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19th PANCHELLENIC ENTOMOLOGICAL CONGRESS

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ABSTRACT BOOK

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Preface

The Hellenic Entomological Society (HES), as non-profit scientific organization, has as its main purpose the expansion and dissemination of knowledge referred to entomological research. As a means to achieve its goals, HES organizes a biennial Panhellenic Entomological Congress, with contribution from both the scientific community and practisioners with geotechnical background.

The main objective of organizing this congress is to exchange ideas and knowledge between researchers and searing the results of scientific research on current developments and new applications with geotechnicians and all those who are involved with the sensitive agricultural sector.

19th Panhellenic Entomological Congress take place in the city of Agrinio, one of the major financial centers in The Western Greece Region.

In the forthcoming 19th Congress 158 papers are going to be presented. Two round tables will be organized. The first one is about the integrated pest management on olive and citrus pests, two the most important cultivations in Western Greece Union. The other round table, refers to new technologies for controlling Insects of Medical Importance.

The Organizing Committee feel the need to express its warmest thanks the Scientific Committee, the invited keynote speakers, the members of the round tables and to all scientists which honor with their participation the works of 19th Congress, in such particular occasion, with Covid-19 pandemic, control health protocols only recently have been streamlined and this caused the annual conference postponement.

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

Finally, the Organizing Committee express its cordially thanks to all Congress sponsors for their significant financial support

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Organizing Committee of
19th Panhellenic Entomological Congress

Opening Session

Session «BIOLOGY - ECOLOGY» Part I

Session «BIOLOGY - ECOLOGY» Part II

Poster Session

Session «Biodiversity, Evolution and Systematics»

Poster session

Session «Chemical Control-Insecticide Resistance»

Poster session

Session «Biological Control-Alternative Control Methods»

Poster session

Poster session

Session «Physiosology-Behaviour-Phenology - Genetics-Biotechnology»

Poster session

Poster session

19th
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Opening Session

Insect pests and climate change: progress, trends, and challenges

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ABSTRACT

Climate change and extreme weather events have major impacts on crop production and agricultural pests. As generally adaptable organisms, insect pests respond differently to various causes of climate change. Because temperature is the most important environmental factor affecting insect population dynamics, it is expected that global climate warming could trigger an expansion of their geographic range, increased overwintering survival, increased number of generations, increased risk of invasive insect species and insect-borne plant diseases, and changes in their interactions with host plants and natural enemies. The effects of climate change on insects are complex because climate change favours some insects and inhibits others while affecting their distribution, diversity, abundance, development, growth, and phenology. Insects would likely expand their geographic distribution. Due to higher overwintering survival rates and the ability to develop more generations, the abundance of some pests will increase. Invasive pest species will likely establish more readily in new areas. As climate change exacerbates the pest problem, there is a great need for future pest management strategies. These include monitoring climate and pest populations, modified integrated pest management, and the use of predictive models. A proactive and scientific approach is needed to address this problem.

From 2020, the European Regional Development Fund has supported the project "AgroSPARC- Advanced and predictive agriculture for resilience to climate change", which will be implemented by the Nikola Tesla Innovation Centre, the Faculty of Electrical Engineering and Computing and the Faculty of Agriculture at the University of Zagreb in Croatia. The goal of this project is to use artificial intelligence to develop mathematical models for different stages of wheat development and use these models to predict yields and plant growth. Artificial neural networks will be developed to classify and select data on climatic conditions, weather forecasts, and indicators of plant development. These neural networks will learn and validate numerical models for different stages of plant development based on large experimental data sets. The models will be used publicly and interactively through an internet application to predict different stages of wheat development under real and hypothetical climatic conditions.

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Session:
Biology - Ecology
(Part I)

Morphology and biology of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), was first reported In Greece in 2014 as a nuisance pest. A few years later, in 2017, adults were observed to cause significant qualitative and quantitative damage on fruits in kiwi orchards in Northern Greece in the prefectures of Imathia and Pieria. This led us to start rearing this species in the laboratory in order to study its biology and morphology. *Halyomorpha halys* is successfully reared in the laboratory (26°C and 60% RH) on green beans. Larvae have 5 instars with a mean duration of 4.08, 9.87, 6.53, 7.62 and 8.80 days from first to fifth instar. Right after their emergence, adults are white colored for a short period of time and immediately after that they obtain their typical brownish color, with characteristic light-coloured bands on their antennae on the base and apex of the second segment and the base of the first segment, white stripes on their legs and alternating dark and light bands on the margin of their abdomen. Females lay 257 eggs on average on the lower surface of the leaf, which initially have a light green color, while over time they become white and smooth. Eggs are about 1.3 mm in diameter and 1.6 mm in height and are placed in groups of 26-28 eggs (egg masses) and hatch within 5.8 days, with a mean mortality of 12.5%. Developmental time from egg to adult takes about 40 to 60 days, while the duration from egg to the death of the adult equals to 96 days for males and 116 days for females.

This research was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK) under the call "Research-Create-Innovate".

Keywords: *Halyomorpha halys*, biology, growth stages, developmental time, egg masses, hatchability.

Development of larvae of the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) on byproduct-based isoproteinic diets

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The valorization of agricultural byproducts as insect feed has lately attracted a lot of scientific interest, as it is aligned with current circular economy strategies, whereas it can substantially contribute to the reduction of insect production cost. In this context, the aim of the present study was to evaluate the potential of eleven agricultural byproducts to support the development and growth of larvae of the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae).

Byproducts stemming from the production of cotton, sugar beet, sunflower, barley, oats, peas and vetch were tested. Initially, byproducts were screened singly. Based on the results of the first screening, isoproteinic diets based on the tested byproducts were designed at two levels of protein (16.7 and 20%) and evaluated as feeding substrates for *T. molitor* larvae in a second series of bioassays.

The results showed the suitability of several of the tested byproducts for the rearing of *T. molitor* larvae. When byproducts were evaluated singly, larvae grew well when fed oats and barley byproducts. Similarly, diets based on the oats and barley byproducts sufficiently supported larval growth. Interestingly, there were significant differences in larval growth and performance among diets with the same protein level, indicating that, apart from protein content, other factors have also to be taken into account when formulating insect diets. These results aim to contribute to the integration of circular economy strategies in the production of insects as food and feed.

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (Waste4Bugs, Project code: T1EDK-1528).

Keywords: agricultural byproducts, circular economy, edible insects, insects as food and feed, insect protein

Effect of host fruit and temperature on the demographic characteristics of different Mediterranean fruit fly*, Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) populations

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Temperature and host are significant predictors of the life history traits of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae). Apples and bitter oranges are key overwintering hosts of medfly larvae in cooler, temperate areas. Despite the high number of studies on the adult demography in controlled laboratory conditions there are only few using fruit hosts and none of them examining how pre-adult (egg to adult) "experience" can affect the demographic characteristics of adult medflies. We used five *C. capitata* populations originated from Zaton (Croatia), and Thessaloniki, Volos, Chios, Crete (Greece) reared in the above two hosts in the laboratory, under constant and fluctuating temperature regimes. To assess the adult lifespan and female fecundity of individuals, artificially infested fruits (10 eggs/fruit) were kept at three constant (15, 20, and 25ºC) and five different thermocycles until pupation and adult emergence. Upon emergence, pairs of adults (1♀ and 1♂) were transferred and kept in individual demographic cages at 25ºC. Longevity and fecundity were recorded daily. Our results demonstrate that the longevity of adults is strongly affected by several factors that immatures experience, such as host fruit and temperature of development. In addition, the population and the sex of the individual were significant predictors of the adult longevity. The population was a significant predictor of the fecundity of females. The above results contribute towards understanding the invasion dynamics of medfly and the biological traits that contribute to invasion success with emphasis on the range expansion to northern, more temperate areas of Europe.

Keywords: Tephritidae, Diptera, demography, fecundity, lifespan, host, invasion dynamics, range expansion

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Contribution to the study of the arthropod fauna of the island of Lesvos

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Lesvos is the third biggest island of Greece, part of the East Aegean Islands and located near the coastline of Anatolia peninsula. In contrast to other islands, such as Crete, Corfu, Samos and Rhodes, the island's arthropod fauna remains understudied. For these reasons, a preliminary research took place on the island of Lesvos, which includes records from 10 different insect Orders - Archaeognatha, Blattodea, Dermaptera, Embioptera, Mantodea, Megaloptera, Neuroptera, Plecoptera, Raphidioptera and Zygentoma.

For the elaboration of the present work, samples were collected from Lesvos Island from April 2019 to January 2022. All collected arthropods were photographed with a camera and, then, the photos were uploaded on the online platform iNaturalist in order to fully record the place and the date of each observation. Finally, hard-bodied insects were dried, pinned and placed in special entomological boxes with camphor, while the soft-bodied insects were placed in 70% ethyl alcohol for better maintenance and study. The specimens are deposited in the entomological collection of the author and in the entomological collection of the Zoological Museum of the National Kapodistrian University of Athens.

In total, 33 different species of Arthropods from 10 Orders were collected, 3 of which are considered new records for Greece and 24 for the island. In addition, 7 of these recordings are alien species for the island of Lesvos, while 2 endemic species of the wider region of Lesvos-Northwest Anatolia were recorded. Finally, the Orders Zygentoma and Megaloptera have been recorded for the first time on the island. Order Megaloptera is recorded for the second time in Greece and simultaneously for the first in the Aegean.

Key Words: Lesvos, Archaeognatha, Blattodea, Dermaptera, Embioptera, Mantodea, Megaloptera, Neuroptera, Plecoptera, Raphidioptera, Zygentoma.

Ground cover flowering plants to attract and preserve insect pollinators in apple orchards, cv. 'Delicious Pilafa'

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Effective pollination, high production and quality of apple fruits depend on insect pollinators, which benefit from the presence of alternative habitats in apple orchards.

The aim of this work is to evaluate the effect of a selected mixture of flowering plants on the attraction of pollinators in apple cultivation, cv. 'Delicious Pilafa'. For this purpose, experiments were carried out in three apple orchards (one of integrated and two of organic management) in Tegea, Tripoli, in the spring of 2020. In each apple orchard, two ground cover treatments were tested: a) a mixture of selected flowering plants b) native vegetation according to the weed management practice followed by the grower (control). The treatments were established between the tree rows in experimental plots of 6.4 m^2 in distinct parts of the fields with six replications each. The plant species in the flowering mixture were *Anethum graveolens* L., *Coriandrum sativum* L., *Eruca sativa* L., *Lathyrus sativus* L., *Vicia faba* L., *Vicia sativa* L. The main flowering weeds in the control were *Calepina irregularis* (Asso) Thell., *Capsella bursa-pastoris* (L.) Medik., *Cardaria draba* L., *Lamium* spp., *Ranunculus* sp., and *Veronica* spp.

The recordings of insect pollinators' visits on the flowering mixture showed greater attraction of honey bees and wild bees compared to the weed vegetation. The main wild bees recorded in the flowering mixture are taxonomically classified to the genera *Anthophora*, *Bombus*, *Eucera*, *Lasioglossum*, while in the weed vegetation to the genus *Lasioglossum*. On the apple blossoms of the adjacent trees to the ground cover plots *Apis mellifera* visits were mainly recorded, as well as wild bees such as *Bombus* spp., which were also attracted by the flowering mixture (*V. faba*). Overall, the number of pollinators' visits on the apple flowers did not differ between the ground cover treatments. The results contribute to the development of good agricultural practices to attract and preserve pollinators in the aforementioned agroecosystems.

Keywords: agroecosystem, apple orchard, flower mixture, integrated management, pollinators.

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Session:
Biology - Ecology
(Part II)

Presence, food preference of Auchenorrhyncha (Hemiptera) in citrus and correlation with chlorotic spots on citrus fruit

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ABSTRACT

Auchenorrhyncha are worldwide known, forming an important part of the world’s fauna, found only in terrestrial ecosystems. They are known to affect crops by harming plants directly through feeding and indirectly due to their ability to transmit plant pathogens. Especially, on citrus trees, Auchenorrhyncha can cause round scars on fruits which reduce their market value, and they can also transmit two harmful plant pathogens; a) Phytoplasma *Spiroplasma citri* Saglio (Mollicutes: Spiroplasmataceae), causing the "Stubborn disease of citrus" and b) Bacterium *Xylella fastidiosa* Wells et al. (Gammaproteobacteria: Xanthomonadaceae), causing "Citrus Variegated Chlorosis [CVC]". This project aims to record and identify Auchenorrhyncha populations; indicate the symptoms caused by them on citrus fruits and examine their food preferences (wild vegetation, leaves, peelings of different citrus varieties and leaves of grapevine varieties).

To this purpose, the writer performed samplings using a sweep-net in the experimental orchard of the Agricultural University of Athens (AUA). The collected insects were placed in cloth cases and tied around citrus fruits. After a while, samplings were transported to the Laboratory of Agricultural Zoology and Entomology of AUA, where both the number and the diameter of the scars were recorded. Moreover, the living individuals were tested in a 4-way olfactometer to examine their food preferences (wild vegetation, leaves and peelings of different citrus varieties and leaves of grapevine varieties), while the dead ones were placed in vials with ethanol 95% and sorted after male genitalia maceration.

The findings showed that there is a strong connection between specific Auchenorrhyncha species and both the number and the surface of scars on infected orange fruits. They also showed statistically important differences in auchenorrhynchan behavior when exposed to three or more different sources of olfactory stimuli (triple and fourfold selection procedures). Further details on the experimental procedure and results, with extensive discussion will be provided in the presentation.

Keywords: Auchenorrhyncha, citrus, wild vegetation, scars, olfactometer

Effect of temperature and host plant species on the juvenile development of *Euscelis ohausi* (Hemiptera: Cicadellidae)

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Euscelis ohausi (Hemiptera: Cicadellidae) of the subfamily Deltocephalinae is a potential vector of phytopathogenic microorganisms. High populations of the species have been recorded on the herbaceous vegetation of olive groves in Crete. To understand the aspects of the biology of *E. ohausi* that could explain its population dynamics in the field, the effects of temperature and host plant on the development and survival of young nymphs were studied under laboratory conditions. Specifically, we studied juvenile development and survival to the adult stage in a range of constant temperatures (15 to 33 ºC) on oat, as well as the effects of host plant species (oat and vetch) at 25 ºC. Temperature was found to affect both developmental rate and survival of *E. ohausi* nymphs. At the higher temperatures tested of 30 and 33 ºC, hatching rates as well as the respective recorded survival to the adult stage were low (32 and 3.4 %, respectively). Optimum temperature for egg hatching was recorded at 25 ºC, and recorded survival to adulthood was also high. The increase in temperature led to a gradual reduction of juvenile developmental time with the maximum value recorded at 15 ºC and the minimum at 30 ºC. At 25 ºC the mean total developmental time on oat was lower compared to vetch without however any significant differences in the respective survival on the two hosts. From the results of our study, the optimal growth temperature, the upper and lower temperature thresholds and the sum of day degrees for the development of the insect were calculated. Assessing the effects of temperature and host plant species on the development and survival of *E. ohausi* is important for understanding the populations dynamics and phenology of this species under field conditions.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT) under the HFRI PhD Fellowship grant (GA. no. 770).

Key words: Auchenorrhyncha, *Euscelis ohausi*, Hemiptera, temperature, oat, vetch

INSECT DIVERSITY IN A COASTAL PINEWOOD AND MARSH AT SCHINIAS, MARATHON, GREECE

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We collected insects in a mixed coastal pinewood and a marsh in Schinias, Marathon, Greece and studied them in relation to the anthropogenic disturbance on the area. A hundred-and-forty insect species were found and 7 insect community types were found. All these types re-emerged in a non-metric multidimensional scaling arrangement of plots. It was also found that insects tend to dwell in different plots even in the same habitat. The invading *Pinus halepensis* which replaces the pine *P. pinea* changed the entomofauna. The seven habitats had different numbers of bioindicators out of 74 insect species not in general correlated with human impact. Three components of insect diversity were measured, namely α (richness), β (species turnover), and γ (landascape) and the temporal species turnover was consistently higher than the spatial one. In terms of biodiversity the impact on the various biotopes was assessed by means of the diversity-equitability index *V* which indicated that the two *P. halepensis* dominated habitats that have negative *V* values together with the habitat dominated by *Tamarix hampeana*. The conservation of *P. pinea* and marsh habitats is recommended for conserving local entomofauna and stopping the progression of *P. halepensis*.

Key words: biodiversity; pinewood; *Pinus halepensis; P. pinea*; diversity partitioning temporal diversity; habitat specificity; anthropogenic disturbance; additive diversity partitioning; insect functional roles

Age of female Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) at oviposition and larval developmental duration affect the biological traits of progeny

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We tested whether the age of females of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) at oviposition and the variability of larval developmental duration affect the demographic traits of progeny. Under constant laboratory conditions, we artificially implanted eggs laid by F_2 generation young and old females (10 and 40 days old respectively) into Golden Delicious apples. Pupae were collected and grouped according to the duration of larval development (short and long-developed larvae). Emerging adults originating from young and old females and from larvae with short and long developmental duration were paired in individual cages with *ad libitum* access to adult diet and water. Adult mortality and female fecundity were recorded daily. Offspring of 40 days old females suffered higher larval mortality in comparison with those originating from 10 days old females. In contrast, pupal mortality was higher for those obtained from young females. Egg to pupa developmental duration was shorter for the progeny of old females, while their size was larger than the progeny of young females. Adult flies with short larval developmental duration lived longer than the long-developed ones, regardless of the age of parental females during oviposition. Neither the duration of larval development nor the age of parental females affected the fecundity parameters of female progeny. Maternal effects on their progeny's fitness parameters are discussed.

Keywords: demography, immature stages, maternal age, medfly, Tephritidae.

Rearing of the insects, *Hermetia Illucens* (Diptera:Stratiomyidae) and *Tenebrio molitor* (Coleoptera:Tenebrionidae) on Substrates Based on plant Residues, from the central market of Athens (OKAA).

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Insects are proposed by the World Food Organization (F.A.O.) as a dynamic alternative source of protein for animal nutrition (Van Huis et al. 2013). In the E.U, specific species (*H. illucens & T. molitor*) of insects are produced for use as animal feed exclusively for pets. A key point in their further usefulness is the recent European Regulation 2017/893 of 24 May 2017, which allowed the use of 7 species of insects (including *H. illucens* and *T. molitor)* as feed in aquaculture.

Given the fact that the use of animal raw materials from ruminants, food scraps and animal feces as component substrates for farmed insects is explicitly prohibited, according to European regulations no. 767/2009 and 2017/893. Therefore, the diet of farmed insects is proposed to consist mainly of plant materials that present an acceptable level of food security to the consumer.

In the framework of the INSECTS for AQUA project, the production of biomass (insect larvae) was studied, using as breeding substrate plant residues (fruits & vegetables) from the Central Market of Athens (OKAA). Residue samples were taken, which were subjected to mild treatment (shredding, drying, milling, sieving, mixing). Three substrate composition methodologies were tested: 1) 100% reference substrate (chicken feed or wheat bran), 2) 100% plant residues, 3) 50% reference substrate & 50% plant residues.

The growth duration and the final weight of the larvae of both species of insects were observed differentiated, depending on the substrates derived from specific types of plant residues. The results show that the available plant organic residues of the Central Market of Athens, can be a satisfactory substrate for the development of larvae of the two insect species. The aim is to contribute to the investigation of the production of nutritionally adequate raw material (insect flour), which can be used in the preparation of diets for aquaculture.

Evaluation of agar as moisture source and nutrient carrier for *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae

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Moisture availability is a crucial factor that enhances the growth of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae. Various vegetables such as carrots and potatoes are commonly used as a source of moisture. However, the nutritional composition of vegetables is not stable and varies, resulting in differences in insect growth. Moreover, since vegetables dry out quickly and are prone to microbial deterioration, they have to be replaced frequently. Agar is the most common gelling agent in artificial insect diets. Nevertheless, it has not been evaluated yet for *T. molitor*. In this framework, the aim of the present study was to evaluate agar gel as an alternative source of moisture and protein carrier for the targeted delivery of nutrients to *T. molitor* larvae. In a first series of laboratory trials, 50 early-instar larvae of *T. molitor* were placed in vials with wheat bran. Three times per week, the vials were supplied with agar cubes (1 cm^3) amended with different concentrations of brewer's yeast (0, 0.5, 1, 2.5 and 5%). In two pilot scale trials, approximately 10,000 larvae were reared in crates with wheat bran as dry feed and agar with different concentrations of baker's yeast or white sugar (0, 2.5, 5 and 10%) as wet feed. In all trials, larval growth was recorded at regular intervals. Based on our results, agar gel proved to be a good source of moisture, as high survival rates were recorded in all cases for *T. molitor* larvae. In the lab trial, the increase of the percentage of brewer's yeast in the agar gel positively affected the larval growth speed, the highest the percentage of yeast, the shortest the development time. Similar results were obtained for baker's yeast in the pilot-scale trial. In contrast, larval growth was negatively affected when larvae were provided with agar with high concentration of sugar (10%). To conclude, our results show that agar can efficiently serve as a moisture source, as well as a nutrient carrier in the rearing of *T. molitor* larvae.

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Key-words: agar, insects as food and feed, edible insects, insect protein, *Tenebrio molitor*

Effect of olive groves cultivation on the abundance of insect vectors of *Xylella fastidiosa*

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Xylella fastidiosa Wells et al. (Xanthomonadales: Xanthomonadaceae) is a bacterium which causes several plant diseases of economic importance and is exclusively transmitted by xylem sap feeding insects, mainly spittlebugs and sharpshooters. During 2018 and 2019 samplings were conducted throughout the year in olive groves in Achaea to investigate the impact of ground vegetation and sowing time on spittlebugs' abundance. An uncultivated olive grove with wild vegetation as ground cover was compared with two neighboring olive orchards which were subjected to co-cultivation. One orchard was cultivated with *Avena sativa* in 2018 and 2019. The soil tillage and the sowing of the field was conducted at the end of October of 2017 and 2018. The other orchard was cultivated with *Vicia sativa* during 2018 and with *A. sativa* during 2019. The soil tillage and the sowing of that field was conducted at the end of October in 2017 and at the end of November in 2018.

Our data showed that cultivation affects the spittlebugs' spring populations depending on the plant species cultivated and the sowing time. In general, early cultivation (end of October) with *A. sativa* had a positive effect on *Philaenus spumarius* Linnaeus (Hemiptera: Aphrophoridae) and *Neophilaenus campestris* Fallén (Hemiptera: Aphrophoridae) while late cultivation (end of November) had a negative effect. The cultivation of *V. sativa* had a rather negative impact only on *N. campestris*. The cultivation of plants of Poaceae family such *A. sativa* under the olive trees may result in increasing spittlebug's abundance as they can be used as hosts by both species. On the contrary, cultivation of plants of Fabaceae family, like *V. sativa*, which are rarely selected as hosts by *N. campestris* may reduce the population density of that spittlebug. Moreover, sowing of *A. sativa* at late November resulted in reduced nymphs' population of both species.

Key words: olive groves, *Neophilaenus campestris, Philaenus spumarius, Xylella fastidiosa.*

Predatory and phytophagous mites on native vegetation of Kea island, Greece

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Mites are found in almost all environments showing great diversity depending on the habitat. Aiming to a preliminary study of phytophagous and predatory species found on native vegetation in Kea island, plant samples were collected from January to August 2020 from many localities of the island.

A total of 314 plant samples from 121 different plant species, some of which endemic of the Cycladic complex, were collected. For the extraction of the mites Berlese-Tullgren funnel was used. Permanent slide mounts were prepared and all individuals were examined by using phase contrast microscope.

The examination of the plant samples revealed 82 taxa belonging to 15 Families and two Orders (Mesostigmata and Trombidiformes). Amongst these, 15 species are new findings for Greek fauna.

Keywords: Acari, native vegetation, new findings, Kea.

Study of reproductive diapause of overwintering female *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northern Greece

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Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is a polyphagous species, native to East Asia, that overwinters as an adult in natural and human-made structures. Adult emergence from overwintering sites starts in spring, whereas females produce offspring in early summer on host plants, where most feeding occurs. In this study, we investigated the incidence of reproductive diapause in overwintering females of *H. halys* in Northern Greece, by determining the preoviposition period and fecundity of individuals. Adults collected from overwintering sites were left to overwinter in natural weather conditions and were subsequently transferred in groups of 10 males and 10 females in laboratory conditions (26 °C, 60% RH, 16:8 h L:D) monthly, from December 2020 to March 2021. For each month, 6 replicates were conducted. The mean preoviposition period of overwintering females that were transferred to laboratory conditions in December 2020 was 29.0 days and significantly longer by 8.3 days than that of females that were transferred to laboratory conditions in March 2021, and by 13.2 days than that of the laboratory colony. No significant difference among the average number of eggs per egg mass laid by overwintering individuals brought in laboratory conditions and the laboratory colony was observed. However, females that were left to overwinter in natural conditions and were brought in the laboratory in March laid a significantly higher number of eggs in total, compared to the ones whose overwintering was disrupted in February. According to our results, overwintering females of *H. halys* experience a facultative reproductive diapause in Northern Greece.

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Key words: reproductive diapause, facultative diapause, preoviposition period, fecundity, egg masses, brown marmorated stink bug.

The genus *Bursaphelenchus* and other nematode groups associated with conifer forests of Northern Greece: a preliminary overview

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The pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Parasitaphelenchidae, Nematoda) is one of the most important pathogens of high ecological and economic importance worldwide, causing the Pine Wilt Disease (PWD). Via the expansion of international trade, *B. xylophilus* has managed to expand beyond its natural range with Wood Packaging Material (WPM). After its first detection in Portugal in 1999, the establishment of national monitoring programmes across European countries and the implementation of appropriate measures were deemed necessary in order to prevent its further expansion. Hence, in the context of the Greek national monitoring programme, 70 wood samples were collected from conifer trees showing symptoms of infestation out of regions of Northern and Central Greece. Nematode extraction was carried out using a modified Baermann funnel technique, and identification was based on their morphological traits. Even though *B. xylophilus* was not retrieved from any of these samples, four other species of the *Bursaphelenchus* genus were identified. In addition to them, other nematode groups that contain phytophagous, microbivorous, predatory, and entomophilic nematodes were also recorded. It is thus evident that besides preventing the introduction of *B. xylophilus* in Greece, the national survey program can substantially contribute to increase our knowledge on the nematode species inhabiting conifer trees in Greece.

Keywords: *Bursaphelenchus* spp., conifers, Greece, nematodes.

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Comparison of Macro and Mesofauna soil fauna in two tree species in Crete

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The use of tree rows is common in urban environments (urban-suburban areas), for shade, ornamental value, and also as windbreaks. Here we investigate the composition and compare the biodiversity of Mesofauna and Macrofauna in native and alien urban tree rows. Two tree species were compared for 14 weeks, the native in Crete *Cupressus sempervirens* Linnaeus (Pinales: Cupressaceae) and the alien *Eucalyptus camaldulensis* Dehnh (Myrtales: Myrtaceae). Sampling was carried out at tree rows established at the experimental farm of the Hellenic Mediterranean University, in a suburban area of Heraklion. Ground fauna was trapped using Berlese - Tullgren funnels, while surface-moving invertebrates were trapped with pitfall traps. Also, above-ground fauna of the trees was monitored at a height of 1 m from the ground, using trunk traps with vinegar.

Comparing the results, there is a statistically significant difference in Berlese traps as Acari were more abundant under *C. sempervirens* (60% vs. 26%), but under *E. camaldulensis,* a class of Collembola - Poduromorpha (186 captures - 48% vs. 92 captures - 12%) was prevalent. In pitfall traps, Collembola outnumbered all arthropods in both tree species. In trunk traps, the value of Shannon index in *C. sempervirens* for biodiversity was lower (0.838) than in *E. camaldulensis*. (0.965).The difference between total animal captures in trunk traps of C. sempervirens and E. camaldulensis is statistically significant (p= 0,019). The results show that, although both plant species have satisfactory biodiversity values based on pitfalls (in anthropogenic ecosystem), the native tree *C. sempervirens* displays higher biodiversity average.

Key words: Acari, biodiversity, Collembola, *Cupressus sempervirens*, *Eucalyptus camaldulensis*, tree rows.

Development of *Tenebrio molitor*, *Zophobas morio* and *Alphitobius diaperinus* larvae in a substrate enriched with functional ingredients of aromatic and pharmaceutical plants

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The inclusion of active ingredients and bioactive compounds in insect diets can be a key factor in their growth and development. In this context, the aim of the present study was to evaluate the growth of larvae of the yellow mealworm, *Tenebrio molitor*, the superworm *Zophobas morio* and the lesser mealworm, *Alphitobius diaperinus*, on a substrate enriched with active ingredients of aromatic and pharmaceutical plants of the Greek flora. Insect diet was enriched with a mixture of (a) aromatic plants of the Greek flora, such as essential oils of oregano, thyme, sage and rosemary, as well as by-products (post-distillation residues) of these aromatic plants; (b) linseed oil, rock-samphire and industrial cannabis and c) olive paste by-product. Insect diet was enriched with various percentages (10, 20 and 30%) of the aforementioned mixture, whereas wheat bran alone served as control. Our results showed that the inclusion of 10 and 20% of the additional dietary ingredients improved larval growth and survival of *T. molitor.* However, developmental time was negatively affected. Similar results were observed for *A. diaperinus* larvae. In contrast, the inclusion of these ingredients positively affected the larval development of *Z. morio* in all three treatments. The data of the present work aim to promote the production of insects enriched with ingredients that impart desirable properties for both animal feed and human consumption.

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Keywords: circular economy, edible insects, insects as food and feed, insect protein

Standardisation of feed experiments with larvae of the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): the Greek perspective

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The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), is one of the most promising and well-studied insect species as food and feed. As larval growth and performance greatly depends on the feeding substrate, a lot of research on this species has been directed on the suitability of various commodities and diets as *T. molitor* feed. However, the wide variation in the experimental setups used for the evaluation of various feeds as feeding substrates for *T. molitor* larvae renders the comparisons among the results of different trials difficult. To address this issue, a consortium of research organizations, as well as commercial insect producers tried to design and develop an appropriate, standardized experimental protocol for feed experiments with *T. molitor* larvae. Subsequently, all partners implemented the protocol to determine the comparability of the results generated by the partners. The Laboratory of Entomology and Agricultural Zoology of the University of Thessaly (UTH) participated as partner into this consortium and the results of the implementation of the standardized protocol in UTH are presented here. Initially, 250 g *T. molitor* adults were allowed to oviposit for 4 d on crates (60 x 40 cm) with wheat bran. Two wheat brans, a local one and a common bran for all partners, were used as oviposition and feeding substrate for the larvae. After 4 weeks, larval density was adjusted to approximately 10,000 larvae per crate. Agar was provided to the larvae as a moisture source and larval growth was estimated at weekly intervals until all feed provided (2.1 kg) was consumed. Based on the results, *T. molitor* larvae grew well in both brans tested, with the common bran giving slightly better results than the local bran. Indicatively, the final individual larval weight was 106.8 and 110.7 mg for the local and the common bran, respectively. All feed was consumed after 10 weeks of larval development. These results will be consequently compared with the results of the rest of the partners that participate in this ring-test taking into consideration the genetic variability among the *T. molitor* strains used by the partners.

Key-words: experimental design, feed experiments, insects as food and feed, edible insects, insect protein, *Tenebrio molitor*, standardization

EVALUATION OF AGRICULTURAL BY-PRODUCTS AS FOOD FOR BEETLE DEVELOPMENT IN THE CONTEXT OF THEIR MASS REARING FOR THE PRODUCTION OF FEED ADDITIVES

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Recently, the Member States of the European Union unanimously decided, based on European Regulation 2017/893, to authorize the use of insect proteins in fish and poultry feed. For this purpose, various species of beetles, flies and crickets are currently being mass reared on a commercial and experimental scale. These include the tenebrionids *Tenebrio molitor* L. and *Zophobas morio* F. (Coleoptera : Tenebrionidae). In the present study, these species were reared on different plant debris. More specifically, the growth duration (egg to adult) and the weight of larva, pupa and adult of *T. molitor* and *Z. morio* were measured in different food substrates (ad libitum supply) derived from by-products of seed production industries (barley, alfalfa, oats, chickpea, etc.). Nutrient-rich feed (feed for laying hens) was used as a positive control, while wheat bran was used as a negative control. The experimental insects were kept at 28^{o}C individually and monitored daily to determine survival as well as changes in growth stage and/or larval instar. The weight of the experimental insects was calculated weekly on a precision balance. The shortest developmental duration was observed in the positive control (78-93 days) for both *T. molitor* and *Z. morio* (119-148 days); the number of larval instars was 9-14 and 13-20, for *T. molitor* and *Z. morio*, respectively. The maximum larval weight of *T. molitor* was recorded 8-11 weeks from egg stage, while for *Z. morio* at 13-19 weeks. Supply of potato pieces (source of moisture) significantly accelerated the developmental rate and increased larval survival and weight in most cases. Increased nutrient content of the food did not always cause a significant increase in developmental rate or larval weight. The results of the study are analysed in the context of the optimal use of agricultural by-products to reduce the time and cost of mass rearing of these insects to produce feed additives (insect flours).

