



ALAEQ

Asociación Latino Americana de Ecología Química
Associação Latino Americana de Ecologia Química
Latin American Association of Chemical Ecology



**III Congress of the Latin
American Association of
Chemical Ecology
~ ALAEQ 2014**

November 18-21
Universidad de los Andes
Bogotá, Colombia

III Congress of ALAEQ
**Latin American Association of Chemical
Ecology**

November 18-21, 2014
Universidad de los Andes
Bogotá, Colombia

Organized by

Latin American Association of Chemical Ecology-ALAEQ

Facultad de Ciencias, Universidad de los Andes
(Bogotá, Colombia)

Corporación Colombiana de Investigación Agropecuaria
CORPOICA (Bogotá, Colombia)

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SPONSORS & PARTNERS ORGANIZATIONS



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PROFESIONAL
DE BIOLOGIA**



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ORGANIZATION

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Dear colleagues,

Welcome to Colombia and to the third Congress of the Latin American Association of Chemical Ecology (ALAEQ)! On this occasion the Universidad de los Andes will open its doors to host researchers from all over the world working in chemical ecology, in such diverse fields as semiochemicals and pest management, insect-plant and plant-plant interactions, semiochemicals identification and synthesis, marine chemical ecology, molecular and neurobiological bases of chemoreception, avian chemical ecology and chemical ecology in medical entomology.

The aim of the Congress is to consolidate the Latin American Association of Chemical Ecology (ALAEQ) in the region and worldwide. To achieve this objective the Organizing Committee, together with ALAEQ's board, has organized a four-day program that includes academic (plenary lectures, symposia, oral and poster presentations), cultural (a visit to the Gold Museum in Bogotá) and social activities.

Like the two previous meetings of ALAEQ in Uruguay in 2010 and Argentina in 2012, this third Congress welcomes approximately 150 participants. We are happy to bring together researchers from 16 countries: Argentina, Brasil, Canada, Chile, Colombia, the Czech Republic, Ecuador, Germany, México, the Netherlands, Panamá, Spain, Sweden, Uruguay, the USA, and Venezuela.

We wish to thank our colleagues at the Universidad de los Andes and Corpoica who helped organizing this event. We extended our special thanks to Colciencias, the Ministerio de Agricultura de Colombia, the Consejo Profesional de Biología de Colombia, the International Society of Chemical Ecology, the Latin American Association of Chemical Ecology ~ ALAEQ, Corpoica and the Universidad de los Andes for the financial support that makes this meeting possible.

We are confident that the presence of the Congress of ALAEQ in Bogotá will not only contribute to boosting this research area in Colombia and to connecting the individual researches working this field in our country, but that it will also increase intraregional collaborations and strengthen the Association.

We wish you a pleasant stay in Bogotá and hope that you will enjoy the interesting presentations and fruitful discussions during this event.

Jorge Molina and Nancy Barreto

PROGRAM



Tuesday, November 18

8:00-9:20 **Registration**

9:20-9:40 OPENING CEREMONY

Silvia Restrepo, Vice-rector of research, Universidad de los Andes
Andrés González, President of ALAEQ
Jorge Molina, President of ALAEQ Congress - 2014

9:40-10:40 **PLENARY LECTURE**

Chair: Jorge Molina

NMR in Chemical Ecology, **Bernd Schneider**
Max Planck Institute for Chemical Ecology, Jena, Germany.

10:40-11:00 **Coffee Break**

11:00-12:30 **SYMPOSIUM: Molecular & Neurobiological bases of Chemoreception**

Chair: Marcelo Lorenzo

Identification of similar odorant-binding proteins among Neotropical phytophagous stink bugs and their egg parasitoid transcriptome annotation and comparative analyses, **Débora P Paula**, Embrapa Genetic Resources and Biotechnology, Brazil

Comparative transcriptome profiling of the antennae of a Chagas disease vector, **Marcelo Lorenzo**, CPqRR-FIOCRUZ, Brazil

Odotopic maps and parallel olfactory pathways in the brain of social insects, **Germán Octavio López-Riquelme**, CINVESTAV, México

Non-elemental olfactory learning in a honeybee brain, **Theo Mota**, Universidade Federal de Minas Gerais (UFMG), Brazil, Centre de Recherches sur la Cognition Animale / Université de Toulouse, France

12:30- 14:00 **Lunch break**

14:00-15:30 **SYMPOSIUM: Semiochemicals identification & synthesis**

Chair: Paulo Zarbin

Structure Elucidation and Synthesis of Semiochemicals, **Wittko Francke**, University of Hamburg, Germany

*Chemical communication in the leafroller species *Proeulia auraria* and *P. triquetra* (Lepidoptera Tortricidae)*, **Jan Bergmann**, Pontificia Universidad Católica de Valparaíso, Chile

*Sex pheromone of the carrion beetle, *Oxelytrum discicolle**, **Paulo H. G. Zarbin**, Department of Chemistry, Federal University of Parana, Brazil

Noneconomic Entomology Sex pheromones of giant silk moths, **Jocelyn Millar**, Department of Entomology, University of California, USA

15:30-16:30 **ORAL PRESENTATIONS 1A/1B**

Chair **1A**: Jan Bergmann

Chair **1B**: Raúl Laumann

1A-1 *Volatile Organic Compounds (VOCs) Related to Chemical Signalization between Guava (*Psidium guajava* L.) and the Guava Weevil *Conotrachelus psidii* Marshall*, **Alicia Romero**, Universidad Nacional de Colombia, Colombia

1A-2 *Methods and natural products applied in the development of pesticides to control of *Xylella fastidiosa* in citrus*, **Danielle F da Silva**, Federal University of São Carlos (UFSCar); Agronomic Institute of Campinas (IAC) - Citrus Center Silvio Moreira and University of Franca (UNIFRAN), Brazil

1A-3 *An Oxidized Monoterpene Library Used to Probe Bark Beetle Chemoreception*, **David Wakarchuk**, Synergy Semiochemicals Corporation, USDA Forest Service, USA

1A-4 *Purification of metabolites from *Duguetia lanceolata* stem barks active for *Spodoptera frugiperda**, **Dejane Santos Alves**, Universidade Federal de Lavras, Brazil

1B-1 *Foliar volatile profiles of *Persea americana* var. Hass and pest incidence*, **García-Rodríguez YM**, Centro de Investigaciones en Ecosistemas, UNAM, México

1B-2 *Flight attraction of Spodoptera littoralis (Lepidoptera, Noctuidae) males and females to cotton headspace and synthetic volatile blends*, **Felipe Borrero-Echeverry**, Chemical Ecology Group, Swedish University of Agricultural Sciences, Biological Control Laboratory, Center for Biotechnology and Bioindustry, Colombian, Colombian Corporation for Agricultural Research, Colombia

