drones) were recorded. The pheromone traps significantly reduced the numbers of wasps in the apiaries. Therefore, the pheromone traps should be further studied as a means to monitor and control wasps in apiaries.

**Back-yard medfly is a key-factor in area-wide management
in southern Europe.
Data from Attiki Greece, 38 North Latitude**

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With the cosmopolitan multi-host Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), the development of an effective area-wide control system involves the prerequisite of successful handling the medfly populations from numerous city and suburb small orchard and back yard fruit trees. These numerous breeding places, difficult to handle for many reasons, can produce massive population outbreaks which will not allow area-wide control in the continuum of fruit tree plantations in the vicinity of small or large cities and suburbs.

A twelve-month back-yard fruit tree Mediterranean fruit fly record was kept from November 2015 till November 2016, in a small back-yard fruit-tree garden about 7 km from the center of Athens. McPhail yellow bottom traps (one per tree) with Biolure (3 separate dispensers of ammonium acetate, trimethylamine, and putrescine) and a water solution of 1.5% sodium tetraborate and few drops of kitchen detergent were used. Traps were examined approximately every week and water solution renewed. The Biolure dispensers were renewed every 3-4 months.

In navel orange tree, a few medflies (3.6-5.3/day) were trapped in November, less than 1 medfly/day was trapped between December-May, except mid-April when 2.1 flies/day were trapped. From June till October high records were detected. In June-July records up to over 90 flies /day were detected. In August till October records up to 66 flies/trap were detected. The sex ratio of trapped flies was almost always in favor of females, usually between 60-100%. An increase of medfly catches was always observed following Biolure dispensers renewal.

Given the huge number of fruit-tree small gardens in and around small or big cities in the Mediterranean, the usually no insect control practices in those areas, and the continuum of extensive commercial fruit tree plantations beyond the suburban areas, we suggest that no area-wide medfly management could be undertaken unless these medfly breeding areas are taken care of. In case SIT practices are considered, the reproductive behavior of released flies in this special environment, i.e. very small and fragmented host tree plantations with many buildings and ornamental plants in-between, continuous illumination from street, security and house lights etc. must be well investigated.

Effect of host plant on the predatory mite *Phytoseiulus persimilis***G. FLOROS, G. BRACHOU, A. KOKKARI, S. KONTZIDOU,
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Host plant chemistry and architecture may influence the development and reproduction of predators, by affecting both the predator and the prey. We studied the effects of bean and tomato leaves on the pre-imaginal development, longevity and reproduction of two populations, a Greek (Larissa) and an imported one (PhytoLine p, Syngenta), of the predator mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). Eggs of the plant feeding mite *Tetranychus urticae* Koch (Acari: Tetranychidae) were offered as prey on the surface of the leaves. The developmental period from egg to adult of the predator mite varied depending on the host plant, being significantly longer on tomato than on bean plants. In addition, predator egg production was significantly higher on bean than on tomato leaves. Moreover, on both bean and tomato leaves, predator females of the Greek population lived longer than the females of the imported population. Our results suggest that plant characteristics along with other factors such as the origin of population, should be considered, in biological control programs.

**Influence of irrigation, nitrogen fertilization
and Prohexadion - Ca applications on infestation
of *Lobesia botrana* and *Frankliniella occidentalis***

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In the current study, we investigated the effect of nitrogen and irrigation on the *Vitis vinifera* var. "Xinomavro" in the vineyard of "Kyr Yannis", in the Yannakohori area of Naousa. The experimental design consisted a two-factorial experiment (3x2) with three treatments of nitrogen [0 (N0), 6 (N6), and 15 (N15) units of nitrogen] and two levels of irrigation (irrigation with water corresponding to 60% of the evaporation and null-irrigation). Each treatment was replicated four times. In addition, the effect of the growth regulator Prohexadion - Ca on infestation of *Lobesia botrana* and *Frankliniella occidentalis* was examined. The experimental design involved three treatments: application of Prohexadion - Ca at the flowering period, after the flowering period and at both periods. Each treatment was replicated eight times. Applications were performed from late May to early June. In mid-July, before the ripening of the berries, we examined 100 bunches of grapes from each plot of all treatments for infestations by *L. botrana* and *F. occidentalis*. Results showed that there are statistically significant differences in infestation by *L. botrana* and *F. occidentalis* as well as between the nitrogen and irrigation treatments. In particular, we observed a favorable effect of the increased amount of nitrogen on the infestation by *L. botrana* and *F. occidentalis* at both irrigation treatments. Application of Prohexadion-Ca at the flowering period resulted in significantly lower injuries by *L. botrana* and *F. occidentalis* compared to the untreated control. Furthermore, damages of the flowering period treatment were lower compared to the after flowering period treatment; however no differences were observed compared to the combination treatment. The management of irrigation and nitrogen fertilization as well as the application of Prohexadion - Ca in relation to the infestation of *L. botrana* and *F. occidentalis* is being discussed.

**Management of the pine processionary moth,
Thaumetopoea pityocampa (Lepidoptera: Thaumetopoeidae),
in urban and suburban areas: trials with trunk barrier
and adhesive barrier trap devices**

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In urban and suburban areas, larvae of *Thaumetopoea pityocampa* (Denis and Schiffermüller), cause serious defoliation to *Cedrus*, *Pinus* or *Pseudotsuga* trees, and health problems to humans and domestic or farm animals. In this study, we present the results of biennial trials (2015-2016) on the management of *T. pityocampa* infestations using commercial or LIFE-PISA prototype trunk barrier and adhesive trap devices in Greece (Attica and Volos), Spain (Valencia), and Italy (Molise). In Attica, the commercial trunk barrier trap devices captured significantly more *T. pityocampa* larvae compared to the adhesive barrier trap devices, indicating also their high capture capacity. The total performance of the trunk barriers trap devices was 99.8% in 2015 and 99.6% in 2016. In Volos and Valencia, no significant differences were recorded between captures in commercial and LIFE-PISA prototype trunk barrier trap devices. In Molise, the commercial trunk barrier trap devices exhibited high effectiveness in capturing the larvae during their procession. Moreover, significantly fewer male adults were captured during summer 2016 in comparison with 2015. Similarly, significantly fewer nests were formed on the experimental trees in winter 2016 and 2017 compared with 2015. The results of the present study show the potential of the trunk barrier trap devices as a control method for the management of *T. pityocampa* infestations after long-term application in urban and suburban areas.

Study of the effect of friendly insecticides to environment on the predator *Hippodamia variegata*

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Hippodamia variegata is a native predator species in Greece, and it is considered a very important biological control agent against aphids in many cultivations. The success of IPM programs requires knowledge regarding the effects of insecticides on the biological traits of the beneficial insects.

This study aimed to determine the effects of four insecticides (kaolin, mineral oil, insecticidal soap and pymetrozine) on the voracity, mortality and developmental time of 4th instar larvae of *H. variegata* under laboratory conditions. The voracity of *H. variegata* was significantly affected after the application of all four insecticides. All insecticides effected the 4th instar larvae duration time. The results show that kaolin and insecticidal soap seem to be safe for 4th instar larvae of *H. variegata*. The two other products were moderately harmful for the larvae.

For an effective IPM program, it is important to apply selective insecticides. Further studies are needed to determine the selectivity of these insecticides under field conditions.

This research is implemented through IKY scholarships programme and co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the action entitled "Reinforcement of Postdoctoral Researchers", in the framework of the Operational Programme "Human Resources Development Program, Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) 2014 – 2020.

**Interaction between the entomopathogenic fungi
Beauveria bassiana and *Isaria fumosorosea* on adults
of *Sitophilus granarius* (Coleoptera: Curculionidae)
and *Sitophilus oryzae* (Coleoptera: Curculionidae)**

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The interaction between the entomopathogenic fungi *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) and *Isaria fumosorosea* (Wize) Brown & Smith (Hypocreales: Clavicipitaceae) on young adults of *Sitophilus granarius* (Linnaeus) (Coleoptera: Curculionidae) and *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) were examined.

To study the interaction, young adults of *S. granarius* and *S. oryzae* were sprayed with conidial solutions of the above entomopathogenic fungi at doses of 10^4 , 10^6 , 10^8 conidia/ml. Three replicates were performed for each dose with 10 individuals each. The mortality of the young heifers was recorded daily for 15 days. The interaction between the pathogens was calculated by the formula of Robertson and Preisler: $PE = P_0 + (1 - P_0) * (P_1) + (1 - P_0) * (1 - P_1) * (P_2) (L_0 - L_E) / L_E + (D_0 - D_E) / D_E$. The effectiveness of each fungus was calculated using the Abbott formula, while the average total survival time of the insect bites by the Kaplan-Meier method (non-parametric) was calculated. The comparison of the survival function results was done with the Breslow-Gehan distribution test.

Overall there was a positive interaction between the pathogenic microorganisms used: at most combinations the positive interaction can be seen as synergistic between pathogens, in combined doses expressed as an additive relationship and some negative interaction between the pathogens.

**Innovative device for the prevention of the infections
by *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)
with the use of electric current and attractive substances**

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Prevention is considered to be the most important measure in limiting the spread of most entomological enemies. In the case of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), given the tremendous difficulty in dealing with it, finding an effective way of prevention is an important issue. The subject of this work is an innovative electronic device which is used to prevent the infestation of *R. ferrugineus*. This device uses electrical current and attractants.

The device consists of a rechargeable battery, a photovoltaic panel, insulated cables, a microcontroller (Arduino/Genuino), a power transformer, a protective cover, a watertight box of the device, a fastening system, pheromone evaporators and rain, temperature and humidity sensors. The device transmits information to the user, through a mobile communication network, about the electrical discharges as well as about the status of its operation.

The palm is pruned and the old leaves are removed from their base. The aperture is placed on the pruned stem of the palm tree and is held by straps. Below the cover there are the attractant vaporizers to ensure that the insects coming from the trunk, as well as the flying ones, will move to the holes and not to the inside of the unprotected crown. The photovoltaic panel and the box of the device are mounted in a sunny position. When an insect attempts to pass through the holes to infect the palm tree, the circuit will close and electricity will penetrate the insect and kill it or cause it significant damage. The device transmits information to the user through a SIM card about the discharges it makes or the problems in its operation.

The description of the above apparatus has been filed for obtaining a patent (Patent Pending Number: 2017-01625) and will be sent for evaluation to a research institute in Greece. This endeavor strengthens a large French company and is expected to be released in France, on a commercial scale.

Use of insects as trapping agents for entomopathogenic fungi in prefecture of Achaia

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This study was realized in the framework/within the Interdepartmental Postgraduate programme: "Agrochemistry and Biological Cultivation" of the University of Ioannina and the Technological Institute of Epirus.

The experimental part of the study was carried out in the Institute of Plant Protection in Patras and its aim is the detection of entomopathogenic fungi in grounds within the prefecture of Achaia via various insect bait methods.

Initially, soil samples from four different regions, Glafkos, Institute (surrounding area in the Institute of Plant Protection), Kastritsi and Zaviani. The samples were placed in Petri dishes where 3rd and 4th stage larvae as well as adults from six different insects: *Plodia interpunctella* (Lepidoptera: Pyralidae), *Rhyzopertha dominica* (Coleoptera:Bostrychidae), *Tribolium confusum* (Coleoptera: Tenebrionidae), *Trogoderma granarium* (Coleoptera: Dermestidae), *Ephestia kuehniella* (Lepidoptera: Pyralidae), και *Tenebrio molitor* (Coleoptera: Tenebrionidae) were inserted.

The specific method is based on the already known bait method of entomopathogenic fungi, the Galleria bait method, yet making use of six different species in this study.

The larvae and the young adults were placed by ten in Petri dishes which contained soil from the olive groves. Measurements of dead larvae and adults were carried out every two days. The measurements lasted 14 days. Then, the dead larvae were placed in moist chamber so as to ensure optimum conditions for the growing of the mycelium of entomopathogenic fungi.

**Evaluation of isolations of the entomopathogenic fungi
Beauveria bassiana, *Metarhizium anisopliae* and *Isaria
fumosorosea* against *Ephestia kuehniella* (Lepidoptera: Pyralididae),
Trogoderma granarium (Coleoptera: Dermestidae), *Tribolium
confusum* (Coleoptera: Tenebrionidae)**

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Ephestia kuehniella (Lepidoptera: Pyralididae), *Trogoderma granarium* (Coleoptera: Dermestidae), *Tribolium confusum* (Coleoptera: Tenebrionidae) are characterized as important pests of stored products. In the present study the effectiveness of isolates of *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Isaria fumosorosea* (Wize) Brown & Smith (Hypocreales: Clavicipitaceae) from soils of the Achaia region, on larvae and young adults of *E. kuehniella*, *T. granarium* and *T. confusum* is reported.

For each isolate, a conidia suspension (10^8 conidia/ml) was prepared. Then the larvae (*E. kuehniella*) and young adults (*T. granarium*, *T. confusum*) are sprayed with a suspension and placed on Petri plates containing 10g of the following individual products (crumb nutty/husked rice/durum wheat/corn flour/Whole meal flour/soft flour) where they were monitored daily for 10 days. The efficacy of each fungus was calculated with Abbott's formula and calculated the average total survival time of flourishing and larvae of insects with the Kaplan-Meier method (non-parametric). The comparison of the survival function results was done with the Breslow-Gehan distribution test. Also, in our statistical approach used as a probe agent, the concentration of the suspension of conidia of three entomopathogenic fungus (10^8 conidia/ml), as the dependent variables, mortality, per item mortality and the effect of the product in the larvae (*E. kuehniella*) and young adults (*T. granarium*, *T. confusum*).

From our results we found significant differences both by product and by isolation that were evaluated. The wide development and utilization of entomopathogenic fungi is a new field of research that will lead to an environmentally friendly management of stored agricultural products having as a guideline the protection of the consumer.