Key words: *Tenebrio molitor, Zophobas morio,* developmental time, edible insects

Survival of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) adults deprived of food at various ages

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The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is a monophagous insect with wide geographical distribution and high economic importance. The present study investigates whether age influences endurance of adults to food deprivation. The experimental insects originated from wild pupae reared on olives in the laboratory. Emerging adults were caged individually with food (a mixture of yeast hydrolysed and sugar) and water. At different ages ten males (0 – 88 days) and ten females (0 – 121 days) were transferred to new cages devoid of food. Survival of these adults – endurance at different ages – following food deprivation was monitored. The results showed that in both sexes endurance dropped significantly with age because of food deprivation. The younger adults (1 – 10 days) endured the most, followed by those of the age class 10 – 20 days. No statistically significant differences in endurance were observed between adults belonging to older age classes (41 – 80 days for males) and (41 – 60, 71 – 90, 121 – 130 days for females). Almost at all ages females were more resilient than males. Statistically significant differences in endurance were observed between males and females at the respective age classes (41 – 90 days) with this difference between the sexes being greater in the age 71 – 80. The differences observed in survival are most likely related to the food reserves of adults at different ages and in the case of females also with their egg maturation cycles. The findings contribute to an understanding of the survival ability of *B. oleae* under unfavorable environmental conditions to find more effective methods of this pest`s management.

Keywords: *Bactrocera oleae*, pests` management, starvation, aging.

Efficiency of *Chrysoperla* (Neuroptera: Chrysopidae) species as biological control agents of pests in pepper

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The family Chrysopidae (Neuroptera: Chrysopidae) includes approximately 1200 species. The larvae of all species are voracious predators and feed on various soft-bodied arthropods. Many chrysopids are well known for their high predation efficiency against several key pests of crops yet, only a few species are currently commercially available, with *Chrysoperla carnea* (Stephens) sensu lato being the species most widely used in biological control. A number of other *Chrysoperla* species may prove more efficient/suitable than the commercial *C. carnea* in controlling different pests under specific conditions. Here, we assessed the ability of two *Chrysoperla* species, namely *C. agilis* Henry et al. and *C. mutata* (McLachlan) to consume aphids and mealybugs and suppress their populations on sweet-pepper plants. We found that 3rd instar larvae of both species were able to consume a high number of aphids and mealybugs within one day. Furthermore, the release of 2nd instar larvae of both *Chrysoperla* species was shown to be efficient in suppressing the pests' populations in greenhouse experiments. Our results highlight the efficiency and biocontrol potential of two widespread *Chrysoperla* species for their use in pest control.

Key-words: aphids, biological control, *Chrysoperla*, mealybugs, pepper

Intraguild predation between the brown lacewing *Micromus angulatus* (Stephens) (Neuroptera: Hemerobiidae) and the phytoseiid *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae)

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Micromus angulatus (Neuroptera: Hemerobiidae) is a brown lacewing species of the family Hemerobiidae, widely distributed in Europe, North America and Northern Asia. It is a promising biological control agent which was recently launched commercially for use in biocontrol practice whose larvae and adults are predatory and feed on several aphid species. On the other hand, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is a predatory mite of the family Phytoseiidae, routinely used in biological control against thrips and whiteflies. Both species are generalist predators and the possibility they coincide in the greenhouse is high. The present work focused on the study of intraguild predation between *M. angulatus* and *A. swirskii* under laboratory conditions. For this, we tested different combinations of the different instars of the two predators i.e. eggs, larvae and adults resulting in a total of six treatments for the non-choice experiments and four treatments for the choice experiments. Our study casts light on the potential of the two predators in intraguild predation and results could be useful in their concomitant use in biological control practice.

Key-words: intraguild predation, biological control, brown lacewing, phytoseiid, predator

Effects of beneficial soil microbes on the biology of natural enemies

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To defend themselves against herbivory, plants have evolved sophisticated mechanisms including a series of constitutive and inducible defense responses. The release of volatiles is one such response which aims to inform natural enemies about the presence of prey and attract them to infested plants. Nevertheless, other plant responses such as those induced by beneficial soil microbes can be harmful to natural enemies if for example, plants are not capable of identifying beneficial arthropods from herbivores or if predators consume prey that live on defended plants. Herein, we studied whether beneficial soil microbes affect the performance of aboveground predators and parasitoids. For this purpose, we recorded the survival and offspring production of the mirids *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) and the phytoseiid *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) on plants infested with different herbivores or non-infested plants which were either inoculated with microbes or not. Furthermore, we assessed prey consumption by the mirids and the phytoseiid, as well as parasitism efficiency of the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae). Our results show that tomato inoculation with microbes results in variable effects on natural enemies' performance on herbivore-infested plants, depending on the microbe, the herbivore and the natural enemy species under study. Overall, we highlight the importance of addressing the plant-mediated effects of beneficial soil microbes on the performance of natural enemies towards the identification of sustainable pest control tools.

Key-words: arthropods, biological control, microbes, plant defense, predators, tomato

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Evaluation of susceptibility of different populations of lentil (*Lens culinaris* Medik.) to aphids

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Acrythosiphon pisum (Harris) (Hemiptera: Aphididae) is a key pest of lentil and a vector of plant pathogenic viruses. The development of plant varieties resistant to *A. pisum* could be an important plant protection tool that would lead to the reduction of the pesticides used. In this context, three lentil (*Lens culinaris* Medik.) landraces, i.e. populations ILL-590, FLIP 03-24L and FLIP 02-1L, were evaluated under laboratory conditions for their innate resistance related with antibiosis to *A. pisum*. In the experiments, aphid population growth on the different lentil populations was used as index of their respective resistance. The lowest intrisic rate of population increase of the aphid was recorded on the FLIP 03-24L population. In a 3-year conventional breeding project, single-plant selection for high yield within the FLIP 03-24L population led to eight progeny lines. Subsequently, the 3rd generation progeny lines were evaluated for antibiotic resistance against *A. pisum*. Intrinsic rates of increase and biomass growth of the aphid populations were used as resistance indices of the progeny lines. The analysis of the results revealed the need of combining antibiosis with plant tolerance effects when assessing the innate resistance of lentil lines to *A. pisum*. Our results may be helpful in the integrated management of aphid populations in lentil production.

Key-words: *Acyrthosiphon pisum*, antibiosis, lentil, tolerance

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Optimisation of protein quantity in larval diets for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Optimization of artificial larval diets for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is related to the quality and quantity of produced adults. Proteinaceous diets are source of essential nutrients that regulate adults' growth, longevity, reproductive capacity and adults' fitness. The successful and economically feasible rearing of the Mediterranean fruit fly requires a right balance between the cost of the protein source in the larval diet and the quality of the produced adults. The main source of protein contained in larval diets is brewer's yeast. The aim of this study was to investigate the optimal content of brewer's yeast on a novel agar-based diet for the Mediterranean fruit fly.

We used as control the standard liquid larval diet (100% brewer's yeast content), containing 50 g sugar, 50 g brewer's yeast, 25 g soy flour, vitamins and preservatives using cotton as diet's bulking agent. We examined the reduction of brewer's yeast concentration on the liquid diet, either using cotton pads or agar (gel) as bulking agents. The experimental trials were carried out in laboratory conditions (25±1℃ and 65±5% RH). The diets were evaluated based on survival and lifespan of immature stages and survival of the yielded adults.

Reducing brewer's yeast to 50% of its initial concentration had no negative effect to the fitness of the produced adults. Egg hatching rates were greater on the gel. Longer larval developmental duration observed in the gel diet compared to the liquid one. On low-protein concentration gel diets, larvae failed to pupate. Our findings can be exploited for the production of high-quality adults of the Mediterranean fruit fly at a lower cost in mass rearing conditions.

Key words: Brewer's yeast, fitness, gel, larval diet, successful rearing

Data on ecology of scale insects (Hemiptera: Coccomorpha) infesting ornamental plants in Messenia

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Data on ecology of scale insects (Hemiptera: Coccomorpha) infesting ornamental plants in Messenian Province (Peloponnese, Greece) during the years 2003 – 2020 were studied.

Five species of the family Diaspididae were recorded. *Aonidiella aurantii* (Maskell) infesting *Citrus sinensis* (L.) Osbeck and *C. aurantium* L. (Rutaceae). It completes three generations per year. Its natural enemies are the parasitoid *Aphytis chrysomphali* (Mercet) (Aphelinidae) and the predator *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae). *Chrysomphalus aonidum* (L.) found on *C. sinensis* and *Citrus limon* (L.) Obseck (Rutaceae), *Ficus benjamina* L. (Moraceae) and *Ligustrum japonicum* Thunb. (Oleaceae). It is a biparental and oviparous species. *Diaspis echinocacti* (Bouché) recorded on *Opuntia ficus-indica* (L.) Mill. (Cactaceae). It is biparental and oviparous species. The main natural enemies of the scale are the parasitoids *Aphytis debachi* Azim (Hymenoptera: Aphelinidae), *Plagiomerus diaspidis* Crawford (Hymenoptera: Encyrtidae) and the predator *Cybocephalus fodori* Endrödy-Youga (Coleoptera: Cybocephalidae). *Lepidosaphes beckii* (Newman) recorded on *C. sinensis* and *C. aurantium*. It completes three generations per year. The natural enemies of this scale insect are the parasitoids *Aphytis lepidosaphes* Compère and *Encarsia* sp. (Hymenoptera: Aphelinidae) and the predators *C. bipustulatus* and *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae). *Lineaspis striata* (Newstead) recorded on *Juniperus oxycedrus* L. (Cupressaceae).

Two species belong to the family Coccidae. *Parthenolevanium corni* (Bouché) (Hemiptera: Coccidae) which found on *Morus alba* L. (Moraceae) and *Protopulvinaria pyriformis* (Cockerell), which recorded for the first time in Greece, on *Laurus nobilis* L. (Lauraceae), *Hedera helix* L. (Araliaceae) and *C. aurantium*. Its natural enemy are the parasitoid *Metaphycus helvolus* (Compère) (Hymenoptera: Encyrtidae).

Two species belong to the family Pseudococcidae. *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), infesting *Aloysia citriodora* Palau (Verbenaceae) and *Osteospermum jucundum* (Phillips) (Asteraceae) and the species *Planococcus vovae* (Nasonov), infesting *J. oxycedrus.*

One species belongs ın the family Kermesidae. *Kermes echinatus* Balachowsky, which recorded on *Quercus ilex* L. (Fagaceae). It is a univoltine, oviparous and biparental species. Its natural enemies are the predator *C. bipustulatus* and the parasitoids *Metaphycus gennaroi* Guerrieri & Noyes and *Cheiloneurus claviger* Thomson (Hymenoptera: Encyrtidae).

Key- words: Messinia, ornamental plants, scale insects.

Macrohomotoma gladiata (Kuwayama) (Hemiptera: Homotomidae) seasonal population fluctuation and its natural enemies in *Ficus microcarpa*

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Macrohomotoma gladiata (Kuwayama) (Hemiptera: Homotomidae) has been recently recorded in the Mediterranean Basin causing serious damages on the widely cultivated ornamental tree *Ficus microcarpa* L.f. The population structure and seasonal fluctuation of *M. gladiata* were studied in *F. microcarpa* in Athens, Greece, from February 2019 to February 2020. Infested twigs of *F. microcarpa* (8-10cm length) were randomly collected in the area of Agios Dimitrios, Athens and placed separately into plastic bags and transferred in the Laboratory of Agricultural Zoology Entomology, in Agricultural University of Athens. Eggs were recorded in May, August and from October to early January, the first two nymphal instars (N1+N2) were recorded throughout the year but not in August and September whereas their densities reduced in early May, the middle-aged nymphs (N3) and the late instars nymphs (N4+N5) were recorded in March and April and then appeared again in June and July. These results indicate that in the area under study: 1) *M. gladiata* overwinters as young nymph 2) Under autumn and winter conditions young nymphs do not develop further and 3) Most likely *M. gladiata* has a bivoltine life cycle. Parasitoids recorded from April to August with the parasitism rate reaching to 81%. The predator *Anthocoris nemoralis* (Fabricius) (Hemiptera: Anthocoridae) was present in low numbers in spring. These results are useful for the rational management of *M. gladiata* in the Mediterranean Basin.

Keywords*:* *Anthocoris nemoralis*, Biological control*, Ficus microcarpa*, Life cycle *Macrohomotoma gladiata*.

Biological observations of *Holotrichapion pisi* (Fabricius, 1801) in alfalfa crops in Greece and DNA barcoding identification.

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Alfalfa plant in many countries around the world like Greece is an important forage crop. *Holotrichapion pisi* (Fabricius, 1801) is a serious pest on alfalfa plant. In the present work the biology and identification of *H. pisi* were studied. The identification of the insect based on adult's morphological characteristics and DNA barcoding. For this, adults of the above insect were collected from different regions of northern Greece.

Two specimens were used for DNA barcoding analysis. Genomic DNA was extracted from whole samples using the PureLink™ Genomic DNA Mini kit (Invitrogen, USA). The *16S rRNA* mitochondrial (mtDNA) gene was chosen as target for the analysis of each sample. The primers for the amplification of a 415 bp fragment of the selected gene were LR-J-13017: 5'-TTACGCTG TTATCCTAA-3' and LR-N-13398: 5'-CACCTGTTTAACAAAAACAT-3'. PCR was performed using the *Taq* 2X Master Mix (New England Biolabs, USA). The purified PCR products were single-stranded sequenced (with LR-J-13017 primer), using BigDye Terminator v3.1 cycle sequencing methodology, on an ABI3500 Genetic Analyzer. The resulting sequences, after manual checking and editing in BioEdit v7.2.6, were compared with those available in GenBank using the standard nucleotide BLAST against the nucleotide collection (nr/nt) database.

The databases (with an identity percentage of 99.2%) and the morphological identification indicated that the insects were of *Holotrichapion pisi* (Fabricius, 1801) (Coleoptera: Apionidae sup. Curculionoidea) species.

The insect recorded two generations per year. The largest populations of the insect were observed from mid-May το early June, in the research areas.

Key- words: *Medicago sativa,* Coleoptera, DNA barcoding.

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Defining virus-carrier networks that shape the composition of mosquito and biting midges core virome of a local ecosystem

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Mosquitoes and biting midges constitute important vectors of emerging infectious diseases. Despite of the diversity of viruses they may carry, most of them infect exclusively insect species and are considered insect-specific. The vast majority of metagenomics approaches and Next Generation Sequencing (NGS) analyses have focused only on specific blood-feeding insect species for the identification of what is called core virome. However, in most ecosystems, multiple species may participate in virus emergence and circulation, while there is lack of understanding on the viruses carrier/host network for both vector-borne and insect-specific viruses. In this study, the core virome of 24 mosquito species and 10 *Culicoides* (Latreille) (Diptera: Ceratopogonidae) midges species was defined, which were all field-collected from the diverse ecosystem of the Eastern Macedonia and Thrace region, an important path of various arboviruses. Utilizing total transcriptome sequencing by the Ion Torrent NGS platform and custom bioinformatics pipelines resulted in identifying and assembling 48 viruses, including 29 novel viruses according to the respective families' species demarcation criteria. More specifically, comparison of the viromes of all blood-feeding insect species revealed a complex interconnected virus-carrier network in the ecosystem and novel relationships between mosquito genera and virus families, as most of the mosquito species had never been analysed in the past. Sample analyses of mosquitoes of variable composition as per species not only showed a stable core virome for each species but also a relationship between mosquito population and virome composition. The proposed study emphasized on the importance of a holistic approach regarding insect viromes in rich and diverse ecosystems. The results further supported the idea of a stable core virome, characteristic of each blood-feeing insect species. The remarkable stability of the core virome seemed to determine the composition of the total core virome in a local ecosystem as it is directly related to the population of the respective species.

Keywords: Arboviruses, ecosystem, metagenomics, mosquitoes, NGS, virome.

Diversity of ground dwelling spiders on different field margin groundcover in orange orchards

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Spiders are generalist predators and important biological control agents of various insect pests of agricultural crops. In the present work the diversity and abundance of ground-dwelling spiders in field margins of citrus orchards (Argolis, Greece) with different groundcover, were studied, i.e., aromatic/medicinal plants (AMPs), weed vegetation, bare soil. The samplings were conducted using pitfall traps along the field margins during orange flowering in two crop years. During the first recording period (April – May 2020) four orange orchards were sampled, three with weed-covered margins and one with bare soil, while in the second (April – June 2021) seven orchards were sampled, five with AMPs (rosemary x 2 orchards, savory, oregano, sage) established on one of the field margins in each orchard, one weed-covered and one with bare soil. A total of 55 species of ground spiders belonging to 16 families were collected. The families with greater numerical abundance in all cases were Lycosidae, Gnaphosidae and Theridiidae. More spider species were collected from the orchards with AMPs and weeds on margins compared to bare soil.

Keywords: spiders, aromatic/medicinal plants, groundcover, citrus, weeds, PLANT-B

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Diversity of mite species on bryophytes

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Bryophytes form an important habitat for smaller invertebrates such as Acari as they provide food, cover, suitable temperature and moisture, and protection from UV exposure. Aiming the study of species diversity and community structure of moss-dwelling mites, 3 sampling efforts (March, May and July 2020) were conducted at the area of Mt. Hymettus Aesthetic Forest. A total of 150 moss samples, each 10 cm^2, were collected from forest floor. Mites were extracted from samples using the Berlese-Tullgren method. Most mites were permanent slide mounted, while adult Oribatids stored in vials with alcohol 70% for further study. In total, 14.342 specimens collected from moss samples belonging to 134 species and 66 Families, 20 species of the Order Mesostigmata, 64 species of the Order Trombidiformes (Suborders Sphaerolichida and Prostigmata) and 50 species of the Order Sarcoptiformes (Suborders Endeostigmata and Oribatida). Sarcoptiformes was the most abundant mite group in all samplings, while the higher number of mite individuals, species richness and biodiversity, were observed in March.

Keywords: Acari, biodiversity, moss.

Preliminary data on the soil fauna of Coleoptera in olive orchards, in Messara, Crete, Greece

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Soil fauna was surveyed in Messara plain, South Crete, Greece. Monthly sampling was carried out by using pitfall traps in twelve olive orchards and three neighbouring natural ecosystems. The olive groves were in six locations with one conventional and one organic neighbouring orchard per location. Three sites were located in hilly and three in the plain agroecological zone. Five traps with propylene glycol were placed in each olive orchard and natural ecosystem.

The Carabidae, Scarabaeidae and Tenebrionidae families of the soil living Coleoptera were studied. The species of family Carabidae found are: *Calathus mollis* Marsham, *Carabus banoni* Dejean, *Lebia cyanocephala* Linnaeus, *Licinus aegyptiacus* Dejean, *Nebria testacea* Olivier, *Ophonus subquadratus* Dejean, *Platyderus jedlickai* Maran, *Tapinopterus creticus* I. Frivaldszky Von Frivald and *Zabrus oertzeni* Reitter. The species of family Scarabaeidae found are: *Elaphocera cretica* Reitter, *Elaphocera insularis* Fairmaire and *Pentodon idiota* Herbst. The species of family Tenebrionidae found are: *Polycoelogastridium sexcostatum* Motschulsky, *Scleron multistriatum* Forskål, *Dailognatha quadricollis* Brullé, *Zophosis punctate* Brullé, *Eutagenia smyrnensis* Solier, *Cossyphus tauricus* Steven.

The two most abundant species of the family Carabidae are *C. banoni* and *T. creticus*. For the Scarabaeidae family the most abundant species found is *E. cretica*, as it has been identified in the majority of sampled areas. Finally, the six species belonging to the Tenebrionidae family were found in similar numbers.

The present work was implemented in the context of the E.U. funded project Life IGIC “Improvement of Green Infrastructure in agroecosystems: reconnecting natural areas by countering habitat fragmentation” (LIFE16 NAT/GR/000575), cofounded by the EU LIFE program and the Green Fund, Greece.

Words-keys: olive orchards, pitfall traps, Carabidae, Scarabaeidae, Tenebrionidae.

Mass trapping of bark beetles (Scolytinae, Curculionidae, Coleoptera) in the pine forest of Samothraki (Greece) – a preliminary insight

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Bark beetles (Scolytinae, Curculionidae, Coleoptera) comprise some of the most notorious pest of forests worldwide. The frequency and intensity of their population outbreaks have been considerably increased in the last decades, resulting in extended damages to forest and natural reservoirs. Due to their biology and intensity of population outbreaks, the only plausible and efficient management approach is to log and remove dead and infested trees. By doing so, both the available food sources and insects' population level are drastically reduced.
Recently, pine trees infested by different bark beetle genera (e.g., *Tomicus* sp., *Orthotomicus* sp., *Pityophthorus* sp.) have been detected in the pine forest of Samothraki (Greece). The unique traits of this forest (natural isolation from other pine-covered areas, relatively small area) allow the establishment and assessment of mass trapping to control bark beetles. In total, 77 Theysohn® traps are installed and equipped with kairomone lures (KaiPin ®), that will be replaced every 40 days to retain their attracting capacity. After only two sampling periods (June 2021 / August 2021), more than 5.000 bark beetle individuals belonging to six different Scolytinae genera have been collected. The ultimate goal of this 2-year project, is to drastically suppress the population of bark beetles. Simultaneously, we will assess the potential of mass-trapping as a complementary and sustainable management tool, that in concert with sanitation loggings, will effectively protect similar forests from bark beetles.

Key words: Scolytinae, Samothraki, *Pinus sp.*, *Tomicus* sp., *Orthotomicus* sp.

Understorey biodiversity management in olive cultivation for integrated management of natural enemies

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The management of natural enemies of perennial tree crops and especially insects is performed with pesticides, which can negatively impact the quality of products, natural resources and biodiversity as well as the health of producers and consumers. An emerging trend focuses alternatively on the use of less or no chemicals and the management of crop pests with natural means. This trend is being promoted by both the EU and the new CAP.

Olive cultivation is one of the most important permanent crops in the Mediterranean area. The most important pest in olive groves is the olive fly [*Bactrocera oleae* (Rossi) (Diptera: Tephritidae)]. In this study we investigate the relationship between the fly population and plant and insect diversity in 15 fields on Lesvos Island and 12 on Kythera Island (project: Terra Kytheria) during 2021.

Specifically, we monitored the population of olive fly with McPhail traps and understorey insects with the use of pantrap and pitfall traps. We also recorded temperature and humidity in the tree canopy. These data are correlated with two different understorey treatments, undisturbed understorey with plant mixture or cleared by mechanical means.

From the first results it seems that in olive groves with undisturbed understorey there is a greater variety and abundance of insects while at the same time the abundance of arthropods has a negative effect on the population of olive fly. The results will be used to formulate guidelines in the context of integrated olive grove management systems.

Key words:

Bactrocera oleae, dacus, olive, pest management, sustainable agriculture.

Molecular taxonomy of species of family Aphrophoridae (Hemiptera: Auchenorrhyncha in Greece and examination of their phylogenetic relationships

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Abstract

Family Aphrophoridae (Hemiptera: Auchenorrhyncha) includes species that are potential vectors of the gram-negative bacterium *Xylella fastidiosa* Wells et al. (Xanthomonodales: Xanthomonodaceae) a very destructive pathogen for fruit trees. Even if it has not been detected in Greece yet, it has been detected in many European countries. The most widespread vectors in Europe are *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) and *Neophilaenus campestris* Fallén (Hemiptera: Aphrophoridae), which are abundant on olive orchards in Greece. The taxonomy for these species was based on morphology, and especially the anatomy of aedeagus of the male adults. Due to the morphological similarities between the species, it is often difficult to distinguish the different species that exist while due to the polymorphism within the individuals of the same species, raised many synonyms.

The aim of the present study is the molecular taxonomy, as well, as the examination of the phylogenetic relationships of the *Philaenus* and *Neophilaenus* species using the sequencing analysis of a mitochondrial DNA gene segment. The sampling was conducted in Lesvos Island during 2017-2019. Samples were collected using a sweep-net from the wild vegetation around the olive orchards and were taken to the laboratory stored in absolute ethanol. Total DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen). Polymerase Chain Reaction (PCR) was performed using BARCODE primers for the mitochondrial cytochrome oxidase subunit I (COI) (5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACC AAAAAATCA-3'). The PCR products were checked using agarose gel electrophoresis 2%. PCR products were purified using the Nucleospin extract II kit (Macherey-Nagel). Individual sequences were determined via automated sequencing of the above mtDNA gene segment provided by CEMIA S.A. Company (Larissa, Greece). For data processing, the packages BioEdit 7.0.9.0, CLUSTALW2 and MEGA 4. BioEdit 7.0.9.0, CLUSTALW2, DnaSP 5.10, MEGA 6.0.0 and GenAlEx 6.5 were being applied. The first results show that genetic variability among the samples analyzed does exist.

Key words: Auchenorrhyncha, Aphrophoridae, Phylogenetics, Molecular Taxonomy

Kalotermes flavicollis (F., 1793) (Isoptera: Kalotermitidae): a new host, in Mediterranean region and its controlling.

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Kalotermes flavicollis can be found in the Mediterranean area, namely in Spain, France, Portugal, Italy, Greece, Slovenia, Near East, and North Africa. This species has been reported to attack a wide variety of ornamental and fruit trees. In Greece, the damage caused to trees and shrubs by termites has been increasing over the last 10 years (Buchelos et al, 2017). In a pleasure craft at Attica beach in Greece, a severe termites attack was observed. The ship was recently hosted in other Mediterranean areas. After its return, it was found attacked by termites in several of its wooden parts. As there are many confused features in the morphology and anatomy of “dump” and “dry wood” *Kalotermes*, apart from the recognition of external morphological features we proceeded to DNA barcoding for species identification. For its control, a trial version was designed using Diatomaceous Earth.

The databases (with an identity percentage of 99.5%) and the morphological identification indicated that the insects were of *Kalotermes flavicollis* (Fabricius,1793) (Isoptera: Kalotermitidae) species (Papadopoulou et al., 2019). Applications using Diatomaceous earth against *K. flavicollis* were effective compared to other alternative means.

Key- words: termites, Diatomaceous earth, DNA barcoding

Alien Chalcidoidea of Greece

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Key words: Chalcidoidea, alien species, invasive species, parasitoids

International trade and globalization have led to the continual accidental introduction of organisms beyond their natural range of distribution. At the same time, the increasing needs for food coverage on a global scale demand the intentional introduction of organisms in order to optimize food production. These often include several species of parasitoid insects belonging to the superfamily Chalcidoidea, which are widely used as "natural enemies" of agricultural pests.

Superfamily Chalcidoidea numbers at least 70 confirmed species from Greece. Within the context of an ongoing investigation focusing on the biodiversity, the ecology, the introduction pathways and the impacts of alien Chalcidoidea in the country, this study briefly presents their systematic taxonomy, their introduction pathway, feeding habits, origin was well as their socio-economic impact.

These alien species are classified into twelve families, with the majority of them belonging to Aphelinidae, Encyrtidae, Pteromalidae and Eulophidae, mostly native to Asian countries and Australia. Most species are parasitoids that are considered beneficial to agriculture. However, a few of them adversely impact the “aesthetics of nature” and agriculture.

The authors address both the biodiversity and the impact of alien parasitoids in Greece, with the ultimate goal of recording and mitigating their negative impacts. The necessity of mapping the distribution and identifying the negative impacts of alien Chalcidoidea in Greece, using both citizen science and conventional scientific approaches, is considered imperative given the continuous increase of imported organisms and their adverse effects upon native biodiversity, society, the economy and human health.

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Session: Chemical Control - Insecticide Resistance

Evidence based plant protection: present and future

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Extensive collaborations among Academic and Research Institutions over the last decade provided the data and the know-how to allow the design of plant protection schemes based on laboratory evaluation of insecticides. Hereby we describe the first systematic efforts to implement evidence-based management of major pests such as the olive fruit fly *Bactrocera oleae* and the tomato pests *Bemisia tabaci* and *Tuta absoluta.* Olive fruit fly control scheme was modified for the first time during 2020-21 cropping season. The scope was to change the synthesis of the mode of actions of the insecticides applied in the prefecture of Crete in collaboration with the Ministry of Agriculture and the regional authorities in order to achieve the highest level of control. Evaluated IPM approaches were also implemented in an experimental greenhouse in the Agricultural Scholl of Messara targeting the optimum control of the tomato pests. The initial results from both approaches were positive, demonstrating that, under working conditions, evidence-based management schemes are providing high levels of crop protection. The use of conventional insecticides in tomato crops, in particular, was minimal, providing emphasis on IPM approaches. Hereby, an optimistic take home message for the future of plant protection is provided, indicating, at the same time, the actions that should be supported in the immediate future.

Ευχαριστίες: Funding, Ministry of Agriculture ‘Comparative Experimental actions of olive fruit fly control’, Prefecture of Crete ‘Specialized scientific support and novel actions for olive crop protection in Prefecture of Crete 2020-2021’ Project Smart Diagnostic tools and database to support precision plant protection in horticultural crops in Crete' 'SmartPP' funded by the Crete Operational Program 2014-2020 and co-funded by the European Regional Development Fund (ERDF), under the Priority Axis “Enhancing the Competitiveness, Innovation, and Entrepreneurship of Crete", Action 1.b.1: Demonstration—Experimental Development Projects, Promoting Research and Innovation in RIS3Crete, Innovative tools for rational control of the most difficult-to-manage pests (“super pests”) and the diseases they transmit. The project has received funding from the European Union’s Horizon 2020 research and innovation program H2020-2016-2017, TOPIC: Innovations in plant protection (SFS-17-2017) under grant agreement number 773902 (SuperPests).

Key words: plant protection, evaluation, resistance, *Bactrocera oleae*, *Tuta absoluta*, *Bemisia tabaci*

Investigation of insecticide resistance levels of Greek populations of insect species belonging to Typhlocybinae subfamily

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Insects of the Cicadellidae family and especially the species of the subfamily Typhlocybinae are major pests of vineyards The development of high populations densities and their constant presence in vineyards require the continuous application of chemical insecticides for their control which has as a result the development of insecticide resistance and consequently difficulties in their management.

The aim of this study was to investigate the insecticide resistance levels in Greek populations of insect species of the Typhlocybinae subfamily to different insecticide compounds, with an ultimate aim the early detection of resistance and the appropriate use of insecticides for pest management.

Within this framework, 16 different insect samplings were conducted from conventional vineyards of the Regional Units of Peloponnese, Attica and Central Greece in the summer of 2020 and 2021. Single dose laboratory bioassays were performed, and five registered insecticides were tested using the recommended dose.

Medium to high mortality rates were observed to the insecticides abamectin and spirotetramat. All collected populations presented low to medium mortality rates to the insecticide pyriproxifen, apart from the population collected from Corinthia. To the contrary, all populations collected from Peloponnese exhibited low mortality levels when tested with the insecticide deltamethrin. Finally, the mortality rates presented by all populations to the insecticide chlorantraniliprole, were extremely low.

To predict the risk for resistance selection, the presence of resistance mutations in insecticide gene targets (VGSC, GluCl and ACCase), previously associated with insecticide resistance in Pyrethroids, Avermectins and Ketoenols respectively, was investigated. The analysis revealed absence of resistance mutations in the aforementioned genes. In conclusion, insecticide resistance doesn't seem to be a major problem for the species of Typhlocybinae subfamily, found in vineyards from Greece.

Keywords: Bioassays Cicadellidae, insecticides, resistance, *Vitis vinifera*.