1B-3 *Effects of the herbivore-induced maize volatiles in the host location behavior of three sympatrical species sesamia nonagrioides, ostrinia nubilalis and Rhopalosiphum padi*, **Diego Cruz**, Department of Crop and Forest Sciences, Agrotecnio Center, University of Lleida, Spain

1B-4 *Time for Outcrossing*, **Felipe Yon**, Max Planck Institute for Chemical Ecology, Germany

16:30-16:50 **Coffee break**

16:50-17:50 **PLENARY LECTURE**

Chair: José Mauricio S Bento

Detection and Management of Xyleborus glabratus and other vectors of Laurel Wilt, a lethal disease affecting avocados in Florida, **Daniel Carrillo**, University of Florida, Tropical Research and Education Center, USA

17:50-20:00 **Welcome Cocktail (Sd exposition room)**

Wednesday, November 19

8:00-9:00 PLENARY LECTURE

Chair: Jorge Molina

Chemical Communication in Songbirds Signal Content, Production, and Inheritance, **Danielle J. Whittaker**, Michigan State University, USA

9:00- 10:00 ORAL PRESENTATIONS 2A/2B

Chair **2A**: Paulo Zarbin

Chair **2B**: Andrés González

2A-1 *Isolation and identification of the sex pheromone components of the Argentine population of *Anastrepha fraterculus* (Diptera Tephritidae)*, **Blanka Kalinová**, Institute of Organic Chemistry and Biochemistry ASCR v.v.i., Czech Republic

2A-2 *Cis-jasmone induces a production of repellent compounds in cowpea, *Vigna Unguiculata**, **Domingos L. P. Macuvelo**, Departamento Química- Universidade Pedagógica de Moçambique- Campus de Niassa- Mozambique, Departamento de Engenharia Química Universidade Federal de Santa Catarina, Brazil

2A-3 *Identification of absolute configuration of two components of the attractant pheromone of *Neomegalotomus parvus* (Hemiptera Alydidae)*, **Oliveira MWM**, Semiochemical laboratory - Embrapa Genetic Resources and Biotechnology, Brazil

2B-1 *Observations on *Heniartes stali* Wygodzinsky (Hemiptera Reduviidae Apiomerini), a resin bug harvesting the trichome secretion from an andean blackberry*, **Jorge Luis Ávila-Núñez**, Grupo de Química Ecológica, Facultad de Ciencias, Universidad de Los Andes, Venezuela

2B-2 *The effect of plant domestication status over tomato herbivore-induced plant volatiles variation*, **Espinosa-García Francisco Javier**, Centro de Investigaciones en Ecosistemas, UNAM, México

2B-3 *Implications of the chemical and genetic variation of *Mikania micrantha* on its specialist herbivore *Stolas punicea**, **Angel E Bravo-Monzón**, Centro de Investigaciones en Ecosistemas, UNAM, México

2B-4 *Geographic structure of chemical variation in wild populations of *Jatropha curcas* L in Mexico*, **Francisco J Espinosa García**, Centro de investigaciones en ecosistemas, UNAM, México

10:00-10:20 **Coffee Break**

10:20-11:50 **SYMPOSIUM: Insect-Plant & Plant-Plant Interactions**

Chair: Andrés González

Chemically-mediated insect-plant interactions in native systems a knowledge base for crop-pest systems, **Andrés González**, Laboratorio de Ecología Química, Facultad de Química, Universidad de la República, Uruguay

Plant volatiles to manage herbivores and natural enemies in arable crops, **Maria Carolina Blassioli-Moraes**, Embrapa Recursos Genéticos e Biotecnologia, Brazil

*Effects of root herbivory on the selection of tomato plants by a leaf herbivore, *Tuta absoluta* (Lepidoptera Gelechiidae)*, **Eraldo Lima**, Departamento de Entomologia, Universidade Federal de Viçosa, Brazil

The Pollination Syndrome Chemical Facts and Evolutionary Speculations, **Wittko Francke**, University of Hamburg, Germany

11:50-13:00 **Lunch Break**

13:00-14:30 **SYMPOSIUM: Marine Chemical Ecology**

Chair: Mónica Puyana

*Variation in the metabolic profile of the octocoral *E caribaeorum* collected at the Colombian Caribbean Sea*, **Freddy A. Ramos**, Departamento de Química, Universidad Nacional de Colombia, Colombia

Microbial interactions of octocoral-associated bacteria, **Marcelino Gutiérrez**, Center for Biodiversity and Drug Discovery, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT), Panamá

Ecological interactions of Caribbean benthic cyanobacteria, **Puyana M**, Universidad Jorge Tadeo Lozano, Colombia

Coral undermining and death by encrusting excavating sponges Is allelopathy involved? **Sven Zea**, Universidad Nacional de Colombia, Centro de Estudios en Ciencias del Mar – CECIMAR, Colombia

14:30-15:30 **PLENARY LECTURE**

Chair: Jorge Molina

Skin microbial volatiles and their function in attractiveness to mosquitoes, **Niels O. Verhulst**, Laboratory of Entomology, Wageningen University and Research Centre, The Netherlands.

15:30-15:50 **Coffee Break**

15:50-16:50 **POSTER SESSION 1 (Sd exposition room)**

16:50-17:50 **POSTER SESSION 2 (Sd exposition room)**

Thursday, November 20

8:00-9:00 PLENARY LECTURE

Chair: Andrés González

Biological Production Of Moth Pheromones In Yeast And Plant Factories: A Synthetic Biology Approach, **Christer Löfstedt**, Department of Biology, Lund University, Sweden

9:00-10:00 POSTER SESSION 3 (Sd exposition room)

10:00-10:20 *Coffee Break*

10:20-11:50 SYMPOSIUM: Chemical Ecology in Medical Entomology

Chair: Pablo Guerenstein

The bad taste governs feeding decisions in a blood feeder, **Romina B. Barrozo**, Laboratorio de Fisiología de Insectos, IBBEA, CONICET-UBA, DBBE, FCEN, Universidad de Buenos Aires, Argentina

The role of epicuticular lipids in chemical communication in Chagas disease vectors, **Alicia N. Lorenzo Figueiras**, Laboratorio de Fisiología de Insectos, Departamento de Biodiversidad y Biología Experimental, IBBEA, Facultad de Ciencias exactas y Naturales, Universidad de Buenos Aires, Argentina

Chemical Ecology of phlebotomine sand flies, vectors of leishmaniasis, **Mara Cristina Pinto**, Laboratório de Parasitologia, Departamento de Ciências Biológicas, UNESP, Brazil

Formulations of oviposition attractants for Aedes mosquitoes: from laboratory to large scale production, **Alvaro E. Eiras**, Chemical Ecology Laboratory Department of Parasitology, Instituto de Ciências Biológicas (ICB) Universidad Federal de Minas Gerais, Brazil

Synthetic host odors could be used to manipulate both the host-seeking and oviposition behavior of triatomines, **Fabio Guidobaldi**, Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CICYTTP-CONICET), Diamante, Entre Ríos, Universidad Nacional de Entre Ríos, Argentina

