International Organization for Biological Control

15th IOBC-MRQA Workshop

DELIVERING ON THE INCREASING DEMAND FOR HIGH QUALITY INVERTEBRATES

Abstracts

Bologna, September 5-9, 2022

Prodi Room, San Giovanni in Monte Complex,
Piazza San Giovanni in Monte, 2, Bologna

KEYNOTE PRESENTATION

History, Purpose and Importance of IOBC, MRQA

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It became evident in the mid-1970s that insects being mass-reared for sterile insect technique and biological control often were not effective. Consequently, expensive autocidal control campaigns against the screwworm fly and Mediterranean fruit fly were failing, along with importation and augmentation biological control efforts. This problem was addressed by a coalition of international scientists who advocated that quality control be instituted as part of insect mass rearing systems. Eventually, in 1980, several of the scientists established the Working Group on Quality Control (WGQC), followed by the first WGQC workshop in 1982. The WGQC evolved into the Arthropod Mass Rearing and Quality Control (AMRQC) Working Group in 2003 and IOBC, MRQA ten years later. At that time, the mission of IOBC, MRQA had expanded to encompass all applications for invertebrate mass rearing. This expansion is exemplified by the program of this 15th workshop: mass rearing and evaluating biological control agents, insects for autocidal control, insects for food and feed, pollinators, and a range of other beneficial organisms. Quality assurance extends beyond quality control to emphasize the importance of accomplishing the purpose for the mass-produced beneficial organisms and satisfying customers who purchase the products. This requires that every mass production program have a quality assurance component. Since the science and practice of quality assurance for mass-produced beneficial organisms has become institutionalized, education and training in the established principles are necessary for existing and new applications. The pioneers who created this field never envisioned the need for quality assurance in so many diverse arthropod production systems or that arthropod mass rearing would evolve from government-funded programs to the private sector.

SESSION: Mass rearing and evaluating biological control agents for crop pests – 1
Moderator : P. De Clercq

Advances in *in vivo* production and application of entomopathogenic nematodes

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Entomopathogenic nematodes (EPNs) are potent biopesticides that are used to control a wide variety of economically important insect pests. Most EPNs are produced using in vitro methods. However, there are several companies that use in vivo methods. In this talk we will present some advanced methods for improving the efficiency of in vivo production. Additionally, some advancements on applying and formulating the nematodes will also be discussed.

Keywords: entomopathogenic nematode, production, pheromone

Liquid fermentation with focus on bacteria and EPN for a sustainable agriculture

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Brazil is the third largest agricultural producer in the world, but the first in the use of chemical pesticides. A major effort to replace pesticides with bio-inputs has been made since the 1970s, focusing mainly on the use of microorganisms to control pests, diseases and nematodes, in addition to other purposes. Fungi began to be used in the 1970s, reaching 8.000.000 ha treated per year today. Viruses began to be used in the 1980s, reaching 2.000.000 ha treated. Bacteria also began to be used in the 1970s, reaching 10.000.000 ha. The largest area with bacteria is promoted especially by the growing interest in the production of biological inputs locally on farms to attend their own needs and use, through simple fermentation techniques called on-farm production. This production takes place from the replication of commercial products

acquired in the market, or through pre-inoculum prepared and sold by specialized companies, which can also commercialize the infrastructure used for this type of production. Findings from researchers and professionals who work in agricultural regions indicate that the practice started in 2013 and intensified in at least 10 Brazilian states, for the growing of soy, corn, fruit, vegetables, sugarcane among other crops. Another microbial agent that have been used in Brazil are entomopathogenic nematodes (EPNs), more recently developed by the Instituto Biológico - Brazil in partnership with the United State Department of Agriculture - USA, to control soil pests. EPNs have been produced by a two-phase system involving firstly liquid fermentation, followed by solid sponge fermentation. However, much still needs to be done to improve the process. The treated area is still small, but it can grow substantially, mainly for the control of soil pests in sugarcane. *Supported by The São Paulo Research Foundation - FAPESP.

Keywords: Bacteria, entomopathogenic nematodes, local on farm production, sustainability, liquid fermentation

Persi+: A novel rearing technology of *Phytoseiulus persimilis*. Implications on predator's quality

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Following an intensive 5-year effort, BioBee has developed and launched Persi+. The predatory mite *Phytoseiulus persimilis* is mass reared on a revolutionary technology, first of its kind, which employs dead Astigmatic mite *Carpoglyphus lactis* as an alternative prey to spider mites, the traditional long lasting food for the predator. The novel technology has yielded another exciting feature, which is a slow release sachet, enabling for the first time ever a preventive introduction of *P. persimilis*. Since the Persi+ is a result of genetic breeding it was imperative to compare the latter's basic traits with those of the traditionally produced *P. persimilis*. Persi+ adult females were compared to the traditional ones in a series of tests on spider mites, using several quality parameters. The fecundity of Persi+ was about 25% higher than the traditionally reared predator, following the standard IOBC method for fecundity test of mass reared *P. persimilis*. In a prey reaching trial conducted on whole cucumber plants under greenhouse conditions, the Persi+ adult females reached the spider mite infested leaves quicker and in higher numbers compared to the traditional predators. In a spider mite control trial, the Persi+ achieved better and quicker control over the

pest compared to the spider mite reared *P. persimilis*. Slow release sachets of Persi+ that were deployed on cucumber plants 13 and 6 days prior to spider mite infestation, gave excellent control of the latter within 10 days following the spider mite occurrence on the plants. The above-mentioned parameters serve as quality control criteria to assure that no genetic drift is occurring in the Persi+ strain due to its non-conventional rearing method. We assume that the fact that adult females of Persi+ are continuously well fed whereas females of spider mite-reared *P. persimilis* are occasionally starved, gives the former a head start while first encountering spider mites on the plants

Keywords: *Phytoseiulus persimilis*, mass rearing, quality control, genetic breeding

Efficiency and health risk evaluation in mass rearing and release of phytoseiids in BC strategies

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The Phytoseiidae, predatory mites, are very important natural enemies used in augmentative biological pest control. A major crucial point in biological control is the discovery and availability of alternative food and/or prey allowing, with sustainable cost, both high populations of these agents and maintaining of their efficiency. To overcome food scarcity, it is important the provision of alternative food sources, as pollen and other factitious (non-natural) hosts offering opportunities as alternative food sources for predatory mite populations in rearing facilities and crops. As situation study, astigmatid mites used as alternative food for phytoseiids were considered as allowing high population levels of predator mites. To gain less time consuming and cheaper products approaches, however, two main aspects are to be considered in mass rearings of phytoseiids conducted on alternative prey/food. First, the quality control: a crucial matter is the evaluation of the maintainment of the real predatory efficiency of the mass reared phytoseiids on the target species. The consideration of the pest kill rate to rank phytoseiids as BCAs, based on the actual killing/predation effect they can determine, can return an effective indication of the potential of control of phytoseiids. Then, evaluation of rearing system includes whether astigmatid mites, largely used alternative food, can pose potential risk to human health because of their association with allergies: although astigmatid mites

are enabling mass production of predators at low cost they can be source of allergens and cross reactivity in exposed people that can persist and accumulate in the environment, and may cause respiratory problems in workers. Highly advisable is a review of monitoring of risk mitigation progress including chances for tracking identified risks, identifying new risks, and evaluating risk process effectiveness throughout the research