This project has received funding from the Flagship Action of Vineyards Roads which is funded by national resources through the Public Investment Program of the General Secretariat for Research and Technology.

Investigation and evaluation of knowledge and practices in the use of plant protection products by producers in Western Greece

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Agrochemicals, including pesticides, are widely used worldwide to increase the efficiency, productivity, quality and variety of products produced on an annual basis, but their misuse poses risks to the health of producers, consumers and the environment. In the present work, the knowledge and practices followed during the use of plant protection products by the producers of Western Greece were studied and evaluated. A synchronous study was conducted from April to July 2021. The tool used a Likert scale self -report questionnaire, in printed and online form, of 30 closed-ended questions, divided into 3 sections (demographics, knowledge of pesticide legislation and adherence to good practices in the use of pesticides). The population of the research was the producers of Western Greece, while the sample with convenient sampling was 150 people. The statistical analysis was performed with IBM SPSS 24 and Microsoft Office Excel 2016.
The research mainly involved producers aged between 31 and 40 years, high school graduates (60%), mainly farmers (61%) based in Western Greece, mainly in the Prefecture of Achaia (56%), with their main crop being grapes (35%), certified in a safe pesticide management program by 54.67%, but not in an integrated crop management program (73.33%). According to the results of the survey, in terms of their knowledge of the legislation governing plant protection products, only 59% answered correctly that they make a decision to use a product after the advice of an agriculture consultant, 54.0% answered correctly that the use of certified products minimizes the adverse effects on the health of the user, the consumer and the environment, while 49% did not recognize the characteristics of a legal versus an illegal insecticide, nor the responsibilities of the user. Regarding the measures needed to control an insect species, only 56% answered correctly that they give priority to biological control means and use low-risk products. On the other hand, in terms of adopting good practices in insecticide application, only 62% were familiar with proper and safe storage and utilize means of personal protection. The study revealed that overall, the respondents' responses were described as unsatisfactory, both in terms of knowledge and practices, as well as in the use of plant protection products. Nevertheless, the majority considered it necessary to undergo training regarding safety and plant protection application good practices.

Key words: Pesticides, Knowledge, Good practices, Producers, Western Greece

Integrated control of the entomological pest problems in potato based on the control of the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) in Greece

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The most serious entomological pests of the potato in Greece which needed direct control are the wireworms, (*Agriotes spp*), the Colorado potato beetle(CPB) and the Potato Tuberworm *Phthorimaea opercutella* (Zeller) Lepidoptera: Gelechiidae. In some regions attacks by cutworms (*Agrotis spp*) can be observed on the young plants and later on the leaves.

In many regions in Greece and wordwide the CPB has developed resistance to many groups of insecticides .It is considered the most serious pest of the Solanaceae (potato, tomato, eggplant).

By observations from extensive network of pheromone traps for different species of wireworms, the species of Coleoptera: Elateridae, *Agriotes sputator, A.obscures, A.brevis,* and *A.lineatus were found.* The larva attacks many crops and particularly produce severe damages to the tubers of the potatoes. In the last years, another serious pest has been the Potato Tuberworm, producing extensive damages to the tubers. From observations on pheromone traps it was found that the catches start at the beginning of June, with maximum number of captures mid of July, mid of August and mid of September.

In the frame of a research program, our study aimed to determine possible development of Colorado potato beetle resistance on insecticide (Confidor, Calypso, Aldverde, Coragen, Laser). lab bioassays were carried out (the method was the topical application using an Arnold hand microapplicator). Thirty- eight (38) populations of CPB, which were collected from different regions in north Greece, were tested (larvae and adults). The insecticides chosen for the bioassays were the most effective and commonly used products for controlling the CPB in Greece. The determination of the lethal doses for mortality ($LD\ 50_S$,$LD\ 90_S$) of the insecticides, it is very important for creation (base-line data), therefore will be able to determine early the development of resistance and to take the proper measures for the management of the applied insecticides.

After the withdrawal of the neonicotinoids insecticides, with the active ingredients (imidacloprid, thiamethoxam, thiacloprid and clothianidin) which have been used extensively for the control of the CPB , aphids and other insects, the only neonicotinoid that is still available in the market is the acetamiprid .It needs to develop resistance management tactics to avoid the development of resistance to acetamiprid and to the other insecticides.

From the used insecticides we found that the CPB had developed resistance only to the imidacloprid with an average resistance ratio 87 resistant/sensitive. For the products Coragen (chlorantraniliprole), Aldverde (metaflumizone), and Laser (spinosad) we did not find any resistance. Also these insecticides were very effective in field experiments. The results from all the lab and field experiments were used to design an effective program to face the existing entomological problems in potato plantations, based on the control of the Colorado potato beetle.

Keywords: *Leptinotarsa decemlineata, Phthorimaea opercutella, Agriotes spp,* Resistance,
Control of Colorado potato beetle.

Insecticide resistance monitoring and frequency of insecticide target site resistance mutations in major agricultural pests of vegetable crops from Crete.

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A vast number of pests infest the vegetable crops in Crete, adversely affecting the production level. Integrated crop protection involves combination of various management approaches including the application of chemical Plant Protection Products (PPPs). However, the intensive use of PPPs resulted in the development of resistance that, in combination with the reduced number of available of PPPs, practicing integrated crop protection becomes extremely difficult.

The aim of the study is the investigation a) of the susceptibility profile through toxicity assays and b) of the presence and frequency of target site resistance mutations via molecular diagnostics of the most important pests (*Tuta absoluta* (Meyrick) (Lepidoptera: Gelehiidae), *Bemisia tabaci* (Genadius) (Homoptera: Aleyrodidae)*, Frankliniella occidenalis* (Pergrade) (Thysanoptera: Thripidae) and *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (Acari: Tetranychidae)) of horticultural crops from Crete. Resistance data were produced for 14 approved active ingredients and for 25 target side mutations in total. The study revealed reduced efficacy to the majority of active ingredients and high mutation frequency in significant number of populations/pests. Resistance data were implemented in upgraded insecticide resistance database Galanthus.

Early and accurate detection of resistance in the field may provide useful knowledge concerning the appropriate resistance control program for the management of two of the most difficult to control pests of vegetable crops in Crete.

Key-words: Vegetable crop pests, bioassays, molecular diagnostics, resistance management, Crete.

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Pesticides and food safety
An investigation of factors affecting consumers' perceptions

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The perception of the concept of food safety by consumers with regard to the use of pesticides is very important and can affect the primary sector at various levels. This study presents the results of an analysis of the factors that affect the consumers' perceptions and attitudes in Greece, regarding the use of pesticides in relation to food safety. It is important to know what factors affect these views so that targeted interventions can be made in the future. For that purpose, in the spring of 2021, a survey was conducted in Greece using an online questionnaire which was answered by 1,846 participants. The questionnaire included demographic questions and five-point Likert scale questions to record the participants' attitudes and views. Exploratory factor analysis was performed on the questionnaire variables. Subsequently, binomial logistic regression was applied regarding the research question, namely, *participants' willingness to accept the statement that the benefits of pesticide use outweigh the risks*. Finally, a non-hierarchical k-means cluster analysis was applied in order to describe quantitatively the structural features of the sample. From the analysis, five influencing factors were extracted: specialized information sources, attitudes towards the contribution of pesticides, confidence in food certification, general information sources and attitudes towards pesticide threats. Among the participants, it was more likely to have a positive attitude towards the research question if they are males [Odds ratio (OR) = 1,737], professional or amateur pesticide users (OR = 2,458), if they positively perceive the contributions of pesticides (OR = 6,346), if they get informed about pesticides by general (OR = 1,452) or specialized information sources (OR = 1,304) and finally, if they recognize the importance of food certification (OR = 1,931). On the contrary, those living in southern Greece (OR = 0.595) and those who perceive the risks of pesticides as more important (OR = 0.535) were more likely to express a negative view concerning the research question. Two main, equal-sized clusters of participants were extracted. The first includes participants who are less positive towards pesticide use and less informed about their use. The majority of them do not use pesticides and are mostly high school graduates. The cluster is comprised mainly of women, residents of urban areas, and includes more civil servants, unemployed persons and college students, as compared to the second cluster. The latter is comprised mainly of participants who get informed about pesticides, mostly men, rural residents, pesticide users, farmers, self-employed, retirees and private employees and are predominantly university graduates.

Key-words: Pesticides, food safety, consumer attitudes, information sources, food certification

Pesticides and integrated crop management food products Factors affecting their acceptance by consumers

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The forthcoming changes in the Common Agricultural Policy of the E.U. are expected to accrue changes to the sector of agricultural food products, in terms of pesticide use and food certification. Knowledge of the factors that affect consumer perceptions of certified integrated crop management food is imperative to making targeted interventions or taking corrective actions in the future. For this purpose, in the spring of 2021, a survey was conducted in Greece using an online questionnaire which was answered by 1,846 participants. The questionnaire included demographic, as well as five-point Likert scale questions. Exploratory factor analysis was applied to the questionnaire variables, followed by binomial logistic regression analysis regarding the research question: *If the participants consider that integrated crop management food products are safe or not, in terms of the presence of pesticide residues*. Five influencing factors were extracted: the specialized information sources, the attitude in favor of the contribution of pesticides, the confidence in food certification, the general information sources and finally, the attitude in favor of the negative effects of pesticides. Among the participants, the likelihood to express a positive attitude towards the research question was greater among those who trust food certification [odds ratio (OR) = 59,357], those who get informed about pesticides from specialized information sources (OR = 2,108), men (OR = 2,077), those who positively perceive the contribution of pesticides (OR = 1,400) and finally, the older aged participants (> 45 years old) (OR = 1,528). The results indicate that consumers consider integrated crop management food products to be safe and acceptable in terms of pesticide use issues. Trust in labeling, traceability and certification procedures is the key factor in accepting integrated crop management products. It also appears that specialized information sources on pesticides (bulletins of public bodies, official websites, agronomists, scientific journals) play a key role in shaping consumer views. Based on these results, we consider important that measures are taken by official bodies for targeted consumer information, in layman terms, on pesticides and food safety through general information sources (television, radio, print and electronic press) so that the consumer audience, to which the valid information will be directed, is expanded. Furthermore, the dissemination of relevant information through social networks would target a substantial consumer group, especially that of a younger age, which uses them as information media.

Key-words: Pesticides, food safety, consumer attitudes, information sources, food certification, integrated crop management

Susceptibility of different developmental stages of the parasitoid *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae) to insecticides commonly used in peach and kiwi orchards in Greece

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Pesticide applications and mass release of parasitoids can be combined into an integrated pest management approach, after careful planning. In this study, we assessed the influence of four insecticides (etofenprox, acetamiprid, spinetoram and spirotetramat) that are commonly used in peach and kiwi orchards, against the egg parasitoid *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae) which was recently found to parasitize the eggs of *Halyomorpha halys* (Hemiptera: Pentatomidae). Application time of insecticides in relation to both parasitoid's development and parasitism rates was also evaluated. Although etofenprox had the strongest impact on *O. telenomicida*, it didn't differ significantly from the other insecticides. On the other hand, the application time of the insecticides significantly influenced the parasitism rate of *O. telenomicida*. According to our results, application of insecticides prior to parasitization resulted in lower parasitism rates compared to the control, whereas application after parasitization caused reduced adult parasitoid emergence. Our results can provide background regarding the integration of insecticides to a potential pest management scheme using *O. telenomicida*.

Keywords: egg parasitoid, active ingredient, brown marmorated stink bug, parasitism, IPM.

Implementation of geoinformatic technologies for the optimization of the National Olive fruit fly Control Program in the Region of Crete "DACUS SOS"

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Bait sprays are a basic control method against *Bactrocera oleae* Rossi (Diptera: Tephritidae) in Greece. However, despite their recognized effectiveness and their environmentally friendly profile, if applied correctly, they do not always ensure high efficiency, resulting in loss of production. For the optimization of bait sprays applied in the frame of the National olive fruit fly control program, new geoinformatic technologies were developed which were applied almost in all olive growing areas of the Region of Crete, at about 200,000 hectares, during 2020 and 2021. For this purpose, a low-cost technological solution and an integrated information system for monitoring and control of bait sprays were implemented using a total of 1,160 smart mobile devices with GPS (650 GPS trackers, 350 GPS trackers with flowmeter and 160 individual recorders for sprayers), and the open-source software application traccar in conjunction with the MySQL database management program. The system allows in real time: a) the entry in a database and the presentation, in a web environment, of spraying means pathways, b) the monitoring of the flowrate of the spray solution and its total consumption per spraying mean, c) the management and the mapping of geospatial data related to the olive fruit fly management program and d) the development of digital maps with sprayed and unsprayed olive growing areas per region or contractor, through the use of an automated model and the QGIS application. During this two-year project, new tools and technologies were integrated in the olive fruit fly management program, assisting all the Directorates of Agricultural Economy and Veterinary of Crete to implement an efficient and economical project. The project is funded by the Region of Crete in the framework of the program: "Application of geoinformatic technologies to optimize the National Olive fruit fly Control Program in the Region of Crete" - "DACUS SOS".

Key-words: GPS trackers, model QGIS, national olive fruit fly control Program *Bactrocera oleae*.

Evaluation insecticide resistance level in grape berry moth *Lobesia botrana* populations from Crete

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Grape berry moth *Lobesia botrana* Den Shif. is the most important pest of grapes in the Mediterranean region. It's damage potential is extensive particularly on table grapes. Control of the pest is based on applications of chemical insecticides. We monitored insecticide susceptibility/resistance against a wide range of insecticides in two populations of grape berry moth collected in 2021 in Crete, Greece. The insecticides tested were etofenprox, alpha- cypermethrin, acetamiprid, emamectin benzoate, spinetoram, spinosad, indoxacarb and chlorantraniliprole, all registered for *L. botrana* control in Greece. The diet incorporation bioassay method (IRAC N17) was used for the evaluation of insecticide toxicity. Initially, a protocol using the recommended label rate (RLR) of each insecticide, as diagnostic dose, was implemented, to evaluate the efficacy of the tested insecticides. Only etofenprox and acetamiprid exhibited low efficacy levels at RLR. The observed mortality did not exceed 80%, suggesting potential control failure likelihood for these molecules. The LC_{95} of the reference strain, as a diagnostic dose for putative insecticide resistance, was subsequently implemented, using the same protocol. Chlorantraniliprole, acetamiprid, alpha- cypermethrin, etofenprox and spinetoram exhibited mortality levels below 80% at this dose, indicating potential cases of resistance. In order to accurately evaluate the suspected resistance levels, full dose response experiments were implemented for those aforementioned insecticides. The responses of the wild populations were compared against the susceptible reference strain utilizing probit analysis which indicated resistance levels (RR) below 5-fold in all cases. In order to predict the risk for resistance selection, the presence of resistance mutations in 4 insecticide gene targets, previously associated with insecticide resistance of arthropods in 5 insecticide chemical classes, was investigated. The analysis revealed absence of resistance mutations in tested populations. In conclusion, with the exception of etofenprox and acetamiprid, the insecticides tested in this study were highly effective against grape berry moth, while only low insecticide resistance levels were detected in the populations from Crete.

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Keywords: bioassay, insecticide, *L. botrana*, resistance, toxicity

Influence of lethal and sub-lethal exposure to sulfoxaflor on the aphidophagous coccinellid *Hippodamia variegata* (Coleoptera: Coccinellidae)

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The predatory ladybeetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is an important natural enemy preying on several aphid species and other arthropods, of which many are pests of cultivated plants. Sulfoxaflor, a sulfoxinine insecticide, is commonly used for controlling sucking insects, however, at the same time, it can cause various sublethal effects to no target organisms. The development of IPM programs against pests requires an evaluation of the side effects of insecticides on natural enemies.

To study the risk of insecticides to *H. variegata*, the neonicotinoid sulfoxaflor was selected for evaluation of its influence on *H. variegata* at lethal and sublethal doses

The LR_{50} (application rate causing 50% mortality) in the exposed larvae decreased from 97.03 σε 35.97 ng a.i. per insect, and the daily HQ (hazard quotient) values increased from 0.4 to 1.4, indicating potential intoxication risks. Our results demonstrate that sulfoxaflor significantly decreased adult female longevity and fecundity. In addition, demographic parameters, such as the intrinsic rate of increase (r), were significantly reduced when exposed to sulfoxaflor.

Conservation of *H. variegata* in agricultural ecosystems thus requires further measures to decrease the applied dosages of Sulfoxaflor.

Keywords: *Hippodamia variegata*, sulfoxaflor, toxicity.

Responses of *Coccinella septempunctata* (Coleoptera: Coccinellidae) to sulfoxaflor exposure

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Coccinella septempunctata is an important predator of several pest species and is part of many Integrated Pest Management (IPM) programs. To assess the risks of pesticide application to *C. septempunctata*, we studied the effects of sulfoxaflor on *C. septempunctata* larvae.

We studied the effect on voracity and developmental timespan, under laboratory conditions, for the generalist predator *C. septempunctata* caused by the exposure to sulfoxaflor, which is routinely used against aphids.

The developmental timespan and voracity differed among the tested concentrations. Also, the adult's weight significantly differed between control and exposed insects. Sulfoxaflor shows no effect on adult female longevity and fecundity.

The results of the present study show that sulfoxaflor can be applied for managing piercing-sucking pests while also allowing lady beetles to survive and develop. However, they also indicate the necessity for field research in order to draw more accurate conclusions.

Keywords: *Coccinella septempunctata*, sulfoxaflor, IPM.

Insecticide resistance in Greek populations of the aphid *Myzus persicae*.

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We have been studying the *Myzus persicae* s.l. (Hemiptera: Aphididae) aphid populations in Greece the last years (2017-2021), analyzing their ability to develop resistance to chemical insecticides. *M. persicae* clones from peach and tobacco were examined in dose-response bioassays with the insecticides flonicamid, acetamiprid, flupyradifurone, και sulfoxaflor. The clones were compared with the standard susceptible clone 4106A. In addition, various clones were examined for the presence of the insecticide resistance mutations R81T (confers resistance to insecticides of MoA4) and A2226V (confers resistance to spirotetramat).

In bioassays with floniocamid, 73.1% of the clones examined (104) showed low Resistance Factor values (RF<5). However, 9.6% of the clones exhibited RF values 10–32. In bioassays with acetamiprid, 75.3% of the clones examined (73) showed low RF values (1–12), while 11.0 % and 13.7 % of the clones moderate (20–48) and high values (100–145), respectively. In bioassays with flupyradifurone 5.9% of the examined clones (68) showed moderate RF values (9–14), while 72.0% and 22.1% of the clones high (19–89) or very high values RF (104–1437), respectively. In bioassays with sulfoxaflor, 80.6% of the examined clones (31) showed low RF values (<5), although 9.7% of the clones exhibited RF values 9–12.

In 313 clones from peach, collected in the years 2017–2021, the frequency of the resistant allele of the R81T mutation was 13.9%. By contrast, the mutation was found only in one out of the 38 clones that were collected from tobacco. The A2226V mutation was not found in any of the 87 clones that were collected from peach (80) and tobacco (7) in 2021.

The results are discussed in relation to the Integrated Resistance Management in *M. persicae in Greece.*

Key-words: resistance, insecticides, aphid, *Myzus persicae*

A five-year monitoring survey on insecticide resistance for cotton whitefly *Bemisia tabaci* in Greece

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Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae), commonly known as cotton whitefly, is one of the most devastating agricultural pests worldwide and is mainly controlled by chemical insecticides. However, the development of resistance to major chemical classes has made control of the pest extremely difficult. Monitoring the effectiveness of insecticides is essential for the implementation of evidence based pest management schemes. Therefore, in the present study we evaluated the resistance levels against five active ingredients used for *B. tabaci* control in Greece, namely flupyradifurone, thiacloprid, sulfoxaflor, pymetrozine and spiromesifen. The survey was conducted between 2017 and 2021 on 19 MED-species (former Q-biotype) populations collected from greenhouses in Crete and mainland Greece. Validated IRAC bioassay method protocols 15 and 16 were utilised, involving classical laboratory leaf –dip bioassays for adults and second-instar larvae respectively. The responses of the collected strains were compared to a laboratory susceptible reference strain (MED-species). Statistical analysis of the probit outputs indicated low resistance to flupyradifurone (Resistance Ratio (RR) <17-fold) and to sulfoxaflor (RR <13-fold) in all cases. Resistance to spiromesifen was first reported in 2018 and it was maintained extremely high (RR> 2500-fold) throughout the survey. High resistance levels were also identified in all cases for pymetrozine (RR >10,000-fold) and thiacloprid (RR: 56 - 3485-fold). *B. tabaci* populations from Greece have evolved resistance to major insecticide classes, while absence of cross-resistance with the new insecticides flupyradifurone and sulfoxaflor was detected. The information of this study can be directly implemented in the design of efficient chemical control programmes within the frame of rational IPM schemes.

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Keywords: *Bemisia tabaci,* resistance, flupyradifurone, thiacloprid, sulfoxaflor, pymetrozine, spiromesifen, IPM.

Current status of insecticide resistance for *Helicoverpa armigera* in Greece

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The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a major pest of numerous crops worldwide. Control of the pest is based mainly on application of insecticides, however in several cases extensive damages have been reported, particularly on cotton crops. Studies on the efficacy levels of chemical insecticides are rather limited in Greece and such information is essential of the implementation of rational IPM schemes. Therefore, the aim of this study was to investigate the status of resistance against key insecticides which are registered for the control of cotton bollworm, namely spinetoram, emamectin benzoate, λ-cyhalothrin, cyantraniliprole and chlorantraniliprole. The survey was conducted on 3 populations collected in summer of 2020, from cotton crops in Central and Northern Greece. The response to the tested insecticides was evaluated *via* laboratory dose-response bioassays using artificial diet with chemical overlay, a modified protocol of IRAC method 017. The LC_{50} ranged between 0.7-0.9 for spinetoram, 0.1-0.2 for emamectin benzoate, 8.3-100 for λ-cyhalothrin, 5.4-6.8 for cyantraniliprole and 0.6-1.1 for chlorantraniliprole (all values in mgL^{-1}). All LC_{50} values of collected strains were compared to a laboratory susceptible reference strain. Analysis of the results indicated low resistance levels to spinetoram, emamectin benzoate, cyantraniliprole and chlorantraniliprole with resistance ratios (RR) up to 3-fold in all cases. In contrast, the pyrethroid λ-cyhalothrin exhibited RR between 4- to 68-fold, suggesting potential cases of resistance development. This study sets the basis for evince-based pest management in Greece and facilitates early detection of incipient resistance cases in the future.

Acknowledgments: The authors would like to thank the Regional Unit of Drama for supporting this research.

Keywords: *Helicoverpa armigera, Resistance*, Greece, spinetoram, emamectin benzoate, λ-cyhalothrin, cyantraniliprole, chlorantraniliprole.

Revisiting of the IRAC 022 methodology for the evaluation of the efficacy of slow-acting molecules in tomato borer *Tuta absoluta*

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The tomato borer, *Tuta absoluta* is one of the most devasting pests of tomato crops. Originally an indigenous pest of South American, it invaded Europe in 2006 and rapidly expanded globally compromising tomato production. It' s control is extremely problematic and relies extensively on the use of chemical insecticides. The evaluation of the efficacy levels of the insecticidal molecules is essential for the estimation of control failure likelihood as well as the resistance levels.

The globally adopted bioassay protocol is currently the IRAC method 022 which was validated back in 2012. Although it is a robust and reliable method, the protocol predicts 3 days exposure interval only. Recently, two rational insecticides, azadirachtin and spirotetramat were evaluated using both IRAC method 022 as well as a modified protocol of method 22 that allows prolong exposure intervals up to pest pupation (12 days after treatment, DAT) and adult emergence (27 DAT). In the case of azadirachtin the LC_{50} gradually decreased with the exposure interval from 244 mgL^{-1} at 3DAT, 17.5 mgL^{-1} at 6DAT, 2.6 mgL^{-1} at 12DAT (pupation) 2.3 mgL^{-1} at 27DAT (adult emergence). More than a 93 -fold difference was observed between the LC_{50} estimated at 3DAT sand 12DAT (pupation) while a minimal deference (less than 1,2-fold) was observed thereafter, up to adulthood. For spirotetramat insecticide LC_{50} was estimated at 18.6, 10.2 and 0.58 mgL^{-1} when evaluated at 3, 12 and 27DAT respectively. Compared to IRAC 022 LC_{50} was reduced 2-fold by pupation (12DAT) and 32-fold by adult emergence (27DAT). These findings indicate that, spirotetramat is highly active against a different developmental stage compared to azadirachtin, thus a different evaluation approach is required. It is suggested that a careful revision on IRAC methods is required based on the activity profile for each insecticide, allowing accurate estimations of the efficacy of slow acting molecules.

Keywords: Azadirachtin, method, Resistance, Tomato, *Tuta absoluta*, spirotetramat

Lessons learned for phosphine distribution and efficacy by using wireless phosphine sensors

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Phosphine is currently the main insecticide used in stored product protection globally. It is related with food safety and food security, through phytosanitary authorities and international trade. However, the extensive and improper use have led to the development of resistance by several major stored product insect species. In the present study, the use of phosphine wireless sensors and computational models have been evaluated in order to illustrate and predict gas concentration in different facilities such as horizontal warehouses, ship holds, silos, containers and tarps. In this context, more than 40 “real world” fumigations were carried out. According to the findings of the study, the distribution of phosphine in large warehouses was often not adequate for a satisfactory level of insect control, and gas concentrations varied remarkably through time and space. In contrast, commercial treatments at containers were sufficient to control the insects tested, even on stored-product insects which were found to be resistant to phosphine. Furthermore, in the cases of silos and ship holds, our work showed that the use of forced recirculation systems is essential to increase phosphine concentration and to ensure uniform distribution, and, as a result, insect mortality. Overall, our tests clearly suggested that the sensors were very effective in measuring phosphine and in combination with forecasting computational models are generally expected to play an important role in the near future in IPM-based programs at the post-harvest stages of agricultural commodities. At the same time, real-time monitoring can be used with success for the prediction of insect mortality in the treated facilities.

Keywords: phosphine, stored product insects, resistance, monitoring, sensors, modeling

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19th PANCHELLENIC ENTOMOLOGICAL CONGRESS

Session:

Insect - Plant - Microbe Interactions

Characterization of the volatile infestation-fingerprint of fruits by *Ceratitis capitata (Wiedemann), Bactrocera dorsalis (Hendel)* and *B. zonata (Saunders) (Diptera: Tephritidae)* and conversion into a detection tool (e-Nose)

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Infestation by true fruit flies (TFF) at early stages is difficult detectable. Inspection of the infestation is destructive for a large proportion of the commodity so there is a demand for the development of rapid, reliable, and cost-effective screening methods for the detection of infestation especially due to restrictions on the trade of products by the Phytosanitary legislation.

In this study, the aim was to determine infestation-specific volatile compounds-indicators emitted by fruits (peaches, pears, apples and oranges) after the infestation by TFF, namely *Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel) and *B. zonata* (Saunders) (Diptera: Tephritidae), in order to develop a rapid, reliable and cost-effective method aiming to reduce the time required for a reliable inspection and to avoid the unnecessary destructive sampling.

For the collection of the volatile organic compounds (VOCs) from healthy and TFF-infested fruits the dynamic headspace sampling technique was used. VOCs were analyzed by Gas Chromatography-Mass spectrometry (GC/MS). The specific volatile profiles were further used as a training and validation set for the e-Nose system that can be deployed as a commercially available tool that could be used to discriminate between FF-infested from un-infested fruits.

Results showed that specific esters are TFF species specific for peaches. In case of pome fruits, esters increase along with the procession of fruit ripening and quantitative differences are observed between healthy and infested fruits. In citrus, the monoterpene, limonene and the sesquiterpene, valencene were the main VOCs detected in healthy and infested fruits. *E*-(*β*)-ocimene and homoterpene *E*-4,8-dimethylnona-1,3,7-triene (DMNT) were also found insignificant quantities in infested fruits. The preliminary results of e-Nose on detection of infestation by TFF are quite promising.

Key words: Fruit flies, VOCs, E-nose, *Ceratitis capitata, Bactrocera dorsalis, Bactrocera zonata.*

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Spatio-temporal analysis of *Halyomorpha halys* populations in Kiwi Orchards

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The import of the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), from Asia to the United States of America in the mid-1990s and later to Europe caused serious damages to fruit, vegetables and high value crops. In Greece, damages are reported at kiwi orchards at the regions of Pieria and Imathia. The aim of this research is to study a) if the nearest to the kiwi orchards crops affect the population of *H. halys* and b) if the vigour of the kiwi plants has an impact on the *H. halys* populations. Thus, two kiwi orchards were selected at the area of Dion and Meliki at the Pieria and Imathia region, respectively. Afterwards, ten traps were installed from early June to late October within each selected kiwi orchard – two types of traps at every side of the orchards and additionally at the center of them. After that, crops that were up to 500 meters far from the selected kiwi orchards were identified. Traps were examined weekly and the number of the captured *H. halys* was recorded. During the same days, Sentinel satellite images were analyzed to calculate the vegetation index NDVI (Normalized Difference Vegetation Index). The results proved that the crops of the nearest fields to the kiwi orchards have an impact on the populations of *H. halys*. Moreover, population of *H. halys* was higher at the areas with high NDVI values and the opposite. Results of this research can be used to reduce the *H. halys* damages in kiwi orchards using different rates of pesticides depending on the prediction of the size of the populations. The benefits of the proposed practice are multiple such as reduction of production cost of kiwifruits, increase farmers' profit and environmental protection.

This research was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK) under the call "Research-Create-Innovate".

Keywords: *Halyomorpha halys*, adjacent crops, vigour, traps, vegetation index, kiwi orchard.

Studies of the symbiosis of *Candidatus* Erwinia dacicola with the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) and characterization of the tissues where it is located

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The olive fly, *Bactrocera oleae* (Rossi) (Dirtera: Tephritidae) is the most important enemy of the olive crops worldwide, causing up to 30% losses of the total olive production. The fact that for decades the control for this pest has been based mainly on chemical compounds, has led to the development of high levels of insecticide resistance, causing huge problems in the orchards of the Mediterranean basin and beyond. In addition, the alternative methods that have been tested, such as the artificial sterilization (Sterile Insect Technique, SIT) and the mass trapping, have not yielded the desired results.

The olive fly larva, after its hatching, begins to feed on the olive flesh. Symbiotic bacteria of the genus *Candidatus* Erwinia dacicola and others, possibly participate in the degradation of the toxic phenolic compounds, allowing the nutrition and therefore the survival of the larva in the acidic environment of the unripe (green) olives. The bacteria are found in four blind-end gastric caeca, which appear greatly swollen until the 2nd instar larval stage. In stage 3, the lobes shrink and the bacteria move on to the midgut, followed by the metamorphosis and the pupation of the insect. During the adult stage, bacteria multiply in the oesophageal bulb, which is located in the head of the fly and connected with the oral parts and the intestine via a channel. The bacteria are passed through the gut and end up in the ovipositor, where they are located in diverticula. When the eggs leave the ovipositor, they pass these diverticula, thus being "covered" with *Ca.* E. dacicola and in this way they are transferred with the egg to the fruit.

Considering all the above, here it is attempted a better comprehension of the olive fly physiology, through the characterization of the tissues where the symbiotic bacteria are located. A deeper understanding of the interactions between the olive fly and its symbiotic bacteria has great scientific and biotechnological interest towards the development of innovative pest management strategies, by targeting the symbionts.

Keywords: *Bactrocera oleae*, *Candidatus* Erwinia dacicola*, olive fly,* symbiotic bacteria, Tephritidae.

Presence, ecological parameters and food preference of Auchenorrhyncha - potential vectors of *Xylella fastidiosa* (Hemiptera) in olive orchards

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ABSTRACT

Hemiptera, one of 5 most important insect orders in terms of economy, includes among others, the suborder of Auchenorrhyncha. From the 42.000 described species, 2/3 of them belong to Cicadomorpha and the rest to Fulgormorpha. The indirect damage of Cicadomorpha in plants through the transmission of pathogens has severe economic impact in crops. Lately, the most serious case of pathogen transmission in Europe is the bacterium *Xylella fastidiosa* (Wells et al.) (Xanthomonodales ; Xanthomonodaceae), which is associated with the olive quick decline syndrome (OQDS). The vector-borne bacterium is exclusively transmitted by sharpshooters and spittlebugs. Before *X. fastidiosa* detection in Palaiarctic region, important facts of bionomics of spittlebugs were not well known. The present study aims to investigate aspects such as ecological parameters and food preference of the potential vectors with special focus on the Aphrophoridae species in olive orchards.

