

12th Workshop of the Arthropod Mass Rearing and Quality Control Working Group of the IOBC

In cooperation with the IAEA



Blueprint for the future of arthropod rearing and quality assurance



IBMA
International Biocontrol agent
Manufacturers' Association



Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture



IAEA



INTERNATIONAL
Standards Worldwide

Vienna International Centre (VIC), Vienna, Austria
Boardroom A (M building)
October 19 - 22, 2010

Joint Meeting in Vienna, Austria of

IOBC Global Working Group on Arthropod Mass Rearing and
Quality Control (AMRQC)

Association of Natural Bio-control Producers (ANBP)

ASTM Subcommittee E35.30 on Natural Multi-Cellular
Biological Control Organisms

International Biocontrol Manufacturers Association (IBMA),
Invertebrate Biocontrol Agents Group

In cooperation with the International Atomic Energy Agency,
Vienna, Austria

Organizing Committee

Tom Coudron, IOBC AMRQC Co-convener, USDA-ARS, USA

Patrick De Clercq, IOBC AMRQC Co-convener, Ghent University, Belgium

Andrew Parker, Conference Host, FAO/IAEA, Insect Pest Control
Laboratory, Austria

Karel Bolckmans, Koppert BV, The Netherlands

Jacques Brodeur, IOBC Global President, Université de Montréal, Canada

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Norman Leppla, IOBC AMRQC former convener, University of Florida, USA

Kim Gallagher Horton, ANBP & Sterling Insectary, USA

Johannette Klapwijk, IBMA & Koppert BV, The Netherlands

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PROGRAM

Monday 18 October

16:00-18:00 Registration at VIC gate 1

Tuesday 19 October

08:00 Registration at VIC gate 1

10:00 Informal reception, setting up of posters

11:00 Opening address – Tom Coudron, Patrick De Clercq, Andrew Parker

12:00 Lunch

Symposium no. 1: The Role of Microbiota in Insect Mass Rearing and Quality Control (chair: Patrick De Clercq, Ghent University, Ghent, Belgium)

13:15 Basic and Applied Aspects of Insect Symbiosis

Kostas Bourtzis (*Department of Environmental and Natural Resources Management, University of Ioannina, Agrinio, Greece*)

13:35 Endosymbionts in predatory bugs of the genus *Macrolophus*

Thijs Machtelinckx¹, Thomas Van Leeuwen¹, Tom Van De Wiele², Nico Boon², Godelieve Gheysen³, Patrick De Clercq¹ (*Department of Crop Protection, Ghent University, Ghent, Belgium;*²*Laboratory of Microbial*

Ecology and Technology (LabMET), Ghent University, Ghent, Belgium;
³*Laboratory of Applied Molecular Genetics, Department of Molecular
Biotechnology, Ghent University, Ghent, Belgium)*

13:55 The intestinal microbiota of tephritid fruit flies as a potential tool to improve rearing and the sterile insect technique

Michael Ben-Yosef, Eyal Ben-Ami, Sagi Gavriel, Edouard Jurkevitch, Boaz Yuval (*The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*)

14:15 Mass rearing Lepidoptera with persistent baculovirus infections

Helen Hesketh, Rosie Hails (*Centre for Ecology & Hydrology, Wallingford, Oxfordshire, United Kingdom*)

14:35 Hytrosaviridae as a threat to the successful application of the sterile insect technique for *Glossina pallidipes*

Adly Abd-Alla¹, Andrew Parker¹, Max Bergoin², Marc Vreysen¹ (¹*Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria;* ²*Laboratoire de Pathologie Comparée, Université Montpellier 2, Montpellier, France*)

14:55 Managing pests and diseases in commercial bumble bee production

Petr Sima, Karel Bolckmans (*Koppert BV, Slovakia & The Netherlands*)

15:15 Coffee break and poster viewing

Symposium no. 2: Entomopathogenic Nematodes: Producing a High Quality, Effective Product for Expanding the Agricultural Market (chair: Lynn LeBeck, ANBP, Clovis, CA, USA)

15:45 Advances in entomopathogenic nematode in vivo production and application methodology

David Shapiro-Ilan¹, Juan Morales-Ramos², M. Guadalupe Rojas², W. Louis Tedders³ (¹*USDA-ARS, SEFTNRL, Byron, GA, USA;* ²*USDA-ARS, NBCL, Stoneville, MS, USA;* ³*Southeastern Insectaries, Inc., Perry, GA, USA*)

16:05 Production technology of entomopathogenic nematodes in China

Richou Han (*Guangdong Entomological Institute, Guangzhou, China*)

16:25 Quality assured mass production of entomopathogenic nematodes

Andrew Brown, Jeremy Pearce, John Godliman (*Becker Underwood Ltd., Littlehampton, West Sussex, United Kingdom*)

16:45 Mass production efficacy and nematode quality - contradicting targets?

Arne Peters (*e-nema GmbH, Schwentimental, Germany*)

17:05 Open discussion

Lynn LeBeck (*ANBP, Clovis, CA, USA*)

Wednesday 20 October**Symposium no. 3: SIT Applications and Other Uses of Irradiation Technology (chair: Andrew Parker, FAO/IAEA, Seibersdorf, Austria)****8:30 Production, shipment and use of natural enemies facilitated by irradiation**

Jorge Hendrichs¹, Kenneth Bloem², Gernot Hoch³, James E. Carpenter⁴, Patrick Greany⁵, Alan S. Robinson¹ (¹*Joint FAO/IAEA Division, Vienna, Austria;* ²*Centre for Plant Health Science & Technology, USDA-APHIS-PPQ, Raleigh, NC, USA;* ³*Department of Forest and Soil Sciences, BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria;* ⁴*USDA-ARS Crop Protection and Management Research Unit, Tifton, GA, USA;* ⁵*2770 Pine Ridge Road, Tallahassee, FL, USA*)

8:50 Use of irradiation for economical production of *Trichogramma chilonis* and its field augmentation to manage insect pests of sugarcane and cotton

Nazir Ahmad, Muhammad Sarwar, Raza Muhammad Memon (*Nuclear Institute of Agriculture, Tando Jam, Sindh, Pakistan*)

9:10 Fruit fly parasitoid mass rearing, quality control and field release

Pablo Montoya, Jorge Cancino, Lía Ruiz, Patricia Lopez (*Programa Moscafrut SAGARPA-IICA, Tapachula, Chiapas, Mexico*)

9:30 Review of largest tephritid fruit fly emergence and release facilities

Pedro A. Rendon (*USDA/APHIS/PPQ/CPHST, Guatemala, Guatemala*)

9:50 Enhancement of sterile male performance: Nutritional, semiochemical, and hormonal pre-release treatments for tephritid fruit flies

Rui Pereira¹, Peter Teal², Boaz Yuval³, Pablo Liedo⁴, Todd Shelly⁵, Jorge Hendrichs¹ (¹*Insect Pest Control Section, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria;* ²*Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL, USA;* ³*Department of Entomology, Hebrew University, Rehovot, Israel;* ⁴*Departamento de Entomología, El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico;* ⁵*USDA-APHIS, Waimanalo, HI, USA*)

10:10 Coffee break and poster viewing

10:40 Field cage assessment of fruit fly competitiveness and compatibility: the example of *Anastrepha fraterculus*

Teresa Vera (*CONICET, Las Talitas, Argentina*)

11:00 The process of revising the FAO/IAEA/USDA manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies

Patrick Gomes¹, Jorge Hendrichs², Rui Pereira², Andrew Parker² (¹*USDA-APHIS-PPQ, Raleigh, North Carolina, USA;* ²*Joint FAO/IAEA Division, Vienna International Centre, Vienna, Austria*)

11:20 Mass rearing and quality control for false codling moth SIT application

Sampie Groenewald (*Xsit Pty Ltd, Citrusdal, South Africa*)

11:40 Development of quality control procedures for Lepidoptera

James Carpenter¹, Greg Simmons², Tom Blomefield³, Stephen Hight⁴
(¹*USDA-ARS, Tifton, GA, USA;* ²*USDA-APHIS, Moss Landing, CA, USA;*
³*Agricultural Research Council, Stellenbosch, Western Cape, South Africa;*
⁴*USDA-ARS, Tallahassee, FL, USA*)

12:00 Lunch

Symposium no. 4: Application of New Technology to Mass Insect Rearing and Quality Control (chair: Tom Coudron, USDA-ARS, Columbia, MO, USA)

13:15 On the genetic improvement of parasitoids: lessons from *Nasonia* wasps

Leo Beukeboom (*Centre for Ecological and Evolutionary Studies, University of Groningen, Haren, the Netherlands*)

13:35 Developments in sexing tsetse pupae and new packing materials for shipping

Andrew Parker (*Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria*)

13:55 On the road to a mosquito SIT programme: Mass rearing tools and quality control

Fabrizio Balestrino, Mark Benedict, Clelia Oliva, Sharon Soliban, Jeremie Gilles (*IAEA, Vienna, Austria*)

14:15 Applications of biomanufacturing and bioreactor technology for developing new insect diets

Allen Cohen (*North Carolina State University, Raleigh, NC, USA*)

14:35 Calculating the costs of rearing: from laboratory to mass rearing for mosquito control

Jack Rhodes, Megan Quinlan, Jonathan Knight, Adrian Leach, John Mumford (*Imperial College London, London, United Kingdom*)

14:55 Standardization of mass-rearing Lepidoptera for evaluation of the efficacy of transgenic crops

J. J. Adamczyk (*Kika de la Garza Subtropical Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Weslaco, Texas, United States*)

15:15 Coffee break and poster viewing**Symposium no. 5: New, Novel, Innovative and Emerging Applications of Insect Rearing (chair: Karel Bolckmans, Koppert BV, Berkel en Rodenrijs, The Netherlands)****15:45 Applications for mass reared arthropods, an overview**

Karel Bolckmans (*Koppert BV, Berkel en Rodenrijs, The Netherlands*)

16:05 Mass production of insects for aquaculture-Part I: market perspectives for a sustainable protein source

Sal Cherch, Ernest Papadoyianis (*Organic Nutrition, LLC, Boca Raton, FL, USA*)

16:25 Mass production of insects for aquaculture-Part II: an innovative solution to the protein bottleneck

Ernest Papadoyianis (*Organic Nutrition, LLC, Boca Raton, FL, USA*)

16:45 *Tenebrio molitor* as a source of insect protein

Juan Morales-Ramos¹, Guadalupe Rojas¹, David Shapiro-Ilan², Louis Tedders³ (¹*USDA-ARS NBCL, Stoneville, Mississippi, USA*; ²*USDA-ARS FTNRRU, Byron, Georgia, USA*; ³*Southeastern Insectaries Inc., Perry, Georgia, USA*)

17:05 Mass rearing insects for the pet food industry

Clay Ghann (*Ghann's Cricket Farm, Inc., Augusta, GA, USA*)

17:25 Mass rearing insects for the production of animal feed from bio-available waste

Hans Wollmann, David Drew (*Agriprotein, South Africa and Germany*)

19:30 Conference dinner

Thursday 21 October

Excursion to IAEA SIT rearing facility, Seibersdorf. A sign-up list for the two groups will be available at registration and during the first day of the workshop.

8:30 First group, return at 14:30

11:30 Second group, return at 17:00

Friday 22 October

Symposium no. 6: New and Future Applications for Mass Rearing Insects and Quality Control (chair: Norman Leppla, University of Florida, Gainesville, FL, USA)

8:30 Growth of insect rearing in the 21st century

Norman Leppla¹, Frank Davis² (¹*University of Florida, IFAS, Entomology and Nematology Department, Gainesville, FL, USA;* ²*Mississippi State University, Department of Entomology and Plant Pathology, MS, USA*)

8:50 A new Canadian Forest Service state-of-the-art insect rearing and quarantine facility

Peter Ebling (*Natural Resources Canada, Sault Ste. Marie, Ontario, Canada*)

9:10 Expansion of screwworm production in Panama

Muhammad Chaudury (*USDA-ARS, Panama City, Panama*)

9:30 Insect rearing and african sugarcane area-wide integrated pest management: challenges and achievements

Des Conlong (*South African Sugarcane Research Institute, Mount Edgecombe, KwaZulu- Natal, South Africa*)

9:50 Increasing production of *Trichogramma* by substituting artificial diets for factitious host eggs

Shoil Greenberg¹, Norman Leppla² (¹*USDA, ARS Beneficial Insects Research Laboratory, Weslaco, TX, USA;* ²*University of Florida, IFAS, Entomology and Nematology Department, Gainesville, FL, USA*)

10:10 Production attributes of *Trichogramma* reared on *Eri* silkworm eggs vis-a-vis *Corcyra* eggs and economics of the rearing system

Yadavalli Lalitha, Sushilkumar Jalali, T. Venkatesan, S. Sriram (*National Bureau of Agriculturally Important Insects, Bangalore, Karnataka, India*)

10:30 Quality and process control in mass-rearing systems for predators of adelgids

Allen Cohen¹, Carole Cheah², Fred Hain¹, Thom Hodgson¹, Kathleen Kidd³
(¹North Carolina State University, Raleigh, NC, United States; ²Connecticut Agricultural Experiment Station, Windsor, CT, USA; ³NCD Plant Industry-Plant Protection, Cary, NC, USA)

10:50 Coffee break and poster viewing**Symposium no. 7: Predatory Mites (chair: Kim Gallagher Horton, Sterling Insectary, Delano, CA, USA)****11:10 Twenty five years of mass production of *Phytoseiulus persimilis* a “bug farm” or an industry?**

Shimon Steinberg (*BioBee Sde Eliyahu Ltd., Kibbutz Sde Eliyahu, Bet Shean Valley, Israel*)

11:30 Life styles of phytoseiid mites: implications for rearing and biological control strategies

James McMurtry (*University of California, Riverside, CA, USA*)

11:50 Trends in predatory mite production and delivery systems

Richard GreatRex (*Syngenta Bioline, Essex, United Kingdom*)

12:10 Demand versus supply in biocontrol: disturbance of natural balance?