Keywords: mass rearing' alternative food, phytoseiids, astigmatid mites, kill rate, human allergy

SESSION: Mass rearing and evaluating biological control agents for crop pests – 2
Moderator: T. Groot

Keynote: Egg parasitoid studies in Brazil: utilization, challenges and future

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The biological control (BC) programs with parasitoids, are related to inter- or multidisciplinary research programs deeply linked to rearing techniques, especially for use in open fields, as the Brazilian case. These programs required broad bioecological studies that provided the basis for both mass rearing and transfer of the necessary technology to farmers. This has allowed Brazil to occupy a leadership position in biological control in open fields. As an example of a successful program will be presented the whole *Trichogramma* program. The *Trichogramma* program began with the identification of the wild species, for example as *Trichogramma galloi* Zucchi (Hym.: Trichogrammatidae), until the cost benefits ratio studies. Surely the *Trichogramma* factitious host studies also have a fundamental importance on the success of use, once mastering this mass-rearing allows the agent to be used/sold for a very competitive cost. Currently in Brazil, expectations are high that the egg parasitoid *Telenomus podisi* Ashmead (Hym.: Scelionidae) can be used to control *Euschistus heros* (F.) (Hem.: Pentatomidae), one of the key crop pests today. This expectation increases in recent years, once a lyophilized green bean-based diet for the host *E. heros*, was developed. Despite the goods results delivery by *T. podisi*, automation of rearing processes still, undoubtedly, provide a huge leap forward in this program, considering that although the 200,000 ha, treated seems to be a large area, it is still very small, compared to the 40 million ha of soybeans in Brazil. The last case to be presented is the *Telenomus remus* Nixon, 1937 (Hym.: Scelionidae), aiming to control *Spodoptera* spp. (Lep.: Noctuidae). Studies aiming to better understand the biology of the parasitoids are being developed. The objective is guaranteeing that they are reared properly without any genetic deterioration or selection of undesirable haplotypes, presenting all the characteristics that an efficient parasitoid must have

Keywords: augmentative biological control, sustainability, IPM

Beneficial insect production and implementation for mealybug biological control

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Mealybug biocontrol is one of the well known application around the world. Since 2004 Biyotar has mass production facility for *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* in Turkey. Annual beneficial production of Biyotar is 25 million *C. montrouzieri* and 45 million *L. dactylopii*. Our production system is based on mealybug production on potato sprouts. Approx. 300 tons potato are used annually. Potato sprouting quality is important for mealybug production which is important for beneficial insect quality too. This process is mainly arrangement of timing of sprouting and infestation of mealybug for prey or parasitoid. These beneficial insects are used for mealybug control mainly on Citrus but also on pomegranates, vineyard as an open field application; banana, ornamentals and vegetables as a cover crop application. As a case study, Citrus open field applications are going to be explained. After flowering season during May scouting starts in Citrus orchards. According to Citrus mealybug, *Planococcus citri*, stage, first *L. dactylopii*, later (when egg masses start) *C. montrouzieri* are relased. If mealybug populations are mixed with adults and small stages and egg masses, both *C. montrouzieri* and *L. dactylopii* are released together. The minimum dosage for annual plants is 10 *C. montrouzieri* and 10 *L. dactylopii* adults per tree. In case of Citrus variety such as grapefruits, starting dosage is 10 *C. montrouzieri* and 20 *L. dactylopii* adults per tree due to heavy mealybug infestation. When these beneficial insects are used to keep orchard under control, 17-21 days after release are necessary to see their activity. Usually one release is efficient for lemon, mandarin and orange mealybug infestations, only grapefruits have heavy infestation and need higher dosage even some times repeated dosages. Mealybug control without chemical residue problem is valuable application for Citrus production either for domestic consumption and/or exportation.

Keywords: *Cryptolaemus montrouzieri*, *Leptomastix dactylopii*, mealybug

The victories, struggles and defeats of rearing tortricids in South Africa

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Lepidopterans are amongst some of the most economically important insect pests of agriculture. In South Africa, three closely related species in the Grapholitini tribe in Tortricidae are important pests in the fruit and nut industries, namely, *Thaumatotibia leucotreta*, *Cryptophlebia peltastica* and *Thaumatotibia batrachopa*. To conduct research towards improved control of these species, laboratory cultures should be established. This study focuses on the establishment of cultures of these species in the laboratory and the struggles and successes experienced during the process. *Thaumatotibia leucotreta* was successfully established in the laboratory and continues to be reared for commercial and research purposes. The protocols for rearing of this culture served as a blueprint for rearing of the other two species. Establishment of the *C. peltastica* culture was not as easy, but after a few attempts was successful, and is now being used for baculovirus production and for research. However, to date, the successful establishment of a *T. batrachopa* culture has proved elusive. This is despite numerous attempts having been made by two research institutions. A key factor that was observed during these attempts was that little or no mating occurred, regardless of changes to the protocol. Thus, further research needs to focus on a mating cue for *T. batrachopa* in order to establish a laboratory culture.

Keywords: laboratory rearing, Tortricidae, artificial diet, *Thaumatotibia batrachopa*, mating cues

Control potential of *Spodoptera frugiperda* by *Trichogramma pretiosum* and *Telenomus remus* isolated or associated in laboratory and semi-field conditions

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Spodoptera frugiperda (J.E Smith, 1979) is a pest originally from the Americas and has been invasive in Africa, Asia, and Oceania. Due its polyphagia and wide distribution *S. frugiperda* has threatening food security worldwide. Currently the methods used to control *S. frugiperda* are insecticides and Bt transgenic plants. However, reports of *S.*

frugiperda populations resistant to both methods are common. The use of egg parasitoids, such as *Telenomus remus* Nixon, 1937 and *Trichogramma* spp. in an augmentative biological control program, could be an efficient control strategy. In this work we selected from 3 different strains of *Trichogramma* the most effective one for the control of *S. frugiperda*; an isoline of *T. remus* was selected to avoid the loss of flight capacity and parasitism efficiency due to laboratory rearing conditions and studies were conducted to evaluate the effects of biotic factors such as temperature and relativity humidity (UR) in the flight capacity of *T. remus* isoline. From the data obtained in these studies, release tests were conducted into semi-field conditions to access the potential control of *S. frugiperda* by *T. remus* isolines and *Trichogramma* spp. used isolated or in association. The *Trichogramma* strain selected was the *pretiosum* AR strain collected in *S. frugiperda* eggs into the field on Piracicaba, SP, Brazil; the selection of an isoline was able to avoid the loss of flight capacity in *T. remus* population reared in laboratory without affecting its parasitism efficiency. *T. remus* isoline have shown better flight capacity at temperature of 25 and 30°C and UR of 90%. In the release test the best control method was the release of 14 female *T. remus* isoline per 100 eggs of *S. frugiperda*. The use of *T. remus* isoline and *T. pretiosum* AR associated did not bring any significant increase in the control potential of *S. frugiperda*. In this way our results shown that the use of *T. remus* isoline alone have high potential control of *S. frugiperda*.