Use of unmanned aerial vehicles (UAVs) in Agriculture: three novel crop protection cases

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The aim of this work is to introduce new applications for Unmanned Aerial Vehicles (UAV's) in Agriculture and especially in crop protection. All tests have been carried out in the island of Crete in Greece. Specific applications are: a) detection of symptoms (canopy discoloration) caused by pest or disease infestation on olive trees, b) mapping of Palm trees and identification of visible signs of infestation by the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in large palm plantations, c) co-operation of UAVs with electronic traps that automatically count insects and send counts to a server which, in turn summons and direct drones to spray in designated areas. Equipment and software, step by step methodology and practical applications of each of the three cases are described. We seek new application of drones in view to expand their possible uses that will allow them to penetrate the business of small to medium sized regional stakeholders. This work puts its emphasis on the practical details of the implementations, serving as a useful reference to relevant field-work. This work was supported by the European Commission Horizon 2020, FTI Pilot-2015-1 under grant agreement n° 691131, project REMOSIS.

Electrospun micro/nanofibrous matrices as sustained-release systems of mosquito repellents

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Competition

Mosquitoes are insects of public health concern. Repellents that are applied on the skin are effective only for a limited time period, while their direct application on fabrics has been proven inefficient in prolonging their repellency. The incorporation of biopesticides in micro/nanofibrous matrices is a promising alternative to other methods, since fibrous systems can extend in time the release of the repellent agent. Electrospinning is a versatile technique for the production of micro/nanofibers from polymeric solutions. Electrospun tailor-made nanofibrous matrices with high surface-to-volume area, high porosity and small pore size can be produced through an electrically driven jet of a polymer solution under the application of high voltage. In this work, micro/nanoelectrospun nonwovens of different biopolymers containing various concentrations of either citronella essential oil or picaridin were prepared. Different formulations of electrospun fiber mats were tested using low-cost biopolymers of hydrophobic and hydrophilic nature, such as cellulose acetate and poly-vinylpyrrolidone, respectively in various layers and blends, for controlling the release rate of the embedded repellents. The fibrous structure of the nonwoven matrices was analyzed using scanning electron microscopy and the release profile of the incorporated repellents was determined by gas chromatography-mass spectrometry. The repellent activity of the electrospun matrices was evaluated in a chamber hosting approximately 50 *Aedes albopictus* female mosquitoes and the number of landings on the hands of two persons was counted in the presence and absence of the tested electrospun matrices. The different formulations exhibited variable release rates, however in all cases the designed micro/nanofibrous systems presented a substantially prolonged repellency for more than 60 days. The present study demonstrates that micro/nanofibrous matrices constitute a promising sustained-release system for mosquito repellents. Selection of an appropriate polymer and the loading level of the repellent agent can have a significant impact on the release rate and the effective duration of such a dispenser.

The effect of wind velocity on the application of mating disruption method in *Lobesia botrana*, using (VP) ISONET L impregnated rings dispensers, in Litochoro Pieria (Greece)

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Competition

Lobesia botrana (Denis and Schiffermuller) (Lepidoptera: Tortricidae) is the most destructive pest of grape cultivation in Greece. In order to achieve integrated pest management (IPM) strategy for *L. botrana*, research on mating disruption was undertaken, where the objective was to evaluate the potential role of the method in *Lobesia botrana*, in a commercial vineyard of E. Tsantalos S.A. in Litochoro Pieria area, concerning the varieties Agiorgitiko, Cabernet, Malagouzia, Asyrtiko and Chardonnay, using (VP) ISONET L pheromone, impregnated rings dispensers of Anthesis (LLC). The dispensers were placed at different densities in the interior and in the periphery of the vineyard. To ensure adequate control of *L. botrana* due to the increased infection in the previous year (20%), an application with an insecticidal formulation was necessary before the emergence of its first generation. In the northern part of the vineyard and in the center, the wind flow velocity was recorded to compare the protection results in the harvest period.

The results showed that the efficiency of the method using ISONET L dispensers within the vineyard and in the periphery, in all five varieties that were present, was particularly satisfactory except for the north (windswept) boundaries, where the results were almost marginal, due to the wind flow velocity which was higher than the rest of the vineyard's areas during the critical months of July and August (1.9 m/s and 1.8 m/s, respectively). Probably, due to this cause, was the greatest damage on the northern (windswept) boundaries of the vineyard, as in the center the values of wind flow velocity were lower in these two months (1.65 m/s and 1.6 m/s, respectively).

Genetic therapy and synthetic biology technologies in the construction of gene silencing pesticides

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The development of insecticidal formulations with new mechanisms of action (MOAs) is a priority for industry. Gene silencing through RNAi technology provides an alternative, environmentally friendly strategy to combat insect pests. Digestion of double-stranded RNAs (dsRNAs) targeting specific developmental insect genes induces RNAi and leads to an inhibition of growth and reproduction, resulting to their death. As with any new technology, the complex nature of RNAi's cellular machinery has created a variety of technical problems mainly related to the stability and residual activity of dsRNA molecules in their target cells, their ability to penetrate the biological membranes, and finally their chemical stability in the environment and inside the insect's circulatory system as well. The above facts generate needs for the development of auxiliary substances which will favor cellular penetration and protect the insecticidal dsRNA molecules from the biochemical environment of their target insects while at the same time will increase gene silencing and mortality levels. Technologies used in the pharmaceutical industry for the treatment of genetic diseases seem to be able to solve the technical problems mentioned previously. These technologies are based on nanotechnology applications using auxiliary molecules that protect dsRNAs and enhance the effectiveness of RNAi. Such helper molecules are protein-chimeras known as PTD-DRBD peptides which have been studied with remarkable results in therapeutic RNAi applications.

In this work we are exploring the possibility of using PTD-DRBD peptides as insecticidal carriers of siRNA and dsRNA molecules to control the Lepidopteran pest of corn *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Our research is expected to provide answers to questions related to the efficiency of RNAi in the target insects, the protection of siRNAs and dsRNAs from problematic environments and the minimization of the production costs of commonly used nanotechnologies.

Contaminated prey toxicity effect on two aphidophagous predators of the Coccinellidae family

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Coccinellids are important predators of many aphid species, but attempts to incorporate them into integrated pest management (IPM) strategies have resulted in varied success. The variable response may, in part, be due to exposure to insecticide residues on plant surfaces and contaminated prey, both through mortality.

In the present study, the sub lethal effect (LD_{10}) of two insecticides is studied, through the consumption of prey contaminated with, imidacloprid (Confidor 20SL, Bayer Crop Science Hellas SA) and deltamethrin (Decis 2,5EC, Bayer Crop Science Greece SA) in 4th instar larvae of *Coccinella septempunctata* L. and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) under laboratory conditions. The aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae) was used as prey.

The results showed that the deltamethrin effected 4th instar larvae developmental time and weight in *C. septempunctata*. In case of *H. variegata*, imidacloprid and deltamethrin found that have no effect on 4th instar larvae developmental time and weight.

The results of the present study, indicates the necessity for field research in order to draw more accurate conclusions.

«This research is implemented through IKY scholarships programme and co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the action entitled "Reinforcement of Postdoctoral Researchers", in the framework of the Operational Programme "Human Resources Development Program, Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) 2014–2020».

Toxicity of two synthetic insecticides on the larvae of *Coccinella septempunctata* and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)

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Predacious ladybirds of the family Coccinellidae are some of the most effective aphid natural enemies. As they are being used as bio-control agents in Integrated Pest Management programs, is important to study the effects of insecticides on these natural enemies.

In the present study, two insecticides that are being used to control aphids -imidacloprid (Confidor 20SL, Bayer Crop Science Greece SA) and deltamethrin (Decis 2.5 EC, Bayer Crop Science Greece SA)- have been tested on 4th instar larvae of *Coccinella septempunctata* L. and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), under controlled conditions in the laboratory. The predator populations were collected from Kalamata. The leaf deep test method was used.

Deltamethrin was highly toxic to 4th instar coccinellid larvae of both predators at the recommended dose. The mortality caused by imidacloprid was 70% and 100% for *C. septempunctata* and *H. variegata* respectively.

The present data should be considered with caution and it is important that it is supplemented with field trials, to provide a final conclusion about the negative effects of the pest control programs on the field populations of the two coccinellid species.

«This research is implemented through I.K.Y. scholarships programme and co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the action entitled "Reinforcement of Postdoctoral Researchers", in the framework of the Operational Programme "Human Resources Development Program, Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) 2014–2020».

Integrated Control Information System with free software for the control of the *Bactrocera oleae* (Diptera: Tephritidae)

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For the optimization of the effectiveness of ground bait sprays, several technologies have been developed, in recent years, towards "Precision Agriculture" for the control of the *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). However, the cost of the materials, the complexity of technological developments, and the difficulty of transforming them into a complete commercial scale product, make it difficult to be disseminated under the framework of a national control program. The aim of the present study was to develop a direct and economical technological solution and to implement an integrated information system for monitoring and control both of olive fruit fly population as well as of the application of the bait sprays that: a) it is not dependent on specific manufacturers, b) it will be implemented on servers that everyone could have access without cost, c) will be use simple free applications and technology that isn't complicated, d) all the recorded information could be transferred immediately (in real time) and could be readily available on-line to each interested person, (e) the mode of implementation could be open to anyone for "copying". The proposed system used mobile phones with GPS for monitoring of *B. oleae* population as well as for the control of the application of ground bait sprays. This system was developed and pilot operated to about 13,000 ha in two regions of Chania during the years 2015 and 2016. The results of the system are presented and commented.

Effect of organic-certified insecticides and copper fungicides on prey consumption by *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae)

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Competition

Macrolophus pygmaeus (Rambur) (Hemiptera: Miridae) is a polyphagous predatory insect that occurs in Mediterranean agro-ecosystems in vegetable crops. The purpose of this study was to evaluate the effect of six widely used pesticides and fungicides in organic farming on the prey consumption and mortality of the predator *M. pygmaeus*. *Bacillus thuringiensis* subsp. *kurstaki*, *Beauveria bassiana*, paraffin oil, copper hydroxide, copper oxychloride and wettable sulfur were used. Deionized water and chlorpyrifos were used as controls. The effects were studied in tomato leaflets by the leaf dip method. The prey [(eggs of *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae)] had been sprayed and placed on the leaflet. Each leaflet was placed in a Petri dish where a 5th instar nymph of *M. pygmaeus* was introduced. The nymph had been sprayed or not. The nymph was starved for 24 hours prior to its use. The dishes were maintained at 25±1°C and 65±5% RH at 16:8 L:D. The prey consumption was recorded after 24 hours. The consumed prey was distinguished in three sub-categories: Little-, half-, and completely consumed. The mortality of the predator nymphs was monitored for a period of 7 days after treatment. Fifteen nymphs were used per treatment. The results showed that copper oxychloride and *B. bassiana* caused significant mortality. Total consumption increased significantly after treatment with paraffin oil and Bt while was significantly reduced by *B. bassiana*. A significantly higher number of half-consumed eggs were found in the treatments where a sprayed nymph had been used as well as after the intervention with copper hydroxide, paraffin oil and wettable sulphur. *B. bassiana* significantly reduced the number of fully consumed eggs in comparison to the control. These results indicate that some organic certified insecticides and copper fungicides can cause mortality and affect the predatory behavior of *M. pygmaeus*. Moreover, the use of paraffin oil caused an elevation in prey consumption but the reverse occurred after applying *B. bassiana*. It was shown that distinguishing the consumed prey in sub-categories may offer useful information on the effects of pesticides on the predatory behavior of *M. pygmaeus*.

Population fluctuation, parasitism and chemical control of *Paraleyrodes minei*, *Dialeurodes citri* and *Aleurothrixus floccosus* (Homoptera: Aleyrodidae) on Citrus in Chania, Crete

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Competition

Whiteflies are a potential major insect-pest for citrus, causing direct and indirect damage. They damage directly by sucking the leaves and secreting honeydew, on which sooty mould can develop, which reduces photosynthesis. Indirectly they can cause damage by vectoring and transmitting plant pathogens.

The aim of the work is to study the population fluctuation, parasitism, and the effectiveness of the chemical control of *Paraleyrodes minei*, *Dialeurodes citri* and *Aleurothrixus floccosus*. Samplings for population monitoring and parasitism were carried out fortnightly in citrus orchards at Platania area of Chania and lasted from April 2016 to June 2017. Collected leaves were sent immediately to the Laboratory of Agricultural Zoology and Entomology of the Agricultural University of Athens, where the individuals of the whiteflies were recorded (live/dead/parasitized). The parasitized individuals, together with a piece of the leaf that were attached, were placed in pharmaceutical *gelatin capsules*, until the adult parasitoid emerged. For the efficacy of insecticides, experiments were carried out in a citrus orchard in the same area, during the period May-July 2017. In these operations approved insecticides with pyriproxifene, spirotetrat, imidacloprid, acetamiprid, thiamethoxam and mineral oil (2%) were used. Foliage sampling was performed at -1, 7, 14, 30 and 45 days after the operation, during which 3 trees of each operation, 20 leaves/tree (60 sheets/total) were collected. The leaves were sent immediately to the Laboratory of Agricultural Zoology and Entomology of the Agricultural University of Athens, where the individuals of the whiteflies were recorded (live/dead/parasitized). The results showed that the most abundant whitefly species was *D. citri*, followed by *A. floccosus*, while *P. minei* was found in low population density. Significant differences in the efficacy of insecticides in the treatment of whiteflies have also been observed. Detailed results and discussion are provided in the presentation.

Evaluation of isolations of various entomopathogenic fungi against *Sitophilus granarius* (Coleoptera: Curculionidae)

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In the present study the effectiveness of isolates of *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metchnikoff), Sorokin (Hypocreales: Clavicipitaceae), *Chaetomium acropullum* (Chaetomiaceae), *Chaetomium globosum* (Chaetomiaceae), *Chaetomium truncatulum* (Chaetomiaceae), *Apophysomyces ossiformis*, *Trichoderma gamsii* and *Aspergillus alliaceus* (Eurotiales: Trichocomaceae) from soils of the Achaia region, on young adults of *Sitophilus granarius*.

For each isolate, a conidia suspension (10^8 conidia/ml) was prepared. Thereafter, they were spraying with suspension and placed in Petri dishes with 10g of product where they were monitored for 7, 14, 21 and 28 days. The efficacy of each fungus was calculated using the Abbott formula and the mean overall survival time of the bites by the Kaplan-Meier method (non-parametric) was calculated. The comparison of the survival function results was done with the Breslow-Gehan distribution test. Also, in our statistical approach, we used the concentration of the conidia of entomopathogenic fungi (10^8 conidia/ml) as an examining factor, and as the dependent variables, the mortality of the young adults.