Pierre Ramakers (*Plant Research International, Wageningen UR, Bleiswijk, The Netherlands*)

12:30 Open discussion

Kim Gallagher Horton (*Sterling Insectary, Delano, CA, USA*)

12:45 Closing remarks**13:00 Lunch****14:00 AMRQC business meeting - Tom Coudron, Patrick De Clercq**

TBD - business meetings

Posters

Symposium no. 1

1.P1 Bacterial community of the spined soldier bug gut

Alejandro P. Rooney, Thomas A. Coudron (*USDA-ARS-NCAUR, Peoria, IL, USA; USDA-ARS-BCIRL, Columbia, MO, USA*)

Symposium no. 2

2.P1 Development of Management Programs for White Grubs in California Blueberries

David Haviland, Natalie Hernandez (*University of California Cooperative Extension, Kern County, CA, USA*)

Symposium no. 3

3.P1 Fecundity and percentage egg hatch of potato tuber moth F1 progeny of 150-Gy irradiated parents crossed with irradiated moths

George Saour, Hayat Makee (*Atomic Energy Commission, Damascus, Syrian Arab Republic*)

3.P2 Thailand mass rearing and quality control of *Bactrocera dorsalis* (Hendel) and *Bactrocera correcta* (Bezzi)

Suksom Chinvinijkul, Supaap Pinkaew, Watchreeporn Orankanok (*Irradiation for Agricultural Development Division, Bureau of Agricultural Product Quality Development, Department of Agricultural Extension, Ministry of Agriculture and Cooperatives, Bangkok, Thailand*)

3.P3 Application of nuclear techniques in the mass rearing of *Nesolynx thymus* (Hymenoptera: Eulophidae), an endoparasitoid of Uzi fly *Exorista sorbillans*

Md. Mahbub Hasan, Md. Rayhan Uddin, Md. Ataur Rahman Khan, Aminuzzaman Md. Saleh Reza (*Department of Zoology, Rajshahi University, Rajshahi-6205, Bangladesh*)

3.P4 VIENNA 7/Mix 99 downunder — the Western Australian experience of rearing a genetic sexing strain medfly for use in SIT programmes

Roselia Fogliani, Bill Woods (*Department of Agriculture and Food Western Australia, South Perth, Western Australia, Australia*)

- 3.P5 Preliminary study of pupal diapause and artificial rearing to Chinese citrus fruit fly, *Bactrocera minax***
 Changying Niu, Yongcheng Dong (*Plant Science and Technology College, Wuhan, China*)
- 3.P6 Feasibility study for the genetic control of *Aedes albopictus***
 Arianna Puggioli, Anna Medici, Marco Carrieri, Romeo Bellini (*Centro Agricoltura Ambiente "G.Nicoli", Med. & Vet. Dept., Crevalcore, Bologna, Italy*)
- 3.P7 Laboratory colonization of *Aedes albopictus* and effect on some fitness parameters**
 Anna Medici, Arianna Puggioli, Marco Carrieri, Romeo Bellini (*Centro Agricoltura Ambiente G.Nicoli, Med. & Vet. Dept., Crevalcore, Bologna, Italy*)

Symposium no. 4

- 4.P1 Olive fly: from small scale production to large scale mass-rearing**
 Sohél Ahmad, Viwat Wornoaayporn, Ihsan ul Haq, Carlos Cáceres, Andrew Jessup (*FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria*)
- 4.P2 Ephestia kuehniella eggs sterilization for *Trichogramma ostrinae* Pang et Chen (Hymenoptera: Trichogrammatidae) mass production**
 Mylène St-Onge¹, Daniel Cormier², Silvia Todorova³, Éric Lucas¹ (¹*Université du Québec à Montréal, Montréal, Québec, Canada*; ²*Institut de recherche et de développement en agroenvironnement, Saint-Bruno-de-Montarville, Québec, Canada*; ³*Anatis Bioprotection, St-Jacques-le-Mineur, Québec, Canada*)
- 4.P3 A new type of solid, semi-solid, and semi-liquid arthropod artificial diets using colloids to replace gelling agents**
 Guadalupe Rojas, Juan Morales-Ramos (*USDA-ARS BCPRU, Stoneville, MO, USA*)
- 4.P4 New frontiers in the biological control of insects**
 Thomas Coudron, Holly Popham, Kent Shelby, David Stanley (*USDA-ARS, Columbia, MO, USA*)

4.P5 Wheat germ oil in larval diet influences gene expression in adult oriental fruit fly

Chiou Ling Chang¹, Thomas Coudron², Cynthia Goodman², David Stanley², Shiheng An³, Qisheng Song³ (¹USDA-ARS-PBARC, Hilo, HI, USA; ²USDA-ARS-BCIRL, Columbia, MO, USA; ³University of Missouri, Columbia, MO, USA)

4.P6 ASTM International Subcommittee E35.30: Supporting development and maintenance of current standards for assessing quality of microbial biological control agents

Carol Glenister, *IPM Laboratories, Inc., Locke, NY, United States*

Symposium no. 6

6.P1 A new world-wide database of insect, mite and nematode cultures available for distribution.

Peter Ebling (*Natural Resources Canada, Sault Ste. Marie, Ontario, Canada*)

6.P2 Artificial rearing of *Anastrepha fraterculus* (Wiedemann 1830) (Diptera: Tephritidae): Egg-viability and models of cages

Juliana García Carrión (*Servicio Nacional de Sanidad Agraria, Lima, Peru*)

6.P3 A global quality index for *Trichogramma*

Shoil Greenberg¹, Norman Leppla² (¹USDA, ARS Beneficial Insects Research Laboratory, Weslaco, Texas, USA; ²University of Florida, IFAS, Entomology and Nematology Department, Gainesville, Florida, USA)

6.P4 Determination of critical storage period of mass reared host eggs parasitized by *Trichogramma evanescens* for efficient adult parasitoid emergence

Md. Mahmudunnabi, Syed Nurul Alam (*Bangladesh Agricultural Research Institute (BARI), Gazipur, Dhaka, Bangladesh*)

6.P5 Artificial rearing of a reduviid predator *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) using meat-based artificial diet

K. Sahayaraj, S. Balasubramanian (*St. Xavier's College (Autonomous), Palayamkottai/Tamil Nadu, India*)

6.P6 Effects of olive oil and yeast in liver-based artificial diet for the production of *Orius laevigatus*

Samira Safarian^{1,3}, Ahmad Ashouri¹, Hamid Reza Sarraf Moayeri², Reza Talaei Hassanlou¹, Sima Kabiri¹ (¹Department of Plant Protection, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran;

²*Department of Plant Protection, Faculty of Agriculture, Zanjan University, Zanjan, Iran;* ³*Gyah Bazr Alvand Corporation, Tehran, Iran)*

6.P7 Developmental and reproductive fitness of *Adalia bipunctata* on factitious and artificial foods

Maarten Bonte, Patrick De Clercq (*Department of Crop Protection, Ghent University, Ghent, Belgium*)

6.P8 A record of three Korea indigenous species newly developed as biological control agents for controlling aphids

Hyunjin Shin, Wooyeun Kim, Taesu Kim (*SESIL Corporation, Nonsan, Chungnam, Republic of Korea*)

Symposium no. 7

7.P1 Mass rearing of *Neoseiulus longispinosus* (Evans) (Acari: Phytoseiidae) under field and laboratory conditions in Himachal Pradesh in India

Usha Chauhan, P.R. Gupta (*Dr YS Parmar University, Solan-Nauni, HP, India*)

1.1

Basic and applied aspects of insect symbiosis

Kostas Bourtzis

Department of Environmental and Natural Resources Management, University of Ioannina, Agrinio, Greece

Arthropods, and particularly insects, have been reported to establish symbiotic associations with a variety of microorganisms which affect many aspects of host biology and physiology. Depending on certain traits of the symbiotic association, insect symbionts are currently artificially divided into three categories: The first category includes symbionts, which provide nutrients such as amino acids and vitamins to their hosts through a mutualistic association. The second category includes symbionts, which provide their hosts with the ability to survive heat stress, to develop resistance to parasitic wasps, and to exhibit altered host plant preference. The third category includes symbionts which manipulate the reproductive properties of their hosts, inducing phenomena such as parthenogenesis, feminization, male-killing and cytoplasmic incompatibility, which is a kind of male sterility. All these phenomena favor the prevalence of infected females. The best-studied symbiont in this group is *Wolbachia*. I will present basic and applied aspects of insect symbiosis, with an emphasis on *Wolbachia* symbiosis and its impact on insect mass rearing and quality control.

1.2

Endosymbionts in predatory bugs of the genus *Macrolophus*

Thijs Machtelinckx¹, Thomas Van Leeuwen¹, Tom Van De Wiele², Nico Boon², Godelieve Gheysen³, Patrick De Clercq¹

¹Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Gent, O-VI, Belgium, ²Laboratory of Microbial Ecology and Technology (LabMET), Faculty of Bioscience Engineering, Ghent University, Gent, O-VI, Belgium, ³Laboratory of Applied Molecular Genetics, Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Gent, O-VI, Belgium

Predatory bugs of the genus *Macrolophus* have been widely used in European protected cultivation for the biological control of key arthropod pests. We have examined the reproductive effect of *Wolbachia* on *Macrolophus pygmaeus*. *Wolbachia* is the best known and most widely spread endosymbiont of arthropods. It manipulates the reproduction of its host by several mechanisms, including cytoplasmic incompatibility, parthenogenesis, male-killing and feminization. Crossing experiments between cured and infected individuals elucidated that *Wolbachia* induces a strong cytoplasmic incompatibility effect in *M. pygmaeus*. This finding may have important consequences for the commercial mass rearing of *M. pygmaeus* and its use in biological control, as incompatible interactions may lead to a suppressed population growth.

Further, the microbial diversity in *M. pygmaeus* and the closely related species *Macrolophus caliginosus* was analysed by denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene sequencing. Besides *Wolbachia*, a co-infection of 2 *Rickettsia* species was detected in *M. pygmaeus*. Based on the 16S rRNA gene, the first is phylogenetically related to *Rickettsia bellii*, whereas the second is closely related to *Rickettsia limoniae*. *M. caliginosus* was infected with the same *Wolbachia* and *Rickettsia limoniae* strain as *M. pygmaeus*, but did not harbour the *Rickettsia bellii* strain. A PCR assay on the ovaries of *M. pygmaeus* suggested that all endosymbionts are vertically transmitted. Interestingly, individuals with a single infection were not detected. The biological significance of these *Rickettsia* species for their *Macrolophus* hosts needs to be clarified.

1.3

The intestinal microbiota of tephritid fruit flies as a potential tool to improve rearing and the sterile insect technique

Michael Ben-Yosef, Eyal Ben-Ami, Sagi Gavriel, Edouard Jurkevitch, Boaz Yuval
The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Many fruit flies (Tephritidae) carry extracellular bacterial symbionts within dedicated structures of their digestive tract. Using the Olive fly (*Bactrocera oleae*) we demonstrated that these bacteria are essential for egg production when females are maintained on a diet devoid of essential amino-acids, or on honeydew - an important component of the natural diet of many fruit flies. These results imply that the intestinal microbiota contributes either protein or amino-acids to these flies, thereby compensating for nutritional deficiencies in the diet. Gut bacteria may perform similar rolls during the larval stage and be an integral part of the nutritional ecology of other fruit flies as well.

During mass rearing the use of artificial diets, microbial control measures and irradiation usually interfere with such associations. Studying the Medfly (*Ceratitis capitata*) we found that the gut microbiota of mass reared V8 males differs from that of their wild competitors, most notably by the increased levels of potentially pathogenic *Pseudomonas* species. Additionally, irradiation was found to decrease the native gut population of *Klebsiella* bacteria. The inclusion of *Klebsiella oxytoca* in the post-irradiation diet regenerated the gut population of these bacteria and resulted in decreased levels of *Pseudomonas*. Moreover, feeding on these diets significantly improved sterile male performance in copulatory tests.

We suggest that implementing bacteria in mass rearing operations may resolve some of the difficulties in rearing flies which currently require special nutritional and microbiological considerations, and promote the production of high quality insects needed for the effective implementation of the sterile insect technique.

1.4**Mass rearing Lepidoptera with persistent baculovirus infections**

Helen Hesketh, Rosie Hails

Centre for Ecology & Hydrology, Wallingford, Oxfordshire, United Kingdom

A large number of pathogens are able to infect arthropods, including fungi, viruses, microsporidia and bacteria. Saprophytic true insect pathogens are generally not problematic in sanitised insectaries although outbreaks may occur under certain circumstances. Of more importance are those pathogens that cause chronic, debilitating disease and have the potential to affect insect fitness. Baculoviruses are DNA viruses that primarily infect Lepidoptera and some Hymenoptera. There are two main groups, the nucleopolyhedroviruses (NPV) and the granuloviruses (GV). These viruses can cause acute infections in mass reared arthropods; examples being Douglas fir tussock moth *Orygia pseudotsugata*, codling moth *Cydia pomonella*, cabbage looper *Trichoplusia ni* and Beet armyworm *Spodoptera exigua*. The Centre for Ecology & Hydrology has reared several species of Lepidoptera for more than 20 years in a specialised insectary. In the early 1990's a persistent baculovirus infection was discovered in a culture of cabbage moth *Mamestra brassicae*. The virus was shown to be actively transcribed but individual insects did not succumb to infection. The culture has been continuously reared since this time with 100% infection in each generation but with extremely rare mortality due to the virus and little apparent fitness costs in the hosts. We discuss the methods by which these insect cultures are maintained and examine another example from a large insect facility, rearing codling moth for an orchard codling moth suppression programme. We will also examine how these persistent infections may be triggered into fully overt disease and the implications that this has for mass rearing of insects.

1.5

Hytrosaviridae as a threat to the successful application of the sterile insect technique for *Glossina pallidipes*

Adly Abd-Alla¹, Andrew Parker¹, Max Bergoin², Marc Vreysen¹

¹*Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria,* ²*Laboratoire de Pathologie Comparée, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier, Montpellier, France*

Hytrosaviridae is a newly proposed family of invertebrate viruses that cause salivary gland hypertrophy syndrome and reduce the fertility in their dipteran insect hosts. *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV), a member of this family, is a linear, double stranded DNA virus with rod shaped particles, 50 nm in diameter and 700 - 1000 nm in length. The success of the tsetse eradication program on the Island of Unguja, United Republic of Tanzania has encouraged other countries to consider the sterile insect technique as one additional control tactic. However, during attempts to colonize *G. pallidipes* in Austria and Ethiopia, colony expansion proved difficult and the virus was thought to be the probable cause. The complete genome of the virus was sequenced and a PCR-based detection method and a qPCR were developed. The PCR studies showed that the virus is widely distributed in several colonized tsetse species, but it only seems to cause sterility in *G. pallidipes*. Under field conditions, the virus seems mainly transmitted vertically whereas in laboratory colonies the mode of transmission is horizontally through feeding. A strategy to manage the virus infection in the colonies is under development and is based on four axes, i) reducing virus horizontal transmission using a strategy of clean feeding (flies are offered clean blood at each meal) ii) neutralizing virus infection using virus specific antibodies, iii) blocking virus replication using commercial antiviral drugs, and ii) inhibiting virus infection by silencing virus specific genes using RNAi technology.

1.6**Managing pests and diseases in commercial bumblebee production**

Karel Bolckmans¹, Peter Šima¹

¹*Koppert B.V., Berkel en Rodenrijs, Netherlands,* ²*Koppert s.r.o., Nové Zámky, Slovakia*

For more than 20 years commercially mass-reared bumblebee colonies (*Bombus* spp.) have been successfully used world-wide for pollination of greenhouse tomatoes and other fruit-bearing crops. Different species of bumblebees are reared for different regions. Bumblebees can suffer from a range of harmful pests and diseases. In order to guarantee the economic and reliable production of high-quality commercial bumblebee colonies and more importantly in order to avoid the inadvertent spread of pests and diseases which could potentially have a negative impact on wild bumblebee populations, it is of the utmost importance to have adequate methods in place to prevent contamination of commercial bumblebee cultures by harmful pests and diseases. The authors will present a hands-on preventative approach which is based on over 20 years of research and practical experience in the world's largest bumblebee production facilities. Preventing contamination of commercial bumblebee colonies by pests and diseases is largely based on the principles of Pasteur's technique for eliminating diseases from the production of silk worms. Additionally careful measures need to be taken to guarantee complete containment. Consequent and rigid implementation of these methods assure the production of pest and disease free colonies.

2.1

Advances in entomopathogenic nematode in vivo production and application methodology

David Shapiro-Ilan¹, Juan Morales-Ramos², M. Guadalupe Rojas², W. Louis Tedders³

¹USDA-ARS, SEFTNRL, Byron, GA, United States, ²USDA-ARS, NBCL, Stoneville, MS, United States, ³Southeastern Insectaries, Inc., Perry, GA, United States

Entomopathogenic nematodes (epns) (genera: *Steinernema* or *Heterorhabditis*) are important biocontrol agents. A variety of economically important insect pests are targeted with epns throughout the world. In North America some of the current or emerging target pests include black vine weevil, *Diaprepes* root weevil, fungus gnats, mole crickets, thrips, white grubs, codling moth, lesser peachtree borer, peachtree borer, pecan weevil, plum curculio, and small hive beetle.