11:50-13:00 *Lunch Break*

13:00-14:00 **ORAL PRESENTATIONS 3A/3B**

Chair **3A**: Pablo Guerenstein

Chair **3B**: Marcelo Lorenzo

3A-1 *Monitoring *Hypothenemus hampei* population using alcohol baited traps as an early alert system to coffee farmers in Colombia*, **Benavides Pablo**, Centro Nacional de Investigaciones de Café, CENICAFE, Colombia

3A-2 *Impact of *S. commersoni* in the incidence of whitefly on tomato crop*, **Umpiérrez, María L**, UdelaR, Facultad de Química, Laboratorio de Ecología Química, Uruguay

3A-3 *The effect of plant volatiles from sunflower, maize and pigeon pea with herbivory damaged made by stink bug *Euschistus heros* on the foraging behavior of the egg parasitoid *Telenomus podisi**, **Aline M.Dias**, Graduate Program in Agronomy / Entomology, Federal University of Lavras, Brazil

3A-4 *Are the wild and mass-reared fruit fly strains differentiable by their sexual behavior and male volatile compositions? The case of the Mexican fruit fly*, **Carlos-Felipe Bosa**, El Colegio de la Frontera Sur, Chiapas, Mexico

3B-1 *A symbiotic strategy for chemical egg defense in insects the *Lagria-Burkholderia* case*, **Laura Flórez**, Max Planck Institute for Chemical Ecology, Jena, Germany

3B-2 *Identification by LC/ESI/MS/MS and LC/DAD of flavonol and isoflavonoid glycosides modulated by solar UV-B radiation and herbivory in soybean leaves at field: effects on *Anticarsia gemmatalis* larvae*, **Francisco M Dillon**, Cátedra de Bioquímica, Facultad de Agronomía, Universidad de Buenos Aires, CONICET, Argentina

3B-3 *Soil microbes influence the above-ground interactions of soybean plants with insects and pathogens*, **Hannier Pulido**, Department of Entomology, The Pennsylvania State University, USA

3B-4 *Effect of phenology on the volatile blends emitted by Uruguayan native host plants of tephritid fruit flies (Diptera: Tephritidae)*, **María Victoria Calvo**, Facultad de Química, Universidad de la República, Uruguay

14:00-15:00 **PLENARY LECTURE**

Chair: Nancy Barreto

Complexity of Multitrophic Interactions mediated by Plant Volatiles,
Maria Fernanda Gomes, University of São Paulo (USP)/Escola
Superior de Agricultura “Luiz de Queiroz” (ESALQ).

15:00-17:00 **GOLD MUSEUM VISIT (PLEASE REGISTER IN ADVANCE)**

Friday, 21 November

8:00-9:00 PLENARY LECTURE

Chair: Paulo Zarbin

*Opening a black box analysis and synthesis of chiral methyl-branched and other cuticular hydrocarbons to assess their functions as signal molecules, **Jocelyn G. Millar**, Departments of Entomology and Chemistry, University of California, USA*

9:00-10:00 Oral Presentations 4A/4B

Chair **4A**: Jorge Molina

Chair **4B**: Nancy Barreto

4A-1 *Expression analysis of transcripts potentially related to olfaction by *Telenomus podisi* (Hymenoptera Platygasteridae) and stimulated by sexual pheromonal compounds of its main hosts (Hemiptera Pentatomidae), **Schimmelpfeng PHC**, Embrapa Cenargen / Universidade de Brasília, Brazil*

4A-2 *Working model (combined strategy) to measure the potential of inhibitors in marine fouling process, **Edisson Tello**, Universidad Nacional de Colombia, Universidad de la Sabana, Colombia*

4A-3 *New compound identified as pheromone component of *Conotrachelus humeropictus* Fiedler (Coleoptera: Curculionidae), **Gabriela G Torrens**, Federal University of Paraná, Brazil*

4A-4 *Extraction, identification and behavioral activity of a male-produced aggregation pheromone in the Lesser Mealworm *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) in Brazil, **Marla Juliane Hassemer**, University of Brasília, Brazil*

4B-1 *Phenalenone-type phytoalexins mediate resistance of banana plants (*Musa* spp.) to the burrowing nematode *Radopholus similis*, **B Schneider**, Max Planck Institute for Chemical Ecology, Germany*

4B-2 *A Multi-species Bait for Chagas Disease Vectors, **Theo Mota**, Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, CPqRR-FIOCRUZ, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas-UFMG, Brazil*

4B-3 *Linking herbivore feeding preference and performance on two *Solanum* host plants, **Paula Altesor**, Facultad de Química, Universidad de la República, Uruguay*

4B-4 *Chemical phenotypes of Persea americana cv. Hass concur with differential incidence of the avocado branch weevil (Copturus aguacatae), Meléndez-González Claudio*, Laboratorio de Ecología Química y Agroecología. Centro de Investigaciones en Ecosistemas. UNAM, México

10:00- 10:20 **Coffee Break**

10:20-11:50 **SYMPOSIUM: Semiochemicals & Pest Management**

Chair: Mauricio Bento

The basis for monitoring coffee leafminer Leucopera coffeella, (Lepidoptera Lyonetiidae) with pheromone traps, Eraldo Lima, Departamento de Entomologia, Universidade Federal de Viçosa, Brazil

Semiochemicals as key tools for pest management The role in the future codling moth management, Esteban R. Basoalto V, Universidad Austral de Chile, Chile

Use of an off-ratio blend for mating disruption of a major citrus pest, Stephen L. Lapointe, ISCA Technologies, Inc., USA

Economic and environmental benefits by using sex pheromone for citrus fruit borer management, José Mauricio S Bento, Universidade de São Paulo, Depto de Entomologia e Acarologia, Piracicaba-SP, Brazil

11:50-13:30 **Lunch Break**

13:30-15:00 **SYMPOSIUM: Importance, regulation and limitations on use of pheromones in Colombia**

Chair: Nancy Barreto

Importance, regulation and limitations on use of pheromones, Nancy Barreto Triana, Researcher Grupo Manejo Fitosanitario, Colombian Corporation of Agricultural Research, Colombia

Limitations on the use of pheromones in Colombia, Emilio Arevalo Peñaranda, Dirección Técnica de Epidemiología y Vigilancia Fitosanitaria, Instituto Colombiano Agropecuario ICA, Colombia

Using pheromones to phytosanitary monitoring: Quarantine pests and low prevalence areas, Jose Roberto Galindo Alvarez,

Director de Inocuidad e Insumos Agrícolas, Instituto Colombiano Agropecuario ICA, Colombia

Regulations for import and use of pheromones in Colombia, **Raúl Laumann**, Laboratório de Semioquímicos, Embrapa Recursos Genéticos e Biotecnologia, Brazil