Exploring the use of entomopathogenic fungi as a biological target is intended to ensure both the quality of the agricultural products produced in our country.

**Economic injury level of *Helicoverpa armigera*
in tobacco cv. Basma****G.D. BROUFAS^{1,*}, K. SAMARAS¹, V. MORAITIS¹, S. KOMPTSIS²,
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The Economic Injury Levels (EIL) of *Helicoverpa armigera* in tobacco cv. Basma were determined under field conditions. The experiment was conducted in the area of Agriani (Northern Greece) during the summer of 2016. Insect cages were established in tobacco fields with six treatments of larval moth densities ranging from 0 to 5 larvae/row meter. The artificially infested plants were marked and inspected till pupation; moth larval survival and developmental stage (according to their approximate length size) were recorded every other day. At the end of the larval feeding period, the leaves from the experimental tobacco plants were harvested and their biomass (g/plant) was recorded. Field collected data and economic parameters of the crop value and the cost of an insecticide curative application was further used for the estimation of the EIL which was estimated to 0,5 larvae/m². We discuss the importance of this value in the development and implementation of an integrated pest management program in tobacco cv. Basma in Northern Greece.

Evaluation of plant derived insecticides and pathogenic fungi against phytophagous Hemiptera (Hemiptera: Hemiptera)

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Competition

Hemiptera (Hemiptera) are major pests of various crop species, causing damage in the yield and the quality of production. Their control is difficult, since the effectiveness of insecticides on them is often quite low, especially in biological farming, when synthetic insecticides cannot be used. The aim of this study was to evaluate several non-chemical insecticides for their effectiveness against heteropterans on goji-berry and capper. For this reason, field and laboratory experiments accomplished in Lechaeo (near Corinth, goji-berry) and the experimental field of Agricultural University of Athens (capper), during 2017. In these experiments, extract from *Melia azedarach*, neem-oil, spinosad, as well as the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were tested for their efficacy on *Spilostethus sp.* (Hemiptera: Lygaeidae) and *Eurydema sp.* (Hemiptera: Pentatomidae). The experiments included foliar applications on goji-berry shrubs, as well as bioassays in petri dishes with capper. Evaluation of the formulations was made by visual observation of the shrubs and the dishes for the presence of insects, alive or dead. Detailed information on the results and discussion is given in the presentation.

Effectiveness of *Melia azedarach* extract against mulberry whiteflies without affecting silkworm survivorship

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Competition

Sericulture is the art of silk production, which comprises cultivation of mulberry, silkworm rearing and post-cocoon activities leading to production of silk fiber. Mulberry is affected by several genera of whiteflies, which feed on the plant sap, excrete honeydew and can transmit pathogens. Recently, two whitefly species infesting white mulberry were recorded in Greece: *Pealius machili* and *P. mori* (Homoptera: Aleyrodidae). Whitefly management is very difficult, since in Greece there aren't any registered insecticides for use on mulberry. Moreover, synthetic insecticides could be harmful for silkworms fed on them.

The purpose of this research was to evaluate the effectiveness of plant-derived extracts against mulberry whiteflies and their side effects on silkworms fed on treated mulberry leaves.

The experiment involved laboratory treatments to test *M. azedarach* natural extract compared to the synthetic insecticide lambda-cyhalothrin. Mulberry leaves infested by whiteflies were placed in Petri dishes and sprayed with the aforementioned substances, while a batch of them remain unsprayed and served as control. At the same time, another group of infested leaves were received the same treatment and they were given to silkworms as feed.

Results have shown that significant differences observed in whitefly mortality among treatments, which was significantly higher in *Melia* extracts and lambda-cyhalothrin compared with the control, 24 hours after application. Although, l-cyhalothrin treated leaves were extremely toxic to silkworms, in contrast to *Melia* extracts in which no negative effect was observed.

These results were quite encouraging and might be considered as parts of a potential integrated pest management program against mulberry whiteflies.

Nymphal development of *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) on the flowering plant *Calendula officinalis*

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Competition

Studies on the potential of flowering plants to support the development of omnivorous predators offer valuable information for the selection of host plants to be used in conservation strategies of those natural enemies. The nymphal development of the predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) was studied on the ornamental species *Calendula officinalis* (Asteraceae). First instar nymphs (<24h) were individually placed in Petri dishes in which were placed: a flower, a flower and food (lepidopteran eggs), a leaf with stem or a leaf with stem and food. In each treatment 15 replicates were used. The Petri dishes were put in a growth chamber at $25\pm 1^\circ\text{C}$, $60\pm 5\%$ R.H. and a photoperiod of 16L:8D. The results showed that *M. pygmaeus* completes its nymphal development in all treatments. Mortality rates ranged below 10% and only in the case of the leaf with stem the mortality rate increased to 26%. The duration of nymphal developmental period was significantly shorter in the presence of flower and food (14.69 days in average) without significantly differing from that in the case of the flower (15.73 days). However, the duration in the case of the leaf with stem and food was significantly higher than the flower with food. The longest duration was recorded when the nymphs were grown on a leaf with stem with no food. These results reveal that the flowers and leaves of *Calendula officinalis* are suitable for the nymphal development of *M. pygmaeus*.

Intraguild predation among three aphidophagous predators

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In biological control the combined use of natural enemies has been suggested as a strategy to enhance pest regulation. Therefore, studying Intraguild Predation (IGP) effects offers valuable information in the optimal use of multiple biological control agents. In this work, IGP interactions and their consequences in prey consumption were investigated among three aphidophagous predators and their instars in combinations of two or three species i.e. *Adalia bipunctata* (2nd, 3rd and 4th larval instar), *Macrolophus pygmaeus* (2nd and 5th nymphal instar) and *Aphidoletes aphidimyza* (2nd and 3rd larval instar) in the absence and presence of extraguild prey (i.e. the aphid *Myzus persicae*), in Petri dishes with eggplant leaves at 25±1°C. The densities of aphids used were similar or higher to the satiation level of the predators. In the absence of extraguild prey, larvae of *A. bipunctata* always preyed upon and nymphs of *M. pygmaeus* killed a high proportion of the larvae of *A. aphidimyza*. Direct IGP between *A. bipunctata* and *M. pygmaeus* was not recorded but evidence for avoidance IGP behaviour occurred and another experiment revealed that *M. pygmaeus* preyed upon the eggs of *A. bipunctata*. In aphid presence, in pairwise combinations there was no adverse effect and even more, when released all the three species together in most cases the observed predation rates were significantly higher than the expected. Partial aphid consumption rates showed evidence for IGP interactions between *A. aphidimyza* and *M. pygmaeus*. Overall, in prey shortage, *A. bipunctata* interactions with *A. aphidimyza* indicate their little potential to be combined but between *A. bipunctata* and *M. pygmaeus* weak interference was recorded. Under high aphid prey densities the results showed that the combined use of these predators can enhance efficacy of biological control.

Effect of insecticides on the prey consumption by *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae)

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Competition

In the current study, the influence of four insecticides to the prey consumption by the omnivorous mirid bug *Macrolophus pygmaeus* Rambur was investigated.

The chemical substances of the insecticides that were examined are the following; *Bacillus thuringiensis* subsp. *kurstaki*, pyrethrins, spinosad and potassium salts of fatty acids. Four different types of treatments for each insecticide were implemented; sprayed insect and sprayed leaflet, sprayed insect and unsprayed leaflet, unsprayed insect and sprayed leaflet and ultimately unsprayed insect and unsprayed leaflet. In the experiment the highest recommended dose for the greenhouse tomato crop was conducted. Each treatment included 12 repetitions. Thus, 12 5th instar nymphs were used in order to examine each treatment. After 24 hours, the number of sucked *Ephestia kuenhiella* eggs was estimated and mortality of the insects was checked for four days.

The results indicated that the egg consumption was highest in the Bt treatments. The number of slightly-sucked eggs was higher after treated with Bt and pyrethrins in comparison to the other insecticides. When the leaf and the predator nymph were unsprayed the egg consumption was higher in the case of Bt than the control but significantly reduced in the treatments of pyrethrins and spinosad. The effect of treatment was significant in the number of half-consumed prey that was increased when the nymph was not sprayed. The nymphal mortality was kept at very low levels. Therefore, insecticides that are considered as safe for *M. pygmaeus* may have effects on its prey consumption.

The use of terpenes against the plant-parasitic nematode *Ditylenchus dipsaci*

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Competition

Management of the plant-parasitic nematodes has become more difficult due to reduced availability of effective nematicides. Alternative nematode control methods need to be established that would be less harmful for the environment and the user. In the effort to develop such methods, research on substances of natural origin with strong biological activity against nematodes has been intensified. Terpenes belong to this category of substances and they have been shown to possess effective nematicidal activity.

The current study is the first attempt to evaluate and investigate the nematicidal activity of terpenes against the stem and bulb nematode *Ditylenchus dipsaci* . (Kühn, 1857) Filipjev, 1936. We conducted laboratory experiments under in vivo conditions, to study possible nematicidal effect of four terpenes (carvacrol, geraniol, eugenol, thymol) against nematodes isolated from infested garlic cloves.

In particular, the effect on the survival of the nematodes using each one of the four terpenes separately in seven different concentrations (62.5–2,000 µg/mL) were studied at three exposure periods. The observed nematicidal activity of terpenes in descending order was carvacrol>eugenol>geraniol>thymol. Additionally, carvacrol exhibited the highest activity showing 100% mortality when it was tested in the concentration of 2,000 µg/mL. Our results indicate that there is a positive correlation between the concentration and the exposure time. In addition, increasing the dose of each terpene increased paralysis rate was recorded either in all biological stages or the larvae of *D. dipsaci*. Our results suggest that carvacrol and eugenol could be used as effective alternative of the management of *D. dipsaci*.

Functional response and multiple predator effects of two generalist predators preying on *Tuta absoluta* eggs

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The objective of our study was to examine the effects of intra- and interspecific interactions between the two predators *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) on the predation rates of eggs of the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Initially, we conducted experiments to identify the functional response of 5th instar nymphs of the predators when single individuals preyed on *T. absoluta* eggs (densities: 10, 30, 50, 70, 90). The main goal was to identify whether these two predators can be used against the eggs of the pest to suppress their populations. To identify the nature of intra- and interspecific interactions, two 5th instar nymphs of the two species were placed together with three combinations (*M. pygmaeus* – *M. pygmaeus*, *N. tenuis* – *N. tenuis* and *N. tenuis* – *M. pygmaeus*) in Petri dishes with *T. absoluta* eggs, at the same densities as previously. The main results were that the two predators exhibited Type III functional response and the combination of the two species gave better pest suppression at high prey densities of the eggs, whereas at low egg densities intraspecific interactions of *N. tenuis* were weaker than the other combinations and resulted to stronger pest population suppression.

**Plant-mediated effects by different populations
of the zoophytophagous *Macrolophus pygmaeus*
on spider mites and aphid pests**

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Zoophytophagous predators are a large group of natural enemies that includes species that are widely used for the biological control of agricultural pests. These natural enemies feed on plant pests, as well as on plant tissue and their phytophagy has been shown to result in the induction of plant responses. For example, tomato plants exhibited an increased resistance to spider mites after having been exposed to the phytophagy by the zoophytophagous predator *Macrolophus pygmaeus*. This response was shown to be associated with the induction of jasmonic acid (JA)-mediated defences (i.e. protease inhibitor activity), analogous to the plant response to phytophagy by herbivores. The fact that omnivorous predators can induce plant defences raises many interesting questions on the ecological implications of this phenomenon. In the present study we assessed the plant-mediated effects of phytophagy by different populations of the zoophytophagous predator *M. pygmaeus* on the two-spotted spider mite (*Tetranychus urticae*) in tomato and on the green peach aphid, *Myzus persicae* in pepper. Tomato or pepper plants were exposed for four days to females of six different *M. pygmaeus* populations. Subsequently, the mirid bugs were removed and plants were infested with spider mites or aphids for another four and eight days, respectively. After this, we recorded spider mite oviposition and survival as well as aphid nymph/adult number. We found all *M. pygmaeus* populations to have negative plant-mediated effects on spider mite oviposition on tomato, while having a positive impact on aphid numbers on pepper plants. This demonstrates that *M. pygmaeus* differentially affects pests depending on the pest species and their feeding type. These effects were expressed irrespectively of the origin of the predator populations. Further experiments are required to assess the effects of the different *M. pygmaeus* populations on the population dynamics of its prey via the host plant.

Prey searching behaviour of the aphidophagous predator *Macrolophus pygmaeus*

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Searching behaviour of predatory insects plays vital role in locating their food resources with implications in their effective use in biological control. In this work the effects of leaf size, leaf trichome density and prey distribution were investigated on the foraging behaviour of the predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae). For this purpose, its prey consumption was searched when prey was offered on different structured prey patches on leaves of variable size of the apex, top, middle and lower plant strata. All the leaves bore 5 leaflets. In each treatment the prey was offered on a pair of leaflets. Four different leaflet combinations (prey patch arrangements) were tested in each leaf size category. As prey, 10 2nd instar nymphs of the aphid *Myzus persicae* were stuck on the underside of each selected leaflet. The leaf with the aphids was enclosed in a muslin cage on the plant. In the cage a starved 5th instar nymph of *M. pygmaeus* was introduced. The plants were placed at 25±1°C and 16 hours of daylight. Prey consumption was recorded after 24h. The highest consumption was recorded on the lower leaves followed by the middle, without differing to each other. The consumption was lowest on the apex leaves that was significantly lower than that on the middle and the lower leaves. The effect of prey patch arrangements significantly affected the results. Finally, prey consumption was similar among all the tested prey patch arrangements in the apex and middle leaves. The lower leaves had a significantly larger surface area than that of the other leaves. The density of the trichomes on the upper surface of the apex leaves was significantly higher than that of the other leaf types, the other did not differ. The trichomes on the under surface were of higher density on the apex leaves but did not differ significantly to the other leaf types. Therefore, prey consumption of *M. pygmaeus* depends on the leaf identities, was highest on leaves of larger surface and should be further studied.