Keywords: augmentative biological control, isolines, fall armyworm, egg parasitoids, quality control

Fecundity and predation activity of *Exochomus quadripustulatus* on *Toumeyella parvicornis*

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The pine tortoise scale *Toumeyella parvicornis* (Cockerell, 1897) is an injurious insect causing several damages to the Italian stone pine (*Pinus pinea* L.). This pest is an alien species for the Italian and European areas: originated from North America, was firstly reported in various Regions of Italy (2014) and in the Southern coastal area of France (2022). Given the high susceptibility of stone pines to the attacks and the diffusion that the host plant has throughout the Italian peninsula, controlling *T. parvicornis* infestations is becoming a must. This insect, in fact, significantly debilitates the host plants until the death. Stone pines are key element in many urban parks and infested

trees may represent a risk for the citizens' health. To date there is not a defined control strategy, but manyfold methodologies and products are still under evaluation. An aspect that is arousing the interest of the scientific community is the biological control carried out by natural enemies, such as predators or parasitoids. In Europe, to date, there are no indications about predators and/or parasitoids that could be effective. This work aims to fill this gap of knowledge: we have planned different experiments about predation activity, fecundity and longevity of some predator species using *T. parvicornis* as a prey. More specifically, we focused on a predator belonging to Coccinellidae: the ladybird species *Exochomus quadripustulatus* (Linnaeus, 1758) (Hemiptera: Coccoidea). Under controlled laboratory conditions of 25±2° C, 65±10% r.h. and a photoperiod of 16L:8D we have evaluated the predation behaviour, the fecundity and the longevity of the larval and adult life stages of *E. quadripustulatus* active on *T. parvicornis* preys. Measurements on the ladybird individuals consisted in reporting, every 24-48h, the weight, the size, and the number of eggs laid by the individuals. The outputs of this research lay the foundations of a further trial in a semi-field and/or open field context

Keywords: pine tortoise scale, stone pine, pine ladybirds beetle, biocontrol, IPM

Can rearing *Sclerodermus brevicornis* (Hymenoptera: Bethylidae) on a factitious host affect behavior?

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Wasps in the genus *Sclerodermus* are quasi-social ectoparasitoids that typically attack coleopteran larvae that live inside the wood. Interest in these species is increasing as they are used in programs to control wood boring longhorn beetles of economic importance in China and could also be used in Europe. However, the mass rearing systems are affected by the host used to produce new offspring and rearing *Sclerodermus* on its natural hosts is time consuming and physically demanding. There is thus a need for factitious hosts with lower production costs and that are easier to rear. The present research focuses on *Sclerodermus brevicornis* which was found in association with *Psacothea hilaris hilaris* and can be reared on both this longhorn beetle and on a factitious lepidopteran host, *Corcyra cephalonica*. As it is known that the biology of natural enemies can be influenced by the host they emerge from, and

that the behaviour of *S. brevicornis* is quite complex due to its degree of sociality (multiple females cooperate to paralyze the host and produce offspring communally), we explored whether and how performance and behavioural traits are influenced by the rearing host. Choice tests were set up using parasitoids with different origins (the host species they emerged from) when offered different host species. Aspects of parasitoid performance, the timing of development, movements between hosts and aggregation on hosts were evaluated. Significant effects were not detected in timing and performance but were found in the number of movements and in aggregation behaviour. As a preliminary conclusion, this research indicates that the overall success of *S. brevicornis* is not influenced by the host of origin, even if some aspects of its relatively complex social behaviours may be.

Keywords: quasi social, choice test, mass rearing, biological control, performance

POSTER SESSION
Moderator: R. Buitenhuis

Rearing of *Aridelus rufotestaceus*, a potential biocontrol agent of stinkbugs

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Aridelus rufotestaceus Tobias (Hymenoptera: Braconidae) is a solitary thelytokous parasitoid, probably originating from Asia and now recorded in different countries, including Italy. It was first detected in Emilia-Romagna region (northern Italy) in 2018, in association with *Nezara viridula* (L.) (Hemiptera: Pentatomidae). A laboratory colony was started with the aim of studying the parasitoid biology and potential as biocontrol agent of stinkbugs. A colony of *N. viridula* was maintained at the Department of Agricultural and Food Sciences (University of Bologna) since 2015. Nymphs and adults were separately kept in plastic boxes (18x10x36 cm) at 25±1°C, 60±5% rh, 16L:8D photoperiod and fed on celery, soybean and sunflower seeds. Oviposition occurred on dry sheets of crumpled paper. The parasitoid colony was started by exposing 3rd instar nymphs to field-collected wasps in a Plexiglas cage (20x20x20 cm), in 10:1 ratio (hosts to parasitoids) for 2 hs. After exposure, nymphs were removed from cages, placed in plastic boxes and maintained as described above. Despite the high number of healthy and active larvae emerged from hosts, the adult yields were very low (about 2.5%), mainly due to larval desiccation. Considerable increased adult yields (up to 36%) were obtained by removing the exposed nymphs from boxes before parasitoid pupation (about 20 days from exposure). Nymphs were moved to new boxes with the bottom covered with wet paper, where the parasitoid larvae could survive, spin their cocoon and pupate. To reduce mold risk, the exposed nymphs were fed on green beans in replacement of celery. Moreover, parasitoid adult yields were influenced by host age at parasitization and increased up to 64% by exposing 2nd instar (instead of 3rd instar) nymphs to female wasps. Following these observations, a continuous laboratory colony of *A. rufotestaceus* has been established at DISTAL and is currently utilized for research on different topics, including the parasitoid capability to attack other stinkbug species

Keywords: *Nezara viridula*, parasitoid, biological control, mass rearing

Pachycrepoideus vindemiae for biological control of *Piophila casei* in ham factories

