International Organization for Biological Control

Mass Rearing & Quality Assurance working group

14th IOBC-MRQA Workshop

Mass Rearing High Quality Invertebrates for Multiple Purposes

Abstracts

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Hotel El Conquistador, Mérida, Yucatán, Mexico

IOBC Global

Mass production and release of ladybird beetles for aphid control in protected culture: search for oviposition stimulants

Session: Mass rearing invertebrates for management of arthropod crop pests

Oral

Eric Riddick

USDA-ARS, Stoneville, MS, USA

One of the major challenges to mass rearing predators of high quality, and in the quantity necessary for augmentative releases, is stimulating females to oviposit their maximum fecundity when fed un-natural foods. Using un-natural foods in lieu of natural prey (aphids) to mass rear ladybird beetles is a cost-effective technique. In laboratory experiments, in replicated cage arenas, plant-derived natural products (three bioflavonoids) were presented to mated, ladybird (*Coleomegilla maculata*) females over consecutive days. Ladybirds often oviposited 80% or more of the time next to the bioflavonoids taxifolin and quercetin, and 60% of the time next to the bioflavonoid naringenin. There was a highly significant negative correlation between the frequency of egg clutches near bioflavonoid powder and the cage wall. Egg clutch number was often 1.5-fold greater in cages with bioflavonoid powder than in control cages (without it). Exposure of freshly-laid eggs to bioflavonoid (quercetin) powder did not affect hatch rate, which ranged from 75-80% in test and control cages. In this study, evidence that bioflavonoids can stimulate oviposition in ladybirds is presented. Further research is necessary to test the stimulatory effect of bioflavonoids at the scale of a mass production system, where ovipositing females are in the company of conspecifics.

Drone-based dispersion of natural enemies in agricultural systems

Session: Mass rearing invertebrates for management of arthropod crop pests

Oral

Christian Nansen, Zhaodan Kong

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I will provide an update on on-going multi-disciplinary collaboration between entomologists, computer scientists, and aerospace engineers regarding development and thorough testing of a prototype to optimize dispersion of natural enemies in agricultural systems

Improvements in the in vitro rearing of the tachinid parasitoid *Exorista larvarum* (L.)

Session: Mass rearing invertebrates for management of arthropod crop pests

Oral

Maria Luisa Dindo[1], Patrick De Clercq[2]

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Tachinid parasitoids are less studied than parasitic hymenopterans. Yet, they play a major role in regulating herbivore insect populations and their importance is increasingly recognized. The tachinid larval parasitoid *Exorista larvarum* (L.), an antagonist of lepidopterous defoliators, is suitable to be cultured both in vivo and in vitro, and yields of fecund adults approaching those usually obtained in host larvae have been achieved on artificial media. A recent study aimed at investigating the effect of supplementing haemolymph of the black soldier fly (BSF) or the Chinese oak silkworm (COS) to an insect material-free artificial medium. The supplementation (20% w/w) was based on the assumption that insect additives, though derived from nonpermissive hosts, may optimize the media. The results suggested that BSF haemolymph positively influenced the *in vitro* development of *E. larvarum* and the quality of the in vitro-reared females. Conversely, COS haemolymph was less adequate. Another study assessed the effects of storage at suboptimal temperatures (20°C for 5 days or until pupation, or 15° for 5 days or until egg hatching) on the in vitro development of *E. larvarum*. For the experiments, parasitoid eggs were removed from host larvae and placed on media. In all the situations tested, the in vitro development of *E. larvarum* was delayed and the parasitoid eventually reached the adult stage, although some negative effects on the quality of the adult flies were observed.