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Effects of the endophyte strain *Fusarium solani* K on the two-spotted spider mite *Tetranychus urticae*

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Unable to run away from their attackers, plants often interact with both arthropods and microorganisms. Besides pest organisms e.g., herbivores and pathogenic microorganisms, plant interactors also include beneficial organisms such as predators and microbes that can act as biocontrol agents against pests. Soil-borne beneficial microbes in particular are known to elicit plant responses or alter plant quality and are therefore capable of protecting plants against herbivores. Furthermore, these microbes may also interact with natural enemies, potentially strengthening biocontrol. Whereas plant-pathogen and plant-herbivore interactions currently form well-established research fields, plant-microbe-arthropod interactions are rarely addressed. The aim of this study was to assess the effects of multi-species above-belowground interactions among herbivores and microorganisms in tomato. Specifically, we focused on interactions among spider mites and endophytic fungi and assessed the effects of the proprietary *Fusarium solani* strain K (FsK), an endophytic fungus isolated from the roots of tomato plants grown on suppressive compost, on the performance of the two-spotted spider mite *Tetranychus urticae*, a highly polyphagous pest attacking tomato and a variety of other crops. In addition, we recorded the plant-mediated effects of FsK on the behavior of *Macrolophus pygmaeus* which is a natural enemy of *T. urticae* in tomato. Tomato plants were inoculated with the endophyte and spider mite oviposition, survival and feeding damage were recorded when plants were five-six weeks old. *Macrolophus pygmaeus* preference for FsK-inoculated over control (non inoculated) spider mite-infested plants was assessed with olfactometer tests. We found that spider mite performance was negatively affected on FsK-inoculated plants and % damaged area was lower on these plants compared to control plants. In addition, *M. pygmaeus* was shown to prefer FsK-inoculated over non-inoculated plants that were infested with spider mites. On the other hand, FsK was shown to confer positive effects on plant growth parameters and that *T. urticae* did not affect its colonization. The results of our study will help better understand spider mite-plant-microbe interactions and explore novel biocontrol strategies for sustainable crop production.

**Investigation on the presence of *Meloidogyne* species
and their virulence on resistant tomato hybrids
in greenhouses of Kyparissia, Greece**

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Competition

Root-knot nematodes (RKN) (*Meloidogyne*, *Nematoda*: *Heteroderidae*) belong to the most important plant pathogens of greenhouse crops worldwide. In the current study soil samples were collected from greenhouses of Kyparissia region (South-Western Greece). RKN second stage juveniles (J2s) were detected in 44 of the 45 collected samples with 38 of them presenting a high density of J2s. PCR amplifications with specific primer sets Fjav/Rjav, Far/Rar and Mi2F4/Mi1R1 were used for the detection of *Meloidogyne javanica*, *M. arenaria* and *M. incognita* respectively. *M. javanica* was detected in 29 and *M. incognita* in 4 of a total of 32 identified populations. Virulence tests with resistant tomato (*Mi*-gene) in controlled conditions revealed the existence of 15 resistant-breaking populations, which are observed in a percentage of 34% of the 44 samples where RKN had been found. The current research demonstrates the problem of RKN in the region of Kyparissia, the absence of efficient control and the necessity of integrated management strategies which have to be promoted to growers. Prevalence of *M. javanica* in the area and the presence of virulent populations on resistant tomato hybrids should be taken into account.



4th Session
Public Health and Stored Product Insects

Efficacy of alpha-cypermethrin, chlorfenapyr and pirimiphos-methyl applied on polypropylene bags for the control of *Prostephanus truncatus* (Horn), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.)

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We examined the immediate and delayed mortality of adults of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on surfaces of polypropylene storage bags under different treatment scenarios with alpha-cypermethrin, chlorfenapyr and pirimiphos-methyl. These scenarios were: only one surface of the bag was sprayed and insects were exposed on sprayed or unsprayed surface or both surfaces were sprayed. We evaluated the immediate mortality for 1, 3 and 5 days (d) of exposure. Then, we transferred the surviving adults to untreated surfaces of the same type of storage bags and measured the delayed mortality after 7 d of exposure. We also evaluated the effect of insecticidal treatments on the numbers of holes/ bites made by the activity of *P. truncatus* and *R. dominica* on storage bags. In terms of immediate mortality, chlorfenapyr and pirimiphos-methyl were very effective against all three species and alpha-cypermethrin against *P. truncatus*. We noticed high mortality values of all species to chlorfenapyr and pirimiphos-methyl, even after 3 d of exposure, reaching 100% in many of the cases examined. For the majority of exposure intervals, insecticides and insects, we did not find significant differences in mortality counts between exposures on sprayed surfaces of the bag. Immediate mortalities of insects exposed on the untreated surface of the bag did not differ significantly with mortalities at the surface single treated with alpha-cypermethrin and pirimiphos-methyl. Concerning delayed mortality, all *S. oryzae* adults were found dead 7 d after their transfer to untreated bags irrespectively of the treatment. The delayed mortality of *P. truncatus* and *R. dominica* adults was either complete (after exposure to chlorfenapyr) or almost complete (after exposure to alpha-cypermethrin). The exposure of *P. truncatus* and *R. dominica* to alpha-cypermethrin almost completely suppressed the numbers of holes/ bites.

Assessment of Carifend® for insect control in stored tobacco**C.I. RUMBOS¹, M. SAKKA¹, S. SCHAFFERT², T. STERZ², J. AUSTIN³,
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Carifend®, an alpha-cypermethrin-impregnated net, was evaluated against adults of the two major insect pests of stored tobacco, i.e. *Lasioderma serricorne* and *Ephesia elutella*, in laboratory, semi-field and field assays. In the field assay, Carifend® Multi-Cubicles (CMC) and pheromone-baited traps were used. The field assay was conducted in a commercial tobacco storage facility and was based exclusively on the naturally-occurring population of *L. serricorne* and *E. elutella*, whereas in the semi-field assay adult beetles and moths were released in the facility. In the first laboratory assay, Carifend® material was adjusted at the bottom of plastic Petri dishes, whereas an additional series of dishes with net material without insecticide was also prepared and served as positive control. Finally, another series of dishes, without net material was used as negative control. Twenty mixed sex adults of *L. serricorne* or *E. elutella* were placed in each dish. Insects were exposed in the treated and untreated substrates for 1, 5, 15, 30, 60 and 120 min and for 12 and 24 h. Mortality and knockdown effect was measured after the termination of each exposure. After these exposure intervals, all alive individuals were removed and placed in untreated plastic dishes and “delayed” mortality was assessed after 1, 3, 5 and 7 d. The same experimental design was followed in the second laboratory assay, in which insecticide-treated nets with different concentrations of alpha-cypermethrin were evaluated against the same insect species.

In the field assay, the number of individuals captured inside the CMCs was minimal, in comparison to the number of insects that were captured outside of the system. Similar results were recorded also in the semi-field assay. In the first laboratory assay, immediate mortality was very low for both species tested, even after 24 h or exposure. In contrast, knockdown was high, especially for exposure intervals longer than 60 min. After insect removal from the treated net, the high knockdown percentages were gradually transformed in most cases to “delayed” mortality. In the second laboratory assay, the increase of the dose rate in the net did not necessarily increase mortality of the exposed *L. serricorne* and *E. elutella* adults. Overall, the results of the present study clearly indicate that Carifend®, at least at the conditions tested here, can provide a satisfactory level of protection for stored tobacco against *L. serricorne* and *E. elutella* adults.

Laboratory evaluation of seven novel pyrrole derivatives as grain protectants against two major stored product insect species *Tribolium confusum* and *Ephestia kuehniella*: influence of temperature, relative humidity and grain

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Members of the pyrrole group are likely to have interesting properties that merit additional investigation as insecticides at the post-harvest stages of agricultural commodities. In this context, the insecticidal activity of seven novel pyrrole derivatives was evaluated (under the trivial names 3a, 3g, 3i, 3k, 3l, 3m and 3h), against adults or larvae of *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) and larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) at different doses (0.1, 1 and 10 ppm), exposure intervals (7, 14 and 21 days or 1, 2, 7, 14, 21 days), temperatures (20, 25 and 30°C), relative humidity (RH) (55 and 75%) levels and commodities (wheat, maize, barley). For *T. confusum* adults, in the case of the pyrrole derivative 3k, mortality reached 67.8% at 30°C and 55% RH in wheat treated with 10 ppm after 21 days of exposure. All *T. confusum* adults were dead at all doses on barley treated with 3i after 21 days of exposure. For *T. confusum* larvae, in the case of 3i, at the highest dose, mortality was 82.2% at 25°C and 55% RH whereas in the case of 3k it reached 77.8% at the same combination. For barley, all exposed larvae were found dead at all doses of both pyrrole derivatives after 7 days of exposure. On maize, mortality exceeded 96% with 3k at 10 ppm after 21 days of exposure. For *E. kuehniella* larvae, on barley, after 14d of exposure all larvae were dead at 0.1 ppm of 3i. For the pyrrole derivatives 3a, 3g, 3l, 3m and 3h, mortality of *T. confusum* and *E. kuehniella* on maize was much lower on treated maize than barley or wheat. However, 100% control of both species was recorded only on treated barley. The results of the present study indicate that the pyrrole derivatives tested could serve as grain protectants against noxious stored-product insects under certain biotic and abiotic conditions.

***Sitophilus oryzae* L. (Coleoptera: Curculionidae):
food preference in different varieties of wheat seeds**

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Sitophilus oryzae L. (Coleoptera: Curculionidae) is an important threat to stored products such as beans, nuts, wide variety of grains (with preference for wheat), even fruit. The adults also eat flour. The rice weevils have a nearly cosmopolitan distribution, occurring throughout the warmer parts of the world. In Europe they are replaced by the temperate Palaearctic wheat weevil. In this work the food preference of wheat seeds by *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in different varieties, was studied. The investigation took place in controlled climatic conditions in the Laboratory of Entomology of the Alexander Technological Educational Institute of Thessaloniki. For the experimental part of the work, two types of wheat, bread and durum, and 10 varieties of wheat harvest 2015, were used. 10 plates for each variety were used and in each plate, 10 disinfested seed of wheat and 5 species *S. oryzae* (from feeding of insects) were placed. The attacked seeds were observed every three days for two months, under a ZEISS stereomicroscope. The SPSS 20 program was used for the statistical analysis. The analysis of variance indicated that $F=3.43 < F_{0.05}=4.41$ ($p=0.05$) (DF 1 and 18). The conclusion was that the two types of wheat (durum and bread) did not show statistically significant differences regarding the level of infestation. From the analysis of variance of the 10 varieties it was indicated that $F=22.55 > F_{0.05}=2.48$ ($p=0.05$) (DF 4 and 72). The conclusion was that there were statistically significant differences between the varieties regarding the level of infestation.

Mosquito's control: Evaluation of the acute and residual toxicity of low doses of temephos (Abate) mixed with certain surfactants for the control of *Culex pipiens* larvae

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We evaluated with laboratory and semi-field experiments the toxicity of certain low doses of temephos in mixture with a surfactant (Agnique[®], Break-Tru[®], Dash[®]) to larvae of *Culex pipiens* Linnaeus (Diptera: Culicidae). Larvae of *Cx. pipiens* were collected from rice fields in central Macedonia, northern Greece, and transferred inside glass vials with water to the laboratory where they were maintained in a climatic room at a temperature of 25°C and a photoperiod of 16L:8D. For the bioassays groups of 20 larvae were transferred and exposed to certain water solutions (concentrations) of the tested products in glass vials. For the evaluation of the residual activity of the tested products we used tin drums filled with water (2 L) treated with the recommended doses. At regular time intervals after the application larvae of *Cx. pipiens* were transferred into the drums in the field and the mortality percentages were determined. Our results show that temephos even at the lowest of the tested rates had a high toxicity for larvae of *Cx. pipiens* which in most cases was maintained for at least 14 days. The surfactants Agnique[®], Break-Tru[®] and Dash[®] increased toxicity and residual activity of temephos.

Assessment of selected larvicides for the control of *Culex pipiens* under laboratory and semi-field conditions

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The common house mosquito, *Culex pipiens* L. (Diptera: Culicidae) is a widespread mosquito species with extremely high medical importance, since it is responsible for the spread of several serious human diseases. For its control, as well as for the control of other relative mosquito species, under an area-wide management concept, larvicides are essential. Currently, the larvicides that are in use in EU belong to two categories, Insect Growth Regulators (IGRs), which are non-neurotoxic compounds and bacterial insecticides, which are based on the toxins of the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti), which acts to the mosquito larvae through digestion. In this regard, mosquito larvae control is relied practically on a relatively narrow number of larvicides, therefore, there is an urgent need to evaluate newer substances to broaden the selection of larvicides available for “real world” applications. Based on the above, the aim of the present study was to evaluate various selected larvicides for the control of *Cx. pipiens* larvae. Hence, the efficacy of temephos (Abate 500 EC), teflubenzuron (NOMOLT[®] 150 SC) and a Bti formulation (VectoBac[™] 12 SC) applied in different doses was evaluated in laboratory and semi-field bioassays. Moreover, the combined application of the aforementioned larvicides with the spray adjuvant Dash[®] HC was evaluated.

Based on the results of the laboratory studies, temephos, applied at half of its label dose, caused 100% mortality of *Cx. pipiens* larvae 1 d post-application. In teflubenzuron-treated water, larval mortality levels of *Cx. pipiens* increased gradually over time and reached 100% after 20 d of exposure. No adult emergence was recorded in all treatments with temephos and teflubenzuron. Similar results were obtained in the semi-field studies. The significance of these results for the implementation of effective mosquito control strategies is discussed.

Effect of mosquito larvicides against mites, thrips and aphids on rice fields

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A large number of studies have taken place on mosquito larvicides and their effect on non target insects. Studies that have taken place are concentrated on aquatic animals, but knowledge on the effect on above-ground animals, is limited.