15:00-16:00 **PLENARY LECTURE**

Chair: Mónica Puyana

The chemical ecology of sponges on Caribbean reefs Natural products shape natural systems, **Pawlik, Joseph R**, University of North Carolina Wilmington, Center for Marine Science, USA

16:00-16:20 **Coffee Break**

16:20-16:50 **JUDGES DELIBERATION**

16:50-17:20 **CLOSING CEREMONY -
STUDENT ORAL & POSTER PRESENTATION AWARDS**

17:20-18:20 **GENERAL ASSAMBLY ALAEQ**

PLENARY LECTURES
Abstracts



Tuesday 18 / 9:40-10:40 / Lleras Auditorium

NMR in Chemical Ecology

Bernd Schneider

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Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans Knöll Str. 8, 07745 Jena, Germany

Chemical ecology investigates the role of natural products in the interaction between microorganisms, plants, animals and their environment, as well as the evolutionary and behavioral consequences of such interactions. Since many ecological interactions are mediated by natural compounds, detailed knowledge about relevant chemical structures and their physical and biological properties are required. Therefore, analytical natural product chemistry is one of the main pillars of chemical ecology. Analytical tools, especially NMR spectroscopy, the most informative method in natural products chemistry, and mass spectrometry, are essential for elucidating chemical structures and stereo configuration, biogenetic and metabolic pathways, metabolic fluxes, tissue-specific localization, the composition in the tissue and other aspects relevant to chemical ecology. Quantitative measures are of special interest in this context and important for understanding the ecological function of metabolites in inter- and intra-species interaction in nature.

This presentation aims to draw attention to recent developments in analytical methods and how they can be used to extend our understanding on the role of natural compounds in the complexity of interactions that occur in nature. The potential and general capabilities of several analytical techniques, with special focus on NMR, will be discussed. Examples of the identification and de novo-structure elucidation of ecologically relevant compounds from living organisms including small molecules obtained as products of isolated or recombinant biosynthetic enzymes will be presented. Further examples are the determination of tissue- and cell-specific occurrence and distribution of natural compounds by NMR and other techniques. Recent developments in the area of biosynthetic and metabolic studies using, for example, retrobiosynthetic approaches are also subject of the talk. Finally, NMR- and MS-based analysis of natural compounds in mixtures as well as their molecular interactions will be subject to the presentation.

References

- Schneider B. Chemical Ecology. *eMagRes* 2013; 2451–466.
- Schneider B. Nuclear magnetic resonance spectroscopic analysis of enzyme products. In Lüttge U, Beyschlag W, Büdel B, Francis D. *Progress in Botany Vol 72*. Berlin, Heidelberg Springer; 2011. p. 183–208.
- Hölscher D, Schneider B. Application of laser-assisted microdissection for tissue and cell-specific analysis of RNA, proteins, and metabolites. In Lüttge U, Beyschlag W, Murata J. *Progress in Botany Vol 65*. Berlin, Heidelberg Springer; 2003. p. 301–322.

Tuesday 18 / 16:50-17:50 / Lleras Auditorium

Detection and Management of *Xyleborus glabratus* and other vectors of Laurel Wilt, a lethal disease affecting avocados in Florida

Daniel Carrillo¹, Wayne S. Montgomery², Rita E. Duncan¹, Paul E. Kendra²

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²*United States Department of Agriculture, Agricultural Research Service Subtropical Horticulture Research Station, 13601 Old Cutler RD, MIAMI, FL, 33158*

The redabay ambrosia beetle, *Xyleborus glabratus*, carries a phytopathogenic symbiont, *Raffaelea lauricola*, which causes laurel wilt, a lethal vascular disease of some Lauraceae species. Both *X. glabratus* and *R. lauricola* are natives of Asia that recently invaded much of the coastal plain of the southeastern USA. This new beetle-disease complex has decimated vast areas of native trees of the Lauraceae in the southeastern USA, and is now threatening the avocado industry in south Florida. Efforts to identify semiochemicals for early detection of this beetle and repellent substances to deter *X. glabratus* from avocado trees are described. Initial studies provided no evidence of an aggregation pheromone or attraction to ethanol, the standard attractant for ambrosia beetles. The existence of a sexual pheromone is unlikely because of the type of reproduction of these beetles. Males of *X. glabratus*, like many other ambrosia beetles, are flightless and never leave the beetle galleries where they mate with sibling or parental females. Females mate before engaging in dispersal and host seeking behaviors, making long range attraction between sexes unnecessary and unlikely. Therefore, research efforts concentrated on identification of economical kairomone-based attractants using essential oils high in sesquiterpenes for early detection of *X. glabratus*. Initially, manuka and phoebe oil lures proved to be attractive to *X. glabratus*. However, tests indicated that manuka lures are not very effective due to short field life, and phoebe lures are no longer available. Distilled cubeb oil lures were identified as a more effective tool for detection of *X. glabratus*, with a field life up to 3 months due to extended, low release of attractive sesquiterpenes, primarily α -copaene and α -cubebene (Kendra et al. 2014). In addition, screening of multiple compounds identified cyclic ketones as potential repellents for *X. glabratus*. However, the epidemiology of the laurel wilt disease in avocado groves is complex, apparently involving other species of ambrosia beetles capable of spreading the laurel wilt pathogen. Research demonstrated that other invasive and native ambrosia beetles also harbor the laurel wilt pathogen, apparently acquiring it when they breed in infected Lauraceae trees (Carrillo et al. 2014). These findings led to increased monitoring and management efforts aimed at resident ambrosia beetles.

Typically, resident ambrosia beetles infest stressed trees; however, *X. glabratus* is a primary colonizer attacking healthy trees. The available evidence suggests that *X. glabratus* transmits laurel wilt to avocados or nearby Lauraceae trees but does not establish in avocado. Then, other ambrosia beetles breed in diseased trees and subsequently transmit the pathogen to new hosts. Current research aims to identify what factors mediate the attraction of resident ambrosia beetles and their activities spreading the laurel wilt disease in commercial avocado groves.