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Phiophila casei (Linnaeus) (Diptera: Piophilidae), is a parasite responsible for a critical hygiene situation in ham factories. The adults are easy vectors for various bacteria, including *Clostridium botulinum*. The ingestion of the larvae, which resist the action of gastric juices, can cause intestinal myiasis due to digestive enzymes. I report the results of laboratory experiments designed to evaluate *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) as pupal parasitoid of *P. casei*. The importance of the pupal parasitoid is due to the characteristic of the fly of pupating in the ground, therefore hymenopterans would not be in contact with foodstuffs. The use of natural enemies, widely used and tested in agriculture, is still little explored in foodstuffs industries, because of possible contamination with food. We prepared 10 Petri dishes with 10 pupae of the fly, aged 1 day, and one parasitoid, less than 3 days old, kept inside for 48 hours with water and honey. One plate was prepared without parasitoids and used as a control. All insect rearing was maintained at 25 °C ± 1 °C, 50-60% RH, photoperiod 16L: 8D. *P. vindemiae* demonstrated a parasitization rate of 23% and pupal mortality of 57%. The development time of the parasitoids was 24 days, and the survival with honey and water was 26 days. The development time of the offspring was also calculated, 23 days, as well as the lifespan of these,15 days. These parameters could be adjusted when rearing these parasitoids for specific aims such as the production of individuals for release. The deepening of this data allows an appropriate choice between inundative releases, where control is determined by the wasps released, or inoculative approach, where control action is determined by the progeny then developed on *P. casei*.

Keyword: Diptera, parasitoids, parasites, foodstuffs

Insects as food for humans: a potential animal protein source in space exploration missions?

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One of the perspective uses of insects as human food may be the utilization of selected species as animal protein source for crewmembers in long-duration space explorations. In these future missions it may be difficult to provide all resources at the beginning (and bring wastes back to earth), as it is currently done. Therefore, food,

including animal protein sources, needs to be produced on board. To find solutions, the MELiSSA (Micro Ecological Life Support System Alternative) project, has been carried out for about 30 years. The project, which is coordinated by the European Space Agency and includes different partners, aims to create an artificially closed ecosystem to recycle wastes to oxygen, water and food with only input of energy. The MELiSSA "loop" is divided in four compartments, one of which includes the sub-compartment of Higher Plants (HPs). To the best of our knowledge, however, little attention is currently given to animal protein sources. The on-board production of insects, to be used for this purpose, may, therefore, represent an interesting field of study. Insect rearing may be easier to be developed in an artificially closed ecosystem than conventional livestock production, because it requires less space, water, labor an implies lower gas emission. As food for insects, plant residues could be used, and insect residuals could in turn be re-cycled as fertilizers. To make this possibility feasible, research should, however, address different issues (first of all, which species of edible insects may be suitable to be cultured in an artificially closed ecosystem, on plant residues, and how these insects behave in microgravity). The results achieved may be of interest also to gain novel information about insect behavior and production, to be applied in different contexts.

Keywords: animal protein source; long-duration space explorations; insect rearing

Importance of rearing alternative insect pollinators to improve environmental risk assessments

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Before approval or renewal, pesticides must be evaluated to identify potential risks for the environment and non-target organisms, including pollinators. Insect pollinators represent a large functional group with about 250,000 species that includes bees, beetles, flies, ants, moths, butterflies and wasps. Despite this large variability, the current risk assessment procedure is focused on a single species, the honey bee *Apis mellifera*, as a model for all other pollinators. However, due to differences in life history traits and potential pesticide sensitivity it is urgent to add other pollinator species in the risk assessment. In the current study we assessed the acute contact toxicity test in three dipteran species, *Exorista larvarum*, *Sphaerophoria rueppellii* and *Eristalinus aeneus*, with double ecosystem services. These three species

are potential alternative managed pollinators of crops during their adult stage and provide other important ecosystem services during larval stages, such as biological control of insect pests and nutrient cycling. For all three species, the individuals were kept in standard conditions with the aim to calculate the LD 50 (lethal dose 50) and female fecundity reduction following a topical exposure to Confidor® (active ingredient imidacloprid). Our results showed high differences of toxicity among the species and the calculated LD 50 decreased in the following order: *E. aeneus*, *E. larvarum* and *S. rueppellii*. In addition, sub-lethal effects were observed in all species. Our results highlight the importance to include other insect pollinators in the environmental risk assessment. However, since rearing conditions may affect the quality of the tested populations, standard rearing methods need to be developed in order to obtain comparable results in pesticide sensitivity between different species.
Keyword: pollinators, ecotoxicology, risk assessment

Indigenous natural enemies in Slovenia with special emphasis on species suitable for mass rearing

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In Slovenia, only indigenous natural enemies that are on the EPPO positive list of safe biological control agents can be used in agricultural production to control plant pests. The list of indigenous species of organisms for biological control, which is an important part of the Rules on biological control (Official Gazette of the Republic of Slovenia, No. 45/2006), currently includes a total of 32 species of beneficial insects, mites and entomopathogenic nematodes. Compared to the first list from 2006, today the number of beneficial species that Slovenian growers of food and ornamental plants can use in everyday practice is twice as high. This is the result of intensive professional and research work in the field of investigation: the occurrence and distribution of natural enemies in Slovenia, which has been systematically supported by the state for many years. In addition to the species of natural enemies, which are an integral part of the EPPO positive list of safe biological control agents and can be included in the List of indigenous species of organisms for biological control by a special procedure, in professional and research work we can also find other species of natural enemies, which are not included on the EPPO positive list (yet). In this poster, we will present the latter, with special emphasis on species that, in our

opinion, have high biological potential and will be suitable in the future for mass rearing and use in augmentative biological control.

Keywords: Indigenous natural enemies, Slovenia, lesser-known species, mass rearing

Rearing and infecting *Anopheles stephensi* mosquitos with *Plasmodium berghei* for malaria challenges

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At the Jenner Institute Insectary, we rear a colony of *Anopheles stephensi* mosquitos which can subsequently be used to test vaccines against malaria, the disease caused by the plasmodium parasites which are transmitted through the bites of female *Anopheles* mosquitos. In our case, the parasite we use most often is the *Plasmodium berghei* parasite, a strain only effective to rodents. Sporozoites, which are the infective agent, are passed to a human or animal via female mosquito saliva upon biting, as they live in the salivary gland. The reason they are passed through is because mosquitos before feeding inject some of their saliva as an anti-coagulant to prevent blood clotting, which allows them to feed on the blood. For a malaria challenge study, researchers will generally prime mice with their vaccine they are looking to test in their chosen regimen, and then infect sporozoites via an intravenous injection and then monitored for parasitaemia (% of infected blood cells) daily

Keywords: mosquitos, malaria, rearing, *Anopheles*, *Plasmodium*

New native biocontrol agents for Canada: opportunities and challenges

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Generalist predators are shown to stabilize and strengthen greenhouse IPM programs as they provide control of multiple pest species. In addition, they can be established early and persist in a crop in a preventative strategy. Regulations in Canada and the US prevent the importation of non-native generalist species due to the risk they may pose to native ecosystems. This created the opportunity to bring new native North

American biocontrol agents to market. At Vineland, we developed the predatory mite *Anystis baccarum* and the damsel bug *Nabis americoferus*, while the Université de Québec à Montréal worked on the syrphid fly, *Eupeodes americanus*. Both *A. baccarum* and *E. americanus* are now commercially available to Canadian growers through the company Applied Bio-nomics. Despite this early success, several challenges still remain including availability outside of Canada.