For this reason , sampling use sweeping net conducted in the olive orchard of Agricultural University of Athens. The insect sampling was conducted from the olive canopy, the ground and the surrounding wild vegetation. They collected insect individuals were placed in vials with ethanol 95%, for taxonomy after male genetalia maceration. Furthermore, for the investigation of their food preferences, Aphrophoridae adults were collected and tested in a four-choice olfactometer. Results showed interesting differences in the preference of nymphs and adults spittlebugs for certain host plants. In the presentation, detailed results and discussion will be given.

Keywords: Auchenorrhyncha, olive, wild vegetation, Aphrophoridae, ecology, olfactometer

Effects of beneficial soil microbes against herbivorous pests

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Plants are often challenged by biotic stressors among them herbivorous arthropods and pathogenic microbes. Nevertheless, they also interact with beneficial organisms such as predators and microbes. Certain soil microbes in particular are known for their ability to improve plant growth, antagonize pathogens and prime plants against future attacks via the elicitation of plant defense responses. Hence, they show potential in protecting plants against herbivores and serve as biological control agents in Integrated Pest Management programs. In this work, we assessed the plant-mediated effects of a series of beneficial soil fungi and bacteria against key pests of tomato belonging to different feeding guilds and feeding styles namely, *Tetranychus urticae* Koch (Acari: Tetranychidae), *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). We recorded life-history traits such as survival, developmental time and egg production depending on the herbivore under study on inoculated plants as compared to control plants. Context-dependency was addressed by recording the effects of selected microbes on the performance of herbivores in different tomato cultivars. Our results show that inoculating plants with different bacterial and fungal strains affect herbivore populations via the plant. Yet, we recorded a considerable variation in these effects depending on the microbes as well as the herbivore species under study. Overall, our work highlights the role of soil microbes as biocontrol agents in suppressing herbivore populations, possibly via the induction of plant defenses.

Key-words: arthropods, bacteria, biological control, fungi, plant defense, tomato

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Domestication, developmental stage and strain contribute to the structuring of bacterial communities associated to Mediterranean fruit fly *Ceratitis capitata*

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Guts microbiota associated with insect species have been known for their ability to affect different aspects of their host physiology and fitness. Insect-gut microbiome interactions can be influenced by host traits like developmental stage, strain and sex, and extrinsic factors such as rearing condition and diet. In the present work, we investigate the changes in the guts microbiota that occur through domestication of two *C. capitata* populations: Vienna 8 Genetic Sexing Strain (GSS) which is a long-established control line and a wild population recently introduced to laboratory conditions. The bacterial profiles were studied for both strains using amplicon sequencing of the 16S rRNA V3–V4 hypervariable region in larvae and gastrointestinal tract of teneral (1 day) and adults (5-15 days) reared under laboratory conditions for 14 generations (F0-F13). Our finding demonstrates the development of distinct bacterial communities across the generations with differences in bacterial composition, suggesting that the domestication has a strong impact of the fly bacteriome. Beside the degree of domestication, different bacterial profiles were observed between domesticated wild and Vienna8-GSS displaying different patterns between developmental stages. Proteobacteria mainly members of the Enterobacteriaceae family represent the major component of the bacterial community followed by Firmicutes (mainly in Vienna8-GSS adults) and Chlamydiae. The distribution of these communities is dynamic across the generation and seems to be strain- and age-specific. In the Vienna-8 GSS population, *Providencia* exhibited high relative abundance in the first three generations and decreased significantly later, while *Klebsiella* was relatively stable. In the domesticated wild population, *Klebsiella* was dominant across most of the generations, indicating that the wild population are more resistance to artificial rearing condition compared with the Vienna-8 GSS colony. The analysis of the core bacteriome shows the presence of nine shared taxa between most of the medfly examined samples including *Klebsiella*, *Providencia*, *Pantoea* and *Pseudomonas*. Other bacterial genera were acquired during the domestication such as *Carninomas* in larvae while others were lost like *Morganella* and *Moellerella*. In addition, the operational taxonomic unit co-occurrence and mutual exclusion networks of the wild population showed that most of the interactions were classified as co-presence, while in the Vienna-8 GSS population, the number of mutual exclusions and co-presence interactions were equally distributed

Curculio elephas (Gyllenhal, 1836) (Coleoptera: Curculionidae): novel tools for Integrated Pest Management on chestnut trees

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Curculio elephas (Gyllenhal, 1836) (Coleoptera: Curculionidae), is an oligophagous pest, attacking several chestnut (*Castanea spp*.) and oak (*Quercus spp*.) species. This weevil is one of the most important pests of European chestnut (*Castanea sativa*) in Europe and it occurs in a continuous range throughout Greece.

A significant limitation for the development of effective Integrated Pest Management strategies is the lack of appropriate tools for the monitoring of insect populations. In particular, neither an attractant has been identified nor a semiochemical trap has been developed for *C. elephas* thus far. As a consequence, preventive measures usually depend on emergence traps or visual inspection of potentially infested chestnuts.

The challenging aspect of this project is to identify the potential VOCs from chestnut acting as attractants for *C. elephas* females that could facilitate the development of novel integrated pest management tools such as the 'attract and kill' method.

Catkins were cut at the beginning of Summer and transferred in the lab for the collection; burs were collected during Summer and at mid of Autumn in situ. Nuts were collected in mid-Autumn and transferred in the lab. For the collection and identification of VOCs, the dynamic-headspace technique combined with gas chromatography-mass spectrometry (GC-MS), was used.

The results, so far, reveal that sweet chestnut releases different VOCs depending on its growth stage, including mainly terpenes, esters and ketones.

Subsequently, the collected plant material will be tested for electrophysiological responses of *C. elephas* female adults using Gas-Chromatography and Electro-Antennographical Detection (GC-EAD). Behavioral responses on both the plant material and individual VOCs will be conducted with the use of Y-tube olfactometer. The key-compounds associated with the host plant-insect interactions will be assessed in order to develop and evaluate traps for monitoring and mass-trapping in different landscapes throughout Greece.

Key words: *Curculio elephas*, GC-EAD, VOCs

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Use of multispectral sensors on the detection of entomological infections in stone fruits and apple trees

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Monitoring a field is a time-consuming and laborious process for the Agronomist, which must be repeated often during the growing season. Remote sensing completes the process of on-site monitoring of a field making it faster, easier and giving better results because of the ability to detect the reflection of light from plants in many spectral bands giving information about their health. As a result, the Agronomist can monitor and perform a more accurate field diagnosis in plants that have been recorded as stressed, based on the reflection of the light.

In the present study, a multispectral sensor was attached to a UAS in order to obtain aerial imagery data of the orchard of the Agricultural University of Athens. Through photogrammetry and GIS programs, these data were used for maps production and the calculation of the Normalized Difference Vegetation Index (NDVI), which might contribute to the detection of stressed trees of the orchard. The results of the remote sensing were compared to these of the on-site observation.

The results of the on-site observation of the orchard showed that many stone fruits and apple trees had symptoms of flatheaded wood borer infestation, *Capnodis tenebrionis* Linnaeus (Coleoptera: Buprestidae), which was reinforced by the presence of adults of the insect on them and it was confirmed by trees uprooting. The values of the NDVI index indicated the trees that were stressed. The results of the two methods were compared.

These two methods of monitoring the health of a crop had statistically significant differences in all of the cases tested. Also, through the remote sensing, early infestations of the insect were detected and the threshold value of the NDVI index was determined. The presentation will be followed by a detailed presentation of the results and an extensive discussion on them.

Key – words: flatheaded wood borer, multispectral imagery, remote sensing, UAS.

Improving Biological Control Agents for Integrated Pest Management

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Integrated management of plant pests (IPM) is based on the application of beneficial arthropods in combination with insecticides. An important biological control agent is the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), which is widely used for biological pest control in various greenhouse crops. On tomato crops, however, *A. swirskii* populations cannot develop and hence, cannot control the pest population. Thus, increasing the adaptability of biological control agents to hostile environments is needed.

Unlike herbivores, the resistance of beneficial arthropods to insecticides is a desirable phenotype. In the present study, firstly, the resistance to insecticides of a commercial and a wild population of *A. swirskii* was evaluated. The wild population was found to be more resistant to the two active ingredients, spinosad and abamectin, compared to the commercial population. Secondly, the wild population underwent artificial selection to promote the increase of the resistance to these active ingredients.

Last, we investigated the possibility of applying *A. swirskii* on tomato crops, which is a hostile environment for the predatory mites of the family Phytoseiidae, due to the lethal effect of the tomato trichomes and their allelochemicals. For this purpose, we investigated the effect of *Fusarium solani* strain K (FsK), an endophytic fungus that colonizes tomato roots, on the trichome density of two commercial tomato varieties that express different trichome composition and also, we examined the dispersal of *A. swirskii* on these plants. In this study, we found FsK-inoculated plants did not express less trichomes and that *A. swirskii* did not disperse more on these plants. We concluded that the characteristic of the plant that causes the trapping and ultimately the death of the predators, is the glandular trichomes type VI. In addition, we found that *A. swirskii* tended to walk longer distances on the stems of tomato varieties with fewer type VI glandular hairs, suggesting better performance of the predators on these varieties. This led us to the investigation, which is in progress, of the existence of genotypes with fewer type VI trichomes among the tomato varieties maintained in the gene bank of the Institute of Plant Breeding and Genetic Resources.

Keywords: resistance, tomato trichomes, abamectin, spinosad, Phytoseiidae

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Irradiation effect on the structure of bacterial communities associated with the quarantine fruit fly, *Bactrocera dorsalis*

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The role of symbiotic microbes in insects, especially the beneficial character of this interaction, has received much attention in recent years as it has been related to important aspects of the host insects' biology such as development, reproduction, survival, and fitness. Among insect hosts, tephritid fruit flies are well known to form beneficial associations with their symbionts. To control these destructive agricultural pests, environment-friendly approaches, like the sterile insect technique (SIT) as a component of integrated pest management strategies, are currently successfully implemented against the Mediterranean fruit fly *Ceratitis capitata*, the Mexican fruit fly *Anastrepha ludens* and Melon fly *Zeugodacus cucurbitae* or are under consideration for other species. In this study, changes in the bacterial profile of mass-reared oriental fruit flies*, Bactrocera dorsalis* (Hendel) (Tephritidae) were examined in both larval and adult stages and also after irradiation by employing a 16S rRNA gene-based Illumina sequencing approach. Proteobacteria were the prevalent bacterial phylum in non-irradiated adults and larvae. Alphaproteobacteria were the most abundant class in larvae but almost absent in adults, which were dominated by Gammaproteobacteria. Firmicutes were present in both developmental stages but at lower relative abundance. At genus level, *Acetobacter* prevailed in the larval stage and members of the Enterobacteriaceae family in adults. Irradiated samples exhibited higher diversity and richness indices compared to the non-irradiated oriental fruit flies, whereas no significant changes were observed between the two developmental stages of the non-irradiated samples. *Lactobacillus*, members of the Orbacecae family and *Morganella* were detected but to a lesser degree upon irradiation, whereas the relative abundance of *Lactococcus* and *Orbus* increased. The bacterial profile of larvae appeared to be different compared to that of adult *B. dorsalis* flies. The subsequent application of irradiation at the pupal stage led to the development of different microbiota between treated and untreated samples, affecting diversity and operational taxonomic unit composition. Controlling and exploiting the microbiota during all developmental stages may improve the performance of irradiated males

Bacteriome analysis in natural populations of *Mayetiola hordei*

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Mayetiola hordei (Barley stem gall midge) is one of the most serious pests of barley in Morocco, causing serious qualitative and quantitative losses in its agricultural production. In this work, we investigate the presence of reproductive symbionts and the effect of geographical origin on the bacterial structure and diversity associated with barley stem gall derived from four major barley producing regions in Morocco, with the aim of exploiting them for the biological control of pest populations. A specific 16S rRNA PCR assay was used to explore the presence of bacteria that may induce reproductive abnormalities in their insect hosts, such as *Wolbachia, Spiroplasma, Cardinium* and *Arsenophonus*. Our results indicated the presence of *Wolbachia* in 8% of barley stem gall midge in only two regions «Rabat and Fes Meknes». Phylogenetic analysis indicated that the *Wolbachia* strain present in barley stem gall midge belongs to supergroup A. High throughput sequencing of V3-V4 region of the bacterial 16S rRNA gene revealed significant differences in the bacterial community between the regions, which may prove that there is an effect of the geographical location on the diversity and composition of barley stem gall midge bacteriome. We recognized that there was a dominant bacterium in all samples, the case for *Pseudomonas*, followed by *Stenotrophomonas*, and *Phyllobacterium*, which belong to the Pseudomonadaceae, Sphingomonadaceae, and Rhizobiaceae families respectively. While *Pantoea* belonged to the family Enterobacteriaceae was present only in the region of Fes Meknes with the highest relative abundance.

Bacteriome analysis of natural and laboratory *Glossina* populations (Diptera: Glossinidae)

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Tsetse flies (*Glossina* spp.; Diptera: Glossinidae) are viviparous flies that feed on blood exclusively in sub-Saharan Africa. They are the only cyclic vectors of African trypanosomes, responsible for human African trypanosomiasis (HAT) and animal African trypanosomiasis (AAT). In this study, we employed high throughput sequencing of the 16S rRNA gene to unravel the diversity of symbiotic bacteria in four wild and laboratory populations of tsetse species (*G. pallidipes, G. morsitans, G. swynnertoni* and *G. austeni*). The aim was to assess the dynamics of bacterial diversity both within each laboratory and wild colony, in relation to the development stage, insect age, gender and location. Our results indicated that the microbiota associated to the four studied *Glossina* sp. were significantly influenced by their region of origin, where the microbiota of the wild samples was more diverse compared to the laboratory samples. We observed also that the larval microbiome was significantly different from the adults. Furthermore, the gender and the species did not significantly influence the formation of the bacterial profile of the laboratory colonies once these populations were kept under the same rearing conditions. In addition, *Wigglesworthia, Acinetobacter* and *Sodalis* were the most abundant bacterial genera in all the samples, while *Wolbachia* was significantly abundant in *G. morsitans* compared to the other studied *Glossina* sp.. The operational taxonomic unit (OTU) co-occurrence network for each locations indicated a high variability between *G. pallidipes* and the other species in terms of number of mutual exclusion and copresence interactions. In particular, some bacterial genera (like *Wigglesworthia*, and *Sodalis*) with high relative abundance were characterized by a high degree of interactions.

Insecticidal effect of *Pistacia lentiscus* (Sapindales: Anacardiaceae) and *Mucor hiemalis* strain (SMU-21) metabolites on the *Lobesia botrana* (Lepidoptera: Tortricidae) larvae.

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Lobesia botrana (Lepidoptera: Tortricidae) is one of the most harmful vineyard pests worldwide with major economic impact on the viticulture industry. To mitigate the negative environmental and human health impact as consequences of the current practices, it is necessary to make the best use of nature-based innovations (the isolation of natural, specialized and biodegradable insecticides from plants and microorganisms) to meet the European Green Deal goals aiming to reduce the overall use and risk of chemical pesticides by 50% and the use of more hazardous pesticides by 50% by 2030.

The objective of this study is to investigate the effects of extracts from the fruits of *Pistacia lentiscus* (Anacardiaceae) and the SMU-21 fungal strain *Mucor hiemalis* (Zygomycetes: Mucorales), which was isolated from larvae of wild populations of the lepidopteran *Sesamia nonagrioides* (Lepidoptera: Noctuidae), on the viability of *L. botrana* larvae, through contact bioassays.

Concerning *P. lentiscus*, following bioassays using fruit extracts derived from fractionation with solvents of increasing polarity, the hexane extract (PLF_{He}) was found to be the most potent and was further fractionated with column chromatography. Of the four main fractions collected and tested for their bioactivity, mortality was only observed in the second fraction (PLF_{He2}), which accounted for 75% of the total initial hexane extract and based on its ^{1}H NMR signal was identified as a triglyceride.

Regarding *M. hiemalis*, the metabolites from liquid culture of the fungus were isolated through several purification steps and their bioactivity on *L. botrana* larvae was investigated. The extract exhibited significant insect mortality in a dose-response manner. Further analysis is required in order to identify the compounds responsible for the extracts' bioactive activity.

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Keywords: fatty acids, *Lobesia botrana, Mucor hiemalis*, natural insecticides, *Pistacia lentiscus*, triglyceride,

Qplots: An interactive web platform for the analysis and visualization of microbial sequencing data based on the R-shiny framework

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The characterization of the structure of microbial communities via the 16S rRNA gene has been greatly advanced in recent years with the introduction of high throughput amplicon sequencing, leading to a better representation of a sample's diversity in a cost-efficient way. The advances in sequencing technologies combined with a significant growth of computational tools and methods have allowed the analysis of complex bacterial communities associated to a wide range of habitats (soil, water, air, humans, animals, etc.). Thus, for the analysis of these expanding data, researchers have to choose among a variety of multipurpose platforms, the use of which can be daunting for non-expert users. Furthermore, complex study designs that include multiple patterns necessitate a flexible analysis tool that can provide dynamic and real-time adjustment of analyses and visualizations. In the current study, we took advantage of the easiness and flexibility of R programming to develop Qplots, an interactive user-friendly platform that enables the analysis and visualization of microbial sequencing data. Qplots constitutes an analytical pipeline for the calculation of the bacterial composition and diversity based on Operational Taxonomic Units (OTUs) tables. Currently, it provides a set of well-documented choices of downstream analysis including taxonomic composition, alpha and beta diversity analysis, statistical comparisons between subsets of samples, as well as a variety of visualization methods including tables, boxplots, heatmaps, bar charts, MDS and NMDS plots. Qplots was implemented entirely in R language using the Shiny framework. It can be easily used locally in any system with R installed, including Windows, MacOS and most Linux distributions or remotely through a web server without bioinformatic expertise. It can also be used as a framework for advanced users who can modify and expand the tool.

Effects of beneficial soil microbes on natural enemies in pepper

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Unable to run away, plants interact with herbivorous pests and their natural enemies, as well as pathogenic and beneficial microorganisms. The latter include soil microorganisms that can modify the physiology and chemical traits of plants by enhancing growth, activating induced systemic resistance, priming plants and activating indirect plant defense. Interactions between beneficial soil microorganisms and natural enemies via the plant are not well studied. Here, we recorded the effects of inoculating pepper plants with beneficial soil microorganisms on aboveground zoophytophagous mirid predators. Specifically, we studied the effects of microorganisms on the performance and behavior of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae). We also, assessed their predation efficiency against aphids as affected by pepper inoculation with microorganisms in the greenhouse. We found that beneficial microorganisms impact predator's behavior towards inoculated pepper plants, but not their survival or nymph production. Similarly, predators release in the greenhouse was not negatively affected on inoculated plants. Our results could be useful for the development of sustainable biocontrol tools and add on the growing literature of plant-microbe-arthropod interactions.

Key-words: bacteria, biological control, fungi, natural enemies, plant defense

Population genetics and bacterial endosymbionts of Aphrophoridae species, putative vectors of *Xylella fastidiosa* in Greece

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Xylella fastidiosa is a Gram-negative bacterium which is responsible for many diseases in economically important crops such as olives. Its vectors are xylem fluid-feeding insects of the suborder Auchenorrhyncha (Order: Hemiptera). The knowledge of the genetic structure of the potential insect vectors of *X. fastidiosa* is important in preventing epidemics related to them. In this study, we investigated the genetic diversity and the infection status by five secondary endosymbionts of three species, *Philaenus spumarius* (Linaeus) (Hemiptera: Aphrophoridae), *Neophilaenus campestris* (Fallen) (Hemiptera: Cercopoidea) and *P. Signatus* (Melichar) (Hemiptera: Aphrophoridae), putative vectors of *Xylella* collected from olive orchards in 19 regions of Greece. The genetic polymorphism was determined based on the mitochondrial (mt) c*ytochrome c oxidase subunit I* (*COI*) and *cytochrome b* (*cytb*) genes. In total, 238 *COI* and *cytb* sequences obtained in the present study were analyzed together with 91 additional from GenBank. Analysis of the mtDNA sequences revealed high polymorphism in Greek *P. spumarius*, (26 haplotypes for *COI* and 52 for *cytb)* and *N. campestris* (13 and 7 haplotypes for *COI* and *cytb,* respectively). In addition, screening for *Hamiltonella*, *Rickettsia*, *Arsenophonus*, *Cardinium* and *Wolbachia* which are known for their interference in shaping the genetic structure of insect species, was carried out. In *P. spumarius*, only *Hamiltonella*, *Rickettsia* and *Wolbachia* were present in equally low frequencies (4%). Regarding *N. campestris, Arsenophonus* had the highest frequency (35%) whereas *Cardinium* was not found in any individual of *N. campestris*. Moreover, to determine the overall bacterial composition of *P. spumarius*, we obtained Next Generation Sequencing data of 16S metagenome of individuals from several Greek regions. The analysis revealed constant differences in bacterial composition between the head and the rest of the insect body.

Key words: Aphrophoridae, endosymbionts, genetic diversity, metagenome, *Xyllela fastidiosa*

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Population genetics and detection of grapevine viruses in Greek mealybug species (Hemiptera: Pseudococcidae).

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Mealybugs (Hemiptera: Pseudococcidae) are insects that feed on plants belonging to more than 250 families. In grapevine, they transmit Grapevine leafroll associated virus -1 and -3 (GLRaVs) and Grapevine virus A and B (GVs) that lead to important economic damages. They are therefore considered important grapevine pests and have caused outbreaks in many important grapevine producing regions of Greece. For their effective control it is important to know the composition of the species of mealybugs that are present in a region. Also, the study of their genetic variability allows the development of molecular diagnostics for their early identification, especially those that may be associated with the transmission of specific GLRaVs and GVs in Greek vineyards.

For the determination of the mealybug species and their diversity, a combination of classic morphological and molecular methods was used. Sequences of *cytochrome - c- oxidase subunit I* (*COI*) of mealybugs collected from grapevine in Crete, Peloponnese, Central and Northern Greece were obtained. As expected, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) was the predominant species (46 out of 51 individuals). Two more species associated with grapevine, *Pseudococcus longispinus* (Targioni-Tozzeti) (Hemiptera: Pseudococcidae) and *Heliococcus bohemicus* (Sulc) (Hemiptera: Pseudococcidae), were also found (4 and 1 individual respectively). *P. ficus*, displayed a high variability as 18 haplotypes were found for *COI*. The mealybugs and the corresponding grapevines that were collected from, were tested for the presence of GLRaVs and GVs. One or more viruses were detected in the samples. Finally, a simple molecular diagnostic tool (PCR-RFLP) was successfully developed based on 28S ribosomal RNA and was applied for the molecular identification of the three species found in Greek vineyards.

Keywords: genetic diversity, grapevine, viruses, mealybugs.

Acknowledgements This work was supported by Greek national funds through the Public Investments Program (PIP) of the General Secretariat for Research & Technology (GSRT), under the Emblematic Action "Routes of Vineyards", Grant No. 6070.03

Effects of beneficial soil microbes on the behavior of natural enemies

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Plants interactors include macro- and microorganisms that can be found above- and belowground. To defend themselves against the harmful of these organisms such as herbivorous arthropods, plants have evolved sophisticated mechanisms aiming to ward off their enemies and to attract predators. The latter can be facilitated by the release of volatiles that inform predators about prey availability on infested plants, thereby increasing the number of natural enemies landing on the plant to feed with pests and suppress their populations. On the other hand, beneficial interactions between plants and predators may be impacted by organisms living belowground such as beneficial microbes. These are of particular interest as they are known to enhance plant responses against pathogens and herbivorous pests. Nevertheless, their impact on the ability of plants to attract predators is not largely explored. We herein present the results of olfactometer experiments performed with natural enemies, namely the mirids *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), the phytoseiid *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) adult individuals when given a choice between all different combinations of plants infested with different herbivores and inoculated with soil microbes or not. We show that the attractiveness of microbe-inoculated tomato plants varies depending on the natural enemy, as well as the herbivore infesting the plant. Overall, our results highlight the need of taking a community perspective in insect-plant interaction studies and may be useful in the identification of sustainable pest control tools.

Key-words: bacteria, biological control, fungi, natural enemies, plant defense

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Biological Control - Alternative Control Methods

The potential of *Neoleucopis kartliana* Tanasijtshuk (Diptera: Chamaemyiidae) as a biological control agent against *Marchalina hellenica* (Hemiptera: Margarodidae)

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Keywords: Classical Biological Control, Chamaemyiidae, Margarodidae

The scale insect *Marcalina hellenica* (Hemiptera, Margarodidae), which is endemic in Greece and Turkey, attacks mostly pine trees (*Pinus spp.*), and, within its native range it is considered an important beneficial insect supporting honey production. However, the introduction of the scale species in novel areas causes ecological and economic problems by jeopardizing pine tree health and insect biodiversity. *M. hellenica* was detected for the first time in Melbourne and Adelaide (Australia) in 2014, causing significant damage to *P. radiata* commercial plantations, threatening Australia's pine forestry industry.

The silver fly *Neoleucopis kartliana* Tanasijtshuk (Diptera, Chamaemyiidae) is considered a crucial factor for the suppression of the scale's populations, as the most abundant and widespread predator. For the assessment of *N. kartliana* as a viable classical biological control agent against *M. hellenica* in Australia, a study on the biology of the fly was carried out, attempting on the same time the establishment of a rearing protocol. The predator was present in all six sampling sites, and it was found preying indiscriminately on every development stage of *M. hellenica*, showing on the same time high prey specificity.

Parasitism rates of *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae) on eggs of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a highly polyphagous pest that is causing severe damage to a wide variety of fruit and vegetable crops by piercing the surface and tissues of the plant . Control of *H. halys* in the newly invaded areas relies mainly on the use of broad-spectrum chemical insecticides, and no information is available regarding the potential for biological control through native parasitoids. The aim of the present study was to assess the parasitism rate of the gregarious egg parasitoid *Ooencyrtus telenomicida* Vassiliev (Hymenoptera: Encyrtidae) on *H. halys* egg-masses in the laboratory at 26 °C, 60 % relative humidity and under a 16:8 h (L:D), depending on i) age of parasitoids, ii) density of parasitoids and iii) age of host eggs. We tested different parasitoid ages (1-2, 3-4 and 5-6 days-old) and densities (1, 2 and 4 pairs) on 1-4 days old *H. halys* eggs. According to our results, there is a strong effect of parasitoid density and age of host eggs and the interaction between them on the parasitism rate of *O. telenomicida*. High parasitoid density along with younger host eggs resulted in higher parasitism rates, with the maximum mean value of parasitism rate being observed at 1 day-old host eggs and 4 parasitoid pairs (57.3%). The lowest parasitism rate was observed at 4 days-old host eggs and 1 parasitoid pair (6.5%). Similarly, a significant interaction was observed between the parasitoid density and their age (highest parasitism rate was 47.18% at a density of 4 parasitoid pairs at an age of 3-4 days). Successful parasitization occurred to host eggs up to 4 days-old, with parasitism rate values decreasing as host egg age increased. We also observed 3-fold higher parasitism rate of *H. halys* eggs that were parasitized by 3-4 and 5-6 days-old *O. telenomicida,* compared to 1-2 days-old ones (31.84, 32.43 and 12.14%, respectively). Our findings provide valuable information on the potential biological control of *H. halys.*

Keywords: *Ooencyrtus telenomicida,* egg parasitoid, *Halyomorpha halys*, biological control, parasitism rate, age, density.

Study of the pathogenicity of *Meloidogyne* species on *Cannabis sativa* varieties and the efficacy of seeds extracts against *M. javanica*

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In the study reported here the host range of five *Meloidogyne* species using eleven *Cannabis sativa* varieties was investigated. The experiments were conducted at the Agricultural University of Athens from April to June for two consecutive years (2020/21). *C. sativa* plants were grown in plastic pots and were artificially inoculated with 500 second stage juveniles (J2s). They were uprooted after 30 days at 25 – 29 ^{0}C. Canopy and root weight, females per g of root, egg masses development and fecundity were evaluated. All *Meloidogyne* species were pathogenic to *C. sativa* plants. The most severe damage of the roots was detected in *M. javanica* infected plants. The other four species in order of pathogenicity were: *M. luci, M. incognita, M. hapla* and *M. arenaria*. The most susceptible varieties were Zenit, SAN70 and USO while Futura hosted the lowest nematode numbers. In a second series of experiments the exposure of second stage juveniles or eggs to different concentrations of aqueous and ethanolic extracts of *Cannabis sativa* seeds was investigated. Both extracts had a marked effect on nematodes survival. High concentrations (250 – 2000 ppm) of both extracts have shown a permanent paralysis of J2s after 24 – 96 h exposure. However, at lower concentrations only the ethanolic extract was effective. The ethanolic extract paralyzed J2s at concentrations higher than 62,5 ppm and reduced the egg hatching up to 76% compared to the control treatment. Aqueous extract was substantially inferior as far as efficacy is concerned compared to ethanolic one.

Key words: *C.sativa*, *Meloidogyne spp,* aqueous extracts, ethanolic extracts

First emerging and treated *Spissistilus festinus (Say) (Hemiptera: Membracidae)* in *Vitis vinifera* cultivation in Ioannina

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Spissistilus festinus (Say) (Hemiptera: Membracidae) was first observed in Peristeri Ioannina in Epirus, Greece. The initial population was collected from local farmers of the area from infected *Vitis vinifera* (Rhamnales: Vitaceae) L. plants, in the province of Ioannina, in the summer of 2020. The infected plant samples (leaf shoots of *V. vinifera* plants) were received at the Plant Laboratory of the Department of Agriculture, University of Ioannina. maintained at 25 ± 1 ° C for observation. Based on the morphological characteristics of adults and nymphs, the species was identified as *S. festinus*. The PCR products were then sent for molecular identification for sequencing where it was confirmed to be the *S. festinus*. Yellow traps were placed to carry out the population and spatial distribution of the insect in a vineyard in Peristeri, Ioannina. The insect pathogenic fungus *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) was used in the laboratory against its nymphs. For each isolation of the fungus a conidia suspension (108 conidia / ml) was prepared. Two biocides were used containing the insect pathogenic fungus *B. bassiana* and they were BotaniGard® ES (strain GHA 10.735%) (K&N Efthymiadis SA) and Velifer® (strain PPRI 5339) (BASF) and *B. bassiana* WildStrain. Mortality recorded for 7 days. Our results led us to the conclusion that the use of insect pathogens and especially *B. bassiana* can play an important factor in the control of the insect *S. festinus*.

KEYWORDS: *Spissistilus fe*stinus, entomopathogenic fungi, *Beauveria bassiana*, first observed

The brown lacewing *Micromus angulatus* (Stephens) (Neuroptera: Hemerobiidae) as a biological control agent of aphids

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The Neuroptera represents one of the smallest orders of Holometabolous insects. Among its families, species of the family Hemerobiidae or brown lacewings are promising biological control agents of crop pests. In the present study, we studied the hemerobiid species *Micromus angulatus* (Stephens) (Neuroptera: Hemerobiidae), an arthropod predator widely distributed in Europe, North America and North Asia. It is a promising biological control agent that was recently launched commercially, whose both larvae and adults are predatory and feed on several aphid species. Two groups of experiments were performed. In the first group, we studied the biology of *M. angulatus* on two prey species, the aphids *Aphis fabae* Scopoli (Hemiptera: Aphididae) and *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae). We recorded juvenile developmental time, survival, female longevity and oviposition, and calculated the values of intrinsic rate of population increase of *M. angulatus* when fed on each aphid species. Furthermore, we studied the efficacy of *M. angulatus* on plants infested with the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). According to our results, *M. angulatus* can efficiently develop and increase its populations with both aphid species as prey. In addition, the release of first instar larvae at the rate of 2 or 10 individuals per plant is effective in reducing the populations of *M. persicae* on pepper plants irrespectively of the initial pest infestation pressure i.e. 10 or 20 aphids per plant. The brown lacewing larvae at the highest release rate tested managed to completely eliminate aphids from pepper plants two weeks after release. Adults release at a rate 1 to 2 females per plant was also effective in drastically reducing *M. persicae* populations. Our results highlight the ability of *M. angulatus* to develop on different aphid species as well as its potential for use in biological pest control.