References

- Carrillo D, Duncan RE, Ploetz J, Campbell A, Ploetz R, Peña JE. Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. *Plant Pathol* 2014; 63 54-62
- Kendra PE, Montgomery WS, Niogret J, Schnell EQ, Deyrup MA, Epsky ND. Evaluation of seven essential oils identifies cubeb oil as most effective attractant for detection of *Xyleborus glabratus* (Coleoptera Curculionidae Scolytinae). *J Pest Sci* 2014 (pub online 30 Jan)

Wednesday 19/ 8:00-9:00 / Lleras Auditorium

**Chemical Communication in Songbirds: Signal Content, Production,
and Inheritance**

Danielle J. Whittaker

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In recent years, birds have finally joined the rest of the world's living creatures as a taxon of interest in chemical ecology. Behavioral, genomic, and neurobiological work has demonstrated that birds are able to detect and respond to odors from plants, predators, and other birds. Many studies have focused on volatile compounds present in preen oil, which is secreted from the avian uropygial gland and spread on the feathers for protection and maintenance. In our work with the dark-eyed junco (*Junco hyemalis*), a North American emberizid sparrow, we have characterized volatile and semivolatile compounds present in preen oil and deciphered some of the information contained within. These compounds vary with many aspects of individuals, including sex, population of origin, and hormone levels. Juncos are able to detect and differentiate among preen oil odors from different individuals, sexes, and species. Furthermore, junco preen oil volatile compounds predict individual genetic reproductive success. These qualities make preen oil volatiles reliable candidate cues for mate assessment and choice, yet their mechanism of production remains unknown. Importantly, many of these compounds, which include linear alcohols, methyl ketones, and carboxylic acids, are known to be end products of microbial metabolism in other environments. The preen gland harbors diverse microbial communities which we hypothesize produce many of the oil's volatile compounds. We characterized the microbial communities in the preen glands of breeding adult dark-eyed juncos, and found that they contained many known odor-producers. In addition to presenting these data, I will discuss how social environment and behavior are a primary driver of an individual junco's microbiome, with important implications for the evolution and development of animal chemical communication.

Wednesday 19/ 14:30-15:30 / Lleras Auditorium

Skin microbial volatiles and their function in attractiveness to mosquitoes

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Diseases transmitted by mosquitoes like malaria, dengue and yellow fever cause millions of illnesses and deaths each year. Mosquitoes use physical and chemical cues to find their hosts, of which chemical cues are considered most important. The malaria mosquito *Anopheles gambiae sensu stricto* and the yellow fever mosquito *Aedes aegypti* are two of the most important disease vectors, because they are anthropophilic and uses human odours to find their host (Takken and Verhulst 2013).

Odours released from the human skin are produced by the conversion of skin gland excretions by bacteria. The human skin contains three types of sweat glands a) Apocrine glands are the main source of human scent, b) Eccrine glands for thermoregulation, c) Sebaceous glands lubricate and waterproof the skin (Stoddart 1990). The excretions from these glands are mainly non-volatile and bacteria convert these non-volatile compounds into volatile compounds with their characteristic smell (Stoddart 1990). These non-volatile compounds are often long-chain fatty acids that are converted by bacteria in short-chain fatty acids. The specific conversion and odours produced is dependent on the type of the bacteria, resulting in a characteristic smell (Verhulst, et al. 2010). The composition of the skin microbiota will depend on many factors including, grooming habits, skin temperature, skin PH, genetics and potential infections with for example *Plasmodium* (Verhulst, et al. 2010).

Since anthropophilic mosquitoes use human odours to find their hosts and skin bacteria play an important role in the production of human body odour we hypothesized that the human skin bacterial composition determines an individuals attractiveness to mosquitoes. To confirm this hypothesis we first showed that bacteria isolated from the skin and grown *in vitro* on agar plates attracted *An. gambiae* in laboratory and semi-field experiments. When 5 species that are common on the human skin were tested, *Staphylococcus epidermidis* was highly attractive to *An. gambiae* and *Pseudomonas aeruginosa* was not attractive (Verhulst, et al. 2010). In order to examine the interaction between the microbiota on the skin and human attractiveness to mosquitoes *in vivo*, skin emanation and skin microbiota samples were taken from 48 individuals.

Pyrosequencing (454) results indicated that individuals with a higher abundance of bacteria on their skin were more attractive to *An. gambiae*, whereas individuals

with a higher diversity of skin microbiota were less attractive (Verhulst, et al. 2011). *Staphylococcus* spp. were associated with individuals that were highly attractive and *Pseudomonas* spp. with individuals that were less attractive to mosquitoes, which confirmed the results of the in vivo experiments. Human Leukocyte Antigen (HLA) genes are considered to influence the human body odour profile and HLA profiling of the individuals suggested that people carrying HLA gene Cw07 are more attractive to mosquitoes (Verhulst, et al. 2013). Interestingly, the frequency of Cw07 is lower in Africa (0.20) compared to western Europe and North America (0.34), which is expected when gene Cw07 could increase the change of becoming infected with *Plasmodium* through a mosquito bite.

When mosquitoes search for a blood hosts, some express preferential behaviour for selected species and those that express such a strong and inherent host-selection behaviour belong to the most important vectors of infectious diseases (Takken and Verhulst 2013). Although previous studies have shown that host preference is often mediated by skin odours, it is not known to what extent host specific bacteria play a role in the production of these odours. To test this hypothesis, first the host preference of several *An. gambiae s.l.* species was tested in an olfactometer and semi-field setup. Secondly skin bacteria of different hosts were collected and individual bacterial species tested for their attractiveness. Mosquitoes with different host preferences responded to different bacteria indicating that skin bacteria mediate host preference.

Analysis of the volatiles produced by skin microbiota grown in vitro led to the identification of several compounds that had an effect on *An. gambiae* behaviour (Verhulst, et al. 2011). Combined with other compounds previously identified, this led to the development of a synthetic odour blend (MB5) that is more attractive to mosquitoes than human odour. Combined with a new mosquito trap, called the Suna trap (Hiscox et al., 2014), we successfully sampled anopheline populations indoors and outdoors and are testing this trap for mass trapping malaria vectors in Western Kenya (Hiscox, et al. 2012). A small modification to the trap and adding CO₂ by yeast-molasses fermentation makes it suitable for trapping both unfed and blood fed mosquitoes outdoors (Mweresa, et al. 2014). Trapping mosquitoes outdoors is essential for measuring outdoor transmission, which becomes more important with increased use of insecticide treated bednets (Takken and Verhulst 2013).

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Thursday 20/ 8:00-9:00 / Lleras Auditorium

Biological Production of Moth Pheromones in Yeast and Plant Factories: A Synthetic Biology Approach

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The use of pheromones for control of pest insects has many advantages over the use of traditional pesticides. The global market for pheromone-based control products is currently estimated to approximately \$200 millions and tons of synthetic pheromones are produced commercially for this purpose.

We currently explore two “green chemistry” alternatives to conventional synthetic production of moth pheromones. One option is the pheromone brewery, a yeast cell factory for pheromone production [1]. Another option is a plant factory, using genetically modified plants for production of pheromones or pheromone precursors [2]. Both concepts take advantage of the successful characterization of genes controlling pheromone production that has taken place during the last two decades. Insect genes, as well as genes isolated from other organisms, contribute to a powerful synthetic biology toolbox, which can be exploited for insect pheromone production.