Bioassays of four larvicides of mosquitoes (diflubenzuron, spinosad, s-methoprene and *Bacillus thuringiensis* var. *israelensis*) in rice cultivation in the region of Anthili Fthiotis against populations of mites, thrips, and aphids were also examined. Samples included 15 above-ground part of rice plants from 3 plots, 3 plots for every application. The samples collected after 7, 14 and 21 days of the application. The insects and the mites were extracted with Berlese-Tullgren method. Statistics were made by ANOVA with the program JMP 7.0.1.

S-methoprene, diflubenzuron and spinosad caused significant reduction to aphids, thrips and some species of mites.

Molecular characterization of insecticide resistance in mosquitoes - disease vectors

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The selection of resistant mosquitoes from the intense use of insecticides in vector and agricultural pest programs poses the biggest problem in controlling their populations and subsequently the diseases they transmit.

We investigate the mechanisms of insecticide resistance, by using a variety of classical and modern molecular approaches, including bioassays, transcriptomic studies, functional expression and characterization of recombinant proteins and immunohistochemical stainings, aiming to understand the role of specific genes and mutations in the resistance phenotype. Recent studies from our team focus on (1) analyzing the role of the cuticle in the impressive resistance of the major malaria vector *Anopheles gambiae*, to pyrethroid insecticides, (2) the characterization and global distribution of temephos resistance in the tiger mosquito (*Aedes albopictus*) and 3) the analysis of the target site resistance of *Culex pipiens* to diflubenzuron. We are also participating in programs monitoring the insecticide resistance of mosquito vector populations in Greece with emphasis on settings of high epidemiological risk. Finally within the framework of European Horizon 2020 programs we are developing automated diagnostic platforms ("sample to answer") for the detection and recording of mosquito species – disease vectors, resistance genes and mutations to specific insecticides and the presence of mosquito transmitted pathogens.

Invasive mosquito species surveillance in point of entries in Greece

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The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse 1894) (Diptera: Culicidae), belongs to invasive mosquito species (IMS). Several IMS have been established in Europe and are well-known vectors of diseases, such as Chikungunya, Dengue and Zika virus. With a view to record the IMS at the main entry points of Greece, LIFE CONOPS project established a co-operation with the Athens International Airport (AIA) and the Piraeus Container Terminal (PCT) in order to conduct an entomological surveillance for IMS.

Mosquito samplings took place monthly in selected open-air areas of points of entry (PoEs) from 2014 until now. Mosquito eggs collected with ovitraps were then transferred to BPI and adult mosquitoes (emerged from egg hatching and subsequent rearing) were morphologically identified with dichotomous identification keys and then stored for subsequent molecular identification. Mitochondrial DNA barcoding confirmed they were all *Ae. albopictus*. This fact indicates the absence of new IMS, such as the *Aedes aegypti*. Subsequent comparison of amplified fragments of the cytochrome oxidase I gene (mtCOI) revealed intraspecific variability among populations and the presence of six different haplotypes. Continuous sampling and screening of more individuals from the PoEs of Greece, where LIFE CONOPS already has established a permanent oviposition network, is undoubtedly necessary to prevent or at least promptly detect the invasion of new IMS.

The LIFE CONOPS project "Development & demonstration of management plans against - the climate change enhanced - invasive mosquitoes in S. Europe" (LIFE12 ENV/GR/000466, www.conops.gr) is co-funded by the EU Environmental Funding Program LIFE+ (Environment Policy and Governance).

LIFE CONOPS project: the surveillance and control of invasive mosquito species

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LIFE CONOPS project aims at the development of integrated management plans for the effective and environmental friendly control of the invasive mosquito species (IMS) spread and establishment across Europe. Within the above-mentioned framework, the LIFE CONOPS project is raising awareness and promoting actions focusing on the early detection and immediate suppression of IMS in Greece and Italy.

The case of *Aedes albopictus*, which already colonized mostly of the favourable regions in the EU, has showed the amplitude of negative effects on the urban habitats as well as the substantial threat to human health. In Greece, several of the new invaded areas experiencing no intense mosquito annoyance in the past are now suffering because of the Asian tiger mosquito.

Controlling *Ae. albopictus* population is challenging since intense chemical control efforts are rather of low efficacy. Operational IMS Management Plans (IMS-MP) have been developed by the LIFE CONOPS team with the aim to provide an easy-to-use-tool serving the local/regional bodies responsible for IMS management. Collaborations with key stakeholders, such as authorities at points of entry (ports - airports) and companies trading used tires have been established and mosquito traps have been activated. Furthermore, an emergency vector control plan is proposed and responsibilities have been clearly assigned to deal with the potential epidemic risk in case of imported Chikungunya, Dengue and Zika cases. One of the main LIFE CONOPS goal is to enhance capacities in the Mediterranean basin for the early detection of possible new IMS, primarily *Aedes aegypti*, therefore making it possible and cost-benefit its immediate elimination.

The LIFE CONOPS project "Development & demonstration of management plans against - the climate change enhanced - invasive mosquitoes in S. Europe" (LIFE12 ENV/GR/000466, www.conops.gr) is co-funded by the EU Environmental Funding Programme LIFE+ (Environment Policy and Governance).

Deterministic and stochastic demographic analysis of the invasive species *Trogoderma granarium* Everts (Coleoptera: Dermestidae)**N.E. PAPANIKOLAOU^{1,2,*}, N.G. KAVALLIERATOS³, M. KONDAKIS⁴,
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Trogoderma granarium Everts (Coleoptera: Dermestidae) is a destructive insect pest of stored-products. It affects a wide variety of basic agricultural commodities with preference to cereal seeds and starchy products. *T. granarium* is also considered as one of the most important quarantine insects in several countries, while it falls among the 100 most important invasive species worldwide. Quarantine insects are of potential economic importance in areas where they are not yet present or where they are present but not widespread. Furthermore, they can seriously damage the trade of agricultural products not only among countries but also among geographical areas within countries. Recently, the presence of *T. granarium* has been recorded in Greece, a fact which is particularly worrying both for the qualitative and quantitative degradation of stored-products.

In the present study, a deterministic and stochastic analysis of the demography of *T. granarium*, fed on wheat, was carried out at 30, 35 and 40 °C. The demographic study can lead to useful conclusions about population variation, as well as on the investigation of several biological features of different insect ages. In the deterministic analysis, various insect population parameters (e.g. intrinsic rate of increase, net reproductive rate, doubling time, mean generation time), as well as the reproductive value and life expectancy of females were calculated. The results showed that the temperature affects the population growth of the insect. The stochastic analysis explored individual variation through a number of distinct hierarchical models based upon variations of zero-inflated-Poisson and Weibull distributions for the number of offspring and the lifetime duration respectively. The results largely agreed with the ones from the baseline analysis, thus suggesting robustness of the findings.

Automated “sample to answer” diagnostic platforms for improving the impact of vector control interventions

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Competition

Vector-borne diseases (such as malaria, dengue, chikungunya and Zika virus infections) are life-threatening conditions that cause thousands of deaths every year. Prevention of the diseases is best achieved by vector control which relies on the use of insecticides. Monitoring mosquito vector populations is an integral component of control programs and a prerequisite for effective interventions. An essential part of vector research is the characterization of field vector populations, such as species/subspecies, insecticide resistance traits and pathogen and symbiote infection status. Several diagnostic methods have been used for this task but all have important limitations including protocol complexity, technical training requirements and high per-assay and platform cost. To address these limitations, diagnostic platforms (MalVecLabDisk / ArboVec-Disk) for monitoring mosquito populations will be developed and interfaced with a Disease Data Management System (DDMS) to collate and manage the data. The integrated system will be evaluated in four malaria endemic countries, to support informed decision-making in vector control and disease management.

Funded by the Horizon 2020 Framework Programme of the European Union.

An alternative food preference of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae)

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Oryzaephilus surinamensis (Linnaeus, 1758) (Coleoptera: Silvanidae) is one of the most common insect pest of grains and a variety of stored products and has been found in high numbers in almost all storage facilities. It is known as Saw-toothed Grain Beetle and is found in granaries, warehouses, elevators and food mills. According the global bibliography, *O. surinamensis* attacks cereals, cereal-based products, but also copra, spices, nuts, dried fruit, cocoa. Both adults and larvae, may feed on grain dust and larvae preferentially fed on the germ. It has also been investigated that insect damaged kernels were more attractive to *O. surinamensis* than whole kernels. Additionally insect damaged kernels elicit more attractiveness than mechanically split kernels. In the present work, another food preference of adults and larvae is presented, namely on dead insects. This insect's preference was observed in a personal entomological collection of the co-author. *O. surinamensis*' adults and larvae (from feeding of insects) were placed in boxes with different species of dead insects. Specifically, in each of 100 cardboard boxes, 10 disinfected dead insects were put from different Orders. Also 30 whole and damaged seeds of rice, 15 adults and 15 larvae of the *O. surinamensis* were put in controlled climatic conditions in the Laboratory of Entomology. The observations were taken twice of the week, for two months. The results showed that both adults and larvae were fed the same from all dead insects' species and seeds of rice. It was observed that the *O. surinamensis*' attack on dead insects was in most cases complete. From the above mentioned results seems that *O. surinamensis* is a dangerous aggressive enemy, against preserved insects, in entomological collection files in Museums and other Zoological exhibitions.

Molecular characterization and geographical distribution of temephos resistance in the major dengue and chikungunya vector *Aedes albopictus*

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Competition

Aedes albopictus is a major vector of diseases and also one of the most invasive species found worldwide. Its control is based on insecticides, however insecticide resistance is a threat for the sustainability of control interventions.

We identified and functionally characterized temephos resistance in *Ae. albopictus*. Combined biochemical and illumine transcriptomic data revealed the up regulation of the *CCEae3a* and *CCEae6a* esterases in the resistant mosquitoes. Both esterases were also found gene amplified and a genetic study associated their gene amplification with the resistance phenotype. *CCEae3a* was heterologously expressed in a baculovirus expression system and shown to strongly interact with temephos, through a sequestration resistance mechanism. Immunolocalization experiments showed the expression of *CCEae3a* in malpighian tubules and the nerve cord, revealing the tissues where detoxification of temephos takes place and providing important information for the optimization of temephos delivery to its molecular target. The geographical distribution of the *CCEae3a/CCEae6a* gene amplification was investigated using samples collected from sixteen countries worldwide. Individuals with amplification were found in Greece and Florida (U.S.A.). All individuals from Greece (Athens) and U.S.A. (Florida) carrying the *CCEae3a-CCEae6a* co-amplicon shared a common haplotype, indicating a single amplification event, that spread between the two countries, highlighting the importance of passive transportation of disease vectors carrying insecticide resistance mechanisms.

**Presence and seasonal distribution of Asian tiger mosquito
(*Aedes albopictus* Skuse 1894, Diptera: Culicidae)
at Technological Educational Institution of Athens**

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The invasive mosquito species *Aedes albopictus* has been spread almost all over Greece since its first record in Corfu and Thesprotia. This species poses an increased risk of human health due to the spread of arboviruses such as dengue and chikungunya, but also because of the numerous bites resulting from its day-time feeding behavior.

The presence and seasonal distribution of this species were studied from November 2015 to December 2016 at Technological Educational Institution of Athens.

Ten (10) ovipositional traps (with tongue depressor as ovipositional substrate) were served every 7 days. Seasonal distribution was estimated through egg-count. *Ae. albopictus* was active from late April to early November. Peak activity was recorded from June to September whereas its activity ceased from December to March.

An effort to correlate egg-count with available climatic data is being made.



5th Session
Physiology and Genetics

Analysis of the reproductive system of the *Bactrocera oleae* aiming at innovating control methods of the insect**M.-E. GREGORIOU, M. ADAMOPOULOU, A. AGGELOPOULOU,
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The olive fruit fly, *Bactrocera oleae*, is the major arthropod pest of commercial olive production. Currently, its control relies mostly on insecticide spraying. Alternative control methods usually target the reproductive system, either through the inhibition of the mating process or through the reduction of the reproductive capacity of the insects. In the olive fly, reproduction occurs when sperm is transferred from the male insect to the female reproductive system. More specifically, sperm develops in male testes and, when mixed with secretions from accessory glands, travels through the female genital tract to the spermatheca, to be stored for later egg fertilization. Given that, we performed genomic analysis and transcriptomic analysis of male and female reproductive tissues, just before and twelve hours after mating. Based on the results, we determined the expression profiles of the genes: *yellow*, *sex peptide receptor* (SPR) and *troponin C*. In order to investigate their functional role, we performed transient silencing of the selected loci through RNAi. Indeed, in all cases silencing of these reproductive genes reduced egg production. Consequently, these genes could be potential targets for the improvement and specialization of the olive fruit fly population control techniques in order to be more efficient and environmentally friendly.

The project is implemented under the "ARISTEIA" Action of the "OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING" and is co-funded by the European Social Fund (ESF) and National Resources.

Functional analyses of olfactory genes in the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae): new targets for the development of insect control methods

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The olive fruit fly causes significant losses to olive cultivation worldwide. While chemical pesticides constitute the major control arsenal, several research efforts focus on alternative approaches. A prerequisite to such approaches is the study of biological systems related to the harmful effect of the olive fly, in order to identify new molecular targets. Genetic manipulation of such targets could lead to the prevention of mating and the disruption of the insect's reproductive success. The study of the olfactory system may contribute to the discovery of such targets, since both survival and reproduction depend on olfactory stimuli.

In the present study, the genes encoding the olfactory co-receptor Orco and the Sensory Neuron Membrane Protein SNMP1 were analyzed. These genes are expressed in the insect's olfactory organs as a response to odorants. In order to investigate their functional role, we generated the respective expression profiles by qRT-PCR analysis comparing the behavioral responses of both sexes at different time points and conditions during the development of the adults. Additionally, we performed gene silencing of their expression through RNAi, to evaluate the effect of silencing during i) mating and ii) oviposition. In the first case we observed low copulation rates when testing the mating ability between dsRNA microinjected insects. Secondly, in the oviposition assay we explored the effect of Orco gene silencing in female olive fruit flies, demonstrating that siRNA reduction of the Orco expression directly affected their post-mating response by inhibiting oviposition. The resulted phenotypes suggest that Orco and SNMP1 play crucial roles in responses related to reproductive behaviour of the olive fly. The observed behavioural changes render these two genes potential targets for the improvement of already established or/and the development of new species-specific population control techniques.