Key-words: aphids, biological control, Hemerobiidae, *Micromus* sp., pepper

Field margin management with aromatic plants to enhance habitats of honey bees, wild bees and natural enemies of insect pests in citrus orchards

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The PLANT-B project examines a mixed farm system that combines the cultivation of citrus and aromatic / medicinal plants (AMPs) with beekeeping. The impact of establishing AMPs in field margins to enhance habitats of honey bees (*Apis mellifera*), wild pollinators and natural enemies of insect pests, was studied in seven orange orchards (*cv.* Merlin, Navalina) in the region of Argolis, Greece, during the crop season of 2021: five orchards with an AMP species (rosemary in organic and integrated management orchard; savory; oregano, sage), and two controls, one with weed vegetation and one with bare soil. During the flowering period of orange trees (mid-April - mid-May) honey bee hives were placed in all orchards and until the end of the flowering of the aromatic plants (end of June) the percentage of vegetation and flower cover in the margins of the fields were recorded as well as the visits of pollinators and the presence of predators and parasitoids on AMPs, weeds and orange blossoms in pre-marked trees adjacent to the margins and within the orchard.

All AMPs except rosemary gave rich flowering and attracted mainly honey bees but also wild bees while weed vegetation (groundcover 30-50%) had low flower cover (up to 5%) mostly by *Anagallis arvensis* L., *Plantago* sp. and *Crepis* sp., and attracted small numbers of pollinators. Sage had the longest flowering period (before, during and after the orange blossom) and high attraction of pollinators while at the end of the orange blossom, savory attracted more pollinators than oregano and sage. The orange blossoms were visited almost exclusively by honey bees (few bumblebees in fields of sage, oregano) while due to uneven flowering as a result of frost in some fields, it was not possible to assess the effect of AMP interventions on honey bee visits.

Establishment of AMPs in the field margins had a significant effect on the abundance of natural enemies, which varied over time. Higher numbers of predators were recorded in oregano (end of April) and savory (beginning of June) compared to sage and rosemary (integrated management orchard), respectively. Only in mid-May the number of predators in weed vegetation exceeded that in AMPs, except for sage. The pattern of parasitoid abundance over time was different with higher numbers in savory compared to rosemary (integrated management orchard) almost throughout the measurements, and in relation to weed vegetation at the end of May-beginning of June. In orange trees, the differences mainly concern the greater presence of parasitoids in the organic rosemary orchard than in the one with bare soil.

Keywords: aromatic/medicinal plants, beneficial arthropods, biodiversity, honey bees, PLANT-B, pollinators, weed management

Financial support has been provided by PRIMA (grant number 1812/PLANT-B), a programme supported by the European Union.

Comparative prey searching efficiency of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae) on eggs of *Tuta absoluta* (Lepidoptera: Gelechiidae) when offered on variable prey densities and distribution patterns

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The efficacy of natural enemies in biological control is determined by several factors including their prey searching efficiency which is affected by the prey density and its distribution. On the other hand, females' oviposition site selection depends on their effort to reduce predation risk of their eggs. The aim of our study was to compare the searching ability of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* on *Tuta absoluta* eggs. Newly laid eggs (24h old) were carefully positioned on a tomato leaflet under different densities (3, 5, 8 or 10 eggs). Eggs were positioned: a) according to the natural pattern followed by *T. absoluta* females for each prey density per leaflet, b) linearly on the central vein, c) in a group and d) close to the leaf margin. Nymphs of *M. pygmaeus* (*Mp*) and *N. tenuis (Nt)* were placed into a Petri dish for 24h without prey. Then, a single nymph was released in a dish with a leaflet on which the eggs had been placed and the number of consumed eggs was recorded after 15′, 30′, 60′, 120′ and 180′, at 25±1°C, 65±5% and 16:8 L:D. According to our results, *Mp* showed a higher prey consumption than *Nt* 15′ after its introduction in the dish when 3 eggs/leaflet were positioned linearly or in group. Also, in the case of 5 eggs/leaflet, *Mp* consumed more eggs in 15′ and 30′ when eggs were positioned in group or in the periphery of tomato leaflet. Finally, when 10 eggs were offered *Mp* consumed more eggs in 30′ and 60′ in all cases except of the natural pattern. In conclusion, *Mp* showed a higher prey searching efficiency than *Nt*. However, in the case of natural egg deposition pattern, both predator species showed a similar prey searching efficiency on *T. absoluta* eggs, independently of their density.

Keywords: foraging efficacy, *Macrolophus pygmaeus, Nesidiocoris tenuis,* searching efficiency, *Tuta absoluta*.

Sublethal effects of oregano essential oil and carvacrol on biological parameters of Asian tiger mosquito

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The overuse of synthetic insecticides against mosquitoes has led to mosquito resistance development, harmful effects on human health and unacceptable environmental effects on non-target organisms. Alternatively, a growing number of plant derived products have been tested as more eco-friendly agents against mosquito larvae, showing high efficacy through various modes of action. In the laboratory, we evaluated the sublethal effects of larvicidal LC_{50} doses of Oregano essential oil and its major component carvacrol, against *Aedes albopictus* (Skuse) (Diptera: Culicidae) (Asian tiger mosquito). Batches of 20 late third to early fourth-instar mosquito larvae were exposed for 24h to LC_{50} doses of oregano oil and carvacrol in 2% v/v aqueous solution of DMSO, and to 2% aqueous solution of DMSO serving as control. Alive larvae 24h post treatment were transferred into beakers with water and fed with powdered fish food until pupation. Alive pupae were kept individually into plastic vials with water until emergence of adults. Adult males and females were paired in plastic cages and supplied with sucrose solution. A blood meal was provided to each female and an oviposition substrate was added into the cage. The eggs were submerged in water with appropriate food for hatching. We determined the effects of LC_{50} doses on survival and longevity of 24h survived larvae until pupation and adulthood, longevity of males and females, fecundity, fertility and adults body size. Results revealed that only half of the 24h survived larvae from oregano oil and carvacrol treatment finally reached adulthood. Carvacrol treatment caused half-day extension of survived larvae development until adulthood. Abnormalities on the formation of dead larvae and pupae, and failed adult emergence were also observed, indicating a potential growth inhibitory activity of the tested materials. No particular effects from LC_{50} doses were recorded on biological parameters of successfully emerged adults. These findings suggest the tested oregano oil and carvacrol as sufficiently effective larvicides against *Ae. albopictus* at lower than the acutely toxic doses, promoting a more eco-friendly and less costly profile for these biopesticides.

Keywords: *Aedes albopictus,* carvacrol, larvicide, mosquitoes, oregano essential oil, sublethal effects.

Tanacetum vulgare essential oil as wheat protectant against four major stored-product insect pests

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The insecticidal impact of the *Tanacetum vulgare* L. (Asteraceae) essential oil (EO) on larvae and adults of four noxious stored-product insect species, i.e., *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) was evaluated. The main chemical composition of the EO includes oxygenated monoterpenes. The EO of *T. vulgare* was applied as wheat protectant at 500 ppm and 1000 ppm. Mortality was assessed after 4, 8 and 16 h and 1, 2, 3, 4, 5, 6 and 7 days. All *T. castaneum* larvae died after a 6-day exposure to 1000 ppm, while adult mortality reached only 25.6% after 7 days at the same concentration. Mortality of *T. confusum* larvae and adults reached 56.7% and 8.9% respectively at 1000 ppm 7-days post-exposure. The EO killed 8.9 and 52.2% of the exposed *T. molitor* larvae at 1000 ppm after 7 days of exposure. *Oryzaephilus surinamensis* larvae exhibited high mortality (>90%) while adults low mortality (<14%) at 1000 ppm 7 days post-exposure. Our results suggest that the EO of *T. vulgare* can be used as an efficient wheat protectant against the tested species. However, the efficacy of this EO depends on the species and its life stage.

Keywords: Essential oil, *Tanacetum vulgare*, *Tenebrio molitor*, *Tribolium castaneum*, *Tribolium confusum*, *Oryzaephilus surinamensis*

Efficacy of insecticides on 2nd instar larvae of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a polyphagous species that flies easily from plant to plant, infesting more than 100 different host plants. So far in Greece it has shown a particular preference for kiwi fruits (*Actinidia deliciosa*), often resulting in substantial economic damage. The extensive damage of kiwi orchards that we observed in the last two years in Pieria and Imathia Prefecture has led us to test the efficacy of certain insecticides on 2nd instar larvae of *H. halys* in the laboratory. The following conventional and biological active ingredients were used: etofenprox, acetamiprid, spinetoram, sulfoxaflor, spirotetramat, *Beauveria bassiana* and zeolite in the following doses 0.75ml / L, 1.5ml / L, 4.8ml / L, 0.2ml / L, 1.0ml / L, 1.5ml / L and 10g / L, respectively. The application of the aforementioned active ingredients was performed with an airbrush (Gravity Dual-action Mini Airbrush) with direct exposure of 10 L_2 larvae of *H. halys* in a solution of a total volume of 1ml per application. A total of 5 replicates were performed per active ingredient. The efficacy of the active ingredients was evaluated after 1, 3, 5 and 7 days. According to our results, efficacy of conventional active ingredients ranged from 52 to 91% after 24 hours, while after 7 days larval mortality exceeded 95%. In contrast, *B. bassiana* and zeolite were less effective displaying a mortality of 66 and 38%, respectively, after 7 days of spraying. In conclusion, all conventional active ingredients showed an adequate efficacy against 2nd instar larvae of *H. halys* followed by *B. bassiana*, while zeolite had no effect.

This research was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK) under the call “Research-Create-Innovate".

Keywords: *Halyomorpha halys*, 2nd instar larvae, conventional και biological active ingredients, efficacy, mortality.

Use of nitrogen for the disinfestation and disinfection of dried Corinthian currants (*Vitis vinifera* L., var. *Apyrena*): effect on the entomological and microbial load and sensory attributes

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The current study aims to evaluate the effect of nitrogen application on the entomological and microbial load and sensory characteristics of Corinthian currants, *Vitis vinifera* L. var. *Apyrena,* in commercial facilities under real field conditions. Nitrogen application took place at two temperatures, i.e. 28 and 40°C, and three exposure intervals- i.e. 2.5, 3 and 9 days, in air tight commercial chambers. Three species of stored product insects were evaluated, i.e. *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). For all species, several populations were tested, with different susceptibility to phosphine. The vials with insects were placed in different locations within the chamber and the commodity, and mortality was recorded after the termination of each trial. Moreover, the vials were kept at laboratory conditions for 65 days in order to evaluate progeny production capacity. For all cases, high mortality was recorded for all species and populations and progeny production was suppressed with few exceptions for some populations. Nitrogen application resulted in the decrease of total viable count, as well as in the decrease of yeasts and mold content in all cases. Total viable count reduction was in the range 0.34 to 0,52-log while for yeasts and mould load the range was between 0.80 to 2.25-log. Neither time nor temperature had a significant effect on the organoleptic characteristics of currants. All currant sensory attributes remained acceptable after the treatments. Our results suggest that nitrogen application could be a valuable tool in the food industry for the reduction of the microbial load and post-harvest insect control of dried fruits.

Keywords: nitrogen, low oxygen, stored product insects, currants, microbial load

The research work was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EΔK-04326).

Insights of *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae) development under laboratory conditions

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Oencyrtus telenomicida (Vassiliev) (Hymenoptera: Encyrtidae) is a minute encyrtid egg parasitoid of several phytophagous species. Recently, the occurrence of *O. telenomicida* was reported for the first time in Northern Greece infesting eggs of the brown marmorated sting bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). However, little is known about this species' biology concerning the developmental rate of its immature stages on *H. halys* eggs. The aim of the present study was to add to the existing knowledge of this species' biology and development. One-day old parasitized eggs of H*. halys* were examined daily under laboratory conditions to assess the duration of each immature stage, while others were dissected to obtain visual data. The results obtained from the visual observation revealed that *O. telenomicida* goes through three distinct immature stages; egg, larva and pupa. The average duration of the total immature development (egg to adult) is 17.1 days. Males emerge after 16.0 days, while females need a significantly longer period to emerge (17.3 days). The egg stage lasts for 2.2 days, the larval stage lasts for 7.6 days and the pupal stage takes 6.3 days for males and 7.5 days for females before adult parasitoids emerge. The sex ratio is 3.8:1 (f:m). Our results can provide information on the development of *O. telenomicida* that can be used for future experiments to evaluate the exploitation of this species as a biocontrol agent.

Keywords: *Oencyrtus telenomicida*, egg-parasitoid, immature stages, developmental time, *Halyomorpha halys*.

Curcumin derivatives as potential mosquito larvicidal agents against two mosquito vectors, *Culex pipiens* and *Aedes albopictus*

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Mosquitoes are responsible for the infection of many people as they are carriers of various diseases. One million deaths per year are due to mosquito infestation, mainly in tropical areas. People emit certain volatile compounds, which attract female mosquitoes. They receive a meal of blood, thus transmitting infectious parasites / viruses. Vector-borne diseases have appeared or re-emerged in many Southern Europe countries making the transmission of infectious diseases by mosquitoes (vectors) one of the greatest worldwide health threats. Larvicides have been used extensively for the control of *Aedes (Stegomyia) albopictus* (Skuse, 1895) (Diptera: *Culicidae*) and *Culex pipiens (*Linnaeus, 1758)*, (*Diptera: *Culicidae)* mosquitoes in urban and semi-urban environments, causing the increasing resistance of mosquitoes to commercial insec-ticides. In this study, 27 curcuminoids and monocarbonyl curcumin derivatives were synthesised and evaluated as potential larvicidal agents against *Cx. pipiens* and *Ae. albopictus*. Most of the compounds were more effective against larvae of both mosquito species. Four of the tested compounds, curcumin, demethoxycurcumin, curcumin-BF2 complex and a monocarbonyl tetra-methoxy curcumin derivative exhibited high activity against both species. In *Cx. pipiens* the recorded LC_{50} values were 6.0, 9.4, 5.0 and 32.5 ppm, respectively, whereas in *Ae. albopictus* they exhibited LC_{50} values of 9.2, 36.0, 5.5 and 23.6 μg/ml, respectively. No conclusive structure activity relationship was evident from the results and the variety of descriptors values generated in silico provided some insight to this end.

Keywords: curcumin, curcuminoids, larvicidal,structure-activity relationships, common house mosquito, Asian tiger mosquito

This research is co-financed by Greece and the European Union (European Social Fund—ESF) through the Operational Programme Human Resources Development, Education and Lifelong Learning 2014–2020 in the context of the project "Monocarbonyl curcumin derivatives as larvicidal agents against Culex pipiens" (Support for researchers with emphasis on young researchers, EDULL-103, MIS 5050593).

Development of tomato leafminer *Tuta absoluta(Meyrick) (Lepidoptera: Gelechiidae)* larvae in tomato plants that have been endophytically colonized by wild strains of entomopathogenic fungi

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The tomato leafminer *Tuta absoluta(Meyrick) (Lepidoptera: Gelechiidae)* is a serious pest of tomato crops worldwide due to its voltinism, feeding habits and fast resistance development to many active ingredients. Many entomopathogenic fungi have been reported as endophytes for various plants providing them protection against plant pathogens and pests. In this study, the endophyticity of *Beauveria bassiana Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae)*, *Metarhizium anisopliae (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae)* from soils of Heraclion and *B. bassiana*, *Metarhizium brunneum (Hypocreales: Clavicipitaceae)* from Achaia against larvae and nymphs of *T. absoluta* in tomato plants.

A conidial suspension(10^8 conidia/ml) for each fungus was prepared. Then tomato plants were drip irrigated with the suspension and were coated with aluminum for 2 days. After 14 dpi(days per irrigation), the distance between egg and 1st instar larvae at the time of gallery construction and nymph weight was counted. The results were compared to the Tukey - Krammer test.

The results showed that the isolations of Heraclion gave significant differences in both the distance of larvae and the weight of the nymph. *Metarhizium anisopliae and Beauveria bassiana* significantly reduced the distance of larvae and the weight of the nymph compared to the control. No significant differences were observed in the isolates of Achaia and the control. Entopathogenic fungi as endophytes produce secondary metabolites that are responsible for reducing the insect's diet and at the same time improving plant growth.

Keywords: *Tuta absoluta*, entomopathogenic fungi, endophytes, *Beauveria bassiana*, *Metarhizium anisopliae*.

Efficacy of three diatomaceous earth formulations against major stored product insects

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Diatomaceous earths (DEs) are promising alternatives over the use of traditional insecticides that are currently in use in stored product protection. Laboratory bioassays were carried out for the evaluation of diatomaceous earths against a wide range of stored product insect species. The commercial DE formulations Diatom Earth (Agroza, Athens, Greece), Silicosec (Intrachem, Athens, Greece) and Solicid (Detia Garda GmbH, Laudenbach, Germany) were used for the experiments at different dose rates: 0 (control), 100, 300, 500 and 1000 ppm. *Sitophilus granarius*, *Sitophilus oryzae*, *Tribolium confusum*, *Tribolium castaneum* were exposed to the aforementioned dust formulations to investigate their susceptibility on the treated substrate. Mortality of the exposed individuals was measured after 3, 7, 14 and 21 days of exposure, while progeny production was counted after 65 days. In all cases, the increase of dose rate from 100 to 1000 ppm notably increased insect mortality. The results of the present study provide data that illustrate the utilization of DE in stored product protection, as an integrated pest management tool.

Keywords: stored product insects, diatomaceous earth, dust formulations, mortality

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Using smart traps and pheromones to control the gypsy moth: ecofriendly control in practice

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The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) is a polyphagous species that infects oak forests in Central and Southern Europe, Asia, Africa and North America. It constitutes a severe environmental problem as it is a voracious eater that defoliates entire trees and causes health problems to humans or animals (e.g., allergies). Various insecticides have been used for its control, such as aerial sprayings, which negatively impact the biodiversity. Recently developed pheromone and non-pheromone traps have been successfully evaluated for the control of moths with different life cycle, such as the processionary pine moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae). These traps allow the continuous monitoring of the insect's population and dispersion, while they substantially contribute to the emergence of an information system in forests, which can lead to increased resilience against this species, especially in protected areas (e.g., Natura 2000 sites). In this context, the LIFE eGymer project aims to utilize non-chemical control by developing and implementing e-traps, novel traps, mass larval trapping and mating disruption techniques to remotely monitor and effectively control more than one life stages of *L. dispar*. In that way, the design and efficiency of pheromone traps are planned to be further improved, the infestation level will be continuously monitored and the damage in specific and diverse target areas will be minimized. Hence, non-chemical control that will be based in the aforementioned techniques will be carried out between 2022 and 2024 in different areas of Slovenia, Spain and Greece. The generated results will provide a replicable, transferable and eco-friendly integrated system for the effective management of *L. dispar*.

Keywords: *Lymantria dispar*, non-chemical control, novel traps, pheromones, mating disruption
The LIFE eGymer project is co-funded by the LIFE Programme of the European Union under contract number LIFE20 ENV/GR/000801

Fusarium species can be used a biological control factor of *Helicoverpa armigera Hübner (Lepidoptera: Noctuidae);*

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Several Fusarium species were collected from soil, isolated, identified, and tested for their entomopathogenicity against *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) larvae on field experiments. Fusarium species are ubiquitous and include phytopathogenic as well as endomopathogenic species. *H. armigera* is described as a major pest in agriculture because of its polyphagy, high mobility, high fecundity, facultative diapause, and insecticide resistance. Seven Fusarium species (Hypocreales: Nectriaceae) were identified and tested including: *F. algerien*, *F. brachygibbosum*, *F. fujikuroi*, *F. longifundum*, *F. pseudoanthophilum*, *F. solani*, and *F. tonkinense*. All the collected fungi showed significant effect on H. armigera larvae on field conditions, while some proved to be notably lethal. Our result indicate that Fusarium species could be implemented as biological control agents against *H. armigera*.

KEYWORDS: *Helicoverpa armigera*, entomopathogenic fungi, Fusarium, Biological Control

Effect of selected bio-insecticides on the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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The present study discusses, through laboratory bioassays, the efficacy of three biopesticides with the active ingredient of azadirachtin and pathogenic fungus *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae), against stored product and quarantine pest, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), in three stages of its biological life cycle. For each stage of its cycle that was examined, there were 4 trials with five repeats each for larvae and 2 for pupae and adults. In one of these trials, the sample was sprayed just with water and treated as a control.

The experiment begins after the maize was placed in 40 individual urine sample containers and subjected to 10.000 ppm concentration. Placement of 10 carefully chosen larvae in 20 of these, 6 pupae in 8 of these and alternating between 12 and 13 adults in another 8 of those followed. Mortality effect was assessed every 7 days up until the 28th day.

Based on the results, there was an apparent tolerance of larvae, unlike the rest of the life stages, especially in azadirachtin. However, pupae appeared to be highly susceptible to fungus *B. bassiana* strain PPRI 5339, after being killed during the first week. Adults exhibited the highest and most immediate mortality rate.

Key-Words: Biopesticides, *Beauveria bassiana*, Azadirachtin

Integrated strategy to address the Mediterranean fruit fly based on mass trapping with the attractant biodelear

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Mass trapping is considered an alternative and environmentally friendly method for controlling the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Diptera: Tephritidae) which is particularly effective when the pest populations are relatively low. Under the framework of the Life Biodelear program (LIFE13 ENV GR / 000414) where field tests carried out in citrus orchards in Chios island during the years 2015–2019, a comprehensive mass trapping strategy was developed against the Mediterranean fruit fly. A key element of this strategy is the use of the biodelear attractant, which is extremely environmentally friendly and particularly effective in luring medfly. The integrated mass trapping strategy is based on the (a) detailed mapping of orchards and the identification of the alternative hosts in the surrounding area; (b) systematic recording of the pest population dynamics; (c) systematic recording of arthropod biodiversity and soil resources (d) continuous monitoring of fruit pesticide residues and (e) collection and analysis of financial data related to the pest control. The density of traps as well as their spatial distribution is adjusted according to the density of *C. capitata* populations while the infected fruits are collected and destroyed. The implementation of this strategy reduced medfly infestation rates on oranges and mandarins at levels below 5 and 2% respectively, had a positive effect on the biodiversity of arthropods and resulted to the production of fruits free of undesirable pesticide residues. This strategy can be adapted and applied to other crops such as stone and pome fruits that are also very susceptible to medfly infestations.

Keywords: Attract and kill, mass trapping, McPhail, control

Effect of Greek isolates of entomopathogenic fungi to eggs and nymphs of *Halyomorpha halys* (Hemiptera: Pentatomidae) under laboratory conditions

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Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is becoming a major pest of Greece's agricultural production, including tree fruit. Up to date, the control of this pest has been based on conventional methods, with the application of chemical insecticides being the only option. However, the wide distribution of *H. halys*, in combination with the large area of tree fruit production in Greece call for alternative control methods. In this study, the possibility of using microbial control agents for managing *H. halys* was investigated. Eggs and nymphs (L_2 and L_4) of *H. halys* were treated with 15 entomopathogenic fungal isolates and their virulence was evaluated in the laboratory. After treatment, egg hatching time was recorded for 7 days and ranged from 4.5 to 7.0 days. Nymphal survival was also recorded daily for 7 days following treatment and ranged from 2.1 to 6.6 days for L_2 nymphs and 3.7 to 6.8 days for L_4 nymphs. According to our results, *Beauveria varroa* (23) isolate displayed the highest toxicity to all *H. halys'* stages that were tested and could be considered a promising biocontrol agent of this insect.

This research was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK) under the call "Research-Create-Innovate".

Keywords: entomopathogenic fungi, brown marmorated stink bug, toxicity, biocontrol agents, virulence, selectivity

The effect of the endophytic insect pathogenic fungus *Beuaveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) on the thysanoptera *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) in strawberry cultivation in Varda.

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Beuaveria bassiana Vuillemin (Hypocreales: Cordycipitaceae) is an endophytic insect pathogenic fungus, which can colonize a wide variety of plants with many ways and in different parts of the plant. This study is a field experiment in the region of Varda, in which three different strains of the fungus *B. bassiana* were applied to strawberry plants, to determine the effect of the fungus: a) on the growth of the melon plant, throughout its biological cycle and b) on the population of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), that infect strawberry. The 80 (eighty) strawberry plants were divided into four groups (Group A: Commercial *B. bassiana*, Group B: Achaias WS, Group C: Velifer, Group D: Control) and treated with 10^8mg/mL conidia of each treatment, four days after the transplanting in the field. Thirty days after the treatment, the successful colonization of strawberry plants with *B. bassiana* detected on SDA plates.

Keywords: *Frankliniella occidentalis, Beuaveria bassiana,* Biological control, Endophytism, Field experiment, Strawberry.

The effect of biological insecticides on three predatory arthropods, commercial biological control agents.

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Predatory arthropods are the cornerstone of integrated pest management (IPM) programs, which often combine biological control with chemical methods, such as insecticides of conventional or biological origin. Insecticides of biological origin are natural toxins used to control herbivores and pathogens and are considered environmentally friendly due to their rapid decay and high selectivity.

The aim of this study was to evaluate the toxicity of four bio-insecticides (Requiem®, Eradicoat®, Prev-Am®, FLiPPER®) on two predatory mites (Acari, Phytoseiidae), *Phytoseiulus persimilis* Evans which is a predator of tetranychids, and *Amblyseius swirskii* Athias-Henriot which is a generalist predator of small arthropods, such as whiteflies, thrips and herbivorous mites. Also, their toxicity was evaluated on the predatory insect *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae), a natural enemy of whiteflies, which is widely used in IPM on tomato crops. We estimated the toxicity of the bio-insecticides on adults and their effect on the fertility of the predators, when exposed directly and indirectly through leaf residues.

The bio-insecticide Requiem® was not harmful to the predators *A. swirskii, P. persimilis* and *N. tenuis* (toxicity <25%), nor did it affect their fertility. Eradicoat® had moderate toxicity to mites (<50%), but was harmless to *N. tenuis*. Prev-Am® had high toxicity (> 50%) on mites and moderate on *N. tenuis*, while FLiPPER® was highly toxic to all predators.

Keywords: bio-insecticides, fertility, toxicity, Miridae, Phytoseiidae

Acknowledgements
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Effects of plant volatiles on the zoophytophagous predator *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae)

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Plants have evolved sophisticated mechanisms to defend themselves against herbivory. These include the expression of direct defenses such as toxins and anti-digestive proteins targeting the herbivore, as well as indirect defenses to attract natural enemies via the emission of herbivore-induced plant volatiles. Headspace volatile trapping from infested plants and subsequent volatile analysis reveals specific compounds with potential in enhancing indirect defense if applied on the plants. In this study, we assessed a series of volatile compounds that were previously identified in volatile blends emitted from plants which were subjected to different stresses. Specifically, we assessed the effects of plant volatiles on the behavior of *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), a zoophytophagous mirid predator which is routinely used in biological control. Our olfactometer experiments revealed a considerable variation among the tested compounds in their effects on the predator ranging from attraction to repellence. The results of our study could be useful in the identification of compounds that could be used to enhance indirect plant defense.

Key-words: behavior, mirids, natural enemies, plant defense, volatiles

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Oberea linearis L. (Coleoptera: Cerambycidae) in the walnut area of Kalavrita

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Around Kalavtyta area, where significant damage is observed on walnut trees, insects mature and exit wings between 10 to the end of May. After a period of adults being fed on new leaves, they mate and lay eggs on wings edge or around the walnut stem. Adults prefer to lay eggs on weak wings with walnuts on. In case of healthy twings, the insect causes perimetrical engraving to weaken the wing and allow larva to survive. The newly hatched lava enters the wing and digs a gallery. If hatchery takes place on stem, gallery is created on the walnut also. Infected wings dry around July, while nuts gradually shrink and dry as well. Damage due to *Oberea linearis* L. (Coleoptera: Cerambycidae), recorded in Kalavryta, regards to significant decrease of walnut production as well as gradual weakening and final drying of trees.

Effective solutions can be achieved through cultivating and chemical means. Proper fertilizing, pruning and adequate irrigation may be combined with removing infected parts of wings intended to be burnt, around July. Same procedure should be followed while pruning the trees. Chemical interventions should include three pesticide sprayings every fortnight, at the period of adult flying, which can significantly limit the problem.

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Keywords: walnut trees, walnut insects, control

Presence and role of aquatic mosquito predators in anthropogenic water bodies in the urban environment

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Mosquitoes are among the most significant problems for citizens in urban areas due to the nuisance they cause and the health risk they pose as carriers of pathogens for humans and animals. A large number of mosquitoes breed in anthropogenic water concentrations in the urban environment, such as ornamental ponds, abandoned swimming pools, and inactive fountains, where the water is naturally or artificially collected and not adequately maintained. These breeding sites are often difficult to detect and inaccessible to implement appropriate mosquito control measures. In those cases, the presence of natural predators of mosquito larvae can be an important biological agent for reducing or eliminating mosquito larvae developing in those water bodies.

In the present study, the fauna of aquatic insects was recorded and evaluated in abandoned artificial water concentrations in the urban environment (South Suburbs of Attica), emphasizing species that are potential predators of mosquito larvae, sampled at regular intervals for two years.

The predatory aquatic insects recorded belong to the orders Ephemeroptera, Odonata, and Hemiptera. The aquatic Hemiptera *Notonecta viridis* (Delcourt) (Hemiptera: Notonectidae) and *Anisops sardeus* (Herrich-Schaeffer) (Hemiptera: Notonectidae) were recorded in significant numbers, with *A. sardeus* being the first record of the species in Greece.

Although there was a high seasonal variation of the various species throughout the year, it is worth noting that the ecosystem was in balance without human intervention as the larvae of the Diptera families Chironomidae, Stratiomyidae, and Syrphidae were probably the primary food source of the insect predators. Mosquito larvae (Diptera, Culicidae) were found in minimal numbers and only at early larval stages, as it was difficult or impossible to complete their development. The remarkable predatory ability of *A. sardeus* against mosquito larvae was also confirmed in the laboratory by preliminary experiments.

Λέξεις-κλειδιά: Aquatic mosquito predators, *Anisops sardeus, Notonecta viridis*.

Treatment of a *Melolontha* sp. infested greenhouse cultivation of strawberry with a combination of biological agents and soil solarization

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In the present work, we monitored the damage of a four acre productive greenhouse with strawberry, Fortuna variety, for which the presence of *Melolontha* sp. larvae was confirmed in November 2019. Monitoring was initiated on the 19th/11 and the number of infected plants was recorded (root system cut at the crown and infestation confirmed by the detection of larvae in the rhizosphere). For this particular crop-enemy combination, there are no approved insecticides for soil application, in Greece. Therefore, in an effort to "rescue" the annual production, a series of approved formulations of biological agents was applied and evaluated for their ability to be used effectively against the given problem. The products Nemabac[3], Nemafelt[3], Biorend R[3], Botaniguard 10,7SC[4] and *Metarhizium anisopliae* var *anisopliae* strain F52[5] were used according to the manufacturers' instructions: 500mi/acre, 500mi/acre, 400ml/acre, 150ml/ acre and 125ml/acre, respectively. The products were applied in various combinations, on the 29th/11, when a rapid and threatening increase in the infected plant numbers was observed. The recordings were carried out at regular intervals until February 2020. Then the heavy rains forbid them, and eventually were postponed, due to the COVID-19 pandemic sanitary measures imposition, in March.
A large reduction in the number of infected plants was observed for all applications, and this was maintained until the last recording. The timing of this reduction is in line with the relevant scientific data, since biological factors do not have a knock-down effect on the insect populations but need some time until establishment and effectiveness of the insect pathogens. The decrease in white grub populations was most likely due to both the reduction of ambient temperatures during this period as well as the effectiveness of the products applied, while any negative correlation can be attributed to irregular watering, application of foliar fungicides and lack of sufficient organic matter.
Unfortunately, the lack of additional recordings in the following period did not serve to draw absolute conclusions. However, it is interesting that the final production was 3 tons/acre, *i.e.* absolutely satisfactory based on the cultivation history. Also interesting is the fact that coming May, no adult flights were observed. Subsequently, in the summer of 2020, soil solarization was applied and since then, no recurrence of the infestation has been observed. The results show a tendency to regulate the problem, which in some areas tends to be intractable due to the species's biology, the long period of deep soil inhabitation and the low control effectiveness of adult spraying, nevertheless, further investigation is needed with respect to the applied doses, frequency of application and form of the aforementioned formulations.