As a proof of the pheromone brewery concept, we co-expressed a $\Delta 11$ desaturase and a FAR in the Brewer's yeast *Saccharomyces cerevisiae* and produced (*Z*)-11-hexadecenol [1]. Using *Nicotiana benthamiana* as a plant factory, we produced several typical ¹⁴C and ¹⁶C moth sex pheromone components by transient expression of up to four genes coding for consecutive biosynthetic steps [2]. The fatty alcohol fractions from the genetically modified plants were acetylated and mixed to mimic the respective sex pheromones of the small ermine moths *Yponomeuta evonymella* and *Y. padella*. These mixtures were very efficient and specific for trapping of male moths and matched the activity of conventionally produced synthetic pheromones.

Work is underway to tailor the production of pure compounds and specific blends and to increase the yields. Semi-synthetic preparation of sex pheromones may be a novel and cost-effective way of producing moderate to large quantities of pheromones with high purity and a minimum of hazardous waste.

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Thursday 20/ 14:00-15:00 / Lleras Auditorium

Complexity of Multitrophic Interactions mediated by Plant Volatiles

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Plants constitutively emit volatiles, however, upon insect attack, they produce a quantitative and qualitative different volatile blend. Many other biotic and abiotic factors can also induce changes in plant volatile blend, such as herbivore species, multiple herbivores, temperature, light intensity, pathogen infection, among others. As plant volatiles serve as important mediators in a multitude of below- and aboveground interactions, changes in the volatile blend composition can establish novel interactions, which can increment plant fitness at some times (Agrawal 1998). Attraction of a wide range of herbivore’s natural enemies (Mumm and Dicke 2010) or repellence of herbivores (De Moraes et al. 2001) to herbivore-induced plant volatiles (HIPV’s) are beneficial for emitter plants and therefore are categorized as indirect induced defenses (Turlings and Wäckers 2004). Nevertheless, in some systems, HIPV’s can attract herbivores looking for suitable host plants or conspecifics for mating (Moayeri et al. 2007). In this scenario, the first role given to HIPV’s as plant indirect defenses against herbivores may be too simplistic considering its complex role on the interactions among several trophic levels, from plant-plant communication (Rodriguez-Saona et al. 2009) to plant-hyperparasitoid interaction (Poelman et al. 2012).

Here, we will discuss plant-insect interactions mediated by plant volatiles at above and belowground level in agricultural systems. Although crops are less complex environments than natural ecosystems, multiple insect attack, root-colonizing microorganisms and plant pathogens, for example, are present and can alter plant volatile emissions in ways to change the frequency and nature of interactions between pests and plants. Insights on the effects of plant volatiles on pest and natural enemy behavior in the agricultural environment may contribute to develop novel strategies for pest monitoring and control using a behavioral approach. Alternatively, strategies based on attractive plant volatiles to egg, larval parasitoids and predators can be harmonically combined with biological control programs.

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Friday 21/ 8:00-9:00 / Lleras Auditorium

Opening a black box analysis and synthesis of chiral methyl-branched and other cuticular hydrocarbons to assess their functions as signal molecules

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Most insects are coated in a complex mixture of straight-and branched-chain alkanes, mono-to polyunsaturated alkenes, and more polar compounds such as long-chain alcohols, aldehydes, carboxylic acids, and wax esters. The primary function of these cuticular hydrocarbons (CHCs) is to prevent water loss, but a number of these compounds have evolved secondary roles as contact pheromones. Thus, for solitary insects, CHC components are used for recognition of species and sex during mating, and possibly as trail pheromones. For social insects such as ants and bees, CHC components have additional functions for nestmate and caste recognition. They also are used by queens to signal fertility and maintain reproductive dominance of the colony, preventing workers from developing their ovaries and beginning to lay eggs. Thus, these fertility signals are critically important for the proper organization and functioning of social insect colonies.

However, because CHCs are complex mixtures of tens or even > 100 compounds with very similar chemical and physical properties, they can be difficult to isolate in pure form for bioassays to determine their functions. The problem is further complicated by the fact that most methyl-branched hydrocarbons (MBHCs) with a single methyl branch can exist in two enantiomeric forms, and compounds with 2 or more methyl branches can have 4 or more different stereoisomeric forms. To date, it has not been possible to determine which stereoisomeric form of an MBHC a particular insect species produces, nor was it known whether insects were able to detect the differences between the enantiomers of MBCHs. This problem has been recognized for several decades, but it has not yet been addressed.

To lay a foundation for attacking this problem, we developed a straight-forward protocol for isolation of individual components from crude extracts of insect CHCs. The protocol first separates the extracts into fractions containing alkanes, alkenes, and more polar compounds. The alkanes fraction is then separated into subfractions containing straight-chain or branched chain compounds, and the alkenes fraction can be separated into subfractions containing monoenes, dienes, and trienes respectively. All of these fractionations can be done with simple and inexpensive materials. Pure compounds can then be isolated from any desired subfraction with reverse phase high pressure liquid chromatography (HPLC) using nonaqueous solvent systems (e.g., methanol and ethyl acetate). Reverse phase HPLC can easily separate CHCs on the basis of chain length, and it can usually

separate compounds of the same chain length but with different methyl branch or double bond positions. Thus, the combination of simple preliminary fractionation steps followed by reverse phase HPLC allows the isolation of many CHC compounds in pure form for analysis or bioassays, and the method can be used for quantities ranging from nanograms to milligrams or more (Bello et al. submitted).

We then used the separation protocol to isolate a total of 36 monomethyl-branched hydrocarbons from adults and nymphs of 20 different insect species from 9 orders, in quantities varying from micrograms to tens of milligrams. Using a digital polarimeter, we measured the optical rotations of the purified compounds. By comparison of the rotations to those of enantiomerically pure synthetic standards, we found that all 36 insect-produced MBCHs had the (*R*)-configuration, indicating that the biosynthesis of these compounds is likely to be highly conserved throughout the class Insecta. Furthermore, all of the isolated compounds were enantiomerically pure (to the limit of detection of the polarimeter), indicating that the enzymatic induction of asymmetry is under tight control.

These straightforward protocols, should now allow researchers to determine the functional roles of individual CHCs or blends of CHCs as various types of signals in any desired insect species. Furthermore, our library of enantiomerically pure synthetic standards should allow us to determine whether insects can distinguish the enantiomeric forms of MBCHs, and possibly to probe the biological receptors to begin to understand how they are able to distinguish long-chain hydrocarbons with no functional groups, that differ only in the placement or configuration of a methyl group somewhere along the chain.