Flight propensity of *Trichogramma pretiosum* Riley isofemale lines at two relative-humidities

Session: Mass rearing invertebrates for management of arthropod crop pests

Oral

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Egg parasitoids of the genus Trichogramma are the natural enemies that are most commonly used to control lepidopteran pests. In the former Soviet Union 17,000,000 ha were treated with this agents, in Brazil nowadays, approximately 750,000 ha of sugarcane and soybeans are treated. For the success of biological-control programs using these parasitoids, the insects must meet minimum quality requirements, especially under field conditions. Little is known about the effect of environmental factors on the flight propensity of Trichogramma, and its variation within the species. This study evaluated the flight propensity of six isofemale lines of Trichogramma pretiosum Riley, using a flight test developed as a quality-control measure for mass-reared Trichogramma spp. The same isofemale lines were used in earlier work to determine their fecundity in the laboratory and their performance in the field. We used five isofemale lines from a Mediterranean climate, and one isoline from a humid-subtropical climate. The flight propensity was assessed with a short-range flight test of the different isofemale lines in 80% and 30% RH. RH affected only the isofemale line from Piracicaba, and about 30% more insects did not fly in low RH conditions. RH conditions did not affect the Californian isofemale lines, which showed a flight propensity above 75% in both RH conditions. These results showed that for some lines, environmental conditions at the release sites might adversely affect the flight propensity of the insects. However, the flight test alone was not sensitive enough to discriminate the lines with different field performances.

Testing nutrigenomics as a new approach to formulating artificial diets for insects

Session: Mass rearing invertebrates for management of arthropod crop pests

Oral

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Formulating and improving artificial diets can be a convoluted, tedious and often underappreciated task associated with mass rearing of insects. The most common method for optimizing artificial diets has been to measure a few preselected biochemical and (or) fitness parameters in order to test the effect of changes in diet formulation on insect performance. The use of gene expression information could be a more direct method to assess and accelerate diet development and identify deficiencies in diet formulations.

We measured gene expression levels in *Arma chinensis,* a beneficial pentatomid, when reared on an artificial diet and a natural prey. We determined genes that were differentially expressed for the two food regimes. Knowing the physiological roles of the differentially expressed genes enabled us to predict specific deficiencies in the artificial diet. Next we altered the formulation of the artificial diet based on those deficiencies and measured changes in a set of life history characteristics. Several characteristics improved while other characteristics worsened. The results are encouraging for the use of gene-directed formulation of artificial diets.

Mass-rearing optimization of the parasitoid *Psyttalia lounsburyi* for biological control of the olive fruit fly.

Session: Mass rearing invertebrates for management of arthropod crop pests

Oral

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The olive fruit fly, *Bactrocera oleae* (Tephritidae), is a direct pest of olives that has invaded the Mediterranean Region and California. *Psyttalia lounsburyi* (Braconidae), an African larval endoparasitoid specific to *B. oleae*, has been approved for release in the USA as a classical biological agent. As this parasitoid is multivoltine and its host develops only in fresh seasonal olives, it has been difficult to rear in laboratory. An innovative method was devised by using a factitious host (the Mediterranean fruit fly, *Ceratitis capitata*), but it needed to be improved to allow mass production of parasitoids for release. We developed a number of ways to increase the efficiency of rearing, including an artificial olive fruit to stimulate oviposition, optimizing host age, density of adult parasitoids, frequency and duration of exposure for oviposition, conditions for holding adults until release, as well as methods to quickly standardize the number of larvae exposed and to count emerging adult parasitoids. We significantly improved sex ratio of progeny, number of progeny produce per female, and survivorship of immatures and adults. In 2017 we produced over 97,000 adults and shipped over 41,000 for release in California.

Mass production and quality control of *Wolbachia*-carrying *Aedes* mosquitoes for the combined IIT/SIT to control dengue, chikungunya and Zika

Session: Mass rearing invertebrates for veterinary and medical field applications