Keywords: *Anystis baccarum*, *Nabis americoferus*, *Eupeodes americanus*, commercialization

SESSION: Mass rearing for SIT and other autocidal pest control
Moderator: N.C. Leppla

Keynote: Ongoing programs and recent advancements in mass rearing and quality assurance for SIT

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The sterile insect technique (SIT) is an integrated pest management strategy based on mass-rearing and releasing high quality insects. The technique was developed initially to control the screwworm fly, tephritid fruit flies, such as the Mediterranean fruit fly, and mosquitoes. The tsetse fly, other flies, a few moths, a weevil, and a tick have been targeted more recently. According to the International Atomic Energy Agency (IAEA), more than 35 SIT programs are being conducted currently in about 31 countries. A recent advancement in SIT, or technically "release of insects carrying dominant lethals" (RIDL), by Oxitec, a biotechnology company, involves genetic methods to produce male *Aedes aegypti* mosquitoes that transmit a self-limiting gene. The gene prevents female mosquito offspring from surviving to the adult stage, whereas males survive for multiple generations. A marker gene also is inserted into the males that produces a fluorescent protein, enabling the number of marked and wild larvae to be tracked in the field. Test releases have been made in Brazil, Panama, the Cayman Islands, and Florida Keys. Another new technique involves infecting male *A. aegypti* or *A. albopictus* with an additional *Wolbachia* bacterium and releasing the mosquitoes to mate with wild females. Two different effects can be induced: hindered human pathogen development or cytoplasmic incompatibility (CI). With CI, the infected wild females oviposit but the eggs are not viable. Product quality assurance includes consistent yield and the pupa size needed for sexing to release only the smaller males. *Wolbachia*-infected *A. aegypti* have been used successfully for SIT in Singapore, Thailand, Mexico, Australia, and California. Gene-drive technologies are currently being laboratory-tested that use the CRISPR/CAS9 system to create persistent deleterious genetic modifications in mosquitoes. Robust rearing and quality assurance procedures are essential for all of these SIT advancements.

The sterile insect technique against mosquitoes: an update

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The increasing concern related to the negative effects of insecticides, together with the widespread resistance developed by target vector populations against the most used active ingredients, are stimulating the investigation on possible new control methods to be deployed against mosquitoes. Among the proposed possibilities, the genetic control-based methods are finding large interest for their high species specificity assuring low environmental and public health impact. The sterile insect technique (SIT) is a well-established technology, included in the biological control methods, applied area-wide since decades to suppress many agricultural pest species. More recently the International Atomic Energy Agency, following the strong demand by member states, has started a program to develop SIT against mosquito vectors of diseases. Mass rearing methods and technologies are under development to standardize the production process while increasing male productivity by facility space; sex sorting methods exploiting dimorphism and protandry are currently used, why genetic sexing systems starting from useful mutations are also investigated; optimal male sub-sterilization level is defined following cost-benefit evaluation; ground and aerial release systems are under testing to achieve optimal sterile males distribution without damaging them; methods to measure the entomological and the epidemiological efficacy are made available. Possible quality control procedures are also proposed to assist the production of vigorous sterile males. While SIT efficacy in mosquito suppression has been already demonstrated in different contexts, the public health outcome, such as the reduction of disease incidence rate, requires more careful analysis on large areas. An important issue on the way of area-wide SIT application on mosquitoes remains the cost which should be reduced to make the technology affordable and sustainable.

Keywords: SIT, mosquito control, genetic control, sex sorting, disease control

Updates and progress on the sterile insect technique against *Aedes albopictus* in Italy

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The current *Aedes albopictus* vector control strategies mainly based on the use of insecticide are insufficient to maintain the mosquito population density below the epidemic risk level or nuisance threshold. The possible integration of the sterile insect technique (SIT) into an area-wide pest management approach is under development and testing to achieve effective suppression. SIT is a biologically-based method of pest control that relies on inundative releases of sterile males to reduce the field population reproduction rate. The continuous release of sterile males could strongly reduce, or even suppress the wild population, especially in those areas where *Ae. albopictus* is isolated or represents a newly imported invasive species. Mass production must be constantly ensured to guarantee the supply of males throughout the release season and encompass several steps for a cost-effective implementation of this method. Larval and adult rearing is essential since the adults provide eggs to sustain the larval colony and the larval rearing supplies males, needful for SIT program, but also females necessary to maintain the adult colony. Sex separation is also an important aspect, indeed, as females' presence inside the sterile males should be reduced to a minimum. The sex sorting process must ensure an acceptable level of females' residual presence but also good male productivity and female recovery to sustain the breeding lines. Nowadays, the available sexing methods are only partially mechanized and are swayed by several problems. Even if they are proficient to provide good results, they are not efficient to afford a fully effective separation without any female contamination. The economical sustainability of SIT programs is then strictly connected to the efficiency of mass rearing. The work, here presented, aims to investigate new methods for efficient and reliable sex separation of *Ae. albopictus* at different stages.

Keywords: SIT, mass rearing, *Aedes albopictus*, sex separation

Developing an automatic mosquito pupae sex sorter for mass production of incompatible males in support of area-wide release for vector control

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Effective mosquito population suppression has been repeatedly demonstrated in field trials through release of male mosquitos to induce sterile mating with wild females using incompatible, sterile insect techniques or their combination (IIT, SIT or combined IIT-SIT). However, it requires for a highly efficient and scalable approach for sex separation of mass-reared mosquitoes to prevent or minimize the risk of the accidental release of females which can lead to population replacement or bite nuisance. Here, we report successful development of an automatic mosquito pupae sex sorter that can effectively separate males from females to mass produce incompatible males for population suppression of *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus*. The production capacity of the automatic sex sorter increases by more than 16 times as compared to the manual sex separation using Fay-Morlan sorter. With ~ 0.5% female contamination, the produced males have high flight ability and great mating performance. The field trial shows that the release of *Ae. albopictus* males produced through the automatic sex sorter causes successful population suppression. These results indicate that the automatic sex sorter has the potential to facilitate mosquito mass rearing and thus deployment of IIT or SIT for area-wide management of mosquito vectors for disease control