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Friday 21/ 15:00-16:00 / Lleras Auditorium

The chemical ecology of sponges on Caribbean reefs: Natural products shape natural systems

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Sponges are now the dominant habitat-forming animals on Caribbean reefs, where the combined effects of climate change, pollution, and disease have decimated reef-building corals. Natural products chemists have been isolating novel secondary metabolites from Caribbean sponges for many decades, but relevant studies of the ecological functions of these compounds have been more recent. Bioassay-guided surveys have revealed sponge chemical defenses against predators, competitors, and pathogens, but many common sponge species lack chemical defenses and appear to have followed a different evolutionary trajectory, investing instead in greater reproduction or growth. Our proposed conceptual model (Pawlik, 2011) predicts that changes in the abundances of predatory fishes on Caribbean reefs will have a cascading impact on the sponge community, with indirect effects on the broader community of corals and seaweeds. A recent test validated important components of this model, with higher proportions of chemically defended sponges on reefs with greater numbers of sponge-eating fishes (Loh & Pawlik 2014), and greater competitive interactions between sponges and corals on those same reefs. Caribbean sponges provide an important alternative to more complicated systems, such as terrestrial plant and insect communities, for testing basic ecological theories about chemical defenses and resource allocation. The tractability of this system may result from its relative simplicity, particularly the apparent absence of bottom-up effects on community structure (Pawlik et al. 2013).

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SYMPOSIA
Abstracts



SYMPOSIUM “Molecular & Neurobiological bases of Chemoreception”
Tuesday 18/ 11:00-12:30 / Lleras Auditorium
Organized by Marcelo Lorenzo

**Identification of similar odorant-binding proteins among Neotropical
phytophagous stink bugs and their egg parasitoid transcriptome
annotation and comparative analyses**

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Olfaction plays a fundamental role for insect survival through resource location and intra and interspecific communication. Stink bugs are major pests in the Neotropics (Schuh & Slater, 1995; Panizzi *et al.*, 2000; Correa-Ferreira *et al.*, 2009) and one sustainable control tactic is biological control. We prospect, characterize and compare transcripts putatively related to olfaction from the antennae of three major stink bug pest species in Brazil *Euschistus heros*, *Chinavia ubica* and *Dichelops melacanthus*, and from the whole body of their parasitoid *Telenomus podisi* through RNA-Seq libraries and Illumina sequencing. Contigs were annotated by BLASTx similarity search and 64 putative Odorant-Binding Proteins (OBPs), 38 Chemosensory Proteins (CSPs) and 23 Olfactory-Receptors (ORs) were found (but no OR from *D. melacanthus*), from which we obtained four full-length putative OBPs from *E. heros* and *C. ubica*, one from *D. melacanthus* and three from *T. podisi*. All full-length putative OBPs had the predicted physicochemical properties expected for an OBP, except for one *T. podisi* OBP with an unusual basic pI. Similarity and phylogenetic analyses revealed low intraspecific similarity of the deduced amino acid sequences of the OBPs, but very high similarity between two OBPs from *E. heros* and *C. ubica* (76.4 and 84.0%) and between two OBPs from the parasitoid and its preferred host *E. heros* (82.4 and 88.5%). This interspecific similarity between OBPs might explain the pheromone cross attraction to several Pentatomidae species, including the attraction of *T. podisi* to *E. heros* sex pheromone.

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Comparative transcriptome profiling of the antennae of a Chagas disease vector

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The advent of NGS techniques has allowed uncovering the molecular bases of diverse physiological processes related to chemically mediated behavior. Our study has performed a mass sequencing of the transcripts produced in the antennae of 5th instar larvae, males and females of *Rhodnius prolixus* and compared their relative abundances.

Three RNAseq libraries from antennae of 5th instar larvae, male and female adults of this species were prepared using Illumina's TruSeq Stranded RNA Sample Prep kit. Illumina HiSeq technology was used to mass sequence these libraries in a single lane generating paired-end reads (350 million). This allowed generating two transcript assemblies (Trinity and Soap), as well as quantifying gene expression levels (BWA and TopHat) to allow a comparative differential expression analysis between libraries.

We have characterized the expression profiles of diverse gene families related to sensory processes and their modulation in the antennae of immature and mature triatomines. Genes classified as differentially expressed in adult bug antennae in comparison to those of larvae were structurally and functionally annotated. A subset of them had the highest expression in male or female antennae, or even in both of them. Conversely, several genes, e.g., some circadian rhythm genes, seemed to have a highest expression in larval antennae.

Our results set the bases for research on the molecular mechanisms underlying diverse well studied triatomine behaviors, particularly those mediated by chemical and thermal sensory modalities. The differences in expression profiles found for immature and mature bugs suggest that several sensory functions are enriched or acquired during triatomine adulthood, while others are apparently impoverished after imaginal molt. The presence of neuropeptide transcripts produced in bug antennae is an interesting finding suggesting that neuromodulation is probably also effected by antennal cells. We will discuss the relevance of these results in the light of the chemical ecology of these bugs.

Odotopic maps and parallel olfactory pathways in the brain of social insects

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The organization of insect societies depends on olfaction the emission and detection of pheromones. Besides, food, prey, enemies, nestmates and chemical stimuli from the environment involved in orientation are also detected by olfaction. This plethora of chemical stimuli generates a constant flow of information that is processed in different centers of the brain. The olfactory system should be able to detect and discriminate the complex mixtures of social pheromones and chemical signals from the environment to generate the appropriate behavior according to the context. One of the main functions of the nervous systems is cognition about the external and internal world by means of perceptual mechanisms, which provides the animal with the wealth of knowledge on the environment by filtering and transforming environmental energies in physiological events, and by giving meaning to such information. The perception of the world in animals is based on two organizational principles of the nervous system 1) sensory world is represented internally as a neural maps, and 2) neurons of different levels along central pathways must extract different kinds of information from these maps. Neural coding of sensory stimuli is represented in spatiotemporal patterns of activity of such maps that finally elicit adaptive behavioral responses. While sensory systems have a neural composition more or less constant due to constraints imposed by physical environment, perceptual mechanisms are variable since they have phylogenetic histories of adaptation to complex specific environmental conditions [1]. Deciphering the neuroanatomical logic of how sensory information in neural maps is relayed and integrated along central pathways will contribute to our understanding of how sensory information is coded. Social insects are very suitable models for the study of olfactory system organization and coding because their lives rely on olfaction, and their olfactory world can be broadly divided into two main sources of information a) information about non-social world (naturally olfactory cues), and b) social information (pheromones). In this work, the general organization of the olfactory pathways from antennal lobes (AL) and its topographic representation in the mushroom bodies (MBs) of hymenopteran social insects are described and discussed to elucidate the organization of the olfactory pathway and the meaning of its specialized structure in social insects.