Oral

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The endosymbiotic bacterium *Wolbachia* is widely recognized for its ability to induce both a reproductive abnormality known as cytoplasmic incompatibility (CI) and a resistance to dengue virus in mosquitoes. *Wolbachia*-based population suppression strategy, referred to as Incompatible Insect Technique (IIT), entails the release of male mosquitoes infected with *Wolbachia*, resulting in sterile matings and a reduction in the mosquito population. Here, we will report an ongoing field trial to control dengue/Zika mosquito vectors through mass rearing and male release to induce female sterility in Guangzhou China. The released *Aedes albopictus* HC strain carry a novel *Wolbachia* from *Culex pipiens* mosquito, which induces both CI toward the wild type mosquito and resistance to dengue virus. Mass rearing capacity has been successfully developed with a production of >5 million males per week. An X-ray irradiator has been developed for mass production to prevent from the potential population replacement caused by the released females escaped from the sex separator. Significant suppression of mosquito population has been accomplished in the release sites compared to the control. Further field trials using different release strategies are also being developed to combat dengue, Zika and Malaria in other countries including Mexico. We will discuss our results in relation to mass production and quality control of Wolbachia infected Aedes mosquitoes and implementation of SIT/IIT for the area-wide suppression of mosquito population for disease control.

Preliminary observations on study results where adults of the U.S. native mosquito assassin, *Toxorhynchites rutilus* were released weekly and their impact measured as population disrupter/reducer of peri-domestic, container breeding pest mosquitoes

Session: Mass rearing invertebrates for veterinary and medical field applications

Oral

Anita Schiller, Rudy Bueno, Mary Allen

Harris County Precinct 4, Spring, TX, USA

Harris County, Texas is situated along the central Texas Gulf Coast and is separated from the gulf waters by adjoining Brazoria-, Galveston-, and Chambers Counties. The municipal City of Houston is centrally located within Harris County and takes up about a quarter of its landmass. Being situated on the crossroads of temperate and sub-tropical zones as well as ecological transitions allows for a myriad of mosquito diversity. Over 55 different mosquito species are recorded for the area and include the non-native species *Aedes albopictus* and *Aedes aegypti*. Both species are identified as competent vectors of Zika, Dengue and Chikungunya viruses and they prefer to live in close proximity to people. They share the ability to breed in containers with *Ae. triseriatus* and *Culex quinquefasciatus*, vectors of La Crosse and West Nile Virus, respectively. As its common name implies, the native mosquito assassin, *Toxorhynchites rutilus* targets other mosquitoes as larval food. Except for sugar, all the adults' nutritional requirements are met during the larval phase, thus the adult does not take nor is capable of taking a blood meal. We initiated a season long study at four similar, yet separate sites in Harris County Precinct 4 and released weekly 200 lab-reared, mixed gender, pre-mated *Tox. rutilus* adults into three sites, leaving one control site monitored but with no releases.

We share the study methods, initial findings and observations on the impact these predator releases alone had on the target pest mosquito populations.

Quality control of sterile male tsetse flies after long distance transport as chilled, irradiated pupae.

Session: Mass rearing invertebrates for veterinary and medical field applications

Oral

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Tsetse flies transmit trypanosomes that cause human and African animal trypanosomosis, a debilitating disease of humans (sleeping sickness) and livestock (nagana). An area-wide integrated pest management campaign against *Glossina palpalis gambiensis* has been implemented in Senegal since 2010 that includes a sterile insect technique (SIT) component. The SIT can only be successful when the sterile males that are destined for release have a flight ability, survival and competitiveness that are as close as possible to that of their wild male counterparts.

Tests were developed to assess the quality of *G. p. gambiensis* males that emerged from pupae that were produced and irradiated in Burkina Faso and Slovakia (irradiation done in Seibersdorf, Austria) and transported weekly under chilled conditions to Senegal. For each consignment a sample of 50 pupae (QC flies) was used for a quality control test. To assess flight ability, the pupae were put in a cylinder filtering emerged flies that were able to escape the cylinder. The survival of these flyers was thereafter monitored without food. Remaining pupae (RF flies) were emerged and released in the target area of the eradication programme. The following parameter values were obtained for the QC flies: average emergence rate more than 69%, median survival of 6 days, and average flight ability of more than 55%. The quality assessment was a good proxy of fly quality of the RF flies.

The quality protocol described here will allow the accurate monitoring of the quality of shipped sterile male tsetse used in operational eradication programmes.