Keywords: mosquito control, sex separation, mass rearing, quality control, IIT, SIT, field trial

SESSION: Mass rearing insects for food and feed
Moderators: L. Pisa, M.L. Dindo

Keynote: The basics of edible insect rearing: Handbook for the production chain

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Looking for a handbook on how to design and upscale edible insect rearing? This easy to read handbook describes the basic knowledge and a detailed step-by-step plan for rearing edible insects. The book presents information essential for further development of the entire insect chain and contains many practical recommendations for the start/setup of professional insect rearing. The reader is first submerged into the fascinating world of edible insects such as black soldier fly, housefly, mealworm, cricket, and locust. A concise overview is provided to get more familiar with the biology and physiology of edible insects. Based on this basic knowledge the book focuses on design principles for large scale insect production, facility design, engineering, insect production management, management of unwanted organisms, economics, and aspects related to the legal framework. The chief editor, co-editors and lead authors of the chapters are from Belgium, Denmark and The Netherlands. These countries have a tradition of professional insect rearing. Reading this handbook will contribute to a successful entrepreneurship in edible insect rearing. This handbook also contains very interesting teaching materials for secondary, higher, and university education.

Keywords: edible insects, insect rearing, insect chain, insect production

Influence of the organic fraction of municipal solid waste on black soldier fly larvae growth and bioconversion

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The generation of organic fraction of municipal solid waste (OFMSW) in Italy amounts to approximately 6,071,512 Mg y-1. Due to its composition, it still represents a valuable material and new strategies for its valorization are needed. Bioconversion by insects has been shown to be a new valuable tool for manage and valorize low valuable substrates recycling nutrients in a circular economy perspective. This work considered the suitability of OFMSW as growing substrate for black soldier fly (BSF) larvae assessing the impact of variation of OFMSW on larval growth and bioconversion ability. OFMSW was collected from two plants over four different seasons and tested as it was (Unpulp) or pulped (Pulp). Experimental trials were conducted with groups of 500 larvae and four replicates were set up. The following parameters were observed: larval growth and survival, final larval biomass yielded, substrate reduction (SR), waste reduction index (WRI) and efficiency of conversion of ingested food (ECI). Data were analyzed with a two-way ANOVA. The results confirmed high survival and growth rates of BSF larvae fed with OFMSW. Survival was high in all tested samples (mean >95%), and the larval growth occurred within 12 days (mean of 9.1±0.1 days). Moreover, no significant differences were found between "pulp" and "unpulp" treatment for larval survival and developmental time. Seasonality did not significantly influence larval survival and developmental time, except for one case. The final larval yield (DM) varied from 9.7 to 40.0g, with a mean 24.7±0.9g. In some cases, pulp treatment had a positive effect compared to unpulped for the final larval yield and the ECI values. WRI did not show differences between pulp and unpulp but some seasonal variabilities were registered. SR presented a mean of 55.5±1.5 %. OFMSW is a suitable substrate for BSF larvae and no clear effects due to season, waste plant or texture were found, although a certain variability was observed.

Keywords: *Hermetia illucens*, larval biomass, OFMSW, seasonality, OFMSW texture

Effect of temperature on storage of the housefly, *Musca domestica*, for mass production

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The mass rearing of the common housefly (*Musca domestica*) for biodegradation and feed production is steadily increasing. For effective and efficient production of housefly on a larger scale, storage of various life stages is beneficial. We investigated the effect of chilling temperatures on the hatchability and survival of housefly eggs and larvae. We will provide results on hatchability of eggs that were exposed to 5, 10 and 25˚C for 1 and 3 days. We will provide results on larval weight, pupation rate and adult emergence of early, mid and late third instar larvae that were exposed to 10, 12, 15, 18 and 25˚C until they developed to adult flies. We will discuss the effect of low temperature for storage of houseflies and provide guidelines for best practices of mass rearing of houseflies.

Keywords: *Musca domestica*, chilling temperature, hatchability, pupation rate, larval weight

Dose-response effects of starch in chicken excreta on housefly larval performance and bioconversion

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Housefly (*Musca domestica* L.) larvae can convert animal manures into valuable protein and fat but little is known about their nutritional requirements. Larval performance and bioconversion might be limited by available energy and competition with microbiota. In this study we assessed the effects of differential starch content in unsterilized (UE) and heat sterilized chicken excreta (SE) on larval performance and bioconversion. Gelatinized corn starch was added to excreta to construct substrates ranging from 0 to 50% starch based on substrate dry mass. Substrates were inoculated with housefly eggs (8/g wet substrate) and larvae harvested by floatation after 5 days. Individual larval mass increased from 12±0.2 (UE) and 13±0.4 (SE) mg at 0% starch up to 18±0.4 (UE) and 18±0.5 (SE) mg at 15% starch and decreased to 5±0.4 (UE and SE) mg in 50% starch. Highest total larva wet yield in UE was 39±2.3 g at 10% starch, 43±2.8 g in SE at 15% starch, decreasing to a minimum of 3±1.5 g (UE) and 4±1.3 g (SE) in 50% starch. The decrease in yield for increasing starch content was faster in UE

compared to SE. Larval survival was highest in 0% starch, 80±8% (UE) and 72±13 % (SE), decreasing to 14±7% (UE) and 18±3% (SE) in 50% starch. Dry matter bioconversion increased from 4±0.1% (UE) and 4±0.3% (SE) in 0% starch to 6±0.2 in UE with 10% starch and 7±0.3 in SE with 15% starch, decreasing to 0.2±0.0% (UE) and 0.6±0.2% (SE) in 50% starch. Highest nitrogen bioconversion was 15±0.5% in UE with 10% starch and 15±0.5% in SE with 15% starch, decreasing for higher starch content. These optimal starch inclusions to UE and SE increased nitrogen bioconversion with 40 and 100% respectively, compared to pure excreta. Pronounced effects of starch on pH and ammonia content were also observed. The results indicate that adding easily digestible energy to chicken excreta can substantially increase larval yield and nitrogen bioconversion while reducing ammonia content in the substrate residue.