Entomopathogenic nematodes are commercially produced using in vivo or in vitro methods. In vivo technology may be appropriate for small or start-up companies, as well as enterprises in developing countries. The primary challenges in terms of in vivo production efficiency include labor and cost of insects. Based on our research and that of others, there are a number of approaches to enhance in vivo production efficiency including improved insect nutrition and production, and optimized inoculation and harvest procedures. Mechanization of the process will lead to a substantial reduction in labor.

Entomopathogenic nematodes are applied using various spray and irrigation systems. Nematode efficacy is impacted by diverse abiotic and biotic factors. Efficacy may be improved through superior application methods (such as application in infected hosts or use of novel formulations) or strain improvement, e.g., hybridization, selected inbred lines, and leveraging genomic studies. In general, aboveground applications have been less successful than soil applications due to environmental degradation in the former, but recent research indicates promise in certain aboveground approaches. Further innovation in application technology will undoubtedly contribute to the expansion of entomopathogenic nematodes as biocontrol agents.

2.2

Production technology of entomopathogenic nematodes in China

Richou Han, Xuehong Qiu

Guangdong Entomological Institute, Guangzhou, China

Entomopathogenic *Steinernema* and *Heterorhabditis* nematodes (EPNs) are the only biological control agents which have the capacity to actively search and infect insect larvae in the soil. These nematodes and their symbiotic bacteria have been demonstrated to be non toxic to vertebrates. Recent advance in the solid and liquid production technology of EPNs has greatly improved the yield, quality, and shelf life of these beneficial worms in China. The factors influencing the production efficiency were explored, including medium development, optimization of the culture parameters (gas supply, inoculum size, bacterial status, temperature, etc), recovery of the infective juvenile (IJ) inocula, formation of the IJs, extraction and harvest of IJs. A company called Century Horse Development Ltd, under the guidance of Guangdong Entomological Institute, is running. Healthy and reasonable products in solid culture system are provided for field trials in China and for internal and international markets. Production cost is still a limiting factor for further commercial development in the world nematode-based market. To maintain and further develop the nematode markets for ideal segments of pest control, it is necessary to realize technology transfer or capital transfer into developing countries with lower labour cost, cheaper materials and equipments, and good transportation system, so as to compete with the chemical insecticides.

2.3

Quality assured mass production of entomopathogenic nematodes

Andrew Brown, Jeremy Pearce, John Godliman

Becker Underwood Ltd., Littlehampton, West Sussex, United Kingdom

Entomopathogenic nematode (EPN) and molluscopathogenic nematode (MPN) biocontrol products are based on a specific nematode life-stage, a modified third stage juvenile (Dauer juvenile). Manufacturing beneficial nematodes at large scale is carried out by liquid fermentation. This process has evolved from *in vivo* rearing inside insects such as waxmoth (*Galleria mellonella*) then to *in vitro* production on sponge cubes in flasks and then bags.

Becker Underwood Ltd. (formerly Microbio) is the largest manufacturer of EPN and MPN. All of Becker Underwoods beneficial nematodes are manufactured on one site; Littlehampton, UK. The site produces all nine species currently commercially available. The first fermenters were installed in 1990, these had a capacity of 180 L. Twenty years later, in spring 2010, a third 25,000 L fermenter was installed.

The process of mass production of EPN presents many difficulties. Strains need to be maintained to ensure virulence traits are not lost during *in vitro* manufacturing. The fermentation is a two stage process, where the bacteria are grown first, followed by the secondary nematode fermentation. The nematode culture has to be synchronised to ensure production of the required infective stage. The nematodes need to be formulated to provide stability in storage and transport, then be easy to apply through a wide range of application equipment and leave no adverse crop residues. To ensure high yields, viability and efficacy it is vital to have rigorous QC procedures from the initiation of the stock cultures to the final packaged product.

2.4

Mass production efficacy and nematode quality - contradicting targets ?

Arne Peters

e-nema GmbH, Schwentinental, Germany

Mass production has a negative image. It is a common belief that with upscaling production, the quality of products declines. Entomopathogenic nematodes can be mass produced in insects or on artificial media in solid state or in liquid culture. The liquid culture technology offers the best economies of scale. While contaminating microorganisms might establish unnoticed in insect cadavers or in patches in solid-state culture, the liquid culture technology requires the absence of any contaminating micro-organism hence comprising an intrinsic quality assurance feature. In terms of the abiotic conditions, the liquid production process can be monitored automatically by e.g. oxygen, temperature and pH-probes. On the other hand, the ingredients of the growing media must be chosen carefully to provide sufficient and balanced food for the formation of the infective juveniles, especially for their stored lipid energy reserves. Sufficient bacteria must be present in the liquid culture at the time they are sequestered by the nematodes to obtain infective juveniles properly charged with entomopathogenic bacteria. The genetic deterioration is of concern in any mass production system and should be mitigated by regularly starting up new lines from a background population parasitizing on the target insect. A final quality assessment is indispensable for all production systems. In conclusion, the mass production of nematodes on artificial media in liquid culture provides a better control of the production process than smaller scale technologies on insects or solid-state culture. In nematode production, quality and production efficacy are compatible and linked optimisation targets.

3.1

Production, shipment and use of natural enemies facilitated by irradiation

Jorge Hendrichs¹, Kenneth Bloem², Gernot Hoch³, James E. Carpenter⁴, Patrick Greany⁵, Alan S. Robinson¹

¹Joint FAO/IAEA Division, P.O. Box 100, 1400 Vienna, Austria, ²Centre for Plant Health Science & Technology (CPHIST), USDA-APHIS-PPQ, 1730 Varsity Drive, Suite 400, Raleigh, North Carolina 27606, United States, ³Department of Forest and Soil Sciences, BOKU – University of Natural Resources and Applied Life Sciences, Hasenauerstrasse 38, A-1190 Vienna, Austria, ⁴USDA-ARS Crop Protection and Management Research Unit, Tifton, Georgia 31793, United States, ⁵2770 Pine Ridge Road, Tallahassee, Florida 32308, United States

An FAO/IAEA Coordinated Research Project (CRP), involving 18 research teams from 15 countries, addressed constraints related to production and handling systems for biological control agents, and the potential presence of accompanying pest organisms during their shipment. These constraints can be alleviated using nuclear techniques, such as ionizing radiation, to reduce production and handling costs (e.g. by expanding the period of host suitability, increasing shelf life, etc.), and to eliminate the risk of shipping fertile host or prey pest individuals or other hitchhiking pests. Applying radiation can also help to reduce the risks associated with the introduction of exotic biological control agents, which can become pests of non-target organisms if not carefully screened under semi-natural or natural conditions. Radiation is also a very useful tool to study host-parasitoid physiological interactions, such as host immune responses, by suppressing defensive reactions of natural or factitious hosts. Applied at a very low-dose, radiation may be used to stimulate reproduction of some entomophagous insects. Additionally, radiation can be applied to partially- or completely sterilize hosts or prey for deployment in the field to increase the initial survival and build-up of natural or released biological control agents in advance of seasonal pest population build-up. Finally, the work carried out under this CRP has demonstrated the feasibility of integrating augmentative and sterile insect releases in area-wide IPM programmes, and to utilise by-products from insect mass-rearing facilities in augmentative biological control programmes. Results have been published in a 2009 special issue of *Biocontrol Science and Technology*.

3.2**Use of irradiation for economical production of *Trichogramma chilonis* and its field augmentation to manage insect pests of sugarcane and cotton**

Nazir Ahmad, Muhammad Sarwar, Raza Muhammad Memon
Nuclear Institute of Agriculture,, Tando Jam, Sindh, Pakistan

Production of natural enemies in an efficient and economical way is prerequisite for biological control programmes. Considerable technological advances have been made in mass rearing of bio-control agents for augmentative releases to manage insect pests. Studies were conducted to economize the rearing of an egg parasitoid *Trichogramma chilonis* and its field releases in sugarcane and cotton crops for management of insect pests. Irradiation of host eggs increased their incubation period which proved useful to enhance the parasitic potential of *T. chilonis*. The use of nuclear techniques in a mass rearing facility of the parasitoid enhanced the fecundity of the females who play an important role in the augmentative biological control programme. Further, storage of the parasitoids at 10⁰C after irradiation at 25 Gy with gamma radiation proved useful for 20 days without affecting quality of the parasitoids. The parasitoids reared on the irradiated eggs successfully managed the insect pests of sugarcane and cotton in the target area of 25,000 and 600 hectares respectively during two crop seasons.

3.3

Fruit fly parasitoid mass rearing, quality control and field release

Pablo Montoya, Jorge Cancino, Lía Ruiz, Patricia Lopez

Programa Moscafrut SAGARPA-IIICA, Tapachula, Chiapas, Mexico

The Mexican campaign against fruit flies uses Augmentative Biological Control (ABC) as part of the strategies to suppress fruit fly populations. *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), a larval fruit fly parasitoid, is mass reared at the level of 25 million pupae per week on irradiated (45 Gy) third instar larvae of *Anastrepha ludens* (Diptera: Tephritidae). The mass rearing of this species is subject to a specific quality control system, where the main parameters determined are: 1) host larvae weight, 2) viability (for the process), 3) percent of emergence and sex ratio, 4) longevity, and 5) flight ability. Later, the adults are released on marginal areas and/or backyard orchards identified as fruit fly reservoirs.

Nowadays, in the Mexican campaign there are plans to integrate the fruit fly pupal parasitoid *Coptera haywardi* (Hymenoptera: Diapriidae), with the aim to reinforce the ABC against these pests, since it is considered that *C. haywardi* could parasitize those *Anastrepha* pupae that escape the first attack of *D. longicaudata* in larger host fruit. *Coptera haywardi* is reared on irradiated (30 Gy) pupae (3 day old) of *A. ludens*, with the potential to reach a production of 5-8 million per week, according to the needs of the national campaign. In this species, the key quality control parameters are: 1) host pupae weight (for the process), 2) percent of emergence and sex ratio, 3) longevity and 4) flight ability. However, the specific environmental conditions where this parasitoid could be successfully released are still under determination.

3.4

Review of largest Tephritid fruit fly emergence & release facilities

Pedro A. Rendon

USDA/APHIS/PPQ/CPHST, Guatemala, Guatemala

The Sterile Insect Technique (SIT) has been adopted as the main tool for control/eradication of several fruit fly species. Worldwide, there are a number of rearing facilities (RF) that produce and deliver sterile insects (SI) locally/internationally to action programs. These programs use SI for various purposes which include preventing establishment of new populations in free areas and the suppression/eradication of incipient populations of these pests. This SI production effort requires of the appropriate conditions for transport, emergence, handling and feeding of sterile fruit fly adults. These activities, conducted at emergence and release facilities (ERF), together with an appropriate aerial release of SI in the target area, contribute to the success of this technique. This paper presents the review conducted by an expert panel at eight of the largest ERF's for Tephritid fruit flies located in: Mexico (3), United States (4) and Guatemala (1).

The total combined sterile fly output of these ERF's, at the time of this review, for two of the most important fruit fly species amounts to ca. 1.4 billion/week for the Mediterranean fruit fly *Ceratitis capitata* (Wied) and ca. 165 million/week for the Mexican fruit fly *Anastrepha ludens* (Loew).

The review identified the following significant needs: i.) Standardization of operating procedures and quality control assessments. ii.) Modernization and implementation of new technologies, efficiencies and worker safety. iii.) The need for periodic reviews by independent international experts as quality assurance of the ERF operations. Findings and recommendations on these subjects are presented and discussed.

3.5

Enhancement of sterile male performance: Nutritional, semiochemical, and hormonal pre-release treatments for tephritid fruit flies

Rui Pereira¹, Peter Teal², Boaz Yuval³, Pablo Liedo⁴, Todd Shelly⁵, Jorge Hendrichs¹

¹*Insect Pest Control Section, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria,* ²*Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL, United States,* ³*Department of Entomology, Hebrew University, Rehovot, Israel,* ⁴*Departamento de Entomología, El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico,* ⁵*USDA-APHIS, Waimanalo, HI, United States*

The application of the Sterile Insect Technique (SIT) against tephritid fruit fly pests as a component of area-wide integrated pest management programmes is gaining momentum, with active programmes targeting major pest species in the Americas, Europe, Middle East, Asia, Africa and Australia. Several billion insects are being mass-reared and irradiated every week in factories in many locations, and shipped (as pupae) to their destinations where emerging flies are fed and prepared for aerial release in fly emergence/release facilities. The exigencies of the industrial process often affect the biological qualities of the final product. Reducing the impact of the process on product quality and improving the sexual performance of sterile male flies released into the field was the objective of a six-year coordinated research project involving the participation of researchers and many collaborators from 17 countries.

Among the studies we highlight:

- (1) effects of nutritional supplements to adult diet on mating success and survival of sterile tephritid males for various species of the genera *Anastrepha*, *Bactrocera* and *Ceratitis*;
- (2) effects of hormone supplements on accelerating reproductive development and improving the mating performance of sterile males for various species of the genera *Anastrepha*, *Bactrocera* and *Ceratitis*; and
- (3) effects of semiochemicals, such as ginger root oil and citrus oils to improve the sexual performance of males of *C. capitata* or methyl-eugenol for *Bactrocera* species responding to this lure.

Several of these advances have been successfully assessed in pilot tests or are already being implemented for some tephritid species in operational SIT programmes.

3.6

Field cage assessment of fruit fly competitiveness and compatibility: the example of *Anastrepha fraterculus*

Teresa Vera

Investigadora Asistente CONICET, William Cross 3150, Las Talitas (4101), Argentina

The success of the sterile insect technique relies on the introduction of sterility in the target population by the release of sterile males. The selection of which insect should be adapted to mass rearing, the evaluation of its quality and the impact of sexual enhancers can be evaluated in field cage tests under outdoor conditions. This test, developed in 1983, proved to be useful to evaluate mating competitiveness and compatibility of fruit flies. A recent example of its continued validity comes from studies in the South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae). Evidence supporting the existence of a complex of cryptic species raised the question whether a single strain would be sufficient to cover demands of sterile insects throughout its distribution. Field cage tests revealed the occurrence of mating incompatibility and showed that even under these artificial conditions, fruit flies still showed aspects of their natural mating behavior like time of mating, location of mating arenas, courtship displays and emission of chemical signals. The evaluation of the impact of refreshing genetic material in the rearing colony and the behavior of hybrid progeny obtained from different regions revealed that deterioration can be restored and that the origin of a new species can be explained by hybridization processes. In all, it can be concluded that efforts to standardize this simple test during these years have resulted in a tool that allows compilation of reliable information relevant both to pest management and to understand processes such as chemical communication and speciation.

3.7

The process of revising the FAO/IAEA/USDA manual for Product Quality Control and Shipping Procedures for Sterile Mass-reared Tephritid Fruit Flies

Patrick Gomes¹, Jorge Hendrichs², Rui Pereira², Andrew Parker²

¹USDA-APHIS-PPQ, Raleigh, North Carolina, United States, ²Joint FAO/IAEA Division, Vienna International Centre, Vienna, Austria

Use of the sterile insect technique to control Tephritid fruit flies began in the mid-1950's and progressed through the 1970's by a series of pilot projects carried out by scientists in a number of countries with support from the Joint FAO/IAEA Division. The technology continued to mature especially in terms of mass-rearing and release, but success in pest control varied from country to country. As activities expanded from research into pilot testing, questions arose over performance of the insects used. In 1975 the Joint Division convened a panel of experts where quality in mass-reared insects was defined for the first time and standards to measure adaptedness, motility and reproductive success were proposed. In 1977, researchers compiled a catalog of procedures published by the International Organization for Biological Control entitled, "Quality Control: An Idea Book for Fruit Fly Workers". During this same period, large-scale operational programs were initiated against New World Screwworm and Mediterranean fruit fly in the US, Mexico and Central America, but these programs lacked consistent, uniform standards for monitoring and assessing insect quality. Collaborative efforts between the Joint Division, IOBC, and fruit fly workers from affected countries succeeded in formulating standards program by program, then on an international level. Today, area-wide SIT programs to control fruit flies are being carried out on several continents based on these international standards. The process for revising these standards will be presented.