Dynamics in the developing insect food and feed industry and opportunities for private/public/academic collaboration

Session: Mass rearing invertebrates for production of insects for feed and food

Oral

Eric Schmitt

Protix, Dongen, The Netherlands

The rate of companies entering the insect food and feed space has grown exponentially since 2008. This has stimulated the first wave of advanced production processes in this field, which promise near-term access to large quantities of product. Protix has been a leader in this field, both in terms of R&D and market impact. This talk will detail some of the key dynamics in the development of the industry, with particular focus on the role that technology and R&D knowledge will play in realizing production and quality. Throughout, the talk will highlight opportunities where academia, government and private companies should work together to ensure that as the industry evolves it protects of consumers and realizes the environmental benefits it is expected to deliver.

Opportunities to bring more native natural enemies to market in North America

Session: Development and validation of protocols for invertebrate mass rearing and quality assurance - novel quality control techniques

Oral

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A reliable supply of natural enemies is essential to support the strong growth in demand for biological crop protection agents. Not only must production of biocontrol agents be scaled up to satisfy demand, but also additional natural enemies are needed to combat an increasingly diverse array of pests. In North America, access to generalist predator species could strengthen augmentative biological control programs in a range of floral and vegetable crops. As regulations in the US and Canada prevent the importation of non-native generalist predators, this opens up opportunities to develop native species.

To guarantee commercial success of a new product, there are several critical steps, including discovery of promising species, testing for host range, and validation of performance on a commercial scale. Further, cost-effective methods must be developed for mass production, harvesting and distribution, while interactions with other control agents (biological and chemical) and their role in IPM programs defined so that their full market potential (and value proposition) can be determined. We are currently investigating a generalist predatory mite as a new control agent for multiple pests such as aphids, thrips and mealybugs. In addition, we are actively searching for native mirid predators that may be able to fulfil the same role as the European Macrolophus in the control of aphids, whiteflies and (when it arrives) tomato pinworm. Collaboration between researchers, biocontrol producers and growers could greatly enhance the process of bringing a suite of new native biocontrol agents to market.

Using proximal remote sensing in studies of insects and in quality control for production of natural enemies

Session: Development and validation of protocols for invertebrate mass rearing and quality assurance - novel quality control techniques

Oral

Christian Nansen

UC Davis, Davis, USA

Through a brief review of proximal remote sensing and description of relevant case stories, I will discuss both challenges and opportunities when this technology is integrated into studies of insects and when being considered for quality control of large-scale production of natural enemies.

The importance of dormancy and dormancy management to biological control

Session: Development and validation of protocols for invertebrate mass rearing and quality assurance - novel quality control techniques

Oral

Dan Hahn

University of Florida, Gainesville, FL, USA

The success of biological control programs relies on producing high-quality insects for release, from programs with sterile insects to natural enemies. I will discuss how an understanding of insect dormancy responses may help improve biological control programs. Insect dormancy responses vary from programmed diapause to environmentally induced quiescence. Obligate diapause can be a hurdle to mass rearing insects and I will discuss efforts to subvert diapause responses. Similarly, mis-matching in the synchrony of seasonal dormancy responses between natural enemies and their prey can be a major hurdle for biological control programs. However, dormancy and other stress responses can also be used to improve the performance of biological control agents. For example, producers may induce dormancy to increase the shelf life of insects so biological control agents may be held longer between production and release without performance loss. These methods may even provide new avenues for cold storage of insect stocks to maintain genetic diversity while reducing rearing costs. Similarly, a class of acclimation responses termed hormesis, where insects are exposed to a mild stress that boosts performance, can also be used to improve biological control agent performance, sometimes in surprising ways. Overall, understanding and managing insect dormancy and acclimation responses offers many new avenues for improving mass rearing, storage and shelf-life, and the field performance of biological control agents used in integrated pest control programs.

New extraction method to assess the amount of predatory mites in a carrier material

Session: Development and validation of protocols for invertebrate mass rearing and quality assurance - novel quality control techniques

Oral

Elmer van Baal, Tom Groot

Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands

Many species of beneficial predatory mites are shipped in a carrier material such as bran, husks or woodchips. The concentration of these mites (and the resulting total number in a package) is a very important aspect of product quality next to intrinsic properties such as predation rate and fecundity.