Key words: White grub, greenhouse strawberry, biological agents, entomopathogenic nematodes

[3]Bio-insecta GR, [4] KNE Certis GR, [5] Met52

Pilot study on the effect of standardized earthworm humus extract on insect populations in a productive olive grove

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In the present study, the standardized, aqueous extract of earthworm humus, Bioremiq™ by Green Arsenal, which in its country of origin, Hungary, is licensed for use in organic crops as a growth enhancer and natural fertilizer. During April and July 2021, the effect of the product on the insect populations, present in an irrigated, conventional, productive olive grove, in Vartholomio, Prefecture of Ilia, was recorded. The product is reported to aid soil fertility restoration, plant growth stimulation, reduction of the negative effects of adverse weather conditions and increase of tolerance to various plant pathogens and pests. The analysis of its components shows a high content of macro- and trace elements, natural enzymes and hormones that contribute to healthy plant growth, while they seem to improve soil structure, improving the ability of plants to absorb nutrients, resulting in a quality and quantity produce increase. It is recommended for frequent use at all kinds of crops, mainly olive and various vegetables, and is applied both by root irrigation and foliarly.
For the requirements of the study, we selected an 11-year-old olive grove, where the variety “Koroneiki” is cultivated, at of 7 x 7m distance. The experimental plot consisted of three rows of ten trees each: row E (application of Bioremiq™), row M (no intervention) and row C (according to the producer's program). In row E, five Bioremiq® applications were performed, every 15 days, the first with root irrigation, the following foliarly, with 3L and 1L / acre respectively, according to the manufacturer's instructions. To record the insect populations, 10 days after each application, 4 branches (~ 20cm) were sampled from each tree, one from each side, a total of 40 branches from each row, 120 from the entire plot. Each branch was placed individually in a plastic bag and stored in the refrigerator until completion of the study. Significant presence of the harmful insects *Prays oleae*, *Palpita unionalis*, *Euphyllura olivina* and *Liothrips oleae* was recorded, while we also detected a scattered presence of *Rhynchites cribripennis*, *Dasyneura oleae*, *Drosophila suzuki*, *Chrysopa* sp. and *Mantis religiosa* and of the mites *Tetranychus urticae* and *Eriophyes oleae*. The results were studied on Excel for trend visualization and then put into SPSS for statistical comparison. There was an obvious trend of population decline, probably also due to climatic factors; nevertheless, a clear statistically significant difference was observed between the Bioremiq™ and conventionally treated rows compared to the control, for all insect species. A statistically significant difference (paired value t-test) * between Bioremiq® and the control was clear for *L. oleae* (p value = 0.033) and *P. unionalis* (p value = 0.317), at 95% significance level.

Key words: compost, growth enhancer, *Prays oleae Palpita unionalis*, *Euphyllura olivina*, *Liothrips oleae*

Impact of mixed farming system of citrus and aromatic plants on predatory mites

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The project PLANT-B tests the combined culture of citrus and aromatic/medicinal plants (AMPs) for conservation and enhancement of beneficial arthropods such as natural enemies of crop pests. Here we present the impact of this mixed farming system on predatory mites of orange orchards in Argolis, Greece. Seven orange orchards were tested, five with AMPs (rosemary x 2 orchards, savory, oregano, sage) established on one of the field margins in each orchard, one weed-covered and one with bare soil. Samples, twigs with leaves, were taken from both orange trees and AMPs during April - July 2021. Preliminary samplings from all experimental fields, were also conducted before the establishment of AMPs (baseline year 2020). Mites were extracted from samples using the Berlese-Tullgren method and permanent slide mounted. A total of 15 predatory mite species belonging to 7 Families and 10 species of phytophagous mites belonging to 5 Families were collected. The dominant species in all fields were *Euseius stipulatus* (Athias-Henriot) and *Typhlodromus* (*Anthoseius*) *kerkirae* Swirski & Ragusa (Phytoseiidae). Predatory mites were more abundant on orange trees in orchards with AMPs (savory and rosemary) and weeds on margins compared to bare soil. Among AMPs, the highest number of predaceous mites were found on rosemary plants.

Keywords: Acari, predators, aromatic/medicinal plants, citrus, weeds, PLANT-B

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The Mediterranean fruit fly, *Ceratitis capitata,* as a model for the comparative study of induced sterility methodologies in populations of insect pests

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The Mediterranean fly (medfly), *Ceratitis capitata* (Diptera: Tephritidae), is a dipteran, holometabolous agricultural pest, with major economic importance and worldwide distribution. Sterile insect technique (SIT) is an environmentally friendly method of insect pest control, where sterile males are mass produced and released into the distribution area to mate with females of the wild population. In medfly, the development of genetic sexing strains (GSS) contributed significantly to the effectiveness of this method through the facilitation of male-only releases. The latest generation of the VIENNA 7 or VIENNA 8 GSS is currently used in all mass rearing facilities worldwide.

The incompatible insect technique (IIT) induces infertility through cytoplasmic incompatibility (CI), which is caused by the *Wolbachia* α-proteobacterium. Incompatibility occurs when infected males mate with uninfected females (unidirectional) or mate with females harboring a different incompatible strain (bidirectional), thus leading to infertile crosses. Medfly is not a typical host for *Wolbachia* and IIT has been proposed as a control method. Over the past two decades, *Wolbachia* strains have been transferred to medfly (*wCer2* and *wCer4*) and 100% incompatibility occurs at crosses between *Wolbachia*-infected males and uninfected females, as well as between medfly strains infected with different *Wolbachia* strains (*wCer2*, *wCer4*).

Medfly is a model species for the implementation of SIT and VIENNA GSS developed for SIT purposes can be used for IIT applications as well. Previous studies have shown that VIENNA 8 GSS with *Wolbachia* a) are genetically stable when filtered in each generation and any recombinants are removed, b) show the expected response to temperature stress of 34 and 35 ^{o}C (emergence of males only), and c) show 100% uni- and bidirectional incompatibility. In the present study it was investigated a) whether these strains could maintain their genetic stability for at least 3 generations if not filtered, a prerequisite for the colony upscaling to produce males for release purposes and b) whether a less stringent temperature stress (lower than 34 ^{o}C) could be used for these strains, which would increase both the production of males and their biological quality.

Keywords: cytoplasmic incompatibility, incompatible insect technique, medfly, sterile insect technique, genetic sexing strains

Efficacy of marjoram (*Origanum majorana*) and pennyroyal (*Mentha pulegium*) essential oils on the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) after their encapsulation in biodegradable and biocompatible carriers

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Our study was focused in the enhancement and extension of marjoram and pennyroyal essential oils insecticidal activity by their encapsulation in natural, biodegradable and biocompatible carriers. The effect of essential oils of different chemotypes before and after their encapsulation in yeast cells and β-cyclodextrin (β-CD), on the survival of the greenhouse pest *Tetranychus urticae* was assessed. *Tetranychus urticae* larvae were sprayed with 1 ‰ (v/v) essential oil emulsion and then placed in Petri dishes on sprayed bean leaf. Mortality was counted daily, for 7 days after spraying. No mortality was recorded when sprayed with deionized water as well as with water and emulsifier, while after treatment with acaricide mortality was 100%. The main components of marjoram oil was terpinen-4-ol and γ-terpinene and the mortality reached 66% in the nymphs, while after spraying with oil encapsulated in yeast cells the mortality increased to 82%. The pennyroyal oil with main ingredients pulegone and isomenthone caused 50% mortality on the nymphs but after its encapsulation in β-CD mortality rate was doubled (100%). In last case, the mortality rate on the 3rd, 4th and 5th day post treatment was 2- or even 3-fold higher than in the case of the oil. Therefore, the encapsulation of essential oils in yeast cells and β-CD significantly improved their efficacy against *T. urticae*. Future studies should be conducted to further evaluate these encapsulation systems in additional *T. urticae* biological parameters under laboratory and field conditions.

The work was implemented in the framework of the RESEARCH-CREATE-INNOVATE Action co-financed by the European Regional Development Fund (ERDF) of the European Union and national resources through the OP. Competitiveness, Entrepreneurship & Innovation (EPANEK) (project code: T2EDK-03105).

Keywords: essential oils, β-cyclodextrin (β-CD), mortality, *Tetranychus urticae*, yeast cells

Genetic mapping and phenotypic analysis of the medfly temperature-sensitive lethal gene exploited in genetic sexing strategies

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The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Diptera: Tephritidae), is an agricultural pest of great economic importance and worldwide distribution. The sterile insect technique (SIT) is an environment-friendly control method of insects of economic and public health importance that is based on mass production and release of sterile insects (males) to mate with wild females thus leading to population suppression. SIT is optimized when male-only releases are possible, a methodology that has been applied in medfly with the development of genetic sexing strains (GSS) and their incorporation in operational SIT programs.

The *white pupae* (*wp*) mutation has been exploited as a morphological marker for the construction of GSSs for SIT purposes, enabling sex sorting based on pupal color. The currently available GSSs (VIENNA 7 and 8 GSS) have incorporated a second mutation named *temperature-sensitive lethal* (*tsl*) allowing elimination of females during embryogenesis.

Accumulation of decades of knowledge from classical genetic and molecular biology has allowed mapping of both *wp* and *tsl* mutations on the right arm of the 5th chromosome (5R) of the Medfly. Recent advances in in genetic analysis, genomics, molecular biology, and bioinformatics enabled identification and mapping of the *wp* locus. The *tsl* locus has not yet been identified, partly due to the difficulty of analyzing lethal mutations, however its possible genomic position has been narrowed down to a small 5R genomic area upstream of the *wp* locus, with few medfly genes identified as *tsl*-candidates in this area. Identification of the *wp* and *tsl* loci, along with availability of the recent advances in good quality genome-wide assembles, bioinformatics, and gene editing technologies allows reproduction of the desirable phenotypes and similar genetic sexing approaches in other insect species that have been identified as candidates of control methods relying on the efficient production and release of males.

The purpose of the present study is to contribute to the genetic mapping and identification of the Medfly *tsl* locus, along with the fine characterization of its role. For this purpose, we created, through genetic recombination, new combinations of the *tsl* marker utilizing the *wp* marker, along with additional morphological markers of the 5th chromosome. Here we present the genetic crosses that led to the development of the new 'tsl' strains plus the results of the thermal stress response experiments that verify that these strains exhibit the typical 'tsl' behavior and, therefore, represent actual recombinant between the *tsl* locus and other available morphological loci.

Keywords: genetic sexing, temperature-sensitive mutations.

Searching for recessive temperature-sensitive lethal mutations in recently colonized *Ceratitis capitata* populations

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Insect control methods for agricultural pests and human disease vectors, that are based on induced sterility, such as the sterile insect technique (SIT) are receiving renewed interest. This is justified since they are considered highly specific and environment friendly. Moreover, advances in mass rearing, quality control protocols, and availability of improved insect strains have revolutionized rearing for release purposes and have made operational sterile-male release projects viable, sustainable, and a reliable alternative with reduced environmental footprint for pest control.

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Diptera: Tephritidae) is the most successful example of pest control using SIT, in the frame of the area-wide integrated pest management (AW-IPM). The development of genetic sexing strains (GSS) that enable labor- and cost-effective male-only releases has been the keystone for elevating medfly as a model insect for SIT. In this respect, the isolation of the *white pupae* (*wp*) mutation, followed by the *temperature-sensitive lethal* (*tsl*) mutation, and their subsequent integration in a genetic sexing strategy, led to the development of the most recent version of medfly GSSs (the VIENNA 7 and 8 GSSs) that are currently used in all mass-rearing facilities globally.

Wp is a spontaneous mutation while the *tsl* mutation has been isolated through extensive screening following chemical mutagenesis. However, it is also possible that *tsl* mutation pre-existed in natural or laboratory populations. *Tsl* mutation is believed that it can act as a 'universal' mutation that can be exploited in genetic sexing strategies in other Diptera species, following similar methodologies. However, given the uncertainties regarding the nature and function of this locus, it may not have the expected phenotype in different species (or at least in some of them that are target for SIT). Therefore, it is important to identify/isolate additional *tsl*-like mutations.

Here we studied the response of recently colonized Medfly populations, to the thermal stress already applied to reveal *tsl*-sensitive and *tsl*-resistant genotypes of the *tsl* gene of Medfly (the only one available and partially characterized up to now). In the greater picture, we want to identify populations (or populations' individuals) with a differential tolerance to the specific thermal stress, and, subsequently, to identify genotypes that we can target for the isolation of new *tsl*-like mutations.

Keywords: Medfly, genetic sexing strains, temperature-sensitive lethal mutations, thermal stress.

Spatial distribution of *Xylotrechus* spp. infestations in mulberry trees

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The invasive beetles, *Xylotrechus* spp. are serious pests of mulberry trees of urban green areas. Aesthetic value of trees and public safety in parks and streets can be problematic due to the damage caused by the beetle infestations. The digitization of the mulberry trees, their symptoms and other elements of the surrounding environment may constitute important parameters in the IPM of these beetles. The main objective of this study was to record and map the symptoms caused by *Xylotrechus* spp. at mulberry trees using smart mobile devices. The digitization of the trees and the symptoms using smart devices can provide rapid representation of the spatial distribution of the infested trees, and also helpful information for the pest management decisions. The study was performed from September 2021 to April 2022 in Pedion Areos park of Athens and in streets of Municipality of Athens. More than 200 mulberry trees were inspected. Most of the trees were located along streets. The main data recorded and digitized were the height and the perimeter of the trunk, the number and orientation of the exit holes and the length and the orientation of the infested branches, the phenological stages of the mulberry trees etc. The results showed that the digitization process can be rapid and ease, the representation of the spatial information of the symptoms can be useful in pest management programs and the dissemination of information can be performed, remotely, using the Internet. The spatial distribution of the symptoms was illustrated in properly adjusted maps.

Keywords: *Xylotrechus* spp., spatial distribution, smart mobile devices.

Monitoring the population of *Grapholita funebrana* in Kavala (Greece) prefecture and trial applications of its control.

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In recent years an important infestation has been observed from the species *Grapholita funebrana* (Treitschke, 1835) (Lepidoptera: Tortricidae) in plum crops, in the region of Kavala (Greece). For the insect's study, a field of 2.2 stremmas was used, consisting of plum trees (Friar variety), with planting distances of 5x3 meters. Funnel pheromone traps were used to monitor the insect's population. *G. funebrana* catches were recorded twice a week and pheromones in insect traps were changed every twenty-five days. An experimental treatment to control the insect was performed with classic and alternative means. For this, a conventional insecticidal formulation with the active substance spirotetramat was used. The biological formulation of *Bacillus thuringiensis* subspecies kurstaki, as well as the zeolite and the diatomaceous earth formulation were used as alternative agents. The "Randomized Complete Design Block" was used with the application of the above formulations including the control, in 4 repetitions. The statistical analysis was performed using the SPSS20 package, for a significance level of 0.05.

Both observations in laboratory (infected fruits) and field showed that *G. funebrana* had two generations per year. For the 1st generation the higher population was recorded in the first days of April to end of the month and for the 2d generation at the end of June.

The results of the experimental application showed that except the conventional insecticide (i.e. spirotetramat), zeolite was superior to the other formulations and it can be used with very satisfactory results, against *G. funebrana*.

Key- words: *Grapholita funebrana,* pheromone traps, zeolite, control.

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Session: Physiosology - Behaviou - Phenology - Genetics - Biotechnology

Behavior of the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) response to volatile compounds from food lure and evaluation of trap effectiveness

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Bactrocera oleae (Rossi) (Diptera: Tephritidae) is one of the major pests of olives, causing both quantitative and qualitative damage. Monitoring of olive fly populations is performed by traps baited with food attractants that are also used in bait sprays against olive fly.

The aim of this study was (1) to collect, detect, and identify volatile organic compounds (VOCs) in existing food attractants, (2) to test the electrophysiological responses of adult females in the volatile extract of the attractant and (3) to evaluate VOCs for their efficacy as fly attractants.

The collection of volatiles released from a commercially available product (ENTOMELA SL75) was performed by dynamic headspace. 20% v/v solutions were prepared and placed at 25 ° C and 35 ° C. The volatiles were collected after 24 hours, 7, 15 and 30 days of incubation and the main compounds identified belong to the pyrazines class. We also identified several terpenes, alcohols, saturated aldehydes, ketones, esters and hydrocarbons.

The electrophysiological response was studied in two age groups of female olive flies: [0-7] and [15-20] days old. Females responded to various classes of compounds including pyrazines, terpenes, ketones, hydrocarbons, and unidentified compounds.

The evaluation of VOCs for their efficacy as fly attractants was done by field trapping experiments with a combination of yellow adhesive traps and an attractant in two different periods with daily monitoring. Results both in the lab and the field were quite promising. Specific compounds were equally attractive to commonly used trap attractants. Investigating the effect of VOCs on the behavior of olive fly can be used to improve or develop new tools for a management plan for monitoring and mass-trapping.

Key words: *Bactrocera oleae*, GC-EAD, VOCs

This work is carried out in the framework of the Flagship Action "Olive Roads" funded by national resources through the Public Investment Program of the General Secretariat for Research and Technology.

Automated electronic recording of the daily activity of adult Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Locomotor activity levels of some organisms are considered biomarkers of ageing, and the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a commonly used model-species in related studies. We tested the daily locomotory activity of adult medflies throughout lifetime, by adjusting the automated locomotor activity electronic device LAM25system (Locomotor Activity Monitor) – Trikinetics. By adding a custom-made apparatus to LAM25system the daily fecundity rates of females were recorded. Male and female medfies were individually kept in glass tubes which were “pierced” by arrays of infrared laser beams located in three different points. Each time a fly crossed the laser beams, its locomotor activity was recorded. Both males and females were kept in the system tubes from adult emergence until death. Flies had access to a gel-based agar diet containing sugar, yeast hydrolysate (protein) and water, which apart from nutrients provided adult flies with water. The adult diet was adjusted to the one end of the tube, while a plastic stopper covered with organdie cloth serving as an oviposition substrate was located at the other end of the tube.

The results showed that the locomotor activity of females was higher than that of males during all age-classes. The highest locomotor activity rates for both sexes were recorded in the first 20 days of adult life and decreased in older ages. The locomotor activity of males was high in the morning and afternoon hours, while females were active throughout the photophase. No activity was recorded in either males or females during nighttime. Males outlived females. Female fecundity was high in young ages. We discuss the adoption of LAM25system in studies addressing the healthy ageing of organisms using medfly as a model organism.

Key words: healthy functional senescence, daily activity, demography, insect movement, LAM25system

Field evaluation of the susceptibility to *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) and its parasitoids efficiency of 27 olive cultivars

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The susceptibility of 27 olive cultivars to *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), as well as its ectoparasitoids efficiency were evaluated during two consecutive years, under field conditions of western Crete (Southern Greece), where environmental conditions are largely favorable for the reproduction and development of its population. Olives were collected from the National Olive Germplasm Bank of Greece of the Institute of Olive Tree, Subtropical Crops & Viticulture, Hellenic Agricultural Organization (ELGO-DIMITRA) in Chania. The samples were collected every fortnight from June to October, during 2019 and 2020. The percentage of infestation was assessed by dissection of all sampled olives under a binocular stereomicroscope to count the sterile oviposition stings, alive, dead and parasitized preimaginal stages and emergence holes. The fruit weight, length and width, were also assessed in 50 fruits per cultivar per date. Adults of *B. oleae* were monitored at weekly interval, during the entire sampling period, by using a network of glass McPhail traps baited with ammonium sulfate 4% and yellow chromotropic sticky traps. The results of this study confirm that female olive fruit flies exhibited a strong ovipositional preference when presented with a choice of different olive cultivars in the field. Among the olive cultivar tested, 'Manzanilla Isr'., 'Manzanilla', 'Konservolia' and 'Picual' were the most heavily infested during the two consecutive years. The next most heavily infested were 'San Francesco', 'Frantoio Rodou', 'Frantoio' 'San Agostino' and 'Kalokairida' while 'Kalamon', 'Kerkyras', 'Koroneiki', 'Tragolia', 'Pikrolia', 'Mavrelia', 'Rachati', 'Myrtolia', 'Koutsourelia' and 'Thiaki' had the lowest susceptibility to olive fruit fly attacks. Oviposition preference was weakly correlated with olive weight but unrelated to length, diameter and volume of fruit. Also, a weak positive correlation was observed between the average number of adults and average total infestation rate of tested cultivars. Ectoparasitism rate of *B. oleae* was significantly different between cultivars, however a weak positive correlation between the parasitism rate and total infestation rate was found. Even though the olive fruit fly infested all tested cultivars, those showing indications of relative resistance could be tested for their suitability in new plantations in areas with high *B. oleae* populations as well as to be employed as prebreeding material. The study was funded by PRIMA Freeclimb. The project is funded by the General Secretariat for Research and Innovation of the Ministry of Development and Investments under the PRIMA Programme.

Key-words: genotype, olive fruit fly, parasitism, susceptibility.

Biologically produced pheromones for *Helicoverpa armigera* and *Ostrinianubilalis:* Laboratory and field tests

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The use of insect sex pheromones is an alternative technology for pest control in agriculture and forestry, which, in contrast to insecticides, does not have adverse effects on human health or the environment and is efficient also against insecticide-resistant insect populations. The development of a novel technology for the biological production of pheromones from yeast cells in bioreactors, reduces significantly production costs..

In order to validate and compare the performance of the produced bio-pheromones with those synthesised chemically, we studied the electrophysiological (EAG) and behavioural response of male adults of two lepidopteran species which are major agricultural pests: the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). In addition, by coupling electroantennography with gas chromatography (GC-EAD), we investigated whether any impurities left in biologically synthesised pheromones after purification can be perceived by the insect's antennae and thus potentially interfere with its normal behaviour. Finally, the efficacy of bio-pheromones was further evaluated by field trials.

EAG experiments with *O. nubilalis* showed that male antennae were responsive to biologically-derived pheromone blend, whilst behavioural bioassays in a wind tunnel revealed attraction of males at a level similar to that of the chemically synthesised pheromone.

Similarly, for *H. armigera*, EAG results showed no significant differences in antennal response between biologically produced pheromones and those chemically synthesised. Also, wind tunnel bioassays using the bio-pheromones blend demonstrated strong attraction and precopulatory and mating behaviour responses comparable to those of the chemically-derived pheromones. In monitoring and sex communication disruption field tests the biologically produced pheromone was equally effective as the commercially available chemically synthesized one.

Pheromone production from yeasts is an innovative, ground-breaking technology expected to strongly influence the plant protection products industry.

This project is implemented under the framework of Horizon 2020/NMBP-BIO-2017 OLEFINE (OLEaginous yeast platforms for FINE chemicals, http://olefine.eu/), which is funded from the European Union's Horizon 2020 research and innovation programme.

Keywords: *Helicoverpa armigera*, *Ostrinia nubilalis*, GC-EAD, bio-pheromones, sex pheromone

Mating between wild and lab-type olive fruit flies, *Bactrocera oleae* (Rossi) (Diptera:Tephritidae)

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Laboratory rearing of insects is facilitating all-year round experiments and is necessary for the implementation of Sterile Insect Technique or the release of genetically modified insects to control damaging insect populations. Laboratory adaptation and mass rearing methods could significantly divert lab-type insects from the wild population. Biotechnological methods for the control of the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera:Tephritidae), like the release of self-limiting engineered strains are being investigated. Released lab-type males will have to compete with wild-type males and successfully mate with wild-type females.

In cages (10x10x10 cm) we placed 10 virgin wild-type female olive fruit flies, 12 days old and reared on olive fruit, with 10 virgin wild-type males of the same age and 10 virgin lab-type males, 8 days old, because they reach sexual maturity earlier. We observed the proportion of matings for each type of males, the time of initiation of each mating and its duration. Following, we measured the size of spermatheca of mated female flies. Wild and lab-type males mated at similar proportions with wild-type females (24%) with no statistical difference between the two types of males. Wild-type males started to mate earlier and had longer mean duration of mating, although no statistical difference was detected. But, the size of spermatheca of wild-type females that mated with wild-type males was bigger that the size of spermatheca of females that mated with lab-type males and the difference was statistically significant ($t=2.572$, $P=0.0146$, n=24).

The results are supportive for the efforts of biotechnological control methods of the olive fruit fly. But, more experiments are needed in semi-field of field trials. Also, focus must be given to other characteristics of lab-type flies, like longevity, dispersal and survival capacity in the wild, which are equally important to the success of these control methods.

Keywords: *Bactrocera oleae*, Tephritidae, SIT, spermatheca

New genomic insight into functional components of *Enterobacter* symbionts of Tephritidae and Glossinidae fly species

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Insects represent the most successful taxon of eukaryotic life, being able to colonize almost all environments. Microbial symbionts associated with insects, impact important physiologies, and influence nutritional and immune system status, and ultimately fitness. A variety of bacterial phyla are commonly present in insect guts, including Proteobacteria, Bacteroidetes, Firmicutes, Spirochaetes, Verrucomicrobia, Actinobacteria, and others. Among them, the genus *Enterobacter* has been recognized as a dominant inhabitant of the gastrointestinal tract for several important insect species, indicating an essential functional role for this taxon. *Enterobacter* strains contribute to the functional diversity among insects with respect to insect development, host exploitation, reproduction, and interactions with other organisms, thus constituting one of the factors that have contributed to the evolutionary success of insects. In this work, we analyzed the genomic background of *Enterobacter* symbionts by performing whole genome sequencing (WGS) of strains isolated from wild Tephritidae fruit flies and one Glossinidae fly. Genome sequencing revealed interesting functional components, including the specialized gram-negative type VI secretion system and effector proteins implicated in pathogenicity, as well as genes inferring antimicrobial resistance (AMR).

Factors affecting trapping of adults of the Mediterranean fly *Ceratitis capitata* (Wiedemann) (Diptera: Tepritidae)

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A set of biotic and abiotic factors may affect captures of adult insects in traps. Such factors include the type of the trap and the attractant, the sex and the age of the insect, but also the prevailing climatic conditions and the host plant where the traps are placed. We studied: 1) the effect of adult acclimation in different temperatures, 2) the effect of prevailing climatic conditions and 3) the host type (host and non-host plants) on captures of adults of the Mediterranean fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tepritidae) in traps. Five days-old adults, having been marked with different colors of a nontoxic fluorescent powder depending on the treatment at the pupal stage, were acclimated to 3 constant temperatures (15, 25 and 30°C) as well as maintained in outdoor conditions for 5 days. Ten individuals of each sex and treatment (acclimation regime) were released, at a short distance (5 m) from a trapping station consisting of a pair of Jackson trap baited with trimedlure and McPhail type trap baited with BioLure that had been established in host and non-host trees. A total of four releases took place, two in early spring, at relative low temperatures and two during the summer months, at high temperatures. All traps were inspected at regular intervals. Captured adults were taken to the laboratory and examined under a stereoscope using ultraviolet light to determine the marking color. Acclimation at different temperature regimes as well as the prevailing ambient temperatures and the type of host plant were significant predictors of recaptures. We discuss the importance of these findings for detecting low *C. capitata* populations.

Keywords: acclimation, *Ceratitis capitata*, recapture, release, temperature, fruit flies, trapping.

This work was carried out in the framework of the FF-IPM Project (Horizon 2020. GA818184).

Novel anosmia-inducing compounds for environmentally friendly mosquito vector control: structural determinants of ORco ligands antagonizing co-receptor function

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Insect olfactory receptors are heteromeric cation channels composed of an obligatory receptor subunit, ORco and one of many variable subunits, ORx, in as yet undefined molar ratios. When expressed alone *in vitro* in various cell culture systems, ORco forms homomeric channels that may be activated by ORco-specific ligands acting as channel agonists. In previous studies, we have used an insect cell-based system as a platform for expressing mosquito odorant receptors and identifying small molecules of natural origin that bind to ORco. Some of these small ligands act specifically as ORco channel antagonists, orthosteric or allosteric relative to the agonist binding site, causing severe inhibition of olfactory function in mosquitoes.

Here, we are reporting on the compilation of common structural features of the identified orthosteric ORco antagonists, resulting in the prediction of a ligand-based pharmacophore. Such modalities are deemed necessary for inhibition of ORco's biological responses. *In silico* screening of an available collection of volatile compounds of natural origin resulted in the identification of several ORco orthosteric antagonist hits. Parallel cell-based screening of the same collection was employed to identify ORco antagonists. The screening resulted in the identification of several ligands capable of inhibiting ORco channel function by at least 40% and inducing anosmic behaviors to *Aedes albopictus* mosquitoes (Skuse) (Diptera: Culicidae) *in vivo*. To test the validity of the pharmacophore model in terms of binding site specificity, binding competition assays were undertaken, able to distinguish orthosteric and allosteric ligands. Direct comparisons of the pharmacophore predictions with the results of the cell-based screening assays revealed that the pharmacophore predicted correctly the identified orthosteric antagonists and none of the allosteric ones, as expected. These results confirm the pharmacophore's value for *in silico* prediction of ORco orthosteric antagonists. Upon combination with the ensuing cell-based screening, the pharmacophore screening provides a valuable tool leading to additional opportunities for fast identification of multiple, ecologically friendly vector control agents.

Keywords: functional assays, insect olfactory receptor co-receptor, receptor orthosteric and allosteric antagonism, *in silico* screening, pharmacophore model.

A low-cost, wireless, multi-sensor apparatus for the monitoring of beehives in the context of the IoT

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ABSTRACT

We present a platform that monitors Carbon dioxide (CO_2), temperature and humidity within a hive and wirelessly transmits all measurements to a cloud server. CO_2 is related to the respiratory emission of CO_2 from bees and its levels inside the hive can change according to the activity of the bees. The other environmental parameters are well known to be related to beehives' health and productivity. While these parameters have been reported before in the literature, in this work, we are interested in introducing a practical, cost-effective solution that would be widely accepted by end-users. Therefore, our aim is to reduce the size and the cost and provide a low-power solution that would allow its deployment in the field for a long time. To this end, we present our own customized electronic board and signal processing software that does not rely on ready platforms that are generic but more power-hungry and costly than the level we target. We discuss about processing the CO_2 timeseries in the context of the IoT, using machine learning techniques to predict future alerting levels of CO2 and critical temperatures that lead to an automatic procedure for real-time, alert issuing of events that are deemed hazardous for the beehive's health.

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Keywords: Bees, carbon dioxide, IoT

Cellular response of *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) during rearing on agricultural residues enriched with a mixture of distillation residues of medicinal-aromatic plants

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The continuously increasing nutritional demands due to the rapid world population growth impose the development of alternative animal protein sources. Insects have a great nutritional value, rich in high-quality protein and nutrients, while their mass farming is associated with a lower ecological footprint compared to conventional animal production industries. In the context of circular economy, various agricultural remnants (wheat bran, rice bran, corn cob) supplied with or without a mixture of distillation residues of medicinal-aromatic plants (DRMAPs) (oregano, lavender, rosemary and olive) at a rate of 10 and 20%, were used as rearing substrate for *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae). The aim of the present study was to investigate the expression of heat shock proteins (HSP60, HSP70 and HSP90) and mitogen-activated protein kinases (p38 and p44 / 42 MAPK) as alternative substrates may affect farmed larvae development through induction of cellular response to potential nutritional deficiencies. Protein expression levels varied depending on the substrate and DRMAPs inclusion percentage. In specific, heat shock proteins expression levels were affected by both the substrate type and the DRMAPs mixture. Regarding mitogen-activated protein kinases, a significant effect of the substrate on the phosphorylation levels of MAPKs was observed, while DRMAPs significantly affected only the p-p38 levels. Furthermore, a significant interaction between the effects of breeding substrates and DRMAPs on both MAPKs and HSP70 and HSP90 protein levels was observed. In conclusion, corn cob, supplied with or without the DRMAPs mixture could potentially be utilized as suitable rearing substrate for *T. molitor* since no apparent induction of cellular response was observed due to nutritional deficiencies. The present research is in full progress to thoroughly assess substrates suitability and DRMAPs contribution to the development of rearing *T. molitor*.