3.8**Mass rearing and quality control for false codling moth SIT application**Sampie Groenewald*Xsit Pty Ltd, Citrusdal, South Africa*

A discussion of the development and implementation, on a commercial scale, of the use of the sterile insect technique to control false codling moth in citrus orchards in the Western Cape of South Africa.

Aspects covered will include the following:

1. Rearing
2. Sterilisation
3. Release
4. Monitoring

Results of the first 3 years of implementation will be discussed in full, with special emphasis on required future development to further improve results.

3.9

Development of quality control procedures for Lepidoptera

James Carpenter¹, Greg Simmons², Tom Blomefield³, Stephen Hight⁴

¹USDA-ARS, Tifton, GA, United States, ²USDA-APHIS, Moss Landing, CA, United States, ³Agricultural Research Council, Stellenbosch, Western Cape, South Africa,

⁴USDA-ARS, Tallahassee, FL, United States

Lepidopteran species are among the most destructive insect pests throughout the world. The sterile insect technique (SIT), within an area-wide integrated pest management (AW-IPM) approach, has proven to be a valuable tactic for controlling and eradicating some important moth pests. However, it is recognized that improving moth quality would increase the efficacy of SIT AW-IPM programs and encourage the continued use and further expansion of the SIT to target key lepidopteran pests. In an effort to meet this challenge, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a Coordinated Research Project, "Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control," in 2009 with participating scientists from 13 different countries. The stated objective of this project is the development and use of improved quality control/management systems for all aspects of the SIT through the following goals: (a) identifying and characterizing factors and variables that affect quality and field performance of released moths; (b) developing and improving tools and methods to assess, predict and enhance the field performance of released moths based on insect quality; and (c) developing new and improved methods for enhancing rearing systems, and facilitating the selection for fitness traits that improve colony establishment and increase field performance of released moths. Currently, our parallel research efforts supporting ongoing SIT projects against three lepidopteran pests (codling moth, light brown apple moth, and South American cactus moth) are focused on relationships between abiotic variables, laboratory parameters and field performance of released moths.

4.1

On the genetic improvement of parasitoids: lessons from *Nasonia* wasps

Leo Beukeboom

Centre for Ecological and Evolutionary Studies, University of Groningen, Haren, Netherlands

Genetics has traditionally been underrepresented in fundamental and applied research on parasitoid biology. This is the more surprising as genetic improvement of other economically important organisms (e.g. crops, life stock) has a long and successful history. With the advent of new genetic and genomic techniques, including the availability of whole genome sequences, there is enormous potential for improving the effectiveness and cost-efficiency of biocontrol agents. This requires a stronger integration of fundamental and applied research on parasitoid biology.

We use parasitoid wasps of the *Nasonia* species complex to answer fundamental genetic questions as they provide unique opportunities to investigate the genetic architecture of life-history traits and parasitisation behavior that differ between and within *Nasonia* species. *Nasonia* are pupal parasitoids of cycloraphous flies that occur in bird nests, carcasses and livestock farms. They are excellent laboratory animals because of their ease of handling and culturing, short generation time and availability of genetic tools including haploidy of males. Artificial selection and QTL analysis are used to investigate the genetic basis of and the genetic trade-offs between life history traits, such as host searching behaviour, sex allocation, mating behaviour and diapause. The publication of the whole genome sequence of *Nasonia* is greatly accelerating the identification and regulation of genes underlying these processes, as well as those that regulate sex determination and locomotor activity. Although our research is not primarily aimed at genetically improving beneficial parasitoids, the fundamental knowledge of genetic processes that we obtain can be exploited for improving biocontrol agents.

4.2

Developments in sexing tsetse pupae and new packing materials for shipping

Andrew Parker

Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria

For the SIT separation of males from females is often desirable. In tsetse flies (*Glossina* spp.) we have shown that males can be distinguished by near infrared spectroscopy 5 days before emergence with 95% accuracy.

Over recent years a number of innovations in commercially available packaging have become available with much better performance than formerly. Vacuum panel insulation shipping boxes have a thermal conductivity up to five times lower than equivalent thicknesses of expanded polystyrene. Phase change materials allow temperatures other than zero to be maintained over extended periods. Semi-permeable membrane sachets of saturated salt solutions absorb excess moisture or release vapour as required. Together, these new materials permit both temperature and humidity to be controlled for an extended period of time, allowing a safety margin for delays during shipping and for extremes of both high and low temperature.

4.3

On the road to a mosquito SIT programme: Mass rearing tools and quality control

Fabrizio Balestrino, Mark Benedict, Clelia Oliva, Sharon Soliban, Jeremie Gilles
IAEA, Vienna, Austria

At the insect pest control laboratories (IPCL), major advances have been made in creating, developing and testing new tools for mosquito mass rearing with the main goal to implement SIT programmes for *Anopheles arabiensis* (malaria vector) and *Aedes albopictus* (Chikungunya vector).

Larval holding trays and a rack system to hold 50 of these trays were designed and tested for mass-rearing of *An. arabiensis* immature stages. The rack capacity should allow the production of approximately 200,000 pupae. A larva-pupa separator has been designed to mechanically separate both stages. This system, which combines cold temperatures and a water vortex, allowed the efficient separation of a 30 000 larvae-pupae mixture in 2 minutes without affecting survival. The adult oviposition cage has also been modified and improved. A new larval diet optimal for all growth parameters of *An. arabiensis* was developed using only ingredients that are widely available and cost-effective.

Standardized protocols for irradiation were developed for the "wild" Dongola *An. arabiensis* strain as well as for the genetic sexing strain. Sterility curves and longevity are now well known parameters and at the moment several tests have been elaborated and conducted to assess the fitness and competitiveness of radio-sterilized males, among which are flight performance and mating competitiveness against wild males.

Finally, a pilot facility design for mosquito mass rearing has been elaborated during a consultants meeting for a production capacity of 1 to 5 millions males per day; designs take into account the use of either a GSS or a "bi-sexual strain"

4.4

Applications of biomanufacturing and bioreactor technology for developing new insect diets

Allen Cohen

North Carolina State University, Raleigh, North Carolina, United States

Bioreactors have been used for production of pharmaceutical and other specialty products on a large-scale. We are applying bioreactor technology to produce custom-made products for use in artificial diets for insects. Starting with a Wheast™ model, where sweet dairy whey, which was a yeast-fermented waste product from cheese manufacturing, we are trying to capitalize on the idea that low cost starting materials can be improved via fermentation or microbial action to become highly nutritious components for insect diets. We are using 3 and 30 liter bioreactors to generate products from various species of yeast, bacteria, and algae (in photo-bioreactors), using a broad range of raw ingredients. The objective of this research system is to produce insect foods that are rich in proteins, suitable lipids, carbohydrates, vitamins, and minerals while having their possible antinutrients (phytic acid, protease inhibitors, lectins, etc.) removed.

Currently, we are using fruit flies (*Drosophila melanogaster*) as initial assay subjects for testing nutritional quality; then we use lacewings and trophically specialized coccinellids to further test novel products. The process is fermentation - post-fermentation processing (centrifugation, differential precipitation, and industrial scale chromatography) - bioassay. Our initial bioassays for overall nutritional value employ *Drosophila* with the putative nutrient and a minimal medium. This process allows high throughput screening of many fermentation and differential processing products. Most promising products from the *Drosophila* assays are then tested with the more nutritionally fastidious species.

4.5

Calculating the costs of rearing: from laboratory to mass rearing for mosquito control

Jack Rhodes, Megan Quinlan, Jonathan Knight, Adrian Leach, John Mumford
Imperial College London, London, United Kingdom

Improved technology raises the prospect of control of vectors of malaria and dengue through releases of sterile male mosquitoes. Consistent with production of other insects for Sterile Insect Technique (SIT), production costs for mosquitoes will be highly affected by the possibility of genetic sexing. If genetically modified, additional costs for biosafety containment also will be incurred.

The *Model Business Plan for a Sterile Insect Production Facility* (IAEA/FAO, 2008) discusses full costing of mass rearing (Sections 6 and 7). Using historic data from fruit fly production facilities, a financial spreadsheet for costs of mass rearing was developed. The spreadsheet covers capital and operational costs, showing international averages such as for equipment. The user is allowed to fill in local costs for labour, diet, utilities, waste disposal, the interest rate for borrowed capital etc.

We adapt the financial spreadsheet to cost mosquito production, starting with laboratory figures and laying out parameters for costs of mass rearing. The resulting template will be shared globally (through the MosqGuide website, www.mosqguide.org.uk) to hold and compare data whilst researchers move from laboratory to mass rearing levels over the next few years. Further data may call for separation by species and/or genetic strategy (sterile, inheritable sterile, self sustaining but non-transmitting, etc). When field control is considered, cost trade-offs between greater numbers for release versus more accurate monitoring, will be informed by more precise costing information as captured in this template.

4.6

Standardization of mass-rearing lepidopteran for evaluation of the efficacy of transgenic crops

J. J. Adamczyk

Kika de la Garza Subtropical Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Weslaco, Texas, United States

Genetically engineered crops have been commercially grown in the United States since 1996. During this time period cotton, corn, and soybean production in the United States has changed dramatically. Assessing the efficacy of transgenic plants under new environment and management regimes is of prime importance to the Companies which produced new or improved existing transgenic products; breeders, which created different varieties stacked with Bt endotoxins; and growers, which used them for production. Laboratory and field performance of cotton containing endotoxins should be standardized. Only this can provide accurate and stable data for insect control with different transgenic technology. In this presentation, we will discuss approaches and criterion for mass rearing standardized laboratory colonies of beet armyworm (BAW), *Spodoptera exigua* (Hübner); fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith); and bollworm (BW), *Helicoverpa zea* (Boddie) for laboratory and field methods for the evaluation of the efficacy of Bt cottons.

5.1

Applications for mass-reared arthropods, an overview

Karel J.F. Bolckmans

Koppert BV, Berkel en Rodenrijs, Netherlands

Arthropods are being mass-reared for many different reasons :

- for biological control of pests (classical biological control and augmentative biological control);
- for the production of insect pathogenic viruses (Lepidoptera) for biological control of pests;
- for Sterile Insect Technique (SIT);
- for pollination of crops (honeybees (*Apis* spp.), bumblebees (*Bombus* spp.), blowflies (Calliphoridae), blue mason bees (*Osmia* spp.), alfalfa leafcutter bees (*Megachile rotundata*), ...);
- for research purposes, such as for example for screening and testing insecticides, including side-effect testing on non-target arthropods;
- as human food;
- as a protein source for animal and fish feed;
- for the production of useful products such as silk, shellac, dyes (e.g. cochénille), honey, royal jelly, wax, pollen, propolis, venom, ...;
- for the production of bioactive/biomedical products (e.g. vaccines, antibodies, recombinant proteins, etc.);
- for medical purposes (e.g. *Lucillia sericata*);
- for bioconversion of manure and other organic waste;
- as pet insects, as pet food (fish, reptiles, birds) and for fishing bait;
- for educational purposes;
- for celebrations (e.g. butterflies for wedding ceremonies).

5.2

Mass production of insects for aquaculture – Part I: Market perspectives for a sustainable protein source

Sal Cherch, Ernest Papadoyianis

Organic Nutrition, LLC, Boca Raton, Florida, United States

World aquaculture production has been projected to grow from 50% to over 75% of world seafood consumption within the next 20 years. With fisheries stocks declining and world population increasing, the pressure on wild stocks, and particularly baitfish stocks, will increase dramatically. The very industry that has been called upon to bridge the gap between demand and wild supply is now fully dependent upon wild stocks for all of its diets. In order to expand and succeed in the future, the industry must develop its independence from fishmeal and wild stocks. Diet ingredients must be produced from sustainable sources to allow the unhindered expansion of global seafood production.

Many different sources of proteins have been explored to date. The difficulty in identifying a suitable source arises from the requirement of most carnivorous species of a high quality animal-based protein source. Insects are naturally consumed in nature by most freshwater species, and represent one of the finest animal protein sources available. Cooperative research conducted at Mississippi State University identified four potential insect species for commercial production based upon a detailed list of production and nutritional criteria that had to be satisfied. Nutritional analyses of these species indicated close proximal analyses to fishmeal. Feeding trials with diets containing insect protein (100% fishmeal replacement) were conducted at Mississippi State University with juvenile hybrid striped bass to assess diet acceptability and off-flavor analysis. Digestibility, food conversion and product acceptance (taste testing analysis) all proved favorable for commercial development.

5.3

Mass production of insects for aquaculture – Part II: An innovative solution to the protein bottleneck

Ernest Papadoyianis

Organic Nutrition, LLC, Boca Raton, Florida, United States

Mass production of select insect species to date has been concentrated around controlling harmful and invasive species, as well as for waste remediation at livestock facilities and landfills. Insects, particularly their larval stages, provide valuable food sources to many fish, birds, reptiles, mammals and other animals, and are rich in quality proteins and fatty acids essential for growth and reproduction.

Following extensive research, Organic Nutrition, LLC has launched a business plan to develop production facilities globally to mass produce insects as a source of sustainable, high quality protein meal. Currently, an integrated pilot production facility has been constructed in order to prove out the economic and mass production viability of the black soldier fly, *Hermetia illucens*. Further, the philosophy of “zero waste” had been employed through the integration of production processes for soil amendments and hydroponic produce as outlets for excess feedstock and insect wastes. The mass rearing process utilizes organic produce waste from local farms, which is brought in fresh, homogenized, and fed to black soldier fly larvae. The life cycle from egg to the self-harvesting pre-pupae is 18-20 days under optimal conditions.

The facilities are located in Florida, USA. South Florida provides an ideal sub-tropical climate where black soldier flies can be produced year round without the necessity of supplemental heating. *H. illucens* is native to the Southeast U.S. area and predominates year round in the wild, providing ample breeding stock to maintain genetic diversity.

5.4

Tenebrio molitor as a source of insect protein

Juan Morales-Ramos¹, Guadalupe Rojas¹, David Shapiro-Ilan², Louis Tedders³
¹USDA-ARS NBCL, Stoneville, Mississippi, United States, ²USDA-ARS FTNRU, Byron, Georgia, United States, ³Southeastern Insectaries Inc., Perry, Georgia, United States

Tenebrio molitor L (Coleoptera: Tenebrionidae) is one of the most commonly mass produced insects in the United States for diverse commercial purposes. The insect's most popular uses include: food for pet finches, reptiles, tarantulas, etc., bait for fishing, and others. The value of *T. molitor* as a source of insect protein has been recognized in areas such as aquaculture, aviculture, dietary supplementation in zoos, and even for human consumption. The value of *T. molitor* has also been recognized in biological control as an easy and cheap source of food to rear predatory Heteroptera, entomopathogenic nematodes, and other natural enemies. We present here a review of the nutritional value of *T. molitor* and we also discuss the potential for improving its mass production by refining the rearing technology and by mechanizing some of the labor intense processes.