To assess the number of mites (both predators and preys) per gram of carrier material, Koppert Biological Systems has been using an internally engineered version of the Tullgren extraction funnel for approximately 20 years. It's basically an old-fashioned light bulb (we use it for its heat production), a mesh screen to hold a sample with mites and a way to collect mites that drop through the screen when the light bulb is on. Although proven very useful, the method has several drawbacks that need to be resolved. For instance, light bulb quality (read: heat production) can be very variable resulting in variable mite extraction results. Moreover, light bulb availability is increasingly scarce nowadays as the EU promotes the usage of power efficient light sources like LED's.

Koppert Biological Systems currently develops a different way to extract mites from a carrier material that overcomes the aforementioned drawbacks and even allows uniform worldwide implementation. The quality of the new method will be assessed using several statistical QC techniques such as Gage R&R and Bland-Altman.

A new initiative for quality control of natural enemies

Session: Development and validation of protocols for invertebrate mass rearing and quality assurance - novel quality control techniques

Oral

Tom Groot

IBMA, Brussels, Belgium

Good IPM programs require high quality natural enemies. Manufacturers of invertebrate biocontrol products have their own quality control procedures to ensure the quality of the product leaving their facilities. In addition to this, there is an increasing demand from growers to have QC tests performed by independent laboratories upon receipt of the products. In the past several laboratories have performed such tests using different protocols. However, the results of the test can differ largely depending on what protocol is used and therefore these results may not be consistent Also the reporting of the results is often incomplete, for example by lacking statistical information or information on the history of the product such as batch numbers and production date . These issues have clearly demonstrated the lack of well-defined protocols for this type of testing. An ideal protocol would yield reliable and consistent results, is relatively easy to perform, includes instruction on reporting of the results, and is widely accepted. This necessity is recognized by the IBMA and we will start developing a protocol for the quantity control of mites. To safeguard the wide acceptance it would be good if other organizations will join in this effort. On the long term, this new initiative on protocols for quality control may result in an update of the IOBC guidelines.

Toward the future: potentiality and challenges of the commercial production of beneficial insects

Session: Breeding of beneficial arthropods

Oral

Marco Mosti

Bioplanet SCA, Cesena, Italy

Augmentative biological control, based on mass reared insects and mites, has a half century history in Europe, stimulated by leverages that have been causing a constant growth, but acting at different speeds according to different countries and crops. More recently, new rules and new commercial standards have been promoting a steadier and more conspicuous increase. A further development is also expected because of greater awareness of consumers and farmers and of established efficiency of Integrated Pest Management, which became more and more cost effective even in tackling the new pest control challenges due to exotic species and climate change. The evolution of biological control along this period and how it is influencing and it is influenced by the new agriculture context will be exposed.

BINGO: Breeding Invertebrates for Next Generation Biocontrol

Session: Breeding of beneficial arthropods

Oral

Tom Groot

Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands

With a growing world population there is an increasing demand for food. There is also an increasing awareness of food safety issues concerning the residues of pesticides. Combined this results in an increasing need for biological crop protection. So far biological control agents have been selected from natural populations and are applied directly. Hardly any attention has been spend on the selective breeding of the beneficial insects and mites. This is quite surprising when one considers the enormous efforts that are made, and results that are obtained, to genetically improve the crops they are to protect. BINGO: Breeding Invertebrates for Next Generation BioControl, is a Marie Skłodowska-Curie Innovative Training Network that develops innovative research training to improve the production and performance of natural enemies in biological control. It aims to exploit natural genetic variation to improve biocontrol agents for rearing, monitoring and performance. It takes an multidisciplinary approach, encompassing a broad range of scientific disciplines in the field of biological control. The program combines integrated training workshops and internship opportunities across the network, with career opportunities in academia, public or the private sectors. BINGO will train 13 PhD students and each with its individual research project. A total of 12 partners are involved from academia, non-profit organizations and industry located in 9 different European countries

Updates on the production process of the mosquito larval predator *Toxorhynchites rutilus* for use in releases in Harris County, Texas.