The project was implemented under the "ARISTEIA" Action of the "OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING" and was co-funded by the European Social Fund (ESF) and National Resources.

The expression of the clock genes *period*, *timeless*, *cycle* and *cryptochrome* have rhythmic patterns and are affected by photoperiod in the moth *Sesamia nonagrioides*

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Insects, like most organisms, have an internal circadian clock that oscillates with a daily rhythmicity, and a timing mechanism that mediates seasonal events, including diapause. The involvement of circadian clock genes in photoperiodism has been supported. In order to understand whether and how the circadian system is connected to photoperiodism, an important piece of information is whether clock genes products oscillate and how they react to a changing photoperiod. In the moth *Sesamia nonagrioides*, which undergoes a facultive diapause controlled by photoperiod, we isolated the clock gene *period* (*per*), *timeless* (*tim*), *cycle* (*cyc*) and *cryptochrome1* (*cry1*). The results revealed that SnPER predicted amino acid sequence contained the functional PAS, PAC, NLS and CLD domains, while the SnTIM revealed the PER interaction site (PIS) and the NLS domain. SnCYC consists of 667 amino acids and structural analysis showed that contains a BCTR domain in its C-terminal in addition to the common domains found in *Drosophila* CYC, i.e. bHLH, PAS-A, PAS-B domains. The sequence of *Sncyc* showed a similarity to that of its mammalian orthologue, *Bmal1*. *Sncry1* consists of 1762 bp encoding a polypeptide of 528 amino acid residues. SnCRY1 consists of 528 amino acid and presented two characteristic conserved domains: the DNA photolyase and the Flavin-Adenine Dinucleotide (FAD) binding domain, which been demonstrated to be sufficient for light detection and photo transduction in *Drosophila*. The four proteins showed significant homology with the clock protein sequences identified from other insects.

We also investigated the expression patterns of these clock genes in brain of larvae growing under long-day 16L:8D (LD), constant darkness (DD) and short-day 10L:14D (SD) conditions using qRT-PCR assays. The results showed that mRNAs of four genes exhibited diel oscillations under different photoperiods. The oscillation of *Snper*, *Sntim*, *Sncyc* and *Sncry1* under short-day photoperiod was quite different from that under long-day photoperiod. Our data on the clock genes *Snper*, *Sntim*, *Sncyc* and *Sncry1* revealed that in the Mediterranean corn stalk borer the expression patterns of these oscillate and affected by photoperiod. Since our experiments were done under LD, DD and SD conditions, the data reflect how the molecular clock adapts to photoperiodic changes. Our results indicate that transcriptional regulation of these four clock genes would play important roles in the diapause programming in *S. nonagrioides*.

Effect of a single nucleotide polymorphism(SNP) on the olfactory response and oviposition behavior of the Medfly

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Olfaction, and the olfactory system, is involved in a series of behavioral responses that are related to mating as well as the search and acceptance of oviposition sites, and therefore plays a determining role in insect biology. The correlation of mutations with such behavioral responses is rarely recorded in literature. Additionally, correlation of mutations, particular of single nucleotide polymorphisms (SNPs), with physiological behaviors could provide excellent diagnostic tools in the effort to understand the structure and dynamics of insect populations and, consequently, of control efforts.

In the current study we describe a SNP in the *scribble* gene of the Mediterranean fruit fly, *Ceratitis capitata* that seems to be associated with the host preference of fly. This particular gene is known to be involved in the olfactory behaviour of *Drosophila melanogaster*. Based on this indication, two Medfly strains (lines) were generated which were homozygous for each of the two alleles of the abovementioned genetic locus. The results of the Electroantennogram (EAG) experiments showed significant variations in the olfactory response of the two strains to a series of odour stimuli derived from specific insect hosts (fig, peach, orange). These variations were also reflected to the oviposition behaviour (fecundity) of these two strains to the different odour stimuli. This study constitutes the first example of an association between genetic background, physiology and behavior of the Mediterranean fruit fly.

Olive fruit fly oviposition behavior in eleven different species of fruit other than olives

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Competition

The olive fruit fly (*Bactrocera oleae*, Diptera: Tephritidae) in its natural environment oviposits only on olive fruits, while in the lab it also oviposits on tomato fruits. In the current experiment oviposition of the olive fruit fly was studied in eleven new fruit-substrates different from those who invade in nature. Rearing population originated from wild olive fruit flies, gathered from affected olives of A.T.E.I. of Thessaloniki farm. The adults, after emergence, were placed in entomological cages for olive fruit fly, containing granulated sugar and liquid protein nutriments. Seven days after the adults transfer eleven new fruit-substrates were placed in the cages, individually or in pairs (different from each other), to determine the acceptance and preference of oviposition of *Bactrocera oleae* adult female on them. The tested fruits for oviposition acceptance were: red and green tomato, green pepper, pink and black plum, "firiki" and "pink lady" apple varieties, "karaliok" Lotus variety, eggplant, avocado and orange. The fruits' puncture resistance was measured utilizing a penetrometer in order to correlate the number of oviposited eggs to the penetration resistance.

Results indicate that most eggs were laid in red and green tomatoes without statistically important differences. Following are black plums and then, pink. Many eggs were also oviposited in green peppers and fewer in eggplants and "firiki". The fewest were oviposited in the fruits that had the highest penetration resistance.

Structural analysis of sex chromosomes: development of a CRISPR/Cas9 synthetic X-distortion system for the genetic control of the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae)

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The olive fruit fly is the most destructive pest of olive cultivation, since olives are the preferred host for insect's oviposition, causing considerable production losses. The use of chemical insecticides for its control has adverse effects on the environment and humans and leads to the evolution of insect resistance. In agreement with the need of new environmentally friendly pest management strategies, the present study aims at the development of an innovative, species-specific genetic control technology for the olive fruit fly. This technology is based on the distortion of the sex ratio in favor of males by selectively destroying the X-chromosome during spermatogenesis. This approach will give new prospects for the development of novel methods of controlling the insect.

The development of such a *B. oleae* X-shredding system requires the deeper insight of the structure of sex determining chromosomes. Presently, potentially sex-linked target sequences have been identified by bioinformatic analysis, which will then be targeted by CRISPR / Cas genetic engineering, ultimately aiming at the control of the insect populations.

This project is implemented under the IKY Scholarships for "Reinforcement of Postdoctoral Researchers" funded by the European Social Fund (ESF) and National Resources.



6th Session
Chemical Control and Resistance

“Use plant protection products safely. Always read the label and product information before use”. Presentation and analysis of questionnaires and official control data for the period 2012-2016

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The sentence “Use plant protection products safely. Always read the label and product information before use” exists in every advertisement of plant protection product. Nevertheless, the question is if and how much is applied. The prevention of harmful effects to human health caused by the intrinsic properties and the use of plant protection products serves primarily the purpose of safeguarding the public interest and in particular the protection of public health. Professional users’ training in the sustainable use of pesticides is the best preventive measure for the results sought. The authorization of plant protection products and the official controls aim at ensuring a high level of protection of human and animal health and the protection of the environment. In the present study, the results and conclusions out of official post-registration controls on pesticides over the past five years are presented focusing on official pesticide residue controls, controls on marketing and laboratory controls on the composition of plant protection products. The conduction of a survey across the country on the use of pesticides through anonymous questionnaires demonstrates the behavior of professional users and the requirements for improving the situation. Tackling the problems requires constant monitoring of the situation and implementation of best control practices as well as the application of proportionate, effective and dissuasive sanctions against any infringement of the law. All participants in the official control system in Greece and in particular the inspectors and the personnel official control laboratories, despite the difficult economic and social circumstances, make every effort and achieve commendable results.

Investigation of the contribution of different molecular mechanisms to insecticide resistance through gene overexpression and targeted genome modification in *Drosophila*

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The characterization of insecticide resistance mechanisms is an important prerequisite for the application of integrated pest management strategies. However, the resistance phenotype may be dependent on the contemporary action of different molecular mechanisms acting either through mutations in target genes or through overexpression of detoxification enzymes. The efficient validation *in vivo* of genes and mutations related to resistance may be hampered by the lack of appropriate genetic or genomic tools beyond of a limited number of model organisms.

Drosophila is a powerful model system, facilitated by a broad repertoire of techniques ranging from classical genetics to cutting edge genomic technologies. Contemporary approaches of targeted genome modification enable rapid investigation of the role of certain SNPs or multiple mutations at the same time, while available transgenic technology enables the overexpression of detoxification enzymes in defined genomic background.

We employ CRISPR/Cas9 genome modification in order to generate recombinant *Drosophila* strains bearing target-site resistance mutations derived from agricultural insect pests and disease vectors. In parallel, we introduce into these strains conditional expression transgenes of characterized detoxification enzymes. Thus, we successfully combine resistance mutations in known insecticide target genes with overexpression of metabolic P450 genes that we have shown that metabolize different substances, as well as overexpression of genes that contribute to resistance through cuticular modifications. Thus, we validate *in vivo* via comparative bioassays the contribution of each mechanism in resistance. Furthermore, we can use the new super-resistant strains as tools for the validation of novel insecticides and molecular diagnostics.

The profile of flupyradifurone: studies on a new tool for whitefly control

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The cotton whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is among the most important pests of numerous crops and a vector of >100 plant viruses, causing significant crop losses worldwide. Its control can be extremely difficult, however new generation insecticides provide novel potential in current pest management.

In a previous study, the insecticide flupyradifurone (Sivanto® prime) inhibited the transmission of Tomato yellow leaf curl virus (TYLCV), one of the most notable viruses in Greece. In the present study the effects of flupyradifurone on *B. tabaci* were investigated. The inhibition in TYLCV transmission by flupyradifurone is potentially related to a strong knockdown effect on the whitefly adults (90% within only 30 minutes after exposure) as well as prolonged antifeedant effect (absence of honeydew excretions on flupyradifurone treated leaves with the recommended rate up to 21 days after exposure).

The effect of the insecticide on the various development stages (eggs/L1, nymphs L2 and adults) of *B. tabaci* was also investigated. Adulticide activity of flupyradifurone was high (LC₅₀: 3.3-15.2 mg/l) as well as its effect on crawlers L1 (LC₅₀: 5.1-9.8 mg/l). True ovicidal activity was not detected. For second instar nymphs (L2) the observed effect was not as strong as in the other development stages (LC₅₀: 10.3-42.3 mg/l). In preliminary studies, cross resistance to neonicotinoid insecticides was not detected, since minimal differences in LC₅₀ levels for flupyradifurone were observed between susceptible (Resistance Ratio, RR=4) and resistant strains (RR=318), to the neonicotinoid insecticide thiamethoxam.

Finally, the residual activity of flupyradifurone was found prolonged, with the observed% mortality in adults on treated leaves being over 90% for up to 35 days after treatment (in bioassays with sprayed leaves at the recommended label rate).

The results of this study indicate that flupyradifurone is a reliable new tool for whitefly control in integrated pest management schemes.

Large-scale applications of phosphine in commercial facilities by using wireless phosphine sensors

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The use of wireless phosphine sensors opens the barriers for extensive and continuous gas monitoring, which was not possible until recently. In this regard, we designed and evaluated wireless sensors in order to illustrate gas concentration in different facilities such as silos, warehouses, ships and containers. These tests were carried out in conjunction with the evaluation of the insecticidal effect of phosphine for the control of insect populations that were susceptible or resistance to this gas. Between 2014 and 2017, there were more than 40 “real world” fumigations which were carried out with the use of sensors, in Greece and abroad. Based on our results, concentration distribution in large warehouses and ships are not usually sufficient for a satisfactory level of insect control. In general, fumigation on containers was found to be effective enough to kill all life stages, in contrast with larger facilities (silos etc). This is particularly important for traditional monitoring techniques, as any measurements correspond to the specific time of monitor and is not transferable to additional intervals, which makes fumigators to either overestimate or underestimate the outcomes of a given fumigation. The reduced success of commercial fumigations in large-scale applications (silo, warehouses, ship,) in relation with fumigation in container is caused by the inability of phosphine to disperse without using forced recirculation. The results are better, when forced recirculation is used. These tests clearly suggest that the sensors were effective in measuring phosphine and are expected to play an important role in the near future in integrated pest management programs at the post-harvest stages of agricultural commodities.

Effect of insecticides (synthetic and biological) and entomopathogenic fungi on the mortality of *Docioctaurus maroccanus* (Thunberg) (Orthoptera: Acrididae)

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The locust species *Docioctaurus maroccanus* (Thunberg), also known as “Moroccan grasshopper” is very common in Greece. For the chemical control of locusts, the only registered insecticide a.i. is lambda-cyhalothrin, as well as deltamethrin for use on fodder plants only. The aim of the present work is to study the effect of insecticides (synthetic and biological) and pathogenic microorganisms on the mortality of *D. maroccanus*. For this purpose, bioassays were carried out using diflubenzuron and spinosad, as well as the entomopathogenic fungus *Beauveria bassiana*. Insects used in the experiment came from a wild population collected from the island of Aghios Eustratios, where excessive population exists. Contact and digestion bioassays were performed in 500 ml glass containers, in which nymphs were placed (together with *Hordeum murinum* parts), and treated with the aforementioned factors in concentrations of 0.3 ml/lit, 0.1 ml/lit, 0.05 ml/lit, 0.025 ml/lit, 0.0125 ml/lit, while a number of containers was sprayed with water and served as control. The nymphal mortality of locusts was recorded daily for three days, based on the mobility of the individuals in the containers. Spinosad, seemed to be very effective against *D. maroccanus*. The mortality of the locusts after 72 hours reached 100% for the most of the contact bioassays of spinosad.