5.5

Mass rearing insects for the pet food industry

Clay Ghann

Ghann's Cricket Farm, Inc., Augusta, GA, United States

Around 1950, a few people in the southeastern USA began to experiment with small scale production of crickets (*Acheta domesticus*) for fishing bait.

Throughout the 1960's and 70's, fishing bait was the primary demand for the insects, and the cricket industry remained relatively small.

In the 1980's and 90's reptiles and amphibians began to gain popularity as pets, and several cricket farms made an unexpected transition into mass producing crickets for pet food. This new direction initiated changes in facilities and production techniques, resulting in a dramatic increase in the number of insects being produced annually.

Many species of insects are now produced commercially for the pet industry, and consumers are always on the lookout for new types of live foods as they become more educated on the nutritional needs of their animals, and as new animals are introduced to the hobby.

Mass rearing that requires commercial viability also poses challenges. The cricket industry is currently facing a serious problem - a densovirus that is crippling many producers of *Acheta domesticus* around the world. Research is needed on this issue, as well as on alternative species that might be able to take the place of *A. domesticus* as food for reptiles should the densovirus problem prove to be insurmountable.

6.1

Growth of insect rearing in the 21st century

Norman Leppla¹, Frank Davis²

¹*University of Florida, IFAS, Entomology and Nematology Department, Gainesville, Florida, United States,* ²*Mississippi State University, Department of Entomology and Plant Pathology, Mississippi State, Mississippi, United States*

Insect rearing programs around the world have expanded significantly during the first decade of the 21st century, within both the public and private sectors. This growth continues within well-established programs through substantial increases in the production of new and existing species. Additionally, entirely new insect rearing programs have been initiated and more are being planned, especially in the private sector. Documented examples of this global escalation in insect rearing include colonies for research and development, pest management (biological control, sterile insect technique), human and pet food, displays and events (butterflies), pharmaceuticals, conservation, and fish bait. We predict that insect rearing, allied industries and markets for insect products will not reach their overall potential for growth within the foreseeable future. This forecast is based on information from our world-wide networks of insect rearing specialists, including those who have graduated since 2000 from the Insect Rearing Workshops at Mississippi State University.

6.2

A new Canadian Forest Service state-of-the-art insect rearing and quarantine facility

Peter Ebling

Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

The Canadian Forest Service, Great Lakes Forestry Centre, has been producing insects at various locations since 1963 in support of nation-wide pest control research activities. In recent years, significant advances in our quality management system have led to the attainment of funding for the construction of a state-of-the-art facility to produce and study invasive forest insect species, while maintaining our core capacity to produce domestic pests.

A 1592 m² facility is currently under construction, bringing together all insect production, quality control and quarantine services. The facility is divided into four zones having different functions and design elements, including 1) a Domestic Species Zone for establishing and maintaining colonies of disease-free domestic insects and for developing and manufacturing artificial diets, 2) an Invasive Species Zone (i.e., quarantine facility) for maintaining colonies of invasive insects and for conducting a wide variety of research activities, 3) a unique Variable Utilization Area, which can be converted between domestic or invasives uses as required, and 4) Offices and other common areas. Laboratory spaces are constructed following Canadian Food Inspection Agency (Biohazard Containment and Safety Branch) PPC-Level 2 facility requirements. "Clean-room" technology is employed, including HEPA-filtered supply and exhaust air, and adjustable air pressurization in each room to maintain various levels of positive or negative pressure for quarantine or domestic species needs. Our computerized integrated control system allows for remote web-based controlling and monitoring of all environmental parameters and historical tracking for all work spaces, including dozens of growth chambers.

Construction and commissioning of the facility will be completed by March, 2011.

6.3

Expansion of screwworm mass rearing in Panama

Muhammad Chaudhury

USDA-ARS, Panama City, Panama

The new world screwworm (NWS) sterile insect technique (SIT) program of the United States Department of Agriculture (USDA) has one of the largest insect mass rearing efforts in the world. At its peak, the program had a budget of more than \$100 million per year. It began in 1957 and moved from Florida to Texas, and later to Mexico, as NWS was eradicated. NWS production in Mexico was at Tuxtla Gutierrez, where as many as 500 million flies were produced per week. Following eradication of NWS from Mexico and Central America in 2004, a new mass production facility was built at Pacora, Panama. The facility became operational in 2008, producing 40 million NWS per week for maintenance of a permanent barrier at the Darien Gap region of Panama between Central and South America. This level of production would not be sufficient if an outbreak of NWS were to occur in the NWS-free area. Therefore, efforts are being made to maximize the production capability of the facility. In the past, the larval diet was switched from meat to an artificial medium and adult diet was changed from horsemeat and honey to a mixture of spray-dried egg and granulated sugar. These improvements, along with semi-automation, reduced costs and improved the efficiency of NWS mass production. Current research and development projects include optimization of nutritional requirements and reduction of ammonia in larval production, and genetic studies to develop and mass rear a male only strain.

6.4

Insect rearing and African sugarcane area-wide integrated pest management: Challenges and achievements

Des Conlong¹

¹South African Sugarcane Research Institute, Mount Edgecombe, KwaZulu- Natal, South Africa, ²Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, Western Cape, South Africa

Sugarcane is exotic to Africa. When first introduced, it was cultivated in wetlands, displacing indigenous vegetation harbouring indigenous arthropod fauna. As plantations grew, more natural habitat was lost, placing pressure on remaining arthropods survival. The indigenous *Eldana saccharina* (Lepidoptera: Pyralidae) has adapted to sugarcane in South Africa, Zimbabwe, Tanzania and Uganda, causing major crop losses. Conventional control methods had limited success, resulting in a new vision of AW-IPM, where sugarcane is regarded as part of the African agro-ecosystem. Emphasis is now placed on indigenous wetland rehabilitation, re-establishing indigenous host plants displaced by sugarcane or invasive plants, and habitat management, thus encouraging indigenous natural enemy population growth and movement. These, especially parasitoids, are not found in infested sugarcane. In addition, *E. saccharina* is showing susceptibility to radiation, thus SIT becomes an option. This may be the case too for the exotic *Chilo sacchariphagus* (Lepidoptera: Crambidae), now present in Mozambique. Increased air travel makes foreign insect incursions easier. Pro-active biosecurity is thus necessary. *Chilo sacchariphagus* is present on two Mozambique estates, and threatens sugar industries in surrounding countries. This is also true for new estates developing, for biomass purposes, in Mozambique. These can be "stepping stones" for invasion of sugarcane plantations within and outside Mozambique. In this bigger AW-IPM vision, biological control of alien plants is included in insect rearing activities, as has modifications to current mass rearing methods for *E. saccharina* and developmental rearing methods for *C. sacchariphagus*, to produce the high numbers of quality moths needed for SIT. Furthermore, X-ray radiation opens the use of mobile radiation units. This paper presents these challenges, and some of the ways they are being met.

6.5

Increasing production of *Trichogramma* by substituting artificial diets for factitious host eggs

Shoil Greenberg¹, Norman Leppla²

¹*USDA, ARS Beneficial Insects Research Laboratory, Weslaco, Texas, United States,*

²*University of Florida, IFAS, Entomology and Nematology Department, Gainesville, Florida, United States*

Trichogramma spp. have been produced on factitious hosts in semi-mechanized rearing facilities at many locations throughout the world. In the former Soviet Union, 4-5 million *Trichogramma* were produced per day in eggs of *Sitotroga cerealella* raised on barley kernels. Production increased from 4.5 to 9.4 g of eggs per kg of grain and the labor required per 100,000 *S. cerealella* eggs ranged from 0.14 to 0.27 man-hours. In the U.S., the production *S. cerealella* eggs on wheat in a semi-mechanized system increased from 6.0 to 12.0 g per kg of grain and 0.239 man-hours of labor were required to yield 100,000 eggs. The cost of raising *Trichogramma* on *S. cerealella* eggs is about \$2.43 per 100,000, whereas only about \$0.06 would be required to produce this number on artificial diet. A model in vitro rearing system for *Trichogramma* based on use of a form-fill-seal machine has the potential to yield approximately 20 billion parasitoids per week. The overall fitness of the mass reared *Trichogramma* could be measured using a recently developed quality index. This standardized assessment of *Trichogramma* is quick and reliable, and estimates generalized criteria for effectiveness in the field. Artificial diets and in vitro rearing systems that incorporate quality control will be required for augmentation biological control to become economically feasible.

6.6

Production attributes of *Trichogramma* reared on Eri silkworm eggs vis-a-vis *Corcyra* eggs and economics of the rearing system

Y. Lalitha, Sushilkumar Jalali, T. Venkatesan, S. Sriram

National Bureau of Agriculturally Important Insects, Bangalore, Karnataka, India

Production attributes of *Trichogramma chilonis* Ishii, a native parasitoid of Southeast Asia and Pacific region, were widely released in different ecosystems in India. Comparisons were made of the parasitoid on Eri silkworm, *Samia cynthia ricini* Boisduval and *Corcyra cephalonica* (Stainton). The developmental period ranged from 9 to 11 days, longevity of adults was 1-8 days, percentage of females ranged from 28.6 to 100% and 7-34 adults emerged from a single parasitized egg. After continuous production on Eri silkworm eggs, all biological attributes were enhanced significantly. The parasitoids produced on Eri silkworm eggs expressed higher speed of travel, searching ability and parasitising ability. The population reared from Eri silkworm eggs parasitized 83.5-90.5% eggs and produced >74.0% females even after shifting them to *Corcyra* eggs for 10 generations.

Endosymbiont yeasts, possibly belonging to the *Candida* group, isolated from the adults of Eri silkworm reared population, appeared to enhance the fecundity and percent female production. In mass production system, percent parasitism and female progeny were 100% and 97.4% respectively, and an average of 29.2 adults emerged from a single parasitized egg.

Cost of production of Tricho cards required for one hectare using Eri silkworm eggs was cheaper at US \$0.81, 47.6% less in comparison to production using *Corcyra*.

Comparatively larger size of *T. chilonis* emerging from Eri silkworms may facilitate better and faster searching of the host. Availability of Eri silkworm throughout the year and lower production cost will enable demand to be met for parasitoids possessing better biological attributes.

6.7

Quality and process control in mass-rearing systems for predators of Adelgids

Allen Cohen¹, Carole Cheah², Fred Hain¹, Thom Hodgson¹, Kathleen Kidd³

¹North Carolina State University, Raleigh, North Carolina, United States,

²Connecticut Agricultural Experiment Station, Windsor, Connecticut, United States,

³NCDA Plant Industry-Plant Protection, Cary, North Carolina, United States

This project was an effort to establish a model for developing quality and process control in insect rearing systems. We developed a system of quality control (QC) and process control (PC) for mass-rearing programs for *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) and *Laricobius nigrinus* (Coleoptera: Derodontidae). The QC system consists of several tiers of observation and decision-making: 1) behavioral, 2) biomass and linear measurements, 3) biochemical assessments, and 4) internal morphology. The PC system is based on measurements of 1) diet quality (natural prey and artificial diet supplements), 2) analysis of process variability, and 3) most influential sources of error and deviations from quality goals (Pareto analysis).

In this study, we compared the value of X-bar charts, R charts, and C charts to determine which of these tools were most helpful in QC and PC systems whose goal was to develop the most reliable controls at the most economical, user-friendly, and biological fitness outcomes. We also made extensive use of regression analysis to help us determine objectively which factors most reliably predict desirable quality of mass-reared predators.

Some of the most important quality and process parameters that we discovered were protein content of predators and diet, free-radical scavenging capacity in predators and diets, lipid content, storage carbohydrate content, predator biomass, and predators' internal condition.

7.1

Twenty five years of mass production of *Phytoseiulus persimilis*: A "bug farm" or an industry?

Shimon Steinberg

BioBee Sde Eliyahu Ltd., Kibbutz Sde Eliyahu, Bet Shean Valley, Israel

The predatory mite *Phytoseiulus persimilis* has been reared at BioBee for 25 years in a "traditional" tri-trophic system under greenhouse conditions, i.e. bean as the plant, red spider mite as the phytophagous prey and the predatory mite. Intuitively, the term "bug farm" relates to mass production systems that are partially controlled or not controlled at all with respect to rearing conditions such as temperature, relative humidity, light, CO₂ as well as demographic indices of the reared organisms. "Industry", on the other hand, has a much stronger grip and control over the aforementioned parameters.

Browsing through the different phases of *P. persimilis* mass production, e.g.: sowing the beans, development of the plants, spider mite infestation, predatory mites' sting stock introduction and *P. persimilis* harvest, shows that with respect to timing and quantitative control, there are phases which are fully controlled yet others are completely uncontrolled. Each of those phases is analyzed according to its degree of control, quantitative consistency and predictability.

There are a few *P. persimilis* producers that aim at alternative "industrial" production utilizing spider mite eggs as a food source for the predatory mites. Others are exploring the possibility of rearing *P. persimilis* on artificial diet. However, mass production of *P. persimilis* in a greenhouse-based tri-trophic system does not automatically mean that the rearing is not industrial. It may well be industrial in parts of its components yet others need more adjustment and improvement in order to shift from a "bug farm" performance to industrial output.

7.2

Life styles of Phytoseiid mites: Implications for rearing and biological control strategies

James McMurtry

Univ. of California, Riverside, CA, United States

Marked specialization for utilizing as prey spider mites that produce copious webbing probably evolved independently in at least 4 of the 16 tribes (taxonomic revision of Chant & McMurtry 2007) across 2 of the 3 subfamilies. The Phytoseiulus species are the most specialized predators (Type I) of Tetranychus spp. The Galendromus spp., e.g. occidentalis and some Neoseiulus e.g. fallacis, prefer spider mites (Type II) but also utilize other prey. Many of these species require spider mites for maximum rearing efficiency, thus making rearing difficult and expensive. Species with generalist feeding habits (Type III) seem to be predominant in most tribes. There exists a wide range of “body plans”, some of which seem more closely related to the plant habitat than to food source. Some genera considered in Type III in which species valuable in biological control have been documented include Typhlodromus, e.g. pyri, Neoseiulus, e.g. cucumeris, Kampimodromus, e.g. aberrans, Amblyseius, e.g. andersoni, Amblydromalus manihoti, Typhlodromalus aripo, and Scapulaseius newsami. Type III generalists can be reared on foods that are easy to produce, e.g. acarid mites or pollen supplemented with prey. Euseius and Iphiseius, in the tribe Euseiini evolved unique characteristics presumably for utilizing pollen as a food source (Type IV). Population increases are sometimes related to airborne pollen fallout on the foliage. These are easily reared on a diet solely of pollen, but they have not been widely utilized. Evaluation of effectiveness in each situation is all-important, and industry probably will play an increasingly major role in this activity.

7.3

Trends in predatory mite production and delivery systems

Richard GreatRex

Syngenta Bioline, Essex, United Kingdom

Plant based production systems for predatory mites have inherent limitations in capacity which place restrictions on their commercial use. Production costs tend to be high, and yields per unit area relatively low. The use of factitious hosts for mass production of polyphagous predatory mites provides many more opportunities for industrial scale production. The pioneering work of Ramakers lead to widespread commercial production and use of *Neoseiulus cucumeris* for control of Thrips. The initial production system using *Acarus siro* has been subsequently improved by use of *Tyrophagus* spp. In parallel with this work, developments in delivery systems produced controlled release products which gave continuous release of predatory mites over extended periods, and effectively removed the need for the predators to establish on the crop in order to give control of the target pest. More recently, the highly competitive nature of this market has lead key players to develop intellectual property to protect their investment in product development. The range of factitious hosts used has increased, and has facilitated an increase in the range of predatory mite species on the market, and the range of pests which can be controlled. Newer delivery systems are increasing the range of crops in which these can be used, and are also protected by intellectual property rights. This trend is likely to continue, as we learn how to economically produce and successfully deliver more species suitable for use in a wider range of crops and environments.