Session: Breeding of beneficial arthropods

Oral

Anita Schiller, Mary Allen, Arielle Fike

Harris County Precinct 4, Spring, TX, USA

Harris County Precinct 4's (Mosquito) Biological Control Initiative called DRAC/MAP has worked to evaluate and develop rearing methods for the Eastern U.S. native mosquito assassin, *Toxorhynchites rutilus septentrionalis* (*Tox. rutilus*) for its use in augmentative releases as part of an Integrated Mosquito Management approach against the Zika Virus and Dengue Virus vectors *Aedes aegypti* and *Aedes albopictus*. Despite utilizing rearing methods shared by colleagues we were unable to achieve the desired adult production numbers. By thinking outside the proverbial box and using selective breeding techniques to tame the fickle *Tox. rutilus* behavior, we've managed to domesticate wild sourced stock and keep this phenotype for over 100 generations. We conducted a release efficacy study at select Harris County sites, that included down-town Houston and Precinct 4, where *Ae. albopictus, Ae. aegypti* and *Ae. triseriatus* were present. The 2016/17 release study, colony maintenance and the select breeding project required approximately 800-900 *Tox. rutilus* adults per week which required culturing their larval prey foods. In collaborations with Purina Mills Nutrition Labs, a promising artificial diet is in development which aim is to replace the need for culturing live prey for larval *Tox. rutilus* and possibly serve as the sole larval diet. We wish to share the trials and tribulations and successes of the rearing methods tried in our efforts.

The International Insect Rearing Workshop at Mississippi State University

Session: Networking and instruction on insect rearing

Oral

Norman C. Leppla

University of Florida, Gainesville, USA

The International Insect Rearing Workshop conducted for the past 17 years at Mississippi State University has been instrumental in preparing insect rearing professionals for current and future opportunities in the field. The workshop has been attended by 460 participants representing 30 countries, 39 states or territories within the U.S., and 5 provinces of Canada. It covers environmental biology, nutrition and diet, quality control, facility management, population genetics, facility design, health and safety/air, quality, and microbial management, along with special topics such as butterfly and black soldier rearing, microbial control in insectaries, and edible insects. The pedagogical approach is to teach principles rather than recipes, cover a wide range of topics in 108 hours of instruction, include a hands-on insect pathology laboratory, and provide basic to advanced information. The workshop is coordinated and led by Dr. Frank Davis and has been delivered by Dr. SenSeong Ng, Dr. John Schneider, Dr. Mohammad Chaudhury, Dr. Norman Leppla, Mr. Peter Ebling, Dr. Michael Caprio, Dr. William Fisher, Mr. Thomas Riddell, and Dr. Louela Castrillo. The instructors have extensive experience in rearing insects as university faculty members, federal researchers, and employees in the private sector. The workshop lectures have been assembled into a widely-disseminated book, "Principles and Procedures for Rearing High Quality Insects." The workshop and associated book are continuing to meeting the increasing international demand for insect rearing education.

The International Insect Rearing discussion group

Session: Networking and instruction on insect rearing

Oral

Léon Westerd

Formerly- Wageningen University, the Netherlands

In November 2011, during the International Insect Rearing Workshop (IIRW) at Mississippi State University, Léon Westerd (at that time head of insect rearing at Wageningen University, the Netherlands) got the idea of starting an email discussion group about insect rearing. In spring 2012, the International Insect Rearing Discussion Group was launched (insect-rearing@googlegroups.com). In 5 years it has grown to 166 members from all over the world, most of them however from the USA. In general the members are highly educated and well experienced in insect rearing. There's an even distribution of men and women in the group. Most of the members are working at universities, in commercial insect breeding companies and research institutes. Most insects are being reared for education, research, biological control and animal feed. Diptera and Lepidoptera are the two main orders of insects that are reared, with black soldier flies (*Hermetia illucens*) being the single species most people are rearing. Some examples of discussions that have taken place in the discussion group are presented.