Keywords: Insect Breeding Substrates, Circular Economy, Alternative Animal Protein, HSPs, MAPKs

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Session:
Stored
Products Pests

Efficacy of contact insecticides for the control of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)

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In the present study, laboratory bioassays were conducted to evaluate the efficacy of three contact insecticides (alpha cypermethrin, spinosad and pirimiphos methyl), against adults and larvae of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae*)*. A total of two bioassays were performed. In the first bioassay, mortality rates were determined after 1, 3, 7 and 14 days of exposure on concrete dishes treated with the above insecticides. The results show that all three insecticides were effective for the control of adults and larvae. After 14 days of exposure mortality exceeded 90 % for both life stages. In the case of spinosad complete insect mortality was achieved after 14 days of exposure. In the second bioassay, the presence of food was evaluated to determine the effects of immediate and delayed mortality. Based on the results, all insecticides the presence of food decreased the levels of delayed mortality of the exposed individuals. All three insecticides showed an increased level of delayed mortality, either with or without food, but mortality did not reach 100 % for all insecticides and combinations. These results are important for the design of a standardized protocol for the control of this pest in different types of storage and processing facilities such as poultry farming, feed mills etc.

Keywords: *Alphitobius diaperinus*, insecticides, alpha cypermethrin, spinosad, pirimiphos methyl, concrete

Susceptibility of *Tenebrio molitor* and *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) larvae meals to infestations by major stored-product insects

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The rapid growth of the human population has significantly increased the need to identify new, alternative nutrient sources. Insects are a promising nutrient source that has attracted a lot of scientific and commercial interest as food and feed ingredient. Currently, insect meal is produced in several commercial facilities worldwide, whereas insect meal production is expected to further rise in the near future. As all agricultural commodities, insect meals may be susceptible to insect infestations during storage. However, available information on the susceptibility of insect meals to stored-product insect infestations is rather limited. Based on the above, the objective of the present study was to evaluate the susceptibility of two insect meals from *Tenebrio molitor* and *Alphitobius diaperinus* larvae to infestations by storage insects.

In a first series of bioassays, the population growth of several major stored-product insects in *A. diaperinus* meal was studied. Furthermore, we investigated the population growth of selected insect species [i.e., *Tribolium castaneum* (Coleoptera: Tenebrionidae), *T. molitor* and *A. diaperinus*] in mixtures of *A. diaperinus* meal with wheat bran at different ratios (0, 10, 50, 75 and 100% insect meal). Similarly, we evaluated the population growth of *Tribolium confusum* (Coleoptera: Tenebrionidae), *Trogoderma granarium* (Coleoptera: Dermestidae), *T. molitor* and *A. diaperinus* in *T. molitor* meal (100%), as well as in mixtures of it with wheat bran at different ratios (0, 5, 10, 25, 50 and 75% insect meal).

According to our results, several of the insect species tested are able to infest *T. molitor* and *A. diaperinus* meal. For instance, *T. molitor* can develop significant populations in both *T. molitor* and *A. diaperinus* meal, whereas *T. castaneum* and *T. confusum* can grow well in *A. diaperinus* and *T. molitor* meal, respectively. In contrast, *A. diaperinus* and *T. granarium* did not manage to grow in 100% *T. molitor* meal, whereas their population growth in the mixture of 75% *T. molitor* meal and 25% wheat bran was poor. This is the first study to show the susceptibility of insect meals to infestations by stored-product insects.

Keywords: *Alphitobius diaperinus*, insect meals, population growth, stored-product insects, susceptibility*, Tenebrio molitor*

Toxicity of natural zeolites and other inert dusts on adults and larvae of bean weevil*Acanthoscelides obtectus* (Say) (Chrysomelidae: Bruchinae)

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The bean weevil *Acanthoscelides obtectus* (Say) (Chrysomelidae: Bruchinae) is a serious pest of stored bean seeds, mainly because of larval feeding inside the seeds. We studied the insecticidal effect of natural zeolites, kaolin and diatomaceous earth on adults of *A. obtectus*. It was found that adult mortality was higher than 80%, after their exposure for 48 hours in bean seeds treated with a dose of 5 gr inert dust / kgr seeds. Furthermore, 100% mortality was found in the bean weevil's larvae when zeolites of different origin were applied on seeds. Our results show that mineralogical and chemical synthesis of natural zeolites affect their insecticidal efficacy

Development and mating compatibility of different strains of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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The fast-growing sector of insects as food and feed stimulates researchers and industries to explore uncharted territories, such as insect breeding. Most research for yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) has been directed to diet improvement, while the strain effect on insect growth and development is often overlooked. The aim of our study was to evaluate the larval development and egg production of seven *T. molitor* strains (i.e., Greek, Turkish, German, USA, Spanish and two Italian strains), as well as the mating compatibility among different strains.

In a first series of laboratory bioassays, the survival, egg production of adults of the tested strains was investigated. While, hatchability, larval weight and survival were also recorded. Taking it one step further, we evaluated 4 cross-mating combinations between two *T. molitor* strains (i.e., Greek and Italian). In a second series of bioassays, cross-mating combinations between four *T. molitor* strains (i.e., “Inagro”, Greek, Italian, USA) were evaluated. The cross-mating was performed either by placing adult beetles into oviposition boxes at a 1:1 ratio (male:female) at lab scale, or by mixing pupae from the different strains in a pilot-scale bioassay. Finally, the larvae were kept for further evaluation of their growth and performance at specific intervals.

Concerning the lab scale bioassays, significant differences among strains were recorded regarding the final individual larval weight, while egg production and adult survival also varied considerably among the seven different strains tested. No significant differences were recorded on the performance of intra- and inter-mated strains in terms of egg production, hatching rate and larval growth. In the pilot-scale bioassays, significant differences were recorded in the case of the hatching rate, but not in the egg production. These results indicate that the insect strain used in *T. molitor* mass-rearing can exert a substantial impact on insect biomass production and should be taken into consideration in commercial insect-producing facilities.

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Key-words: insects as food and feed, insect rearing, *Tenebrio molitor*, cross-mating, insect strains

Factors influencing male mating success of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae): the role of lateralization

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In the present study, we examined the impact of laterality behavior on male mating success of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), by investigating the presence of population-level behavioral asymmetries. For the laterality behavior, we studied which side of female's body was preferred by male for mounting. Males of this species showed right-biased population-level copulation attempts, followed by left-biased and backside copulation attempts. Right-biased males achieved higher mating success over left-biased and backside males. We also recorded the duration of: (i) mate recognition, (ii) precopula (the characteristic tapping performed by the male before the genital contact with the female), and (iii) repeated copulation (copulation duration and number of successful copulations for each pair until pair got separate). Concretely, the duration of mate recognition and precopulation tapping differ significantly between the right-biased and left-biased males, while no significant differences were noted during copulation among the males that performed mounting from different sides of the females' body. The number of repeated tappings (precopulation tapping) was significantly lower in backside approaching males than the left- and right-biased males, while the number of repeated copulations did not differ significantly among the tested males. Overall, this study shed light on the mating and reproductive behavior of *O. surinamensis*. Moreover, these findings may be helpful for optimizing rearing techniques of this species that are necessary to large-scale laboratory or semi-field experiments, as well as in contributing to the development of control tools based on insect's behavior.

Keywords: lateralization, behavior, stored-product insects.

This work was supported by a Short-Term Scientific Mission (STSM) from the Cost Action OC-2015-1-19590 Mathematical and Computer Science Methods for Food Science and Industry.

Interactions of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) with *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) under different abiotic conditions

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The Gaussian and Poisson regression models were used to examine the associations of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) with *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) alone and in all possible combinations, at 25, 30 and 35 ºC, 55% and 75% relative humidity (RH), after 65 d, 130 d and 195 d of storage on maize. When the temperature increased from 25 to 30 ºC, the emergence of insects alive as well as the damaged kernels increased, while from 30 to 35 ºC the aforementioned parameters decreased. The increase of RH from 55% to 75% increased both the emerged insects alive and the damaged kernels. When *O. surinamensis* was examined alone outnumbered all the other insect species alone and their combinations, except the combination of *P. truncatus* alone. The combination of *O. surinamensis* - *P. truncatus* caused the most damage to maize kernels, while the combination of *O. surinamensis* - *T. confusum* caused the least damage. Regarding the frass production, *P. truncatus* alone, *O. surinamensis* - *P. truncatus*, and *O. surinamensis* - *T. confusum* - *P. truncatus* combinations were the most damaging. Future analyses (for this type of data) could be based upon the Poisson model, as it demonstrated better performance than the Gaussian model. Our results could be useful for the prediction of damage when different species co-infest the same commodity. Also the reveal of those combinations that lead to elevated damage potential, may differentiate the management strategies in storage facilities.

Keywords: *Oryzaephilus surinamensis, Tribolium confusum, Prostephanus truncatus,* temperature, relative humidity, stored maize

Demographic response of *Tribolium castaneum* (Coleoptera: Tenebrionidae) to constant temperatures

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The present research is related with the impact of different constant temperature levels (i.e., 20, 25, 30 and 32.5 °C) on the demographic parameters of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Temperature exhibited a profound effect on the estimated life table values. The net reproductive rate increased from 0.0784 females/female at 20°C to 11.7665 females/female at 25 °C and 102.0678 females/female at 30 °C, whereas a decrease was noted to 10.7310 females/female at 32.5 °C. The lowest values of the intrinsic rate of increase and the finite rate of increase were observed at 20 °C (-0.0105 females/female/day and 0.9895, respectively) and the highest at 30 °C (0.0348 females/female/day and 1.0354, respectively). The mean generation time did not differ significantly between 20 and 25 °C (249.9 and 225.5 days, respectively), while it decayed to 132.8 and 115.1 days at 30 and 32.5 °C, respectively. The value of the doubling time was negative at 20 °C (-67.5 days), but it increased to 19.9, 34.0 and 63.9 days at 30, 32.5 and 25 °C, respectively. By using the non-linear Briere model, the lower threshold for *T. castaneum* population increase was estimated at 22.2 °C, the upper threshold at 33.2 °C, and the temperature for maximum growth rate at 30.1 °C. Survival analysis indicated that temperature also affected the risk of death of *T. castaneum*. The mean survival time increased from 112.1 days at 20 ºC to 462.4 days at 25 ºC, followed by a decrease to 206.5 and 64.5 days at 30 and 32.5 ºC, respectively. These results are expected to serve on the prediction of the population growth of *T. castaneum*, the potential of its expansion and its management.

Keywords: *Tribolium castaneum,* Soft wheat flour, Survival analysis, Briere model, Intrinsic rate of increase

Evaluation of various feed additives and wet feeds for the larval development of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)

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The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is among the insect species that are registered in EU as alternative protein feed sources [Commission Regulations (EU) 2017/893 and 2021/1372]. However, the success of its mass-rearing depends greatly on the optimization of the dry and wet feed provided as a growth substrate. It has been shown that the addition of yeast as feed additive can greatly enhance larval growth. Little is known though about the effect of other protein sources as feed additives on the larval development of *A. diaperinus*. Similarly, limited information is available on the effect of different wet feeds provided as a moisture source on larval growth. Taking these into consideration, the objective of the present study was to evaluate, in laboratory trials, the effect of various protein sources as feed additives, as well as different wet feeds as a water source on the growth and development of *A. diaperinus* larvae. In a first series of bioassays, wheat bran was supplemented with protein concentrates of pea, soy, brown rice or linseed flour, brewer's yeast and baker's yeast as a protein source to form isoproteinic diets with 27.5% protein. Agar slices were provided as a moisture source. In another set of bioassays, larvae were grown on wheat bran and baker's yeast with 27.5% protein and different wet feeds as a moisture source, i.e., carrot, potato, apple, agar and gelatin slices, as well as water crystals. Based on the results, the faster larval development (time to pupation) and highest survival rate were recorded when wheat bran was supplemented with brewer's yeast. However, larvae fed on bran with baker's yeast had the highest individual larval weight at the end of the bioassay. All wet feeds tested supported larval growth, with the faster larval development and survival rates being noted for carrot, potato and agar slices. These data aim to contribute to the optimization of the mass-rearing of *A. diaperinus* to meet the industrial demands.

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Keywords: feed additives, insect nutrition, larval growth, lesser mealworm, wet feed

Development of *Zophobas morio* and *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) on agricultural byproducts

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The interest for insect utilization as a source of nutrients for animal feed and human food has been steadily increasing in recent years. Insect are highly nutritious, whereas their rearing has low environmental footprint (low GHG emissions, low water needs and land use). The environmental and economic costs of insect production can be further reduced when organic side-streams and agricultural byproducts and wastes are used as feeding substrate. In this context, the aim of this research was to evaluate the suitability of a variety of agricultural byproducts for the rearing of larvae of the superworm, *Zophobas morio*, and the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae).
The substrates tested were byproducts of the cleaning process of oats, barley, vetch and pea seeds, as well as byproducts of the sunflower, sugar beet and cotton processing and production. A mixture of wheat bran and dry yeast (9:1) was used as control. In a first series of bioassays, larval growth was evaluated in each byproduct alone, while in a second set of experiments byproduct-based isoproteinic diets (at two protein levels, i.e. 16.7 and 20%) were evaluated. In all trials, larval survival and development over time were evaluated and feed utilization parameters were calculated.
Both *A. diaperinus* and *Z. morio* larvae grew well in barley and oat byproducts, as well as in sunflower meal, giving results similar to control. Regarding the diets tested, most diets were suitable for the rearing of *A. diaperinus* larvae. In contrast, low survival of *Z. morio* larvae was recorded for all diets evaluated. Our results aim to contribute to the implementation of circular economy strategies for insect farming.

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Key-words: agricultural byproducts, circular economy, edible insects, insects as food and feed, insect protein

Effect of various biotic and abiotic parameters on the growth and development of *Alphitobius diaperinus (*Panzer) (Coleoptera: Tenebrionidae) larvae

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The rapid growth of the world's population and the increasing demand for food and feed have increased the interest for alternative nutrient sources. Insects appear to be a promising food and feed source alternative, as they are rich in nutrients, while their rearing has low water and carbon footprint, fully aligned with circular economy practices (valorization of organic byproducts as insect feeding substrate, exploitation of insect frass as biofertilizer). One of the insect species reared as food and feed is the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). In this study we tested the effect of various biotic and abiotic factors on larval development and growth of *A. diaperinus*.

Initially, the effect of the presence or absence of a moisture source on larval growth was evaluated, while in a second series of bioassays the effect of three temperatures (25, 30 and 32 ° C) and two levels of relative humidity (R.H.) regimes (55 and 75%) was examined on larval development. Finally, in a third series of trials, the growth of *A. diaperinus* larvae was studied on wheat bran-based substrates with different percentages of dry yeast (0, 10, 17.5, 25, 32.5 and 40%) and subsequently different protein content (16.7, 20, 22.5, 25, 27.5 and 30%, respectively).

The presence of a moisture source is a determining factor for the development of *A. diaperinus* larvae, since without moisture provision all larvae were dead five weeks after the initiation of the bioassay. The effect of temperature also proved to be important, as the higher the temperature, the better the insect growth. The best results in terms of survival, growth and feed conversion ratio (FCR) were recorded when the highest temperature (32 °C) was combined with the highest R.H. level tested (75%). Finally, it was observed that the higher the yeast and protein content in the diet, the better the larval growth. These results aim to contribute to the optimization of the rearing of *A. diaperinus* as food and feed.

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Keywords: *Alphitobius diaperinus*, insects as food and feed, circular economy, edible insects, insect protein

Evaluation of critical factors during the long-term storage of agricultural products in storage facilities of Thessaly with emphasis on stored product insects

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Insects are the most important pests of stored agricultural products, as they can cause significant quantitative but also quality degradations in the product. Today, the protection of stored products is mainly based on suppressive methods, in contrast to the lack of preventive measures. At the same time, the continuous and improper use of contact and gaseous insecticides has led to resistance development in several major insect species, and is posing a continuous threat to the sustainability of these key treatments. In the present work, the University of Thessaly in collaboration with a cooperative of agricultural products, carried out on-site inspections, indicating all the critical points in storage facilities and products of the cooperative, which can contribute to the increase of entomological and fungal infestations. The inspections were accompanied by various sampling methods in order to collect and identify at the laboratory scale the existing species and pathogens in the stored products, as well as to evaluate the existence of resistance to the most widely used insecticides. Various insect species and fungi were found in all the warehouses. The majority of insect species found were major pests of stored products worldwide. Saprophytes that cause serious degradations during storage and can produce mycotoxins were found in the cereals. Most of the insect species collected from stored cereals and legumes were found to be resistant to phosphine. At the same time, the test populations did not indicate significant traits of resistance to the active ingredients deltamethrin, cypermethrin and pirimiphos-methyl, but in many cases, the recommended label doses were not able to completely control some populations of these species.

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Key words: Stored product insects, mycotoxins, stored cereals, insecticides, resistance phosphine.

Insecticidal effect of diatomaceous earth applied under field conditions on wheat

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Diatomaceous earth (DE) is probably one of the safest and most effective natural insecticides, as its toxicity primarily depends on its physical properties. However, different factors contribute in the DE insecticidal efficacy, such as the target species and its developmental stage, the product to which DE will be applied and the temperature and relative humidity levels of the storage facility. Thus, the current series of field bioassays were performed to evaluate the insecticidal efficacy of different doses of DE against adults and larvae of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Trogoderma granarium* Everts (Coleoptera: Dermestidae). These trials were conducted at the Agro-Farm facilities of the Department of Agriculture Crop Production and Rural Environment, University of Thessaly, in industrial-scale storage conditions. Bags with durum wheat were mixed with 0 (control), 500 and 1000 ppm (mg insecticide / kg of wheat) of DE, and were subsequently artificially infested with stored-product beetles of the species above. Sampling was carried out by taking wheat samples, and also by recording the insects that had been captured in probe traps, that had been placed inside the grain bulks. This procedure was carried out for a period of 4 months (August - December). Based on the samplings, fewer insects were found in the bags treated with DE in comparison with the control bags. At the same time, trapping was much more effective than sampling, in terms of insect detection and population estimation. The results of the present study illustrate the factors that affect the efficacy of DE for the control of stored-product insects in bulked wheat, in realistic conditions.

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Key words: Stored product insects, natural insecticides, stored cereals, inert dusts.

Seasonal visualization of insect distribution in feed processing facilities

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In the present study, the seasonal distribution of stored product insects was recorded, spatially visualized, and evaluated in a feed production facility in Greece. The installation of the trapping network took place in July 2021 and the insect recording lasted from August 2021 until April 2022. A total of 30 Dome traps (Trécé Inc.) were installed for trapping crawling insects and 8 aerial sticky Delta traps (Trécé Inc.) for trapping Lepidoptera. Oil (Storgard Oil, Trécé Inc.) was used as an attractive source in the case of the Dome traps, while Delta traps were baited with lures containing the ZETA pheromone, which is the male attractant for stored product Pyralidae. All insect samples taken were transferred to the Laboratory of Entomology and Agricultural Zoology for counting and identification. Furthermore, using relevant Python libraries, the data were spatially visualized based on the building floor plan. This process, enabled the identification of zones/areas in the building with the highest insect activity. The results showed that in the summer months the captures were significantly higher than in the winter months, with the highest number of insects being recorded on 02/09/21 (approx. 1000 individuals). The dominant stored product species for the summer period were certain stored product moths, including *Plodia interpunctella* (Lepidoptera: Pyralidae) and species of the genus *Ephestia*, as well as the tobacco beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). In contrast, during the winter months the populations of the aforementioned species decreased significantly, with the exception of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *Tribolium confusum* Jacquelin duVal (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). The recording of insects' number and the spatial visualizations will continue for another year in order to quantify the levels of insect populations and therefore to apply focused solutions depending on the insect species and the area of infestation.

Keywords: stored product insects, feed, traps, seasonal distribution, visualization

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Population growth of major stored product insects and quality characteristics on rice fortified with spearmint and basil

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Rice is the most important durable food product for more than half of the world's population, as it is still a very nutritious food in terms of the contained carbohydrates and it can meet large part of the human caloric needs on a daily basis. The aims of the present work were to evaluate the fortification of rice with nutrients coming from various herbs, that give flavor, aroma, color, while at the same time could significantly improve the antioxidant content in the final products. The sensitivity of the new fortification product with spearmint or basil were evaluated against stored product insects. The insects used were *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Five different containments of fortified rice were used (0, 25, 50, 75 and 100%) separately for each species. In light of our findings, the data of the present study show that the new product, has some certain characteristics in terms of infestation patterns by stored product insect species, since the fortification can protect the commodity from insect infestations. Moreover, the effect of the two herbs (spearmint – basil) used in rice, suppressed the progeny production. The results of the present study are certainly encouraging for further utilization of spearmint and basil in rice.

Keywords: rice, stored product insects, spearmint, basil, population growth
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Insecticidal effect of a botanical enhanced attapulgite for the control of four stored-product beetle species

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We examined the insecticidal effect of three different dust formulations, which were attapulgite, attapulgite mix with oregano essential oil with 52% carvacrol and attapulgite mix with oregano essential oil with 75% carvacrol, in four major stored-product beetle species. The insect species tested were: larvae of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Tribolium confusum* Jacquelin duVal (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Three different concentrations (500, 1000 and 2000 ppm) were evaluated for each of the dust formulations, while the exposure period of the aforementioned species in these formulations was 1, 3, 7 and 14 days. Among the insect species, *S. oryzae* was the most susceptible at all three formulations, followed by *T. confusum* and *R. dominica*. In contrast, *T. granarium* larvae showed the lowest mortality rates even on the 14th day of observation and at the highest concentration (2000 ppm). Progeny production was particularly reduced for all species relative to the controls. Nevertheless, complete suppression of the offspring was observed only in the case of *T. confusum* and *S. oryzae*, while *R. dominica* showed the highest tolerance to all three dust formulations, giving an average of up to 20 individuals per vial at 2000 ppm. To our knowledge, this study is the first that has examined the insecticidal activity of *Origanum vulgare* (L.) essential oils in combination with attapulgite for the control of stored-grain insect species. The data of the work showed that additional experimentation is required to understand in depth the logic of using materials based on natural resources in the context of a non-chemical control strategy in the post-harvest stages of agricultural commodities.

Keywords: essential oils, botanical formulations, attapulgite, stored product insects

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Tribolium confusum du Val (Coleoptera: Tenebrionidae) larvae as potential enemy of stored industrial hemp

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Following the rapid development of the cannabis industry (*Cannabis sativa* L.) the cultivation and storage of the plant has increased accordingly. Therefore, new issues and research fields have emerged, one of them being the interaction of post-harvest cannabis with storage insects. The experiments were executed on the larvae of two major storage pests; *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). The assays were carried out in four hemp varieties (Finola, CBD, CBG and Carboxylated CBD) and two tea-hemp mixtures. Third instar larvae were placed in mixed nourishments with a content of 0 (control), 25%, 50%, 75% και 100% (n=5) of each sample. The purpose of the study is the observation of the growth and the mortality rate of the larvae. At all concentrations, the life cycle was successfully completed and in samples with <75%, progeny appeared.

Keywords: cannabis storage, larvae, mortality, progeny, storage beetles.

Evaluation of graphene for the control of stored product insects

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The necessity to ensure the quality and the sufficient quantity of food production with a view to reducing the environmental footprint has led to the development of new non-chemical, friendly to humans and the environment, active ingredients. Graphene is the most important discovery of the 21st century so far as it is the thinnest and strongest material on the planet with a thickness equal to one single atom. Graphene is also widely known as "super carbon", due to its unique physical and electrical properties, which are exploited in multiple technological fields. From the review of the available literature and despite the countless applications of graphene, there is no research work on the evaluation of its insecticidal action on stored product insects. Therefore, the aim of the present study was to evaluate the effectiveness of graphene against major stored product insects. For this purpose, three graphene formulations were tested, each with a different particle size (5, 10 and 25 μm) in order to examine the particle size as a factor that affects its insecticidal action. The insects that tested in the experiments were *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on soft wheat and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) on maize. These species were exposed to three graphene formulations at three different concentrations (100, 500 and 1000 ppm) and at four different exposure intervals (3, 7, 14 and 21 days after the application). In addition, progeny production capacity was also evaluated. The results of the present work have shown that graphene is effective in controlling the specific stored product insect species, and can contribute to the inclusion of graphene in stored product pest management programs, as an alternative method over the use of traditional insecticides, encouraging the production of graphene-based insecticides that can be commercialized for this purpose in the near future.

Keywords: graphene, stored product insects, particle size, nanoparticles, alternative control methods

Insecticidal effect of graphene against stored product beetle species on wheat

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In the current study, the efficacy of graphene was evaluated against *Rhyzopertha dominica* (F.) (Coleoptera: Bostryhidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) on wheat. Insecticidal effect of graphene was evaluated with two different graphene materials, at different concentrations (0, 250, 500 and 1000 ppm) on wheat kernels. Adult mortality was recorded after 7, 14 and 21 days of exposure and progeny production was recorded after 65 days. Our results indicate that high mortality was recorded for all species and formulations tested. *Rhyzopertha dominica* and *S. oryzae* were found to be the most susceptible species with 100 % mortality after 21 days of exposure to 250 ppm. Application of 500 and 1000 ppm caused 100 % mortality after 7 days of exposure. For *T. castaneum* some survival was recorded after 7 days of exposure at 500 and 1000 ppm, but mortality was 100 % at the 21-day exposure interval. For both materials, progeny production was negligible for 500 and 1000 ppm. The findings of the present study showed that the tested graphene materials showed high efficacy and could be used to control stored product species and additional experimentation is needed for the evaluation of this material.

Keywords: graphene, nanotechnology, stored-product pests, wheat

Carob (*Ceratonia siliqua*) flour as substrate for rearing *Tenebrio molitor* (Coleoptera:Tenebrionidae) larvae

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Tenebrio molitor (Coleoptera:Tenebrionidae) larvae, besides their use as animal feed, are now available for purchase and human consumption in the countries of the European Union (EK 2022/169) as novel food (frozen and dried larvae or in powder/flour form). The aim of this study was to study the effect of partial or total substitution of wheat bran with carob flour originating from Keratokampos, Crete (pilot scale production by grinding whole carob pods) to the weight, the survival rate as well as the developmental time of *T. molitor* larvae. The pilot usage of carob flour as substrate for *T. molitor* is intended for insect feed with high nutritional value and functional characteristics. First instar larvae were weighed and placed in groups of 50 in cylindrical vials containing substrates with 25, 50, 75 and 100% carob flour tested against a control substrate (100% wheat bran). Larvae were left to feed for 4 weeks, after which they were weighed and counted biweekly until the appearance of the first pupa. The experiment consisted of six replicates per diet. According to our preliminary results, *T. molitor* larvae showed enhanced development in the 25% carob flour substrate displaying higher final body weight, increased survival rate and shorter developmental time. On the other hand, larvae fed on the 100% carob flour substrate displayed the lowest total final body weight, lower survival rate as well as longer development time. The study is in progress, including additional biochemical analyses of the substrates and larvae.

Key words*:* insect meal, carob flour, larval weight, survival rate, developmental time

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Session:
Insects of Medical Importance - Social and Productive Insects

Detection of deformed wing virus (DWV) in Apis mellifera and its relation with the applied beekeeping practices.

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Deformed wing virus (DWV) is possibly the most investigated virus that affect *Apis mellifera*, due to its high prevalence and close association with colony losses induced by *Varroa destructor*. The aim of this study, was the investigation of virus-varroa relationship, in combination with treatment using one conventional and one organic acaricide. Knowing that a honey bee colony is a complex superorganism with heterogeneity between its individuals, it was initially considered necessary to determine a representative honeybee sampling, in order to estimate the viral load at colony level. Firstly, bees were collected from different sampling points, particularly from brood frames and honey super of the bee colonies ($p = 0.551$ and $p = 0.353$, $\alpha = 0.05$). Subsequently, the viral load was quantified in samples consisting of bees collected from the whole range of the hive, specifically 10 and 50 bees per every frame (CV = 5.3% and CV = 6%). The sampling of 10 bees/frame was selected as the most appropriate, as the sample consists of individuals from all over the hive, while the size is proportional to the strength of the colony. To control varroa, oxalic acid and amitraz were used. The treatment efficacy was 94.6% and 91.3% respectively ($p = 0.213$, $\alpha = 0.05$). A significant reduction of the viral load was observed in the oxalic acid group after treatment ($p = 0.021$, $\alpha = 0.05$). On the contrary, the viral copies showed a slight increase after treatment with amitraz or without treatment at all. However, statistically significant differences were not observed at any case ($p= 0.079$, $p = 0.639$, $\alpha= 0.05$). Inferentially, while both acaricides showed similar efficacy against varroa, a significant decrease in the viral load was only recorded when oxalic acid was used. This difference is probably due to the formulation itself; nonetheless further investigation is needed.

Keywords: *Apis mellifera*, Deformed Wing Virus, *Varroa destructor.*

Quantitative and qualitative traits of reared hybrids and pure races of the silkworm, *Bombyx mori*, in Greece

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Silkworm rearing in Greece relies exclusively on imports of silkworm hybrids' eggs from countries such as Italy, Bulgaria, China, Turkey or Japan. Greek silkworm farmers select the hybrid they will rear on the basis of hybrid stock availability, not taking into account the cost of purchasing the silkworm eggs, the productivity of the hybrids and the quality of the produced cocoons. The present study is a step towards rectifying this situation by presenting evidence of quantitative and qualitative analyses of the produced cocoons, aiming to rationalize the import of silkworm hybrids' eggs in relation to their cocoon yields.

Random sampling of cocoons was carried out in May-June 2021 from various farms that were rearing silkworm hybrids imported from the above-mentioned countries. Upon analyzing the quantitative characters of the sampled cocoons, the qualitative characters of the produced silk were also analyzed by cocoon quality testing.

We mapped the quantitative and qualitative traits of the produced cocoons from a variety of hybrids and pure races in relation to the market price of the silkworm eggs, withholding the specific names of the silkworm hybrids for obvious ethical and market concerns. The results show that the Italian hybrids had the best cost-effectiveness relationship at the level of cocoons produced, despite the higher quantitative yields of the Japanese hybrid. The Chinese hybrids had the lowest performance at both the qualitative and quantitative traits. Pure silkworm races, which, by their non-commercial nature, can not be considered in any cost-effective relationships, yielded equally satisfactory results compared to imported silkworm hybrids. Lastly, our data highlight the absence of any correlation between the hybrids' rearing site and the hybrids' qualitative and quantitative traits.

Field evaluation of eight repellent products against mosquitoes in the area of Macedonia airport

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Eight commercially available mosquito repellents, as well as a repellent mixture based on a formula of our laboratory research, were tested in the Aristotle University of Thessaloniki Farm, near Macedonia airport, in a self-funded research. For the evaluation of the repellent effect of the tested products, we used CO_2 traps and scored the number of mosquitoes' catches. We tested the following products: Pyrox spiral® (prallethrin 0,093% w/w), fleriana wax® (geraniol 5% w/w) and citronella wax (lemongrass oil 5% w/w) as well Autan Family Care Lotion® (icaridin 10% w/w), Autan Junior Gel® (icaridin 10% w/w), Autan Defence Plant Based Spray® (citriodora oil 30% w/w), Hansaplast® (Ethyl Butylacetylaminopropionate 15% w/w). The tested products were placed in front of each trap. Liquid products were applied in a piece of sponge and placed in front of the trap. Among the tested repellents, Pyrox spiral®, fleriana® and citronella wax, Autan Family Care Lotion®, Autan Defence Plant Based Spray®, Autan Junior Gel®, as well as our mixture reduced significantly the number of mosquitoes' catches and showed a higher repellent effect than Autan Defence Plant Based Spray® and Hansaplast®. In all the cases, adults of *Culex* sp. (Diptera: Culicidae) and *Aedes* sp. (Diptera: Culicidae) were trapped. The repellent effect of most of the tested products was higher for *Culex* sp. than for *Aedes* sp. adults. By contrast, our formula had a high repellent effect for both *Culex* sp. and *Aedes* sp. adult mosquitoes. Further experiments are now running to verify the present results and improve repellency and human protection.

Keywords: mosquitoes, repellent effect, repellent substances, traps.