Keywords: housefly larvae manure bioconversion

Impact of starving time on the fitness of Black Soldier Fly (Diptera: Stratiomydae) neonates

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The mass production of *Hermetia illucens* (Diptera: Stratiomyidae) (BSF), for use as a composting agent, waste management and alternative protein source has gained attention in different regions of the globe. Within the production cycle, the fattening stage of the larvae is the one that most demands food and draws the attention of the producers of this insect, since the prepupa is the final product with the highest commercial value. However, the success of this stage is extremely related to the fitness of the neonates that will be part of the fattening process. Newly hatched larvae (neonates) represent the most sensitive phase of the BSF biological cycle, and require great efforts to understand, within the management, how much the lack of food at the right time can interfere with its development and performance. Therefore, this study aimed to evaluate the biological characteristics (final weight and viability) of neonates in different starving periods. The experiment consisted of 3 treatments and 10 repetitions, as follows: < 24 hours (control), < 48 hours and <72 hours. The effect of starving time on the viability of neonates was analyzed by fitting a linearly generalized model, assuming a binomial error, and multiple comparisons among treatments were performed by obtaining 95% confidence intervals. The effects of starving periods on weight of neonates was analyzed by ANOVA. The results found in this study indicated that the weight and viability of neonates starved for 24 and 48

hours do not differ, but both differ significantly ($p<0.05$) from those starved for 72 hours. Understanding how stress due to food and nutrient restriction biologically interferes with this insect is essential to meet the daily needs of its mass production, since it can change the neonates collection routine or even interfere with the estimation of viable neonates that will be transferred to the fattening stage, and thus contribute to the successful establishment of new production systems.

Keywords: *Hermetia illucens*, nursery, fasting, performance, mass production

Special session on the book "Mass Production of Beneficial Organisms"
Moderators: M.L. Dindo, P. De Clercq

Mass Production of Beneficial Organisms, Second Edition: a synopsis

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The challenges of mass-producing beneficial organisms, particularly arthropods and pathogens for biological control, are addressed in this book. Production technologies for beneficial organisms often are based on systems originally developed to mass produce insects for pest management, but they have improved with advances in related science and technology. The systems created by these innovations have many comparable production processes, although each is unique to the biology of the species being mass produced. The new methods and materials incorporated into a production system for one species often can be adapted for use with another, thereby advancing the entire field of mass producing beneficial organisms. Thus, the primary purpose of this book was to assemble examples of production systems for arthropods, pathogens, and other beneficial organisms that can be compared and adapted to develop efficient mass production systems. The book consists of three sections: I) production of arthropod biocontrol agents; II) production of entomopathogens, and III) production of invertebrates for food and pollination. In the second edition, new material has been included in section III dealing with the new emerging field of insect production for food and feed. The potential of insects as animal feed is particularly discussed as ingredients for poultry and aquaculture feeds. Also three new chapters have been included in section I dealing with hymenopteran parasitoids, mite predators, and insects for biological control of weeds.

Keywords: rearing, culturing, biological control, insects as food, pollination

Mass production of predatory mites: state of the art and future challenges

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Predatory mites, and phytoseiids in particular, are among the most important biological control agents used in integrated pest management. The ability to mass-produce these predators at high density on inexpensive food sources is a key driver of their success. In this presentation, we provide an overview of the rearing systems currently in use by biocontrol producers. Moreover, we discuss the biotic and abiotic challenges that are associated with the rearing systems. Finally, we highlight possibilities for further improvement of the efficiency of mass production of predatory mites.

Keywords: mass-rearing, Phytoseiidae, biocontrol, factitious prey, artificial diets

What's new in the mass production of heteropteran predators?

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The presentation will highlight several key aspects of rearing procedures for predatory bugs. The focus will be on factitious and artificial foods used for the mass production of heteropteran biological control agents. Whereas several types of factitious foods are routinely used for the mass rearing of heteropteran predators, the adoption of artificial diets in mass production systems has remained negligible. Attention will also be given to the implications of zoophytophagy for production of predatory bugs. The use of plants and plant materials as sources of water and supplementary nutrients, and as living and oviposition substrates will be discussed, as well as the potential of alternative substrates. The main gaps in our ability to produce heteropteran predators will be identified, as well as new technologies that may assist in advancing the practice of rearing high quality predatory bugs.

Keywords: mass production, Heteroptera, facititious prey, artificial diets, predators

Production of dipteran parasitoids

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Tachinidae and other dipteran families (Sarcophagidae, Phoridae, Cryptochaetidae and Bombyliidae) include many parasitoid species of interest for applied biocontrol and, consequently, rearing. Yet, their role as beneficial insects is often underestimated and, to date, "mass" production has been performed for only a few species. With the purpose to bring to light this group of biological control agents, examples of species successfully used in biocontrol strategies are presented in our book chapter, as well as the most important characteristics of their biology relevant for rearing. Different aspects of the in vivo and in vitro techniques of these entomophagous insects are illustrated, e.g., host type (natural vs factitious) and age, infestation mode, abiotic conditions, parasitoid nutritional needs, and continuous in vitro culture. Sterilization and antimicrobial agents for diets/media, adult maintenance, quality control, storage and shipment procedures are also discussed. On all topics, emphasis is especially given to Tachinidae (the largest and most important family of non-hymenopteran parasitoids), mainly for research performed and goals achieved in the last decade. Some perspectives are finally presented with the aim of stimulating new ideas and new research efforts, even addressed with novel approaches.

Keywords: biological control; *in vivo* rearing; *in vitro* rearing; quality control; Tachinidae

Concepts and methods of quality assurance for mass-reared parasitoids and predators

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This chapter describes the principles of quality assurance for mass-produced arthropods, especially as they apply to producing parasitoids and predators. Quality assurance encompasses every aspect of arthropod mass production and utilization for augmentative biological control. Quality assurance for mass-produced arthropods was created to improve the reliability of producing and using products that meet required specifications and standards. The nucleus of a quality assurance program for

mass-reared arthropods is based on total quality control developed for industrial management, including the production capability and associated production, process and product quality control. The application of quality assurance principles and methods can accelerate the market for commercial natural enemies and enable increased growth of the industry.

Keywords: quality assurance, mass-reared arthropods

Insects as feed for aquaculture: a concrete solution to solve the worldwide protein hunger

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The use of insect-derived products in animal nutrition attracts great attention and shows a great opportunity for meeting the increasing feed raw materials demand. Not only the protein fraction is of interest, but also fat and bioactive compounds present in insects. In the aquaculture sector, the interest about the use of insects as feed pushed the European Union to allow, in 2017, the inclusion of insect-derived protein from seven insect species in aquafeeds. A synthesis of the available literature about the dietary use of insect protein, fat and bioactive compounds in freshwater and marine fish species with a special focus on fish performance, immune response, oxidative status, gut microbiota and gut morphology is reported. On the other hand, the challenges to be solved to allow a high inclusion of these products in aquafeeds are highlighted as well. Insect-derived products have a great potential to mitigate the shortage of conventional protein and lipid source in aquafeeds. By using a correct balance of nutrients, the partial or total replacement of fish meal or fish oil has already been achieved in some fish species without compromising the growth or health. For other species, on the other hand, the situation seems to be more complex, and it is not clear yet if this depends on dietary nutrient imbalances or on factors intrinsic to the insect meal. Moreover, the current product availability, consistency and price result in negligible use of these products. The role of scientific research in conjunction with the insect production industry and legislator remains essential in order to allow the full development of the sector.