7.4

Demand versus supply in biocontrol: Disturbance of natural balance?

Pierre Ramakers

Wageningen UR Greenhouse Horticulture, Bleiswijk, Netherlands

When biological pest control in protected cultivations was initiated half a century ago, it was a merely technical issue for entomologists, horticultural advisors and vegetable growers, hardly noticed by the outside world. Commercialization was typically supply-driven.

Today, biocontrol has become far more of a societal subject. This has created a marked increase of the demand for biocontrol products since the beginning of this millennium. It involves not only the traditional market of fruiting vegetables, but also cut flowers, potted plants, nurseries, semi-protected crops and soft fruits.

The problem is in the limited assortment. For most pest-crop combinations, effective biological products are simply not available. This gap between demand and supply has created a commercial vacuum, attracting dubious products of unproven efficacy.

Biocontrol is suffering from a notorious lack of innovation. Scientists produce an overwhelming amount of publications about pest predator interactions, but development into a both effective and marketable antagonist is a rare event. Biocontrol companies spend most of their energy on mutual price competition (read: reduction of mass rearing costs), trying to cover as much market as possible with one and the same natural enemy. It should be recognized that innovation is of crucial importance for the biological industry as a whole rather than for an individual company. If (industrial and independent) researchers will not succeed in bridging today's gap between demand and supply, policymakers may conclude that biocontrol has come to the bounds of its possibilities.

1.P1**Bacterial community of the spined soldier bug gut**

Alejandro P. Rooney¹, Thomas A. Coudron¹

¹*National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, United States*, ²*Biological Control of Insects Research Unit, USDA-ARS, Columbia, MO, United States*

Cost of mass rearing beneficial insects remains a major impediment to their deployment for pest insect control. Potentially, the microbiota of the digestive track may be altered during mass rearing and subsequently performance impacted. Dietary supplements of probiotics may be a way to address some of the effects of mass rearing and diminished performance. We analyzed the bacterial community in the gut of field-collected and domesticated colonies of *Podisus maculiventris*. Overall levels of bacterial diversity were low. Several previously unknown species were detected. A potential symbiont was found in the field-collected samples. Additionally, we found a shift in bacterial content from one year to the next. Collectively, these findings are cause for optimism for the development of probiotics that will improve mass rearing of insects and potentially the field performance of beneficial species.

2.P1

Development of management programs for white grubs in California blueberries

David Haviland, Natalie Hernandez

University of California Cooperative Extension, Kern County, CA, United States

During the past few years white grubs have become recognized as a pest of southern highbush blueberries in California. White grubs feed on plant roots, causing the plant to be stunted. In some cases plant death has occurred when large grub populations attack newly planted fields. The predominant white grub species in California blueberries was identified as *Cyclocephala longula*. Research on flight characteristics determined that grubs are primarily in the third instar in April, pupate in May, and fly from mid-June through mid-July. Egg hatch begins in mid-July. Adult beetles begin flying about 30 min after dark and can be collected for a period of about 2 hours with black-light traps. Evaluation of control methods found that the entomopathogenic nematode *Heterorhabditis bacteriophora* and the insecticide imidacloprid can both provide control of the grub. Applications of *Heterorhabditis bacteriophora* on 1 April initially only provided 8.3% control, but resulted in secondary spread that led to an epizootic within the grub population. Applications of *Heterorhabditis bacteriophora* and imidacloprid in August resulted in 81.6 and 71.1% control, respectively, the following June.

3.P1**Fecundity and percentage egg hatch of potato tuber moth F_1 progeny of 150-Gy irradiated parents crossed with irradiated moths.**

George Saour, Hayat Makee

Atomic Energy Commission, Damascus, Syrian Arab Republic

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is one of the most important insect pest of cultivated potato. F_1 sterility in Lepidoptera provides advantages for the use of inherited sterility in a sterile insect technique (SIT) program. Newly emerged PTM males and females were irradiated with a dose of 150 Gy and either inbred or outcrossed with fertile counterparts. Inherited effects resulting from irradiation of males and females were expressed in the resulting F_1 generation. The percentage of egg hatch declined significantly (with almost zero emerged adults) when the irradiated females were crossed with either irradiated or non-irradiated males. In addition, the crosses of surviving F_1 -generation offspring originating from irradiated male x non-irradiated female with normal and 150-Gy irradiated moths were determined. Fecundity and percentage egg hatch were the lowest when F_1 males were crossed with 150-Gy irradiated females. Our data suggest that 150-200 Gy would be an appropriate dose for using a sterile insect technique (SIT)- F_1 program against PTM.

3.P2

Thailand mass rearing and quality control of *Bactrocera dorsalis* (Hendel) and *Bactrocera correcta* (Bezzi)

Suksom Chinvinijkul, Supaap Pinkaew, Watchreeporn Orankanok
Irradiation for Agricultural Development Division, Bureau of Agricultural Product Quality Development, Department of Agricultural Extension, Ministry of Agriculture and Cooperatives, Bangkok, Thailand

Fruit fly mass rearing facility of Thailand is located in Thanyaburi district, Pathumthani province, 34 km from Bangkok. The original target was mass rearing the sterile Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). More responsibility has occurred since 2003 after *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) was reported as one of the serious insect pests in commercial fruit crop in fruit fly control area in 2002.

Ten years mass rearing data from 2000-2009 demonstrated that the highest total weight of diet preparation for both species was 323.38 tons in 2008 while the lowest amount was 60.08 tons in 2006. The maximum number of irradiated pupae released for both species was 1311.33 millions in 2007 and the minimum was 312.81 millions in 2009. Quality of eggs, pupae and adults were assessed continuously. Average quality of *B. dorsalis* and *B. correcta* respectively were egg hatch: $80.01 \pm 5.60\%$ and $83.78 \pm 2.21\%$; pupal weight: 11.07 ± 0.51 mg and 11.02 ± 0.21 mg; pupae number per 10 cc: 463.88 ± 17.70 and 477.17 ± 7.79 ; pupae recovery: $37.41 \pm 8.19\%$ and $21.70 \pm 3.08\%$. Average adult eclosion and fliers percentage before and after irradiation process of *B. dorsalis* in respective were $90.66 \pm 5.01\%$, $83.95 \pm 7.88\%$, $88.85 \pm 5.58\%$ and $76.95 \pm 7.24\%$ meanwhile for *B. correcta* were $90.09 \pm 3.76\%$, $85.67 \pm 4.52\%$, $87.88 \pm 3.84\%$ and $81.06 \pm 4.47\%$, respectively.

3.P3**Application of nuclear techniques in the mass rearing of *Nesolynx thymus* (Hymenoptera: Eulophidae), an endoparasitoid of Uzi fly *Exorista sorbillans***

Md. Mahbub Hasan, Md. Rayhan Uddin, Md. Aatur Rahman Khan, Aminuzzaman Md. Saleh Reza

Department of Zoology, Rajshahi University, Rajshahi-6205, Bangladesh

The Uzi fly, *Exorista sorbillans* Weidemann, is an endoparasitoid of the the silkworm moth, *Bombyx mori* L., and can impact commercial sericulture. Effects of gamma radiation (^{60}Co) on the mass production of the hyperparasitoid *Nysolynx thymus* (Girault) were investigated. To assess the potential value of nuclear techniques in improving host suitability, two cohorts of early (2-4 day-old) and late (6-7 day-old) host puparia were selected for irradiation. The host pupae were irradiated with 0 (control), 0.5, 1, 2, 4 or 8 Gy for early pupae and 0 (control), 10, 30, 50, 70 or 90 Gy for late pupae. Gamma irradiation significantly ($P < 0.001$) increased the progeny production of *N. thymus* when reared either on early or late irradiated host puparia, particularly in the parental generation, but irradiated early host pupae were more suitable for mass production of *N. thymus* than the irradiated late pupae. The sex ratio of parasitoids developing from gamma irradiated host pupae varied significantly. Higher proportions of females were observed for all the dose and host-age groups. The present finding leads to the conclusion that ionizing radiation offers a reliable means to achieve developmental arrest of insect hosts for use in in vivo rearing prior to mass production of the parasitoid *N. thymus*.

3.P4

VIENNA 7/Mix 99 downunder — the Western Australian experience of rearing a genetic sexing strain medfly for use in SIT programmes

Roselia Fogliani, Bill Woods

Department of Agriculture and Food Western Australia, South Perth, Western Australia, Australia

Mediterranean fruit fly (medfly), *Ceratitis capitata*, has been present in Western Australia since the late 1890s and occurs in established populations wherever fruit is grown, with the exception of the tropical north. It does not occur elsewhere in Australia. The male-only, white pupa/temperature sensitive lethal, genetic sexing strain medfly, VIENNA 7/Mix 99, was introduced in late 1999 for use in a trial eradication programme in the coastal town of Broome, north of Perth, to provide data for a benefit/cost analysis into eradication from the whole state. It has since been used in SIT campaigns to eradicate medfly outbreaks in neighbouring South Australia on five occasions between 2001 and 2010, and in a suppression programme in combination with a baiting scheme in the inland town of Katanning, south east of Perth.

The maximum weekly production of male medfly pupae in the Western Australian facility is 10 million. Constant monitoring of, and improvements to, the rearing process over the past 11 years have resulted in a stable egg-to-male pupa efficiency of approximately 20%. Key quality indicators (pupa weight >8mg; post-irradiation emergence >90%; post-irradiation flight ability >80%) exceed accepted international standards for mass-reared medfly. An effective continuous filter rearing system, amplified weekly into the main colonies, has ensured strain integrity with a low recombination rate (<1%). Gamma irradiation of pupae in a nitrogen atmosphere, and pre-release exposure of sterile male flies to ginger root oil have helped to maintain fly competitiveness and extend the useful life of this genetic sexing strain medfly.

3.P5**Preliminary study in artificial rearing of Chinese citrus fruit fly, *Bactrocera minax* (Enderlein)**

Yongcheng Dong¹, Changying Niu¹, Peng Han¹, Zhou Chen¹, Chaoliang Lei¹, Rui Cardoso Pereira¹

¹*Plant Science & Technology College, Huazhong Agricultural University, Wuhan, Hubei 430070, China, China,* ²*Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear, Techniques in Food and Agriculture, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria*

The Chinese citrus fruit fly (*Bactrocera minax*) is a univoltine frugivorous specialist. Recorded most from China, the host range is restricted to species of citrus. Adults emerge annually in early May. Females lay eggs in the fruits in July, and larvae develop inside until they drop to the soil, and pupate in early November. Pupal stage enters diapause for overwinter and last 6 months in the field. To study the pupal diapause, thermal responses controlling adult eclosion were evaluated. When pupae were exposed to various temperatures (5°C, 10°C, 15°C, 20°C) and different time intervals (30d, 60d, 90d, 120d, 150d, 180d), and then transferred to 25°C, the highest percentage of emergence was obtained at 5°C and 60d. At constant 25°C, few pupae emerge as adults. However, there are about 10% non-diapausing individuals in the studied population. Preliminary studies for semi-artificial rearing of *B. minax* were conducted. Adult flies were fed with white sugar and yeast at a ratio 4:1, aside from the availability of water. Green oranges were supplied in cages for the females to lay eggs, and then transfer the infested oranges to the incubator at 26°C to trigger eggs hatch. Larvae were removed from the oranges and then transferred to the artificial diet, including sodium benzoate, sugar, yeast, HCl, orange juice and water. Pupae were stored in vermiculite at ~15% relative humidity. Laboratory rearing and knowledge of diapause of *B. minax* are key aspects to a potential development of SIT for this species.

3.P6

Feasibility study for the genetic control of *Aedes albopictus*

Arianna Puggioli, Anna Medici, Marco Carrieri, Romeo Bellini

Centro Agricoltura Ambiente "G.Nicoli", Med. & Vet. Dept., Crevalcore, Bologna, Italy

SIT application against *Aedes albopictus* is based on the mass rearing, sterilization and release into the natural habitat of large numbers of males of the target species. The released sterile males must be able to fly, to disperse enough from the release station to cover the target area, to survive and be sexually active long enough to cover the time between successive releases, to locate the virgin wild females and successfully compete for mating with the wild males. The CAA laboratory, under standard conditions, has the capacity to produce 100,000 sterile males/week. An isolated village and a closeby control area were chosen to carry out release trials for three consecutive years. From April to September, male pupae were irradiated with Gamma rays and released in fixed stations. Ovitrap were placed in the release area and in the control area to collect the eggs and check for their hatching rate. From mark-recapture tests we were able to investigate the male longevity in urban areas during the summer months and we estimate that during the harsh periods the longevity of the males may be a limiting factor. R.H. in particular resulted to play a major role strongly influencing male survival. The number of the release stations was reduced during the three year study, introducing a new special release station to supply the irradiated males with an energetic source. A significant reduction in the eggs fertility level and density in the release area when compared with the control area were obtained, supporting the possibility of scaling up the system.

3.P7**Laboratory colonization of *Aedes albopictus* and effect on some fitness parameters**

Anna Medici, Arianna Puggioli, Marco Carrieri, Romeo Bellini

Centro Agricoltura Ambiente G.Nicoli, Med. & Vet. Dept., Crevalcore, Bologna, Italy

Aedes albopictus is the most dangerous mosquito species recently imported in Europe. In Italy, its density in the inhabited areas is too high despite the application of the currently available control technique, thus making large areas at risk of epidemic (Italy has recently experienced an outbreak of Chikungunya virus) and requiring the development of new tools. *Ae. albopictus* has different aspects which could be regarded as positive for SIT application: urban distribution, low active dispersal capacity, natural mortality during the winter period and the possibility to be mass reared. CAA is currently running a small pilot mass rearing system for experimental purposes, which shows the opportunity to scale up with a larger pilot facility. One of the main factors to be considered in order to guarantee a sufficient level of competitiveness of the reared sterile against fertile wild males is the negative effects of colonization. To evaluate the effect of mass-rearing, trials to measure the fitness of two strains reared for 18 generations and of two hybrids obtained crossing the two strains were conducted. One of the two strains was then maintained in lab for other 15 generations and, on it, the same fitness analysis was conducted. On the F33 both males and females seem more adapted to the cage, showing a better longevity and a better capacity to mate but a reduction in the wing length and a reduction in the fecundity of the females. The hybrids gave good results but the convenience to adopt this option remains to be determined.

4.P1

Olive fly: from small scale production to large scale mass-rearing

Sohel Ahmad, Viwat Wornoyporn, Ihsan ul Haq, Carlos Cáceres, Andrew Jessup
FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria

The olive fly, *Bactrocera oleae* Rossi (Diptera: Tephritidae), is considered an ideal candidate for control by the sterile insect technique (SIT) as part of an integrated pest management approach because it attacks only olives and does not disperse naturally great distances unlike other *Bactrocera* spp. One major constraint in the development of a successful and cost-effective SIT programme for olive fly is the large scale production of high quality mass-reared flies. The aim of this work was to develop cost-effective methods for mass-rearing. The following three basic parameters in mass-rearing were examined: Cage density of adult flies, size and design of the adult oviposition cage, and egg collection methods. The results showed that an adult fly density of 4.1 cm² (internal surface area of cage) per fly in a medium-sized cage (0.036m³) produced up to 11.5 eggs/female/day over the life of flies. Further research on other cage sizes ranging from 0.015 m³ to 0.4 m³ resulted in egg volumes from only 2.8 to 6.8 eggs/female/day. Our newly developed method of egg collection using a flat egg panel proved cost-effective, more efficient, and enabled us to elevate the colony size to a level that mass-production can be started. As a consequence of this work, we are now rearing the flies in large cages (0.4 m³) formerly used for mass rearing of Mediterranean fruit fly and we are now optimizing the density of olive fly in cages that are more acceptable for cost-effective mass rearing of this insect.