Tuta absoluta*: A European perspective to a global problem*E. RODITAKIS^{1,*}, M. STAVRAKAKI¹, E. VASAKIS¹,
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The tomato borer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most important invasive species and a major pest of tomato that is mainly controlled by chemical insecticides. Extensive use of insecticides has resulted in the development of resistance, causing significant problems in the management of the pest. Hereby, the current insecticide resistance status will be reviewed at a global scale and the results of a four-year resistance monitoring survey for the most important chemical insecticides (chlorantraniliprole, indoxacarb, spinosad and emamectin benzoate) in the European/Asian region will be presented. Briefly, resistance to diamides (chlorantraniliprole) is expanding (Italy, Greece, Israel) with the exception of Spain; a contrasting situation highlighting the benefits of IPM that is extensively adopted in this country. The first cases of resistance to indoxacarb (Italy, Greece, Israel) and emamectin benzoate (Italy, Greece) were detected, but reports of poor field performance were absent for these chemicals. Analysis of the resistance mechanisms for diamides and indoxacarb revealed the presence of four and two mutations, respectively, in the target sites of these chemicals (ryanodine receptor for diamides and sodium channel for indoxacarb). Finally, the efficacy levels of thirteen commercially available pesticides against *T. absoluta* were evaluated using diagnostic concentrations. Our aim is to establish a rapid diagnostic process to support advice with high regional accuracy for the chemical control of *T. absoluta*, in order to sustain tomato production in Greece.

This work was supported by STOMP project that is funded through the ARIMNet2 2015 Call. ARIMNet2 (2014-2017) is an ERA-NET coordinated by INRA (France). It has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 618127. This work was also supported by DuPont De Nemours and BAYER-AG research grants. Emmanouil Vasakis received a scholarship (MSc 2017) by the Hellenic Entomological Society.

Study of imidacloprid and spirotetramat resistance in *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in Greece

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Myzus persicae s.l., a major aphid-pest, has evolved resistance to a range of insecticide classes, making its control increasingly difficult in some areas. Here we compare bioassay monitoring data for two important compounds, imidacloprid and spirotetramat, on field samples/clones collected in Greece.

Dose-response bioassays with imidacloprid were performed to 122 aphid samples/clones from central and northern Greece. There was an overall increase in the level of resistance (RF=15–40) within tobacco-collected samples from 78.7% in 2007 to 86.7% in 2015. The corresponding frequencies for peach samples were 13.3 and 6.7%. These results were however confounded by the first identification of the R81T target mutation in Greece in 2015 (4.3% as heterozygotes in peach) and 2016 (21.3% as heterozygotes in peach). No resistance to spirotetramat was found at the 60 clones collected in 2015.

Resistance to imidacloprid is continuing to increase within Greek *M. persicae* s.l. populations and the recent identification of the R81T resistance mutation in Greece will only worsen the situation. Spirotetramat is a good alternative to neonicotinoids for resistance management.

Characterization of acaricide resistance mechanisms of *Varroa destructor* (Acari: Varroidae) resistance in Greece

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Beekeeping provides nutritional, economic and ecological security globally. Crop pollination is valued at >215 billion euros per year. However, bee colony collapse is on the rise and represents a major problem globally. This devastating event for apiculture might be attributed to *Varroa destructor* infestation. Only limited options of synthetic registered acaricides for *Varroa* are available and these are beginning to lose efficacy as *Varroa* mites develop resistance. A major challenge for the management of resistance, as well as the introduction of novel compounds/biopesticides to tackle the problem is the understanding of acaricide resistance mechanisms. These mechanisms have not been yet analyzed in *V. destructor*. According to our data, resistance of *Varroa* to Coumaphos reaches more than 660 fold. Additionally, the presence of pyrethroid resistant mutations (kdr) has also been recorded in several geographical regions in Greece. Taking into consideration our preliminary data and the urgent need for the judicious use of acaricides to manage resistance, we aim to characterize resistance mechanisms and develop and apply multiplex diagnostics, for known kdr mutations and novel molecular markers (SNPs, gene overexpression). For this reason, we have already preformed RNAseq analysis of resistant *versus* susceptible mites in order to find genes implicated in Coumaphos resistance that could be candidates for functional validation. These genes could also be the basis of novel molecular diagnostics to monitor resistance, as well as targets for future acaricide/synergist development.

Evaluation of phosphine resistance in different populations of stored product beetles originated from different geographical regions

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The present study aims to evaluate the level of phosphine (PH₃) resistance and assess the biological parameters and molecular indices of different species of stored product beetles. More than 400 different populations were evaluated from different geographical regions of Europe, America, Asia and Africa. Approximately, 20 species were examined. However, in this work, we present the results from *Rhyzopertha dominica*, *Sitophilus oryzae*, *Lasioderma serricorne*, *Tribolium confusum* and *Tribolium castaneum*. From the protocols that were followed to indicate phosphine resistance, two are presented here: (a) exposure of insects to 30 ppm for 20h (FAO protocol) and (b) exposure of insects for 5-90 min to 3000 ppm [DetiaDegesch Phosphine Tolerance Test Kit (DDPTTK)]. Phosphine resistance was also analyzed at the molecular level, by sequencing the gene *dld* (*rph2* genetic locus), the putative target of phosphine where mutations associated with resistance have been previously reported. The findings suggest that the characterization of resistance is similar among protocols, with some deviations. Walking behavior and population growth was different among populations with different levels of resistance. Several known resistance mutations were also found in the *dld* gene. Overall, the results of the present study are expected to contribute to the improvement of phosphine resistance management, in "real world" applications.

Genomic and functional characterization of acaricide resistance in *Tetranychus urticae*

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Spider mites such as *Tetranychus urticae* are among the most important agricultural pests worldwide and well-known for the striking resistance levels they develop to acaricides of different mode of action. Towards this direction, we aim to understand the role of individual genes in resistance phenotype by using a number of approaches such as functional expression and characterization of recombinant proteins, immunohistochemical staining, ectopic expression of *T. urticae* detoxification genes in *Drosophila melanogaster* and the role of previously known mutations in resistance phenotype by multiple genetic backcrosses in *T. urticae*. Several detoxification enzymes, such as cytochrome P450s, of *T. urticae* were functionally expressed and characterized successfully indicating their ability to detoxify acaricides to less toxic metabolites. By employing the GAL4/UAS system in *D. melanogaster* we expressed ectopically detoxification genes of *T. urticae* and validated their role in resistance *in vivo*. Through immunohistochemistry in *T. urticae* we aim to study the physiology of detoxification and identify possible routes of insecticide uptake. Recently, by multiple backcrossing, we introgressed target-site alleles bearing known resistance mutations in a susceptible reference background. Our results allowed interpreting the relative effect of target-site mutations in highly resistant strains.

Analysis and monitoring of insecticide resistance in the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae)

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In the past few years, we study insecticide resistance levels and mechanisms in the olive fruit fly, *Bactrocera oleae*, in the frame of a Hellenic Ministry of Rural Development and Food project and in collaboration with several Directorates of Rural Economy and Veterinary**. Data from bioassays and molecular methods, which are applied for the evaluation of resistance, indicated elevated levels of pyrethroid resistance in different field populations from Greece and especially in those from Crete.

The fact that several cytochrome P450 genes were found to be over expressed in pyrethroid resistant populations, led to the development of new molecular diagnostic tools. The use of these tools will contribute to monitoring and management of resistance. In parallel we are trying to assess the impact of resistance levels to the efficacy of applied insecticides in the field. In particular, we are conducting laboratory bioassays to field-collected flies, and correlate the results with those of recommended dose application in field experiments.

Monitoring data are imported to GALANTHOS Database, which facilitates the correct and prompt management of olive fruit fly, through the open access of scientific data of resistance.

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“Invasion” of rodents in citrus crops of Argolida**D. DIMOU^{1,*}, K. SPANOU¹, B. TOMPRAS²,
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During the conduct of official surveys for the Closterovirus Citrus tristeza virus, the most devastating virus of Citrus, in locations of the local communities of Koutsopodi, New Iraion, and Shoinohorio, fields were detected with certain trees carrying small size leaves, reduction of the leaf surface and production, extensive chlorosis exhibiting a general aspect of dieback, while in other cases trees had declined. Because of the similarity of the symptoms of citrus trees infected by the CTV virus, plant samples were collected and surveys were carried out. The samples were tested with Direct tissue blot immunoassay (DTBIA) method and no positive results occurred. According to our observation and farmers' testimonies, the status of trees is due to rodents attack. Careful monitoring showed typical feeding activity (gnawing) of the rodents against the base of the trunks and the main roots. What is highly spectacular is the creation of innumerable underground burrows, causing water loss. Samples of rodents were sent to the corresponding laboratory of the Agricultural University of Athens for species identification. It was the rodent species *Pitymys subterraneus* (Rodentia: Arvicolidae). This species is of small size and low weight of brown and gray color. The problem is serious and has been extended in an area of approximately 100 ha of citrus crops. Control methods applied such as: a) placing baits (improvised or commercial types) in the underground burrows -always according to label instructions b) grain seeds mixed with rodenticide placed in the burrow c) irrigation with extensive flooding that will cause suffocation of rodents d) careful and deep digging around the trunk base and filling it with gravels. Worth noting that these control methods not definitely solve the problem. The determinant factor in the severity level of the problem seems to be the soil identities and climate conditions with clay soil and claysandy soils to be the most susceptible.

Isoclast™ Active: A valuable tool for the control of sap feeding insects in the Mediterranean region

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Sponsor

Oral presentations

Isoclast™ Active, is a new insecticide active ingredient which has been discovered and developed by Dow AgroSciences, for the control of sap feeding pests in various crops. It is classified as the first and sole member of the Sulfoximines, Group 4C of the IRAC classification scheme, and is a valuable rotational partner for other insecticide groups as all relevant studies showed lack of cross resistance with neonicotinoids, pyrethroids, organophosphates and other insecticides. Isoclast™ Active is a neurotoxin which kills insects on contact and through ingestion providing excellent knockdown and residual control against aphids, whiteflies, mealybugs and scales in pome and stone fruits, vines, citrus, cotton, leafy and fruiting vegetables, potatoes and legumes. Isoclast™ Active which has an excellent systemic and translaminar activity, is applied through foliar applications at rates from 24-48 gr ai/ha depending on the type of the crop and the insect and is formulated in as Suspension Concentrate (Closer™ 120 SC) and Wettable Granules (Transform™ 500 WG).

Isoclast™ Active is expected to be a valuable tool for Greek farmers for the control of economically important sap feeding insect pests in all major crops with a minimal impact on beneficial insects. Isoclast™ Active is already registered in several countries including USA, Canada, Australia, S. Korea, Israel and South Africa while it was registered in Europe on August 2015. The first official registration of a formulation of Isoclast™ Active in Europe (Transform™ 500 WG) has been granted in June 2017 in Ireland concerning the use in cereals against aphids. More registrations in other EU countries, including Greece, in various crops are anticipated in 2017 - 2018.

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Determination of the lethal doses (LD_{50} and LD_{90}) of the insecticides spirotetramat, thiamethoxam and deltamethrin, against the aphid species *Aphis pomi* de Geer, *Hyalopterus pruni* Geoffroy and *Myzus persicae* Sulzer (Homoptera: Aphididae)

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Aphids are considered to be among the most serious pests of tree crops as their infestation can result in growth inhibition, deformations in plant parts and quality degrading of the harvested product. Chemical applications against aphids on time, are essential for the vigor and good growth of crops. For this purpose, the efficacy of the insecticides Movento Gold 100 SC (spirotetramat: Tetramic acid), Actara 25 WG (thiamethoxam: neonicotinoid) and Decis 2.5 EC (deltamethrin: pyrethroid group) against three of the most important aphid species infesting trees was evaluated in laboratory conditions. The median lethal dose of the formulations was also determined 48 hours after application. The bioassay method applied was as follows: IRAC Susceptibility Test Methods Method No: 001 Version: 3 (June 2009). According to the results, all three formulations presented high efficacy against aphids at the recommended dose (mortality 92-100% at 48 hours), with the exception of the treatment of spirotetramat against *Myzus persicae*, where the efficacy reached moderate levels (50% mortality at 48 hours). Data was analyzed using probit analysis and no significant differences were observed between the species *Aphis pomi* and *Hyalopterus pruni* regarding the median lethal dose of the tested insecticides. *Myzus persicae* showed a higher median lethal dose in the tested formulations compared to the other two aphid species. Indicatively, the LD_{50} of thiamethoxam against *Aphis pomi*, *Hyalopterus pruni* and *Myzus persicae* was estimated at 4.04, 5.6 and 30.76 ppm, respectively at 48 hours. Estimation of lethal doses is an important parameter for the timely determination of the occurrence of aphid's resistance to insecticides, which can contribute to a more effective control of the pests of the crops.

Chemical control of red palm weevil *Rhynchophorus ferrugineus* using the trunk injection method

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Poster presentations

The endotherapy method for the chemical control of red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) was adopted. Trunk injection using approved insecticide active ingredients (authorization for limited and controlled use in emergency situations) for use against this pest was conducted in the Paphos and Limassol districts, for two consecutive years. The active ingredients applied were the following: imidacloprid 20% SL, (Confidor® 200SL), abamectin 1.8%EC (Vertimec® 1.8 EC) and thiamethoxam 25% WG (Actara® 25 WG). Twenty one date palms of the *Phoenix dactylifera* species and 10 palms of the *P. canariensis* species, all of them 25-30 years old were used. The specifically-designed trunk injection plastic pegs (25cm-length for *P. canariensis* and 15cm-length for *P. dactylifera* trunks) were obtained from PROVEFE, S.A. Company, Spain. All instructions regarding installation, preparation of insecticide mixture, and application specifications and schedule were proposed by the company (www.sospalm.com). Two 15cm-length pegs were used on *P. dactylifera*, while three 25cm-length pegs were used on *P. canariensis*. Pegs were inserted in previously drilled trunks in an angle of 45-60°, approximately 1.5-2m above the ground level. The application programme started in late March and on a rotation basis every 40-45 days, 30-45ml/palm (depending on palm species) of the approved against the pest insecticides were applied. Application of insecticides was based on: a) RPW's population density in the area, b) pheromone trap captures, and c) the intensiveness and frequency of palm damage in the area.

The 2-year results showed that, 8 (38.1%) out of the 21 date palms of the *P. dactylifera* species with visible signs of severe damage at the beginning of applications couldn't recover and finally died, while 2 (9.5%) palms managed to recover from slight damage. Eleven (52.4%) palms without any visible signs of damage were healthy throughout the study.