From qualitative to quantitative insect metabarcoding: an in tandem multilocus mosquito identification methodology

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High throughput trap-based insect monitoring is essential for the study of insect populations and the identification of invasive species. Insect DNA metabarcoding technology has emerged as a highly promising methodology for unbiased and large-scale surveillance. Although significant attempts have been made to apply DNA metabarcoding within mosquito or other insect surveillance, the combined application of qualitative and quantitative metabarcoding has remained a challenge. In the present study, we developed a methodology of in-tandem identification and quantification of samples, using cytochrome oxidase subunit I (COI) combined with a secondary multilocus identification and quantification involving three loci of 28S ribosomal DNA. Using the methodology developed here, we were able to identify individual species in sample pools of mosquitoes with 95.94% accuracy and resolve with high accuracy ($p = 1$, $\chi 2 = 2.55$) mosquito population composition species by species, thus providing a technology capable of revolutionizing mosquito surveillance through metabarcoding. The results obtained here show that the methodology has the potential to be applied widely, including to various invertebrate and non-invertebrate populations made up of small-sized individuals.

Keywords: Metabarcoding, mosquitoes, NGS, monitoring.

Effect of water salinity on immatures' survival and lifespan of emerged adults, of *Aedes albopictus* (Skuse) (Diptera, Culicidae)

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The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera, Culicidae) is an important vector of deadly diseases such as, Dengue and Yellow fever viruses. Various biotic and abiotic factors affect the biological traits of both aquatic immatures and adult vectors. Reports of larval presence in both fresh and brackish waters, have increased the interest on possible effects of salinity on mosquito's biology. We examined the possible effects of different salinity concentrations on mosquito's survival including both immatures and the obtained adults. First instar larvae were placed in small glass tubes containing 3ml of water and kept at 25°C. Water of different salinity, representing fresh (0 ppt, 0,5ppt), brackish (2ppt, 5ppt, 10ppt, 15ppt, 20ppt) and salty waters (30ppt) was used. Larval survival, moltings, pupation and adult emergence were recorded daily. Emerging adults were placed in individual plastic cages (400ml capacity) to record lifespan. Larvae reared at 15, 20 and 30ppt salt couldn't survive. In contrast, a positive effect on larval survival, was found at the minimum concentration of 0,5ppt. In intermediate concentrations, the higher the accumulation of salt in the water, the higher the mortality was. There were no apparent effects of salinity on the duration of larvae and pupae development periods. Only larvae reared in salinity up to 5ppt managed to reach adulthood. The impact of salinity, during the development of immatures, on the demographic traits of adults and on the population dynamics of *Ae. albopictus* is discussed.

Key-words: mosquitoes, lifespan, vectors, demographic traits

Sterile Insect Technique (SIT) against *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae): A roadmap and good practice framework for designing, establishing, and evaluating pilot field trials

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Aedes albopictus (Skuse, 1894) (Diptera: Culicidae) (also known as Asian tiger mosquito) is an invasive mosquito species that impose a substantial risk to human health. To control the abundance and spread of these arboviral disease vectors, sterile insect technique (SIT) is emerging as a powerful complement to conventional approaches. The SIT is recognisable for agricultural pest management and is now being increasingly applied to mosquitoes as part of integrated vector management programs. A successful implementation of SIT project relies on the mass production and high-quality sterile males. The new project “moSquITo” will invest on the production of sterilized mosquito males in Greece in all stages (mass rearing, sterilization and releasing) contributing to the integrated management of Asian tiger mosquito in agreement with the EU Regulation 528/2012. For the proper design and implementation of SIT, under different environments, several key elements are required which focus on how to (1) ensure wide stakeholder support; (2) select suitable field sites; (3) shape a locally adapted integrated vector management strategy; (4) warrant high-quality releases; and (5) evaluate the results of the field trial.

Keywords: Mosquito Control; Integrated Vector Management; Stakeholder Engagement; Pilot Trial.

Within the framework of the project «moSquITo»: Innovative approaches for monitoring and management of the Asian tiger mosquito with emphasis on the Sterile Insect Technique (ΤΑΕΔΚ06173- National Recovery and Resilience Plan, “Greece 2.0” & EU Funding – Next Generation EU) and the programs financed by International Atomic Energy Agency (IAEA): TC Project RER/5/022 “Establishing Genetic Control Programmes for *Aedes* Invasive Mosquitoes” & Programme of Coordinated Research Activities “Quality control bioassays for irradiated *Aedes albopictus* males”(IAEA-CRP Contract No: 23915).

Selection of representative mosquitoes' (Culicidae) trap locationsand first results of entomological surveillance

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Both native and invasive mosquito species (Culicidae) are important vectors of pathogens. Entomological surveillance and population data collection are particularly important both for determining the risk of disease transmission in an area but also for taking timely action to reduce the risks.

In the present study, the methodology for the selection of representative sites for mosquito traps in the Attica Region is presented. The geostatistical method of stratified random sampling was used to select the sites. The method was applied to selected land uses, based on entomological requirements, throughout the Attica Region (except of the islands). For the selected land uses we used Corine Land Cover (CLC 2018) and initially 50 representative sites were chosen (trap locations).

For the determination of the final trap locations, we evaluated entomological data collected in previous years, while all the Municipalities (58 in total) were also asked to suggest available appropriate sites. In almost all cases, the initially selected locations were adopted, ensuring an accurate geostatistical representation. In cases that it was impossible to establish a trap in the initially selected locations (e.g. military camps) a nearby location was selected ensuring the accurate entomological monitoring.

Totally 57 adult traps were established, and weekly results are presented with online thematic maps. In collaboration with the relevant stakeholders both in Region and in Municipalities, the results are constantly evaluated to take appropriate measures for proper mosquitoes' management.

Keywords: *Aedes albopictus*, *Culex pipiens*, entomological surveillance, stratified random sampling.

The present study was carried out in the framework of the project "Recording the presence and seasonal variation of mosquitoes (Culicidae) in the Attica Region", which is funded by the Attica Region.

Presence and seasonal variation of mosquitoes (Culicidae) in the Region of Attica for the year 2021

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Entomological surveillance of mosquito populations is critical for the design and implementation of effective mosquito management programs and also plays a significant role in the surveillance of vector-borne diseases by contributing to the timely implementation of targeted response and preventive measures at regional and local level. Within the framework of the systematic entomological surveillance of mosquito populations in the Region of Attica, a total of 55 BG-Sentinel 2 (BGS2) traps and 110 oviposition traps were installed. For the selection of the trap locations the geostatic method of stratified random sampling was used, based on land use criteria. The BGS2 traps were continually operated equipped with BG-Lure and constant flow rate of CO_2 (gas cylinders). Specimens from all traps (BGS2 and ovitraps) were collected weekly throughout the year. Sixteen mosquito species were recorded in the adult traps and the largest populations were recorded for *Culex pipiens* and *Aedes albopictus*. The study of seasonal variation for 2021 revealed that the maximum number for *Culex pipiens* adults recorded the 23rd to 26th week and for *Aedes albopictus* both for adults and eggs (ovitraps) the 27th to 29th week. The results were collected and communicated weekly to the relevant stakeholders, both at Regional and local level, for the implementation of appropriate and timely decisions regarding interventions.

Keywords: Attica, Mosquitoes, Regional Unit, Entomological surveillance, stratified random sampling.

The present study was carried out in the framework of the project "Recording the presence and seasonal variation of mosquitoes (Culicidae) in the Attica Region", which is funded by the Attica Region.

A case for a systematic quality management in mosquito control programmes in Europe

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The recent spread of invasive mosquito species, such as *Aedes albopictus* and the seasonal sporadic transmission of autochthonous cases of arboviral diseases (e.g. dengue, chikungunya, Zika) in temperate areas, such as Europe and North America, highlight the importance of effective mosquito-control interventions to reduce not only nuisance, but also major threats for public health. Local, regional, and even national mosquito control programs have been established in many countries and are executed on a seasonal basis by either public or private bodies. For these interventions to be worthwhile, funding authorities should ensure that mosquito control is (a) planned by competent scientific institutions, (b) addressing the local demands, (c) executed following the plan that is based on recommended and effective methods and strategies, (d) monitored regularly by checking the efficacy of the implemented actions, (e) evaluated against the set of targets, and (f) regularly improved according to the results of the evaluation. Adherence to these conditions can only be assured if a formal Quality Management system is adopted and enforced that ensures the transparency of effectiveness of the control operation. The current study aims the two major components of this Quality Management (QM) system are presented, the Quality Assurance and the Quality Control for mosquito control programs. Moreover, challenges for QM implementation over temperate areas, with special emphasis on Europe, are explored.

Keywords: Mosquito Control; Mosquito Management; Vectors; Public Health

This study was supported by the European project AIM COST - Action Aedes Invasive Mosquitoes (CA17108; http://www.aedescost.eu).

Effect of food availability and crowding during larvae development on some traits of immature and adults of the Asian tiger mosquito, Aedes albopictus

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Aedes albopictus, widely known as the Asian tiger mosquito, is an invasive mosquito species of major importance to public health and a vector of lethal human diseases. *Aedes albopictus* utilizes ephemeral and relatively small aquatic habitats for its reproduction. Consequently, larvae often face intraspecific competition and shortage of food availability under crowding conditions that may affect immature survival and development as well as the size and performance of yielded adults. We studied, in laboratory conditions, the effect of crowding and food availability during the larvae developmental period on survival and development of immature stages as well as lifespan and size of obtained adults. Larvae were reared at 3 different densities (100, 300, and 600 larvae/lt water) with the food availability of 1mg/lt and 2 mg/lt for all the densities above under controlled temperature (25^0C), humidity (65 ± 5% RH) and photoperiod (14:10 L:D). Our results demonstrate that the larvae crowding and the food availability were significant predictors of the survival and larvae developmental period. Moreover, crowding and sex were important estimators of the adult lifespan longevity. Food availability affected the wing size of males while food availability and crowding affected the wing size of females. The importance of the results for populations of *Ae. albopictus* is discussed.

Keywords: nutrition adequacy, overcrowding, lifespan, physiometric characteristics

Hunting temperature-sensitive lethal mutations: Response of *Aedes albopictus* to thermal stress

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The mosquito *Aedes albopictus* is a vector of several diseases, including Yellow and Dengue fever, as well as Zika and Chikungunya. However, current vector control programs are either heavily based on chemical insecticides, partially effective, costly or time consuming. Population suppression strategies through the release of sterile males are re-emerging to suppress wild populations and subsequently reduce disease transmission and bite annoyance. Such an example is the Sterile Insect Technique (SIT), which relies on the mass production and release of sterile males to mate with females of the wild population.

The SIT is successfully applied to control the Mediterranean fly, *Ceratitis capitata*, which is a model for the implementation of the SIT due to the possibility of releasing only males. This has been achieved through the discovery of the *temperature-sensitive lethal (tsl)* mutation that is genetically linked to the *white pupae (wp)* mutation and led to the development of Genetic Sexing Strains (GSS). In these strains, the males emerge from brown pupae and are resistant to temperature stress (34 – 35 °C), while the females emerge from white pupae and are sensitive. Different such mutations have been found in *Drosophila melanogaster* but still none in insects of economic or public health importance that could be potential targets for SIT.

Improving the sex separation methods is essential for the application of SIT to mosquitoes. This research studies the response of *Ae. albopictus* in temperature stress, at early developmental stages, with the ultimate goal of finding/isolating mutations related to sensitivity or resistance that could, following the Mediterranean fly model, be integrated into a sex separation strategy.

In this study, groups of twenty larvae on the first stage (L1) were placed at five different temperatures - 27 °C (control), 37 °C, 39°C, 41°C, and 43 °C -, for 6, 12, and 24h. Our results show that the potentially diagnostic temperatures are between 37 °C (almost zero mortality) and 43 °C (almost complete mortality), for exposure to stress from 12 to 24 hours. The analysis of additional populations will show whether it is possible to isolate existing mutations or new ones should be induced by irradiation or chemical mutagens.

Keywords: Aedes albopictus, genetic sexing strains, population suppression, sterile insect technique, tiger mosquito, vector control.

Investigation of susceptibility of *Culex pipiens* Linnaeus (Diptera: Culicidae) populations to larvicide B.t.i. (*Bacillus thuringiensis* var. *israelensis*)

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Mosquitoes have been counting as an object of study for many years due to their great public health importance as in addition to the nuisance they cause to humans, their ability to transmit pathogens of very serious diseases is widely known. They can be treated either at the larval or adult stage with natural, biological, or chemical measures. Chemical measures seem to be more efficient than the others, but the emergence of resistance due to repeated use of the same or similar chemicals is a very common situation. In Greece, the available larvicides are quite limited as there are only three (3) active substances registered against mosquito larvae. *Bacillus thuringiensis* var. *israelensis* (Bti) seems to be the most effective due to its unique mode of action and its impact to the environment.

In the present study, the susceptibility of three larval populations of *Culex pipiens* to the Bti was investigated. Initially, larvae were collected from natural habitats in city. More specifically: from the Filothei stream, Vrysaki stream (Agios Stefanos I) and Agia Paraskevi stream (Agios Stefanos II). The collections took place on different dates in June and July 2021. Larval rearing was occurred at the Agricultural University of Athens (AUA - Laboratory of Pesticide Science). Egg rafts and first or second instar larvae (L1-L2) were selected for breeding. When larvae reached the appropriate age (late 3rd – early 4th) were bioassayed according to World Health Organization protocols using the bacterial larvicide Vectobac12AS (Bti 11.61% w/v). A range of concentrations was tested in order to determine mortality between 10% and 95% and to define LC_{50} and LC_{95} values.

The results showed that all 3 populations were particularly susceptible to the larvicide. Especially the larval population from Agia Paraskevi (Agios Stefanos II) showed the greater susceptibility (LC_{50}: 0.0008mg/l) compared to the other two tested populations. The population from Filothei showed the lowest susceptibility (LC_{50}: 0.002mg/l). Finally, the population from Vrysaki stream (Agios Stefanos I) showed an intermediate response (LC_{50}: 0.001mg/l) compared to the other two populations.

Keywords: resistance, susceptibility, *Bacillus thuringiensis* var. *israelensis*, *Culex pipiens*, LC_{50}.

Presence and contribution to the management of the invasive mosquito species *Aedes albopictus* (Skuse) (Diptera: Culicidae) in the Evrotas Municipality (Lakonia, Peloponnese) during 2021-2022

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In an earlier study of the mosquito fauna in the Municipality of Evrotas, Laconia, in 2012-2013, in the framework of the MALWEST project, two individuals of the invasive mosquito species *Aedes albopictus* (Skuse) (Diptera: Culicidae), commonly known as the Asian tiger mosquito, were detected for the first time in this area without any evidence of population establishment. *Aedes albopictus* is an invasive mosquito species and a competent vector of some of the world's most serious arboviruses (Dengue fever, Chikungunya virus, Zika virus, among others). Its presence has been recorded in Greece since 2004. Since then, populations of the species have been established in many country areas, posing a considerable health risk.

As local malaria cases have been reported in that particular region, the presence of an additional invasive mosquito vector is of great importance for assessing the risk of disease transmission and the proper design and implementation of mosquito management programs. In the present study, mosquito surveillance was conducted in urban and semi-urban areas in the greater area of the Municipality of Evrotas, Laconia (Skala, Asteri, Stefania, Dafni, Leimonas, Krokaies, and Vlachioti) to investigate the presence and possible establishment of *Ae. albopictus* in the area. Specifically, a network of ovitraps was installed and monitored in sites that are typical habitats of the particular mosquito species. Additionally, during the same period, mosquito collections were carried out using adult mosquito traps at regular intervals from 2021 to the present.

According to the results, *Ae. albopictus* is widespread in the wider region of Evrotas, and its populations have been successfully established as they are reproducing at relatively high rates. Biocides registered in Greece could be effectively used for the management of *Ae. albopictus* populations in the area as no evidence of resistance development was found in laboratory efficacy bioassays using the diagnostic dose.

Keywords: *Aedes albopictus,* Municipality of Evrotas Laconia, invasive mosquito species.

Comparative study of ovitraps for monitoring *Aedes albopictus* (Skuse) (Diptera: Culicidae)

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Aedes albopictus (Skuse) (Diptera: Culicidae), commonly known as the Asian tiger mosquito, is an invasive mosquito species of significant public health importance as it is a known vector of some of the world's most dangerous arboviruses (Dengue fever, Chikungunya virus, Zika virus among others). Its presence in Greece has been confirmed since 2004, but its spread, seasonal occurrence, and population dynamics are of great importance for the prevention of disease risk and the integrated management of mosquito vectors in general.

The establishment and monitoring of oviposition traps are among the most common and effective ways to study the presence of *Ae. albopictus* in an area and its population dynamics. The method involves the placement of a container that resembles the larval development habitat of the particular mosquito species and an oviposition substrate. However, there is variation in the recommended container size and the suitable type and material of the oviposition substrate in the literature.

In this study, field experiments were conducted in order to compare three different container sizes (350 ml, 600 ml, and 1100 ml) in combination with two different types of oviposition substrates (tongue depressors and expanded polystyrene pieces) to determine which of the combinations offers the most advantages based on the following criteria: a) reliability of the results and sensitivity of observations, b) ease of use and recording of results, and secondarily, c) low cost and convenience of supply and d) ease of carrying and installation.

The statistical analysis of the results and consideration of overall evaluation criteria showed that combining a medium-sized container (600 ml) with tongue depressors as an oviposition substrate is the most efficient combination for studying the occurrence and assessing the density of *Ae. albopictus* populations in an area.

Keywords: *Aedes albopictus,* invasive mosquitoes, ovitraps.

Efficacy evaluation of Long-Lasting Insecticidal Nets after their use in malaria prevention and management programs in Greece

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As part of the integrated response to malaria transmission cases in an area of the Municipality of Evrotas, Laconia, in 2011-2012, the national public health authorities purchased and distributed Long-Lasting Insecticidal Nets (LLINs) among migrants from malaria-endemic countries. These mosquito bednets were impregnated with the insecticidal active substance deltamethrin or alpha-cypermethrin and were used during the warmest months of the year, between 2013-2020, and were subjected to one wash per year. To determine whether the used bednets remain sufficiently effective, a representative sample was evaluated in the laboratory in 2021.

Bioassays were performed on a total of six (6) used mosquito bednets, according to the method recommended by the World Health Organization (WHO) for testing the efficacy of impregnated mosquito bednets (mosquito mortality >80% after 24 hours), with minor modifications. The bednets were tested against mosquitoes of the species *Culex pipiens* biotype *molestus* (Forskal) (Diptera: Culicidae) from laboratory rearing and mosquitoes of the species *Ochlerotatus caspius* (Pallas) (Diptera: Culicidae) from wild natural populations. The efficacy of the used mosquito nets was compared with the efficacy of new unused impregnated bednet, while a negative control, a bednet not impregnated with insecticide, was also included in the bioassays.

According to the results, the mosquito bednets were effective - meeting WHO criteria - in the tests against *Cx. pipiens* biotype *molestus,* while in the bioassays against *Oc. caspius* from wild natural populations, their efficacy varied depending on their previous use.

Keywords: Mosquitoes, malaria, long-lasting insecticidal mosquito nets.

Intraspecific and interspecific competition between larvae of two mosquito species, *Aedes albopictus* (Skuse) and *Aedes cretinus* (Edwards) (Diptera: Culicidae)

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Aedes albopictus (Skuse) (Asian tiger mosquito) and *Aedes cretinus* (Edwards) (Diptera: Culicidae) are closely related mosquito species sharing morphological and bio-ecological traits. After the invasion and establishment of *Ae. albopictus* in Greece, it has been observed that these species co-occur in specific areas of Attica, Greece, with *Ae. albopictus* developing significantly higher population densities than the native mosquito *Ae. cretinus.* In the current study, inter- and intra- specific competition between larvae of these species was investigated under laboratory conditions. In containers with 250 ml water, groups of 20 or 60 1st instar larvae of one species or two species at a ratio 1:1 were placed providing 0.1 or 0.3 mg fish food/larva/day. The longevity of larval stage, the survival until adulthood and the wing length of female mosquitoes as an indicator of female body size were recorded for each species. Data for each species were analyzed with Generalized Linear Models (α=0.05). At low food supply, the time until pupation when these species grew up together was 1.3 to 2.3 days shorter for *Ae. albopictus*, and 1 to 1.4 days longer for *Ae. cretinus,* compared with single species development, indicating a trend of inter-specific competition in favor of *Ae. albopictus*. Interspecific competition resulted in the production of larger *Ae. albopictus* females at limited food availability and low larval density, in contrast to concomitant species development. Survivorship to adulthood for both *Ae. albopictus* and *Ae. cretinus* was generally high and was not significantly influenced by competition. When these two species developed on their own at low larval food provision, larvae developed slower and the females were smaller, indicating intraspecific competition. These overall findings are evidence that *Ae. albopictus* is a superior competitor to *Ae. cretinus*, primarily at limited larval food resources, and this may account for the expansion of *Ae. albopictus* and the limited presence of *Ae. cretinus* in areas of Attica, Greece, where these related species co-exist.

Keywords: larval competition, *Aedes albopictus, Aedes cretinus.*

Operational integration of the Unmanned Aircraft Vehicles – UAVs in the wide area integrated mosquito management programs

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Ecodevelopment S.A., since 2018, has invested in intensively implementing Unmanned Aerial Vehicle (UAVs), in order to upgrade its efficiency for mosquito control applications. Thus, several Multicopters with multispectral, thermal and RGB cameras for monitoring and Agricultural UAV sprayers with liquid tanks and granule dispensers were gradually added at the service of mosquito control.

These UAVs have a tank with a capacity of 10lt and 20lt respectively and are capable of spraying inaccessible mosquito breeding grounds with the ability to cover up to 18 ha in an hour. When combined with photographic UAVs and georeferenced image processing platforms, the spraying can be done with an accuracy of a few centimeters, thus increasing the effectiveness of the application. Compared to the use of other spraying methods, the use of UAVs in mosquito control programs, in addition to the possibility of approaching areas to which traditional vehicles do not have access, also offers the controlled and uniform diffusion of the spray solution by reducing the total amount of larvicide used.

Specifically, for the mosquito control program of Western Greece Region, in the years 2020 and 2021, Ecodevelopment S.A. carried out spray applications in mosquito breeding sites of a total area of 3,500 ha of which 1.300 ha were sprayed using UAVs, 1,400 with the use of a helicopter and 1,150 by ground vehicles crews. 77% of the areas sprayed with UAVs are areas of natural wetland systems and the remaining 23% concerns mosquito breading sites in the peri-urban system and flooded arable land (rice fields). All the spraying interventions were carried out with the biological larvicide *Bacillus thuringiensis subsp. israelensis* at a dosage of 1000 - 2000ml/ha.

The effectiveness of the applications ranged from 78% to 89% depending on the parameters of the flights and the characteristics of the vegetation that surrounded the mosquito breeding site. We conclude that use of UAVs in mosquito control programs can indeed fill the gap between ground and manned aircraft applications, especially at water surfaces between 5 ha – 200 ha.

Detection and characterization of *Wolbachia* infections in wild populations of *Culex pipiens* and *Aedes aegyptis*

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Mosquitoes (Diptera, Culicidae) are one of the most diverse arthropod groups in the world and their feeding behavior as hematophagous gives them the ability to transmit an enormous number of pathogens from one host to another. Among the most well-known arthropod-borne viruses or arboviruses are West Nile, Zika, Dengue and Chikungunya, thus are responsible for several cases and deaths every year. *Wolbachia* is a genus of endosymbiotic α-Proteobacteria, which is able to induce reproductive abnormalities such as cytoplasmic incompatibility (CI), thelytokous parthenogenesis, feminization and male killing, thus affecting biology, ecology and evolution of its hosts. Recently, this bacterium has been used as biocontrol strategy for their ability to induce CI. In the present work, we investigated the presence of *Wolbachia* infection in wild populations of *Culex pipiens* and *Aedes aegypti*. Wild populations of mosquitoes were collected in Bangladesh and stored in absolute ethanol in -20°C until further use. DNA isolation was performed based using CTAB (cetyltrimethylammonium bromide) protocol. PCR amplification with the set of specific primers WspecF and WspecR was used in order to identify the presence of *Wolbachia* in wild populations of *Culex pipiens* and *Aedes aegyptis*. Preliminary results indicate that 87.5% of the wild population of *Culex pipiens* were infected with *Wolbachia*, against 80% of *Aedes aegypti*. The *Wolbachia* strain is further characterized using a Multi Locus Sequencing Type (MLST) approach. Phylogenetic links with known *Wolbachia* strains will be presented.

Operational use of early risk warning systems in large-scale mosquito control programs – the award-winning EYWA system

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The wide area integrated mosquito management programs are characterized by the systematic implementation of a plethora of actions aimed at reducing mosquito abundance and protecting public health. Deciding on the type, area and frequency of implementation of specific actions is a complex process that must be based on data.
The systematic collection of entomological and environmental data (raw, processed and open source data), from 2010 onwards in all mosquito control projects undertaken by Eco-development S.A., in combination with the building artificial intelligence techniques and the unhindered cooperation with research institutions and control organizations from Greece and abroad, allowed the creation of a series of innovative digital tools that were operationally integrated from 2020 in the large-scale mosquito control programs carried out by the company in 5 Regions of the country:

- Restricted access electronic platform "eBite" for the recording, monitoring, visualization and real-time analysis of mosquito control applications performed and the data collected in the field
- The BAd model, a predictive model for the abundance of mosquitoes at the settlement level that provides a five-day forecast of abundance for *Culex spp*. and *Aedes caspius* (operating in 2415 settlements in four Regions of Greece since the summer of 2020)
- The BAR model, a weekly predictive model for assessing the risk of transmission of West Nile virus at the settlement level (operating in Central Macedonia since 2020 for 1,025 villages)
- Open source application –Mosquito Vision, which is based on the predictions of the Bad model, and provides a daily forecast for mosquito nuisance at settlement level in the Regions of Central Macedonia, Western Greece, Thessaly and Crete.

The functional integration of the above digital tools in mosquito control programs, in addition to contributing to timely decision-making for the intensification of specific actions in space and time, facilitates quality control and the supervision of the services offered, as well as the early warning of citizens and the public administration for increased mosquito populations in order to take measures to protect individual and collective Public Health.

The above digital tools form the basis on which the EYWA – **E**arl**Y** **WA**rning System for Mosquito - system was built borne Diseases which brought together 15 partners from five countries and which was awarded the EIC Horizon Award on 17 January 2022 by the European Innovation Council (EIC). This award is one of six total awards awarded since the establishment of the EIC in 2015.

Education needs of Greek beekeepers, a case study

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The aim of the present work was to cluster beekeepers, taking into account their educational needs and their professional and demographic characteristics. A questionnaire was developed on a multivariate five-point Likert scale and distributed to 338 respondents. The statistical analysis of the data includes a reliability analysis on the five-point Likert scale, exploratory factor analysis with varimax rotation, and a two-step cluster analysis considering the factor scores and beekeepers' demographics. The SPSS v.26 was used for the statistical analysis of the data.
The results showed that the majority of the sample were males (92,9%), and the mean age was 44,2 years old (SD=10,141). The scale of the self-assessment of beekeepers' knowledge on enemies and diseases was highly reliable (Cronbach's Alpha = 0.944), and the exploratory factor analysis indicated that three distinct factors were underlying. The two-step cluster analysis showed that 225 individuals in the sample could be distinguished into two groups of educational needs. The first group (70.7% of the participants in the clusters) consisted mainly by holders of 10-100 hives (89.9%), that produced 494.52 kg of honey/year. These beekeepers practiced beekeeping as a secondary occupation for 7.04 years, and 35.8% were higher education graduates. The 50.9% of the first cluster had attended a seminar on bee attacking. The second group concerned 29.3% of the participants in the clusters and consisted of the sample that are beekeepers as a main occupation, aged 45-54 years, owned 101-200 hives, produced 3,531.24 kg of honey/year, and had an experience of 16.48 years in beekeeping. 45.5% were graduates of higher education and 68.2% had attended a seminar on bee attacking.
In conclusion, while the second group shows a higher average score than the first on knowledge related to the protection of the hive from enemies and diseases, the group's knowledge regarding the sample is characterized in general as mediocre. Therefore, the need for continuing education and training programs for beekeepers is imperative.

Key words: *Apis mellifera*, beekeepers training.

Food preferences of Diptera species and its identification

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Species of the families Muscidae and Calliphoridae, except to being carriers of many diseases, contribute to the decomposition of organic matter by helping agricultural practice. In the present work, the preferences of *Musca domestica*, *Chrysomya albiceps* and *Calliphora vicina* on different nutrient substrates and temperatures was studied. The identification of the insects based on adult's morphological characteristics and DNA barcoding. For this, adults of the above insect were collected from different regions of Thessaloniki (Greece).

Three specimens were used for DNA barcoding analysis. Genomic DNA was extracted from whole samples using the PureLink™ Genomic DNA Mini kit. The *16S rRNA* and *COI* mitochondrial (mtDNA) genes were chosen as target for the analysis. The primers used were the following: SJerryF (5'- CAACATYTATTYT GATTYTTTGG-3') and SPatR (5'-GCACTAWTCTGCC ATATTAGA-3'), LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCGGGTGACCAAAAAATCA-3'), Forward (5'-TTA CGCTGTTATCCCTTA-3') and Reverse (5'-CGCCTGTTTATCAAAAA CAT-3') (*16S* gene. PCR was performed using the *Taq* 2X Master Mix. The purified PCR products were single-stranded sequenced, using BigDye Terminator v3.1 cycle sequencing methodology, on an ABI3500 Genetic Analyzer. The resulting sequences, after manual checking and editing in BioEdit v7.2.6,were compared with those available in GenBank.

The morphological identification and the amplification and sequencing were successful for all DNA samples. The three submitted sequences were identified as follows: *Musca domestica* (Diptera: Muscidae) (99,73% percentage of identity), *Chrysomya albiceps* (Diptera: Calliphoridae) (99,76% percentage of identity) and *Calliphora vicina* (Diptera: Calliphoridae) (99,84% percentage of identity).

The food preferences of the dipterans showed that *M. domestica* had a preference mainly for plant organic matter and secondarily for animal one.

C. albiceps chose to feed on decomposing animal mainly organic matter and secondarily on plant, while *C. vicina* was found to select mainly organic matter of animal origin and was active at lower temperatures.

Key words: *Musca domestica, Chrysomya albiceps, Calliphora vicina.*

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Special Session

The impact of Drosophila research in biomedical sciences

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The use of Drosophila a research model in biomedical research was introduced during the early years of the 19th century. A few decades later the first Noble prize related to Drosophila research was awarded to Thomas Hunt Morgan (The Nobel Prize in Physiology or Medicine – 1933) *"for his discoveries concerning the role played by the chromosome in heredity"*).

During the first half of the previous century Drosophila research was exclusively based on traditional genetic tools and approaches aiming to the elucidation of the heredity principles. Following the introduction and establishment of both state-of-the-art imaging modalities and advanced molecular techniques the biomedical research using Drosophila as a model organism has been rapidly expanded. Nowadays it embraces topics such as immunology, microbiology, metabolism, neurosciences, sleep, memory, behavioural, stem cell and cancer studies.

In early 2000s a landmark in Drosophila research was the first detailed sequencing of its full genome which enhanced the validation of Drosophila as an advanced research model by revealing the high homology with the human genome and the existence of a series of so far unknown biochemical pathways. As a result, Drosophila was introduced as a suitable model for the study of a series of human diseases including dementia diseases and inflammation bowel diseases (IBD)

In the current talk the latest experimental tools in Drosophila research will be highlighted followed by a demonstration of a series of in house publications related to the use of Drosophila as a model for the topics below: i) the study of the structure and composition of the gut microbiota of flies with various levels of activated gastrointestinal immunity, ii) the impact of the activated or supressed brain immunity into the lifespan, nutrients storage, hormonal profile and activity of adult flies, and iii) the use of *in vivo* and *ex vivo* Drosophila models for the recognition and following clearance of human pathogenic microorganisms.

In the last part the use of professional phagocytes from Drosophila larvae for the development of advanced correlative microscopy schemes will be discussed. For the correlation soft X-rays data from a tomograph connected directly to a nanoparticle accelerator (originating from Diamond Light Source, the UK's National Synchrotron) will be complimented with data from a bespoke cryo-Structured Illumination Microscope (cryo-SIM). The above correlative microscopy approach is used for the study of the phagocytosis of fungal species belonging to the Candida genus by circulated Drosophila phagocytes extracted from the haemolymph of 3rd instar larvae.

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