4.P2

***Ephestia kuehniella* eggs sterilization for *Trichogramma ostriniae* Pang et Chen (Hymenoptera: Trichogrammatidae) mass production**

Mylène St-Onge¹, Daniel Cormier², Silvia Todorova³, Éric Lucas¹

¹Université du Québec à Montréal, Montréal, Québec, Canada, ²Institut de recherche et de développement en agroenvironnement, Saint-Bruno-de-Montarville, Québec, Canada, ³Anatis Bioprotection, St-Jacques-le-Mineur, Québec, Canada

Trichogramma ostriniae a parasitoid of *Ostrinia furnacalis* was introduced to the United States in 1990 to evaluate its potential to parasitize eggs of the European corn borer *Ostrinia nubilalis*. *Trichogramma ostriniae* appeared to be one of the most effective species against this pest. The rearing host *Ephestia kuehniella* needs to be sterilized because the larvae are very voracious and can affect the quantity of the trichogramma produced. Consequently, in this study, three different sterilization modes were evaluated: UV irradiation, freezing at -15° C and freezing in liquid nitrogen at -196° C. For each sterilization mode we determine the minimal exposure time to prevent the *E. kuehniella* larvae emergence. Our hypothesis was that the sterilization by liquid nitrogen would provide the best parasitism rate by *T. ostriniae* since the vitrification generated by this temperature cause less damage than the freezing process at lower temperature and the UV irradiation. In order to obtain 100% eggs mortality, we used an UV irradiation period of 15 minutes, a freezing at -15° C period of four hours and a freezing in liquid nitrogen period of 30 seconds. The parasitism rate of the eggs sterilized with UV and at -15° C were respectively 0,78 and 0,80, whereas among eggs sterilized with liquid nitrogen only 0,30 were parasitized. The emergence rate of *T. ostriniae* was also significantly lower for eggs that have been sterilized with liquid nitrogen, 0,86 compared to 0,98 for the other two methods of sterilization.

4.P3

A New Type of Solid, Semi-Solid, and Semi-Liquid Arthropod Artificial Diets Using Colloids to Replace Gelling Agents

Guadalupe Rojas, Juan Morales-Ramos

USDA-ARS BCPRU, Stoneville, Mississippi, United States

A new type of artificial diets for arthropods has been developed that incorporates a colloidal component. The physical consistency of these colloidal artificial diets can be adjusted from semi-liquid to solid by varying the water content. The colloidal components do not need to be heated during the mixing process allowing sensitive nutrient ingredients to be mixed simultaneously. The colloidal particles provide a substrate that enhances extra-oral digestion making this type of diets suitable for a large range of predatory arthropods. Colloidal diet formulations have been developed with satisfactory results for a variety of arthropods including, *Trichoplusia ni*, *Helicoverpa virescens*, *Lygus hesperus*, *L. lineolaris*, *Coleomegilla maculata*, *Hypothenemus hampei*, *Tetranychus urticae*, and *Phytoseiulus persimilis*. Solid formulations were developed for the two lepidopterous species. Results showed similar development time and fecundity as existing agar-based diets. *Trichoplusia ni* was reared for 10 generations in the new colloidal formulation with survival higher than 85% and insignificant changes in development time and fecundity. The diet formulation for *C. maculata* has a semi-solid consistency, but adults and larvae feed directly on the diet. This coccinellid has been reared in the new artificial diet for 3 generations without changes in development time and fecundity. The two *Lygus* species have been reared in a semi-liquid colloidal diet for 10 generations. Development time has decreased significantly in the 5th generation as compared to the first generation in the colloidal formulation.

4.P4**New frontiers in the biological control of insects**

Thomas Coudron, Holly Popham, Kent Shelby, David Stanley

USDA-ARS, Columbia, Missouri, United States

The greening movement, food security and safety, climate change and expanding applications provide opportunities for growth of the biological control industry. Plant biofortification, cost of biocontrol agent production, regulatory restrictions and non-producer decision-making present challenges to the industry. These opportunities and challenges are influencing research efforts intended to improve biological control efforts. As a result, biocontrol is undergoing a seismic shift from classical control toward conservation strategies. Two additional concepts are breeding of native beneficial agents for improved performance and increasing the susceptibility of pest insects to beneficial agents. The Biological Control of Insects Research Laboratory is developing these two ideas and partnering with producers to test the feasibility and effectiveness of their application.

4.P5

Wheat germ oil in larval diet influences gene expression in adult oriental fruit fly

Chiou Ling Chang¹, Thomas Coudron², Cynthia Goodman², David Stanley², Shiheng An³, Qisheng Song³

¹USDA-ARS-PBARC, Hilo, Hawaii, United States, ²USDA-ARS-BCIRL, Columbia, Missouri, United States, ³University of Missouri, Columbia, Missouri, United States

Culture media supplemented with wheat germ oil (WGO) exerts observable physiological actions, such as increased fecundity and mobility, in some insects. Although the impact of WGO on insect physiology is important, the mechanisms of these actions are poorly understood. Here we report on the outcomes of experiments designed to test our hypothesis that the addition of WGO into medium developed for larval oriental fruit flies modulates gene expression in the corresponding adults. We separately reared immature *Bactrocera dorsalis* on diets lacking, and supplemented with, WGO and analyzed expressed proteins in the resulting adult males and females by 2-D electrophoresis. Analysis of the gels revealed significant changes in expression levels of >70 proteins, 64 of which were identified by mass spectrometric analysis on MALDI TOF/TOF. The apparent changes in expression levels of 6 proteins were confirmed by quantitative real time PCR or qPCR, showing that the changes in mRNA expression were reflected in changes in protein expression. These findings support the hypothesis that one mechanism of WGO actions in insect nutrition is the modulation of gene expression.

4.P6**ASTM International Subcommittee E35.30: Supporting development and maintenance of current standards for assessing quality of macrobial biological control agents**

Carol Glenister

IPM Laboratories, Inc., Locke, NY, United States

ASTM Subcommittee E35.30 on Natural Multi-Cellular (Metazoan) Biological Control Organisms is active in creating, commenting on, and voting on quality standards for commercial natural enemy products. Members include producers, researchers and users. As a full consensus organization, all opinions on standards under development are valued and considered. Balance of member users and member producers is a requirement in the voting process. However, one does not have to be a member to comment on either published standards or standards in process. All comments must be addressed by the subcommittee. To date, quality standards have focused on methods of product enumeration upon receipt by the buyer. Each standard is reviewed for relevance every 5 years and it is voted whether to maintain, revise or drop it. ASTM is a non-profit international organization with the purpose of "the development of standards on characteristics and performance of materials, products, systems and services; and the promotion of related knowledge." More information on ASTM International can be found at www.astm.org.

6.P1

A new world-wide database of insect, mite and nematode cultures available for distribution.

Peter Ebling

Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

The Canadian Forest Service, Natural Resources Canada, has established a comprehensive world-wide listing of producers and suppliers who are willing to sell or donate live insects, mites or nematodes. This database is intended to provide current sources of live cultures and to give producers and suppliers a no-cost opportunity to expand their client base. **We are currently soliciting the enrolment of additional insect, mite and nematode producers and suppliers.**

Producers and suppliers are required to submit contact information, identify the cultures they have available for sale or distribution, select target pests for biological control agents, and provide the geographical locations of the cultures. This information can be entered by selecting appropriate information from our drop down menus.

The database can be sorted by producers or suppliers; taxonomic order, family, and genus; scientific or common names; use categories, and geographical region. In addition, this database will soon be searchable for target pests and sources of biological control agents for their control.

We manage the database so that submissions are screened and the information is verified. Registrants may review their information at any time and submit revisions. Also, registrants are contacted annually to update their submissions and assure that the cultures remain available. The database is designed to be accurate and available in perpetuity.

This database will become more useful as additional producers and suppliers participate by having their cultures listed. **We hereby solicit your enrolment and encourage you to make your colleagues and peers aware of the database:**
www.insect.glf.cfs.nrcan.gc.ca/prod/index.cfm?lang=eng.

6.P2**Artificial rearing of *Anastrepha fraterculus* (Wiedemann 1830) (Diptera: Tephritidae): Egg-viability and models of cages**Juliana García Carrión*Servicio Nacional de Sanidad Agraria, Lima, Peru*

The South American fruit fly, *Anastrepha fraterculus* (Wiedeman 1830), is one of the most important agriculture pests in Peru. The Sterile Insect Technique (SIT) is a powerful no-pollutant method for its direct control. There has been reported the efficacious control of a parental species *Ceratitis capitata* in Peru (Wiedeman 1829). The present work aims to contribute to the improvement of the SIT against *A. fraterculus*. Selection of the period of egg collection was based on viability percentage and volume of collected eggs during 21 days. Moreover, three models of cages called "big", "medium" and "mission" were compared to evaluate the number of eggs/female/day in order to improve mass rearing and SIT. The results showed a positive correlation between % viability and egg volume ($R^2=0.83$). The suitable collection period was 10 days with viability (67.4%) and total egg produced (22.4 ml). Furthermore, when the eggs produced and the number of eggs/female/day was evaluated among the three models of cages, the "medium" showed the highest values (11.4 eggs/female/day) while the "big" one produced 8.6 eggs/female/day. However, there was no significance difference between the models. These values are very important in mass rearing. The "missions" showed an average value (4.6 eggs/female/day) and exhibited significant difference compared to the other two models. Thus, the best model of cage to improve the mass rearing of *A. fraterculus* is the "medium".

Key words: *Anastrepha fraterculus*, SIT, egg viability, Number of eggs/female/day, cages.

6.P3

A global quality index for *Trichogramma*

Shoil Greenberg¹, Norman Leppla¹

¹*USDA, ARS Beneficial Insects Research Laboratory, Weslaco, Texas, United States,*

²*University of Florida, IFAS, Entomology and Nematology Department, Gainesville, Florida, United States*

Reliable supplies of superior quality *Trichogramma* spp. are required for their effective use in biological control programs. While research has been concentrated primarily on maximizing the yields from rearing facilities, both quality and quantity are crucial. Consequently, mass-rearing must be accompanied by the development of quality control methods. A quality index for mass reared *Trichogramma* has been developed that measures their overall vitality and productivity. It predicts their ability to perform the functions for which they are produced. This standardized assessment of *Trichogramma* is quick and reliable, and estimates generalized criteria for effectiveness in the field. The quality index provides a means of calculating release rates based on pest density, weather conditions, plant phenology, and application methods. It could be used as a global standard for mass-produced *Trichogramma*, particularly for those produced commercially. Ultimately, production, quality control and utilization technologies are interdependent and require interdisciplinary input, e.g., biology (behavior, physiology, ecology), industrial chemistry and engineering, and food processing technology. The goal is to optimize rearing systems designed for both high capacity and the production of high quality insects.

6.P4**Determination of critical storage period of mass reared host eggs parasitized by *Trichogramma evanescens* for efficient adult parasitoid emergence**

Md. Mahmudunnabi, Syed Nurul Alam

Bangladesh Agricultural Research Institute (BARI), Gazipur, Dhaka, Bangladesh

This experiment was undertaken at IPM laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur to determine the critical storage period of mass reared parasitized host eggs by *Trichogramma evanescens* for its efficient release. Mass rearing of the egg parasitoid, *Trichogramma evanescens* was done on *Sitotroga cerealella* (Olivier) eggs. Mass reared parasitized eggs of *Sitotroga cerealella* were kept in desiccators at 3-4°C and 75-85% relative humidity for 3 months. In this study the number of *Trichogramma evanescens* adults emerging from 30 parasitized host eggs/test tube was observed, counted and recorded at 0, 3, 5, 10, 15, 20, 30, 45, 60, 75 and 90 days after storage. In each case, 5 test tubes were used as a replicate and 3 generations of *Trichogramma evanescens* were observed. Results indicated that up to 20 days after storage more than 90% of the adults emerged from the parasitized eggs. Adult parasitoid emergence below 80% was observed after 45 days. It was also found that with the increase of storage period of parasitized egg, percent adult parasitoid emergence was gradually reduced. A negative correlation was observed between the storage period of parasitized egg and percent adult parasitoid emergence from the parasitized eggs. This suggests that for ensuring better performance of the parasitoid *Trichogramma evanescens* the parasitized egg should be stored not more than 45 days.

6.P5

Artificial rearing of a reduviid predator *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) using meat-based artificial diet

K. Sahayaraj, S. Balasubramanian

St. Xavier's College (autonomous), Palayamkottai/Tamil Nadu, India

Reduviids are the important predator against many cosmopolitan insect pests. However, very limited information is available about their utility under field conditions. Because of the non-availability of an artificial rearing method. In this study, we tried an artificial rearing method based on a meat-based diet for rearing a zoophagous reduviid, *Rhynocoris marginatus* (Fab.). *Rhynocoris marginatus* continuous completed three generations were with meta-based oligidic diet without any insect food. Total nymphal period of *R. marginatus* was gradually diminished from F1 (70.1 days) to F2 (68.8 days) and F3 (65.1 days) with survival rate of 68 to 78 percent. However, when factitious host, *Corcyra cephalonica* Stainston was provided as prey, the total nymphal period was ranged from 44 to 47 days. The sex ratio of oligidic-diet (0.62, 0.79 and 0.68 for F1, F2 and F3, respectively) and factitious groups (0.72, 0.65 and 0.82 for F1, F2 and F3, respectively) was female biased. Male reduviid lived longer than females in both categories. Artificial rearing of reduviid with oligidic diet reduced the adult longevity of both male and female. Factitious host reared reduviid laid a maximum of 177 eggs/female. Whereas artificial diet reared reduviid laid 53 eggs per female. Nearly 6 - 8% of adults are deformed while reared with meat-based artificial diet. Artificial rearing of reduviid is possible either in small or large scale and utilizes them in pest management programme. We suggest supplementary feeding with live prey during the development can reduce nymphal developmental period and deformities, enhanced survival, fecundity and hatchability.

6.P6

Effects of olive oil and yeast in liver-based artificial diet for the production of *Orius laevigatus*

Samira Safarian^{1,3}, Ahmad Ashouri¹, Hamid Reza Sarraf Moayeri², Reza Talaei Hassanloui¹, Sima Kabiri¹

¹Department of plant protection, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran, Islamic Republic of, ²Department of plant protection, faculty of agriculture, Zanjan University, Zanjan, Iran, Islamic Republic of, ³Gyah Bazr Alvand Corporation, Tehran, Iran, Islamic Republic of

Effects of four artificial diets containing D1) ground beef, beef liver, sucrose solution and egg yolk (as a base diet); D2) first diet plus olive oil; D3) first diet plus yeast and D4) first diet plus olive oil and yeast on life history traits of the predaceous bug, *Orius laevigatus* were studied under laboratory condition. Nymphal development time of bugs reared on D4 was significantly lower in comparison to other diets (14.2±0.2, 14.1±0.1 and 14.1±0.1, 13.7±0.1 days respectively for D1 to D4) but was not significantly different from conventionally reared individuals on *Ephestia kuehniella* eggs (13.0±0.1 days). Although, nymphal survivorship for D2 and D4 did not show a significant difference (75±4.3% and 83±4.8%) from nymphs reared on *E. kuehniella* eggs (87±5.5%), the nymphal survival of D2 and D4 was significantly higher than D1 and D3 (37.33±5.7% and 57.5±7.7% respectively). Another study was conducted to compare the biological characteristics of adult bugs reared on D4, as the most efficient diet for nymphs, and *E. kuehniella* eggs. Fecundity of females fed with D4 (126 eggs) did not reveal any significant difference compared to females reared on *E. kuehniella* eggs (118 eggs). The egg hatch and oviposition rate of bugs reared on the aforementioned diets were similar. The results of this research suggest that adding olive oil, as a resource of fatty acids, and yeast can improve the nutritional value of the artificial diet for *O. laevigatus*.