On the other hand, 4 (40.0%) palms out of 10 *P. canariensis* species with or without visible signs of damage at the beginning of applications were totally destroyed by the pest during the 1st year of applications, while visible signs of damage appeared on the other 6 palms during the 2nd year of treatments and eventually were not able to recover and died, indicating once again the preference of the pest to this palm species.

The most frequent problem appeared using the trunk injection method was the clogging of pegs with gum mainly on date palms, as a result of the injuries caused by gallery drilling in the trunk, preventing this way the insecticide from flowing within the trunk and transferred throughout the palm tissue.

Susceptibility data for deltamethrin and spinosad for *Ceratitis capitata* (Wiedemann) populations from Greece

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Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) causes severe damages to citrus and other fruits in Greece. For many years its control has been based on organophosphates insecticides, but there has been a switch to pyrethroids recently.

In the present study, the resistance status to deltamethrin and spinosad in 27 *C. capitata* samples from various hosts and regions of Greece was examined with dose-response bioassays. The bioassays did not reveal significant resistance in neither of the two insecticides. All but one samples tested with deltamethrin showed significant higher LD₅₀ than the laboratory susceptible strain (LS) but the Resistance Factor (RF) values were low (1.2–4.6). In support to this, no significant over expression of *CYP6A51* gene (associated with pyrethroid resistance in other studies) was detected in the samples examined compared to the susceptible strain, also indicating the absence of incipient pyrethroid resistance. The spinosad bioassays showed lower LD₅₀ variation among the samples compared to deltamethrin, 23 out of the 27 field samples had LD₅₀ significantly higher than the LS and RF values of 1.1–2.4.

In conclusion, the results suggest that deltamethrin can effectively control *C. capitata* in Greece and spinosad -once it gets registration for the control of this pest in Greece- would be a valuable alternative. However, continuous monitoring of resistance in the medfly populations is essential, given that P450-mediated metabolic resistance has been demonstrated for *C. capitata* populations in the Mediterranean basin.

The European network TOPPS: Best management practices in using plant protection products

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Best Management Practices (BMPs) in plant protection products (PPPs) should always be in accordance with the relevant European Union legislation, such as the Water Framework Directive (WFD) and the Directive on Sustainable Use of Pesticides (SUD). The European network TOPPS (from the initials of Train Operators and Promote Best Practices and Sustainability) aims at educating applicators in relation with the promotion of sustainable practices in crop protection. Since 2015, Greece participates with 11 other European countries in the TOPPS network, and implements various activities such as trainings, workshops, booklets, leaflets and digital material on BMPs. In general, the contribution of point sources to water pollution by PPPs is greater than that of diffuse sources, indicating the need for further training towards this direction. For this purpose, an online spray drift mitigation tool was created that is freely available and can be used with success by farmers. Another action was to conduct a stakeholders' survey with e-questionnaires, in order to assess the perceptions of professionals on the use of PPPs in relation with water protection. Finally, a demonstration farm has been established since 2015 by the University of Thessaly, to study runoff of certain selected PPPs, in relation with the presence or absence of vegetative buffer strips (VBS) in maize and cotton.

Investigating the mechanisms and the resistance levels to indoxacarb in Greek populations of *Tuta absoluta*

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Competition

The tomato borer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is considered the most important pest for tomato crops. Its management is primarily based on the use of insecticides, however development of resistance to specific chemical classes has made control of the pest extremely difficult. Indoxacarb belongs to the oxadiazine mode of action (MoA) group, acting on insect voltage-dependent sodium channel. It is one of the most effective insecticides for the control of *T. absoluta*. Within the frame of proactive resistance management, resistance levels to indoxacarb were closely monitored along with studies investigating the potential mechanisms of indoxacarb resistance.

During the 2015 -16 sampling period, eight Greek populations of *T. absoluta* were collected. Evaluation of the resistance was based on the IRAC method 022. Although no problems in the field performance have been reported so far, indoxacarb resistance was detected in four cases (Resistance Ratio, RR from 15- to 91- fold). One of the populations was subjected to selection with indoxacarb for two sequential generations, resulting in very high resistance levels (RR: 1794-fold). Cross-resistance among the main registered insecticides for *T. absoluta* management (chlorantraniliprole, spinosad, emamectin benzoate and metaflumizone) was not detected. Indoxacarb resistance, in absence of selection pressure, was unstable, with a negative rate of change, indicating an associated fitness cost. The mechanisms of resistance to indoxacarb were investigated. The presence of target site mutations F1845Y and V1848I, in segment6, domain IV of the sodium channel was detected. The presence of the mutation was strongly associated with the resistant phenotype. Synergism bioassays with enzyme inhibitors (DEF, PBO, and DEM) and subsequent biochemical analysis (activity levels of esterases, oxidases and glutathione S-transferases), indicated that detoxification enzymes were not a major component of resistance to indoxacarb.

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Investigating ketoenol toxicity in Mediterranean populations of the whitefly *Bemisia tabaci*

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The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is one of the most important pests worldwide. Here we monitored the susceptibility of 15 *B. tabaci* MED populations collected all over the Mediterranean Basin to two insecticides, spirotetramat and spiromesifen, that belong to spirocyclic tetroneic acid derivatives (ketoenols) as well as their role in the neonicotinoid resistance management. Full dose response leaf-dip bioassays on nymphs were performed with spirotetramat and spiromesifen, while diagnostic leaf bioassays on adults were performed with the neonicotinoid imidacloprid.

Populations from Greece and Italy displayed the lower LC₅₀ values, for both spirotetramat (LC₅₀<7 mg/L) and spiromesifen (LC₅₀<8.25 mg/L). Populations from Spain however, exhibited variable LC₅₀s ranging from low (LC₅₀=1.8 mg/L) to high values (LC₅₀=250 mg/L) for spirotetramat and from low (LC₅₀=1.4 mg/L) to extremely high values (LC₅₀>4,000 mg/L) for spiromesifen. Although the correlation among the LC₅₀s for ketoenols was not very strong, the higher LC₅₀s for both ketoenols were exhibited by the same populations. In addition, among the populations that exhibited reduced susceptibility to imidacloprid none displayed reduced susceptibility to ketoenols, suggesting absence of cross resistance between the two groups of insecticides.

Genetic studies using a highly resistant spiromesifen strain revealed that resistance was inherited autosomally and incompletely recessive. Results from F1 back-crosses suggested that resistance is polygenic.

In conclusion our results show the development of resistance in *B. tabaci* towards both ketoenols, which is geographically restricted to the South Spain. The use of ketoenols in alternation with insecticides with different mode of action classes as well as continuous monitoring of the efficacy should improve the sustainable chemical control of this major pest.

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The Achilles' heel of *Anopheles gambiae*? (LEGOmics)**V. BALABANIDOU¹, M. AIVALIOTIS¹, J.R. GIROTTI², A. KAMPOURAKI^{1,3},
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The most effective way to eradicate mosquito-borne diseases, as malaria is still remaining the use of insecticides. However, currently used insecticides are under the threat of rising resistance in vector populations. A very recent study of our team revealed a novel mechanism of pyrethroid resistance by reduced uptake of the drug through the legs – probably the most relevant for insecticide uptake body part. We demonstrated that resistant mosquitoes possess thicker leg cuticles, mainly because of enriched deposition of hydrocarbons to their outermost cuticular layer.

We first investigated if this mechanism is expanded to other mosquito populations from Africa and indeed, the hydrocarbon levels of highly resistant *An. gambiae* strains were found significantly higher compared to susceptible strains.

We subsequently conducted targeted (leg-specific) proteomics, to identify novel proteins implicated in cuticular resistance. Indeed 12 cuticular proteins found enriched in the resistant legs. An integrated multi-omics approach focusing on the resistant mosquitoes “super-legs” is currently underway (LEGOmics), aiming to identify the specific mechanisms mediating insecticide uptake and resistance in highly resistant *An. gambiae* legs, as well as possibly identify novel insecticide targets.

Levels and mechanisms of resistance in natural populations of the insect pest *Drosophila suzukii* (Diptera: Drosophilidae) from Greece**J. MOUSTAKA^{1,2,*}, V. DOURIS^{1,2}, A. ILIAS¹, I. LIVADARAS¹
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The fruit fly *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is one of the most polyphagous and destructive invasive pest species that infests grapes and several fruit. It has been first described in Asia, in the autumn of 2008, was reported in Europe (Tuscany, Italy), while since August 2014 there are recordings also in Greece. Although most fruit flies (*Drosophila* spp.) are not considered especially harmful pests, since they infest only rotten, fallen fruit, *D. suzukii* females possess a strong ovipositor that enables them to deposit their eggs at fruit which are still on the plant. A number of methods, including traps and insecticides, have been used for the control of *D. suzukii*.

In the present study, the resistance status of natural *D. suzukii* populations is compared with a laboratory population that has never been exposed to insecticides, in toxicity bioassays for a series of insecticides that are used or might be used in the field for insect control. These insecticides (active substances) include the pyrethroids β -cyfluthrin and λ -cyhalothrin, the neonicotinoid thiacloprid, the spinosyn spinosad, the diamide cyazypyr and the organophosphate chlorpyrifos. Furthermore, the sequences of the target genes of these active substances (sodium channels, nicotine acetylcholine receptor, ryanodine receptor, acetylcholinesterase) are examined in natural populations in comparison to the sensitive laboratory strain, for the detection of the presence and relative frequency of known resistance mutations, in order to assess the risk of development of resistance against the specific insecticide classes.

This knowledge will enable the design of molecular diagnostics for resistance that will contribute to efficient integrated pest management strategies for *D. suzukii* in Greece.

Effects of lemon balm essential oil, pennyroyal essential oil and pennyroyal essential oil encapsulated in microparticles of yeast cells (*Saccharomyces cerevisiae*), on insect and mite crop pests

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The purpose of the current study was to examine the effects of the essential oils extracted from the aromatic plants *Melissa officinalis* L. (lemon balm) and *Mentha pulegium* L. (pennyroyal) on the greenhouse pests *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). In addition, encapsulation of the pennyroyal essential oil in yeast cells of *Saccharomyces cerevisiae* was performed aiming to extend the duration of its effect. Microencapsulation was succeeded by mixing an aqueous dispersion of yeast cells with the essential oil. Extraction of the essential oils was performed via the technique of hydrodistillation using Clevenger apparatus while the separation and identification of the substances was performed using Gas Chromatography - Mass Spectrometry (GC-MS). The percentage yield of essential oils was calculated to be 0.2% (v/w) for lemon balm and 4.0% (v/w) for pennyroyal. The chromatographic analysis of the essential oils showed lemon balm to contain two main constituents, geranial (38.0%) and neral (26.9%) while pennyroyal contained mainly pulegone (43.1%) and piperitenone (37.4%). Emulsified lemon balm essential oil produced a significant repelling effect on the adults of *T. vaporariorum*. The pennyroyal essential oil caused high egg mortality and significantly reduced the fecundity of *T. urticae*. The residual effect of the encapsulated essential oil on the apterous adults of *M. persicae* in the first two days after treatment was similar to that of the essential oil, however the effect of the encapsulated oil was increased during the third day, in contrast to the effect of the essential oil that was eliminated.

Evaluation of insecticide effectiveness against the European grapevine moth

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Competition

Lobesia botrana (Lepidoptera: Tortricidae) is considered one of the most important pests of the vine, because it feeds on flowers and rails in all the vine varieties. Due to the damage this pest causes and the economic importance it has for the cultivation of the vine, its effective control is of great importance. The aim of this work is to study the effectiveness of registered insecticides on the European grapevine moth. For this purpose, insecticides with BACTOSPEINE WG (*Bacillus thuringiensis*), XELGAR (fexoxycarb), LAMBADA 10 CS WITH ZEON (lambda cyhalothrin), CYPERKILL MAX2 (cypermethrin), RELDAN (chlorpyrifos methyl), LASER (spinosad) were tested. These insecticides were applied at the label rates in "Thompson seedless" vineyards in Stimaga, Corinthia in a 2-ha vineyard. Every row of the vineyard received randomly different insecticide application. A total of five treatments were applied in fortnightly intervals with a high-pressure motorized sprayer. Evaluation of the effectiveness of the sprayings was made with optical observation for larval presence. Detailed results and discussion are given in the presentation.

Effect of spinosad in various physiological olive growth parameters when applied for olive fruit fly control

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Competition

Since the antiquity in the Mediterranean region and especially in Greece, the olive fruit fly *Bactocera oleae* (Rossi) (Diptera: Tephritidae), has coexisted with the olive tree and is so far a scourge for the olive-growing across Mediterranean regions. The protection of the olives requires treatments with insecticides whose effect on the various physiological mechanisms of the olive tree has not been studied.

The purpose of this research is to study the influence of insecticide treatments for olive fruit fly control on the physiology of the olive tree. Two treatments with spinosad were applied and 14 days past spray, leaf and fruit branches were collected from the treated trees and from unsprayed control trees. Different physiological parameters in olive trees were recorded such as photosynthesis, maximum photochemical yield and concentration of the amino acid proline, (usually accumulated in plants after the effects of various types of stress such as salinity, low temperatures, drought and contributing to face extreme environmental factors).

The results showed that application of the spinosad insecticide (Success 0.24 CB), improved the quality of olives and did not cause any changes in the photosynthetic mechanism of the olive leaves. The maximum photochemical yield (the ratio of variable chlorophyll fluorescence / maximum chlorophyll fluorescence) was similar to that of untreated olive trees, resulting in the ability of the plants to bind CO₂ efficiently, promoted the deposition of dry matter, and the quantitative and qualitative improvement of fruit yield. From the photosynthesis assimilation rate of CO₂, no reduction in the photosynthetic yield of the olive trees applied with spinosad, compared to the control, resulted in the photosynthesis being carried out without interruption. No reduction in transpiration rate was noted nor any adverse effects in stomatal conductance (stomatal opening/closure mechanism) compared to control trees. The percentage of proline concentration, was observed to increase relative to the control, which helped the olive trees acquire resistance mechanisms against stress. The response mechanism of trees after spinosad treatment may contribute to a satisfactory way of protecting against pests of the olive fruit fly.

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