Development, reproduction, and flight of the mealybug destroyer, *Cryptolaemus montrouzieri*, when reared on an unnatural food and an artificial oviposition substrate

Session: Mass rearing invertebrates for management of arthropod crop pests

Poster

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The mealybug destroyer, *Cryptolaemus montrouzieri*, has been used in different parts of the world as a biological control agent of mealybug pests. Whereas it was previously thought to be a specialist predator of mealybugs, we developed a rearing system in which larvae and adults are exclusively reared on eggs of the Mediterranean flour moth, *Ephestia kuehniella*, as an unnatural food and polyester wadding as an oviposition substrate.

Developmental and reproductive performance of the ladybeetle on *E. kuehniella* eggs and the artificial oviposition substrate was compared with that on citrus mealybugs, *Planococcus citri*. In addition, the effect of the unnatural food on the flight performance of the adults was investigated in a flight mill.

Ladybeetles reared on flour moth eggs developed faster and weighed more than those reared on citrus mealybugs. Females offered flour moth eggs and the artificial oviposition substrate had a longer preoviposition period, but deposited similar numbers of eggs as compared with those maintained on mealybugs. In the flight mill study, ladybeetles reared on flour moth eggs outperformed their counterparts fed on mealybugs in terms of flight distance and duration.

The findings from this study demonstrate the potential of this semi-artificial rearing system for the mass production of *C. montrouzieri.* Furthermore, our findings have bearing on the environmental risk assessment of non-indigenous arthropods used in augmentative biological control, as they suggest that rearing conditions can affect the establishment and dispersal potential of these species if they were to be released in areas outside their native range.

Cover crop and *Trichogramma brassicae* interactions

Session: Mass rearing invertebrates for management of arthropod crop pests

Poster

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Trichogramma species are the most widely used natural enemies for biological control of Lepidopteran pests worldwide. The species of laboratory host used for its rearing is an important issue in the improvement of mass rearing techniques. However, it is not only the maternal host who determines the fitness of reared wasps but also the presence of flowering plants can improve efficiency of biological control agents by supplying sugar resource for parasitoids. The present study was undertaken to determine the effect of maternal host on *T. brassicae* and cover crop interactions. For this purpose, two experiments were performed under laboratory conditions. In the first experiment, the wasps were provided with five different flowering plants including: buckwheat, coriander, alyssum, white mustard and red clover to choose the best cover crop which cause more fecundity and longevity of adult parasitoids. In addition, 10% honey-water and control (no feeding) were also considered for comparison. In the second experiment, biological characteristics of *T. brassicae* reared on *Ephestia kuehniella* and *Sitotroga cereallela*, with continuous feeding on buckwheat flowers during their adulthood were studied. Since buckwheat flowers caused higher survival (5.35±1.04 day) and fecundity (34.8±1.42 eggs per each female) in *T. brassicae*, therefore the buckwheat plant was chosen for the second test. The results showed that *T. brassicae* reared on the flour moth eggs in presence of buckwheat flowers has more longevity and parasitism rate. Our results clearly showed significant effect of flowering buckwheat (*Fagopyrum esculentum*) on the efficiency of *T. brassicae* reared on *E. kuehniella* eggs.

Seasonal diversity, mass rearing and application of trichogrammatids in India

Session: Mass rearing invertebrates for management of arthropod crop pests

Poster

Yadavalli Lalitha, Jalali, S. K., Chandish R. Ballal, Navik, O. S., Richa Varshney

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Diverse agro climatic conditions prevailing in states of India favours not only cultivation of crops but also insects and their natural enemies diversity round the year. Diversity of egg parasitoids, trichogrammatids across the seasons were quantified by collecting parasitized insect eggs or through keeping sentinel cards.

Widely occurring lepidopteran eggs were of noctuiid, pierid, sphingid, *Danus* moths and sentinel cards of *Corcyra cephalonica* Stainton and *Phthorimaea operculella* (Zeller) eggs were found to attract these egg parasitoids in different ecosystems. Host plant *Crotalaria* was attracted most of the lepidopteran insects and parasitoids like *Trichogramma* Westwood and *Trichogrammatoidea* Girault species. *Trichogramma chilonis* Ishii, *Trichogrammatoidea armigera* (Manjunath), *Trichogramma achaeae* (Nagaraja & Nagarkattti) and *Trichogramma japonicum* (Ashmead) were most frequently collected throughout the year. High searching ability strain (HQS) of *T. chilonis* was developed by rearing them in insect rearing cages continuously and was used effectively on tomato for suppression of *Helicoverpa armigera* (Hübner).