Keywords: black soldier fly, fish gut morphology; fish health; fish microbiota; yellow mealworm

SESSION: Mass rearing and evaluating pollinators
Moderator: G. Burgio

Keynote: Quality control in populations of solitary bees and dipterans used in ecotoxicological studies

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Global agriculture is becoming increasingly pollinator-dependent. At the same time, agricultural intensification is considered one of the main drivers of pollinator declines. The use of pesticides affects pollinator populations and compromises pollination services. Most ecotoxicological studies use honey bees, *Apis mellifera* L., and bumblebees, *Bombus* spp., as species models. However, different pollinator species differ in sensitivity to pesticides and in levels and routes of exposure. For this reason, it is necessary to develop standard pesticide risk assessment protocols for other pollinator species. A first step towards the development of these protocols is the establishment of standard rearing methods that ensure good population quality. Rearing conditions may affect the viability and other fitness parameters of insect populations altering their susceptibility to pesticides. In this presentation we will describe some case studies with solitary bees of the genus Osmia and dipterans of the families Tachinidae and Syrphidae. Knowledge on pesticide sensitivity of these species is fundamental to understand the full extent of pesticide use on both managed and wild pollinators.

Keywords: bees, dipterans, pollinators, ecotoxicology, pesticides

Development and application of genomic approaches to identify *Apis mellifera* subspecies

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A growing concern across Europe is emerging to preserve the genetic integrity of *Apis mellifera po*pulations and subspecies that might be adapted to local environmental conditions and climates. Subspecies integrity is also important for the beekeeping

sector i) that in many countries or regions requires certified queens of authorized subspecies, according to local regulations, or ii) that needs different genetic resources to design selection and crossbreeding programs. In this study we developed and applied DNA based approaches to identify the honey bee subspecies, combining information at the mitochondrial DNA and nuclear genome levels. These methods were applied both indirectly, using environmental DNA (eDNA) present in the honey, and directly, using DNA extracted from honey bee workers of different colonies to obtain genetic information on the *A. mellifera* populations of the whole Italian peninsula, Sardinia and Sicily. In addition, results obtained with the traditional morphometric method, usually applied to identify honey bee subspecies, have been compared with results obtained from genomic analyses. Genomic data revealed a broad genetic diversity in the Italian honey bee populations. Genomic approaches resulted much more informative than morphometric analyses. We also demonstrated that eDNA from honey can be exploited to design a cost-effective population genetic study in honey bees. The obtained results can be useful to design conservation strategies of *A. mellifera* genetic resources in Italy.

Keywords: conservation genetics; environmental DNA; genomics; honey bee; population genetics

SESSION: Mass rearing and evaluating biological control agents for crop pests – 3
Moderator: A. Coelho Jr.

Selective breeding of biocontrol agents; lessons learned from the BINGO project

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In horticulture the crops that are grown are the result of long running and highly advanced breeding programs. These programs are very successful and have a very large impact throughout. In contrast, for the biological control agents that are applied to protect these crops from pests, breeding has hardly been done at all. From 2015 to 2018 the BINGO-ITN project was developed. BINGO is the acronym for Breeding Invertebrates for Next Generation BioControl and hosted 13 ESR's that each worked on a project related to the breeding of biocontrol agents. The project was successful in training the ESR's and establishing an international network. It also successfully generated genomic data for some important biocontrol agents and much more valuable insights. However, actually generating new varieties by selective breeding turned out very hard. It requires establishing genetically diverse populations to start the selection process, phenotyping for complex characters on very small and short-lived animals, and the maintenance of many different populations and selection lines. In this talk I will discuss why each of these requirements are challenging, and how they may be resolved in the future.

Keywords: improving biocontrol agents, selective breeding, experimental evolution, genetic variation, phenotyping

Optimization of rearing techniques for *Trissolcus japonicus* (Hymenoptera: Scelionidae) release in the Emilia-Romagna region

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Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive and polyphagous pest of Asian origin, responsible of severe damage in Italian agriculture. *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), an exotic egg parasitoid, seems to be the best promising solution. In Italy, field releases of *T. japonicus* were authorized in 2020 as part of a national release program. In Emilia-Romagna region the production goal was of 60000 individuals in 2020 and of 20000 in 2021. In this study, some parameters that could influence the rearing of *T. japonicus* were investigated. A higher production of progeny was observed on host eggs stored at 6 °C (86.5%) compared to −24 °C (48.8%) for up to two months prior to exposure to parasitism. There were no significant differences in progeny production from single females in a vial provided with only one egg mass (83.2%) or 10 females inside a cage with 6 egg masses (83.9%). The exposure of parasitoids to refrigerated (6 °C) egg masses of *H. halys* for 72 h led to a significantly higher production of progeny (62.1%) compared to shorter exposures for 48 (44.0%) or 24 h (37.1%). A decline in production of progeny by the same female was detected between the first (62.1%) and the second parasitization (41.3%). Adult parasitoids stored at 16 °C for up to 90 days had an 87.1% survival rate, but a significant decrease in progeny production was detected. These parameters could be adjusted when rearing *T. japonicus* for specific aims such as the production of individuals for field release or colony maintenance. Our parasitoid production met the release program goals. Field monitoring, which was carried out during the release of the parasitoid to verify its impact, demonstrated the establishment and the overwintering of *T. japonicus*. However, its parasitism rates on egg masses of *H. halys* was very low (0.55% in 2020, 0.83% in 2021).

Keywords: *Trissolcus japonicus*; biological control; release program; rearing parameters; parasitism rates

DIY mass rearing of biocontrol agents, an alternative or supplement to commercial products?

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Biological pest control strategies require a preventative approach. In greenhouse horticulture, the most common practice is to purchase natural enemies from a commercial insectary and release them into the crop on a regular (e.g. weekly) basis. In addition, the use of banker plants and supplemental food can promote the establishment and persistence of biocontrol agents in the crop. Yet, some commercial greenhouse operations go one step further and mass rear their own biocontrol agents on-site to fulfil some of their pest control needs. Do-it-yourself (or DIY) mass rearing should be considered for biocontrol agents that are either not commercially available or if significant cost savings can be achieved. In some cases, on-site rearing is the best option when long shipping times or an uncertain supply chain are affecting the quality of the natural enemies and the regularity of their releases in the crop. Finally, DIY mass rearing can result in biocontrol agents that are adapted to the host plant, host or prey species and to local environmental conditions.This presentation will highlight the experiences of several commercial greenhouse growers, identify the research and actions needed to improve DIY mass rearing methods and argue how this practice can improve the successful adoption of biological control in general.

Keywords: on-site rearing, grower case studies, biocontrol success and adoption

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