6.P7

Developmental and reproductive fitness of *Adalia bipunctata* on factitious and artificial foods

Maarten Bonte, [Patrick De Clercq](#)

Department of Crop Protection, Ghent University, Ghent, Belgium

The aphidophagous ladybird *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) is native to Europe and may have potential for the biological control of aphid pests in different crop systems. The availability of adequate factitious or artificial foods may contribute to enhancing the cost-effectiveness of its mass production. This study examined the nutritional value of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and pollen, pea aphids, *Artemia franciscana* Kellogg (Branchiopoda: Artemiidae) cysts, a lyophilized meat-based artificial diet, and mixtures of pollen with lyophilized artificial diet and/or *A. franciscana* cysts. Immature survival, developmental time and fecundity of ladybirds fed *E. kuehniella* eggs and pollen (94.9%, 15.3 days and 1864 eggs, respectively) were better than that of those fed aphids (84.5%, 16.9 days, and 796 eggs, respectively) or any of the other tested foods. The mixtures could, however, support survival (40.4-74.1%) and sustain development (taking 20.7-25.1 days) and reproduction (264-889 eggs) of *A. bipunctata*. Using mixtures of certain animal and plant components as foods for this and other insect predators may help increase the cost-effectiveness of their mass production by reducing the inputs of nutritious but expensive foods, like *Ephestia* eggs.

6.P8**A record of three Korea indigenous species newly developed as biological control agents for controlling aphids**

Hyunjin Shin, Wooyeun Kim, Taesu Kim

SESIL Corporation, Nonsan, Chungnam, Korea, Republic of

It is well known 4 parasitoids (*Aphidius colemani*, *Aphidius ervi*, *A. matricariae*, *Aphelinus abdominalis*) and 3 predators (*Aphidoletes aphidimyza*, *Chrysoperla carnea*, and *Harmonia axyridis*) as the major natural enemies of aphids all over the world. In addition to above mentioned natural enemies, novel aphid natural enemies include two parasitoid species (*A. gifuensis*, *Aphelinus asychis*) and two predator species (*Micromus angulatus*, *H. yedoensis*) which have been developed are used in Korea. Especially, *A. gifuensis*, *A. asychis*, *H. yedoensis* are widely used for aphids biological control and were the first commercial native natural enemies developed by SESIL of Korea.

In the biological characteristics, it was confirmed that *A. gifuensis* was similar with *A. colemani*, *A. matricariae*. But *A. gifuensis* have high adaptability in higher temperatures, it was more available to use these conditions that *A. colemani*, *A. matricariae*. In general, aphid parasitoids have an advantage that can be used for effective control of aphids compensating disadvantages such as decrease of activity during high temperatures periods.

The Biological characteristics of *A. asychis* are similar with *A. abdominalis*. However, in the reason that *A. asychis* have high parasitism to *Myzus persicae* and *Aphis gossypii* as well as potato aphid and greenhouse potato aphid, it can more effectively control aphids when aphids density is low and simultaneously occurred different aphid species at the control area.

7.P1

Mass rearing of *Neoseiulus longispinosus*(Evans) (Acari: Phytoseiidae) under field and laboratory conditions in Himachal Pradesh in India

Usha Chauhan, P.R. Gupta

Dr YS Parmar University, Solan-Nauni, HP, India

Various vegetables, fruits and ornamentals are being grown in the polyhouses in Himachal Pradesh in India, which experience heavy damage due to the attack of two spotted spider mite *Tetranychus urticae* (Koch). Indiscriminate use of pesticides against this pest has led to development of resistance and residues problems which are harmful to the human health and causing environmental pollution. So the best available option for the management of mite is the use of natural predators and botanicals. *Neoseiulus longispinosus* (Evans) (Acari: Phytoseiidae) is a one of the natural and potential predator of this mite which can be successfully used to control its population. For maintaining culture of prey and *Neoseiulus longispinosus* under laboratory conditions, excised mulberry leaves were used on wet sponge sheet in Petri plates/trays. In the summer month's multiplication of predator was faster than in the winter months. Total life cycle egg to adult of *N. longispinosus* was between 7 to 14 days in the winter months of November and Decembers. During summer, mass multiplication was done on raised potted bean plants. The predator:prey ratio was found to be 1:30 during July-August to wipe out population of *T. urticae* in a week. During winters, when no mulberry leaves are available and bean plants also die due to cold weather, then culture of the predators was multiplied on the strawberry plants. So the present work is an small effort towards developing some of the economical mass rearing methods of this potential predator.

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Abdalla, Adly, *FAO/IAEA, Vienna, Austria*

Adamczyk, John J., *USDA, Weslaco, TX, United States*

Ahmad, Nazir, *Nuclear Institute of Agriculture, Hyderabad, Sindh, Pakistan*

Ahmad, Sohail, *FAO/IAEA, Vienna, Austria*

Andermatt, Martin, *Andermatt Service AG, Grossdietwil, Switzerland*

Arijs, Yves, *Biobest, Westerlo, Belgium*

Barcinas, Joe, *Foothill Agricultural Research, Inc, Corona, CA, United States*

Ben-Yosef, Michael, *The Hebrew University of Jerusalem, Rehovot, Israel*

Beukeboom, Leo, *University of Groningen, Haren, Netherlands*

Bolckmans, Karel, *Koppert BV, Berkel en Rodenrijs, Netherlands*

Bourtzis, Kostas, *University of Ioannina, Agrinio, Greece*

Brown, Andrew, *Becker Underwood, Littlehampton, West Sussex, United Kingdom*

Burri, Patrik, *Andermatt Biocontrol, Grossdietwil LU, Switzerland*

Cahn, Daniel, *Syngenta Bioline, Oxnard, CA, United States*

Carpenter, James, *USDA-ARS, Tifton, Georgia, United States*

Chang, Chiou Ling, *USDA-ARS-PBARC, Hilo, Hawaii, United States*

Channappa, Ravi, *Monsanto Research Centre, Bangalore, Karnataka, India*

Chaudhury, Muhammad, *USDA, Panama City, Panama, Panama*

Chauhan, Usha, *Dr Y.S. Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India*

Cherch, Sal, *Organic Nutrition, LLC, Boca Raton, Florida, United States*

Chinvinijkul, Suksom, *Irradiation for Agricultural Development Section, Bureau of Agricultural Product Development, Bangkok, Thailand*

Conlong, Desmond, *South African Sugarcane Research Institute, Mount Edgecombe, KwaZulu-Natal, South Africa*

Cortes Ortiz, Juan Antonio, *Biocolor, S.L., Almeria, Almeria, Spain*

Coudron, Thomas, *USDA, Columbia, Missouri, United States*

Couwels, Peter, *Koppert B.V., Berkel en Rodenrijs, Zuid Holland, Netherlands*

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De Clercq, Patrick, *Ghent University, Gent, Belgium*

Demirbas, Halil, *Biological Agriculture Consulting and Engineering Co, Hatay, Turkey*

Ebling, Peter, *Natural Resources Canada, Sault Ste. Marie, Ontario, Canada*

Erkilic, Lerzan, *Biological Agriculture Consulting and Engineering Co, Hatay, Turkey*

Fogliani, Roselia, *Department of Agriculture and Food Western Australia, Perth, Western Australia, Australia*

Fru, Gideon Azie, *Greenbelt, Inc Cameroon, Yaounde, Centre, Cameroon*

García Carrión, Juliana, *Servicio Nacional de Sanidad Agraria-SENASA, Lima, Peru*

Ghann, Clay, *Ghann's Cricket Farm, Inc., Augusta, GA, United States*

Gilles, Jeremie, *FAO/IAEA, Vienna, Austria*

Glenister, Carol, *IPM Laboratories, Inc., Locke, NY, United States*

Gomes, Patrick, *United States Department of Agriculture, Raleigh, NC, United States*

GreatRex, Richard, *Syngenta Bioline, Little Clacton, Essex, United Kingdom*

Greenberg, Shoil M., *USDA, Weslaco, TX, United States*

Groenewald, Sampie, *X Sterile Insect Technique, Citrusdal, South Africa*

Groot, Tom, *de Groene Vlieg, Nieuwe Tonge, Netherlands*

Häckermann, Johanna, *Andermatt Biocontrol AG, Grossdietwil, Switzerland*

Hale, Angela, *The Bug Factory Ltd, Nanoose Bay, British Columbia, Canada*

Hale, Chris, *The Bug Factory Ltd, Nanoose Bay, BC, Canada*

Han, Richou, *Guangdong Entomological Institute, Guangzhou, Guangdong, China*

Hasan, Md Mahbub, *Rajshahi University, Rajshahi, Rajshahi, Bangladesh*

Haviland, David, *University of California, Bakersfield, CA, United States*

Hayder, Ghulam, *BCP Certis, Ashford, Kent, United Kingdom*

Hendrichs, Jorge, *FAO/IAEA, Vienna, Austria*

Herz, Annette, *Julius Kühn-Institut, Darmstadt, Germany*

Hesketh, Helen, *Centre for Ecology & Hydrology, Wallingford, Oxfordshire, United Kingdom*

Horton, Kimberly, *Sterling Insectary, Delano, California, United States*

Jans, Kris, *Biobest Belgium NV, Westerlo, Belgium*

Jessup, Andrew, *FAO/IAEA, Vienna, Austria*

Klapwijk, Johannette, *Koppert B.V., Berkel en Rodenrijs, Zuid Holland, Netherlands*

Knight, Jonathan, *Imperial College London, Ascot, Berkshire, United Kingdom*

Lalitha, Yadavalli, *National Bureau of Agriculturally Important Insects, Bangalore, Karnataka, India*

Lasater, Charles, *Beneficial Insectary, Inc., Redding, CA, United States*

LeBeck, Lynn, *Assoc. Natural Bio-control Producers, Clovis, California, United States*

Leppla, Norman, *University of Florida, Gainesville, Florida, United States*

Levi, Ofir, *Bio-Fly Ltd., Kibbutz Sde Eliahu, Israel*

MacDonald, Tom, *MGS Horticultural, Leamington, Ontario, Canada*

Machtelinckx, Thijs, *Ghent University, Gent, Belgium*

Mahmud, Md. Mahmudunnabi, *Bangladesh Agricultural Research Institute (BARI), Gazipur, Dhaka, Bangladesh*

McMurtry, James, *Univ. of California, Riverside, Sunriver, Oregon, United States*

Medici, Anna, *Centro Agricoltura Ambiente G. Nicoli, Crevalacore, Bologna, Italy*

Montoya, Pablo, *Programa Moscafrut SAGARPA-IICA, Tapachula, Chiapas, Mexico*

Morales Ramos, Juan, *USDA-ARS-MSA-BCPRU, Stoneville, ms, United States*

Niu, Changying, *Plant Science and Technology College, Wuhan, Hubei Province, China*

Papadoyianis, Ernest, *Organic Nutrition, LLC, Boca Raton, Florida, United States*

Parker, Andrew, *FAO/IAEA, Vienna, Austria*

Penn, Sinthya, *Beneficial Insectary, Inc., Redding, CA, United States*

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Pereira, Rui, *FAO/IAEA, Vienna, Austria*

Peters, Arne, *e-nema GmbH, Schwentimental, Germany*

Piedra, Freddy, *Bio Control; S.A., Cartago, Costa Rica*

Puggioli, Arianna, *Centro Agricoltura Ambiente, Crevalcore, Bologna, Italy*

Ramakers, Pierre, *Wageningen UR, Bleiswijk, Netherlands*

Rendon, Pedro, *USDA/APHIS/PPQ/CPHST, Guatemala, Guatemala*

Rhodes, Jack, *Imperial College London, Sunningdale, Berkshire, United Kingdom*

Riley, Kim, *Biobest Canada, Leamington, Ontario, Canada*

Rojas, Guadalupe, *USDA-ARS-MSA-BCPRU, Stoneville, ms, United States*

Sahayaraj, K., *St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India*

Saour, George, *AEC of Syria, Damascus, Syrian Arab Republic*

Shapiro-Ilan, David, *USDA-ARS, Byron, GA, United States*

Shin, Hyun Jin, *SESL Corporation, Nonsan Si, Chungnam Do, Korea, Republic of*

Sijperda, Tjalling, *de Groene Vlieg, Nieuwe Tonge, Netherlands*

Spencer, Brian, *Applied Bio-nomics Ltd, North Saanich, BC, Canada*

Steinberg, Shimon, *BioBee Sde Eliyahu Ltd., Emeq Hamaayanot, Israel*

St-Onge, Mylène, *Université du Québec à Montréal, Montréal, Québec, Canada*

Timmer, Radbout, *Koppert Biological Systems, Berkel en Rodenrijs, Netherlands*

van Baal, Elmer, *Koppert Biological Systems, Berkel en Rodenrijs, Netherlands*

Vera, Teresa, *Facultad de Agronomía y Zootecnia, UNT - CONICET, San Miguel de Tucumán, Tucuman, Argentina*

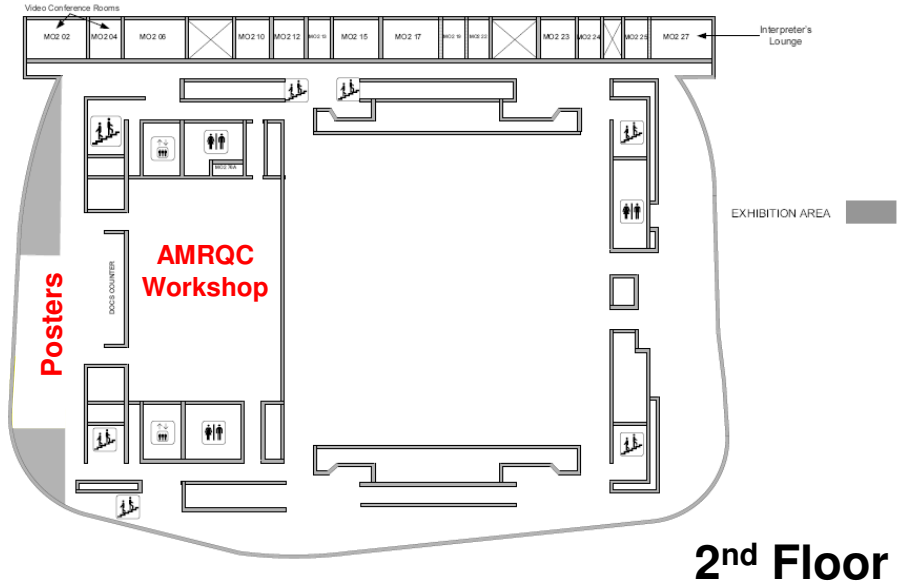
Walker, Phil, *BCP Certis, Ashford, Kent, United Kingdom*

Wandeler, Heiri, *Andermatt Biocontrol AG, Grossdietwil, Switzerland*

Ward, Richard, *Biobest Canada Ltd., Leamington, Ontario, Canada*

White, Jennifer, *ILE, Indian Queens, Cornwall, United Kingdom*

Yang, Sung, *SESL Corporation, Nonsan, Chungnam, Republic of Korea*



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