A mass rearing unit (3×1.5×1.5 feet) was designed to meet the increasing demands for supply of tricho cards for field releases. In such cages, more than 200 numbers of egg cards were exposed in one time and over 95% parasitism was obtained. The area covered under trichogrammatid was remarkably widened from 4891.5 hectares in 2003-2008 to 38951.7 in 2008-2013 and 42926.5 hectares during 2013-2017 by ICAR-NBAIR, Bangalore. Besides, several state Departments of Agriculture and Horticulture, sugar factories and non-government organizations in different states of India are also covering vast areas under *Trichogramma* release.

Comparative assessment of four Steinernematidae and three Heterorhabditidae species for infectivity of larval *Diabrotica virgifera virgifera*

Session: Breeding of beneficial arthropods

Poster

Ryan Geisert[1], Bruce Hibbard[2], David Shapiro-Ilan[3], Kent Shelby[1], Thomas Coudron[1]

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Diabrotica virgifera virgifera LeConte larvae were exposed to six different entomopathogenic nematode species to test their potential infectivity in a laboratory setting. Known *D. virgifera* infecting nematode species *Heterorhabditis* bacteriophora Poinar, *H. megidis* Poinar, Jackson & Klein, *Steinernema feltiae* Filipjev, and *S. carpocapsae* Weiser were tested in a concerted experiment alongside *S. diaprepesi* Nguyen & Duncan, *S. riobrave* Cabanillas, Poinar & Raulston, and a Missouri wild type *H. bacteriophora* which have not been previously tested on *D. virgifera*. Third instar *D. virgifera* were exposed to either 60 or 120 nematodes per larvae for six days. Following exposure, mortality was recorded and larvae were dissected to determine the presence of active nematode infections. Results indicated a significantly higher proportion of larvae with active infections from the *Heterorhabditidae* species and *S. diaprepesi* than the other *Steinernematidae* species for both exposure rates; morality data indicated a similar trend.

Diversity of bacteria composition of spined soldier bug, *Podisus maculiventris* (Heteroptera: Pentatomidae), digestive system

Session: Breeding of beneficial arthropods

Poster

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Spined soldier bug is an important biological control agent that preys on eggs and larvae of forest and agricultural pests. Attempts at mass production of the insect have been limited due to the lack compatible artificial diets used for long-term colony rearing. One possible solution is to incorporate probiotic microorganisms into artificial diets. Unfortunately, virtually nothing is known concerning which microorganisms are effective probiotic agents in insects. To address this question for the spined soldier bug, we analyzed the gut microbial community of wild collected and laboratory reared populations using Illumina MiSeq 2×250 bp paired-end reads of 16S rDNA V4 hypervariable region amplicons. We found that the bacterial communities of both populations possessed low levels of microbial diversity. These results support testing whether the addition of probiotic strains of bacteria to the food stream might have a positive effect on mass rearing of *P. maculiventris*. Potential symbionts were identified for further investigation.

Diet improvement for rearing larvae of western corn rootworm (Coleoptera: Chrysomelidae) using response surface methodology

Session: Networking and instruction on insect rearing

Poster

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera LeConte*, is the most serious insect pest of corn (*Zea mays* L.) in the United States and parts of Europe. Several diet formulations are currently used by industry and researchers to evaluate WCR larvae for diet-toxicity bioassays. However, a publicly available diet that is comparable with a variety of Bt proteins will accelerate development of improved management technologies. We used response surface methods (RSM) and multi-dimensional mixture designs to identify principal ingredients and then optimized a four-component blend that resulted in improved larval development compared with the only public diet and four proprietary diets. This approach enabled more rapid progress in diet optimization than what would have been possible using traditional factorial or one-ingredient-at-a-time designs. This new formulation provides a standardized growth medium for WCR larvae that will facilitate comparison of research results from various working groups and compliance with regulatory requirements.

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