The main olfactory pathway is conserved among neopteran insects. Chemicals first interact with olfactory receptor neurons (ORN) located on the antennae altering the electrical properties of ORNs, transducing the chemical stimulus into action potential trains that run through the antennal nerve (AN) towards the AL, the region in which olfactory information is first processed. In the AL, axons from ORNs segregate from the AN as separated tracts each of which going to one or few

glomeruli, spheroidal structures of neuropil where axons from ORNs end to form synapses [2]. Glomeruli are grouped into clusters, each of which receives one of the tracts from the AN [3, 4, 5]. In glomeruli, ORN axons form synapses with different kinds of neurons such as projection neurons (PN). PN connect the AL with higher brain centers, mainly the mushroom bodies (MBs), which are centers where multisensory information converges [3]. The general organization of AL in hymenopterans is conserved. In ants, the AL is organized in six groups of glomeruli, each innervated by its own tract [3, 4, 5, 6]. In turn, glomeruli clusters are organized into two efferent regions, anterior and posterior, according to the type of PN that connects AL with the MBs via one of the two parallel pathways, which are the two main antennoprotocerebral tracts (APT) [3, 4, 5, 6]. Calyces of the MB are divided into layers the collar (receives visual input), basal ring (receives olfactory and visual input), and the lip (receives olfactory input). PN axons are segregated when they leave the AL, and follow one of the two main parallel paths through the brain until they end in the different layers in the lip and basal ring of MB calyx. Thus, the layered calyces of MBs receive segregated olfactory information PNs innervating anterior glomeruli clusters send axons through lateral-APT to the lateral horn (LH) and then to inner layer of the calyx, while PNs innervating posterior glomeruli clusters send axons through medial-APT to inner layers of the calyx and then to the LH. In this manner, AL organization is topographically represented in the MB forming a coarse odotopic map [3, 4, 5, 6].

The dual pathways connecting AL with MB could represent two channels for separately processing different kinds of odors (differential processing) or different attributes of behaviorally relevant olfactory stimuli (parallel processing) [7] that arrive from antennae. The subsequent convergent processing would be intergated in the MB along with other sensory modalities that would allow the associative function that they have been assigned such as linking sensory modalities as a colour with a smell [3], or a pheromone in a specific context [8]. This segregation of the olfactory tracts in parallel pathways present in several groups of insects [7] could represent an adaptation to environments with olfactory complex demands, such as life in society which is based on the detection and processing of pheromones. It is possible that social odors are processed separately through one of these channels, while non-social odors are processed through the other channel [9]. Thus, this functional organization would imply a high specialization of the brain of social insects to social life, as suggested by the hypothesis of social brains [10].

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Non-elemental olfactory learning in a honeybee brain

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*Insects have traditionally been considered small and simple reflex machines, but this particular view overlooks the fact that they process information flexibly in order to produce adaptive responses to their environment. Despite their small brain size, honeybees *Apis mellifera* are equipped with sophisticated sensory systems and evolved amazing learning and memory capabilities. Honeybees can learn to associate a floral odor with a nectar reward and store this information in long-term memory. However, changes in food source profitability occur rapidly in nature, so that bees have to change their acquired odor valences and quickly switch to another flower to ensure efficient foraging. In this scenario, bees may solve floral switch requirements by mastering non-elemental discriminations, in which the reward or its absence is not associated univocally with a single odor or set of odors. We used the conditioning of proboscis extension reflex to study how honeybees can deal with changing environments through non-elemental olfactory learning. In *multiple reversal olfactory conditioning*, successive valence reversals were performed using the same olfactory stimuli (e.g. A+ vs. B-, A- vs. B+, A+ vs. B-). This protocol is useful to determine whether or not animals “learn to learn” and solve successive olfactory discriminations faster with increasing reversal experience. In *occasion setting olfactory conditioning*, animals had to disambiguate an uncertain olfactory conditioned stimulus (A) using alternative visual stimuli (B, C) that do not enter into direct association with the reward (e.g. B A+ vs. C A-). We show that bees can master the multiple olfactory reversals, but instead of improving their successive discriminations, they tend to generalize their choice to both odors at the end of conditioning. In the occasion setting paradigm, we found that bees can learn to discriminate odor rewarded from odor non-rewarded trials if these two situations are indicated by different colors that do not themselves become associated with the reward. These cognitive neuroethological studies open new doors for understanding complex olfactory learning in insects, and to identify the potential neural substrates underlying non-elemental olfactory processing.*

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SYMPOSIUM “Semiochemicals identification & synthesis”
Tuesday 18/ 14:00-15:30 / Lleras Auditorium
Organized by Jan Bergmann and Paulo Zarbin

Structure Elucidation and Synthesis of Semiochemicals

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The lecture will deal with the identification and assignment of the absolute configuration as well as the synthesis of chiral volatile compounds from insects and plants.

1. Cephalic glands of the endohyperparasitoid wasp *Alloxysta victrix* contain several *trans*-fused iridoid lactones. For unambiguous structure assignments, pure reference compounds were needed. Starting from pure enantiomers of limonene, the 8 stereoisomers of *trans*-fused dihydronepetalactones and the 8 *trans*-fused iridomyrmecins were synthesized.^{1,2}
2. The composition of the unique scent of *Yucca* flowers will be presented. A couple of new volatile terpenoids form species specific mixtures that are attractive to *Yucca* moths.³
3. The Dufour glands of workers of the myrmicine ants *Tetramorium* spp. contain a long known, however, structurally undetermined group of terpenoids.⁴ These compounds that we like to generally call “tetramorines” were identified to be (2*S*)-2,3-dihydrofarnesal, two corresponding homo-sesquiterpenoids, a bishomo- and a trishomoterpenoid.
4. Two female-specific volatiles released by the parasitoid wasp *Trichogramma turkestanica* were shown to be (2*E*,4*E*,6*S*,8*S*,10*S*)-4,6,8,10-tetramethyltrideca-2,4-diene and (2*E*,4*E*,6*S*,8*S*,10*S*)-tetramethyltridecadien-1-ol or their enantiomers. These are the first sex specific compounds identified from a *Trichogramma* species and may be biosynthesized during a sequence including 5 propionate- and 1 acetate-unit.

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Chemical communication in the leafroller species *Proeulia auraria* and *P. triquetra* (Lepidoptera: Tortricidae)

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The leafrollers *Proeulia auraria* and *P. triquetra* are polyphagous insects native to Chile. They are distributed mainly in the central-southern region, and the host plants include economically important species, such as fruit trees, grapes, blueberries, and other crops. The aim of this work was to identify the sex pheromones produced by females, in order to provide information for the development of efficient lures for monitoring and/or control strategies.

The calling behavior of virgin females was studied under natural light conditions. The biologically active compounds produced in the female sex pheromone gland were extracted with hexane and identified by means of gas chromatography (GC)-electroantennographic detection (EAD), GC-mass spectrometry, and comparison of the analytical data of the natural compounds with those of authentic reference substances. Field tests were carried out for confirmation of biological activity.

The majority of females of *P. auraria* initiated calling between 5 and 7 a.m. The stereotyped behavior includes activation of resting females, wing fanning, antennal movements, curving of the abdomen, and protrusion of the pheromone gland while lifting the wings. The EAD-active compounds present in the gland of virgin females of *P. auraria* were tetradecyl acetate (14OAc), (*E*)-11-tetradecenyl acetate (E11-14OAc), (*Z*)-11-tetradecenyl acetate (Z11-14OAc), and (*E*)-11-tetradecenol (E11-14OH) in a relative ratio of 14100430. Field tests showed that all four compounds are behaviorally active. The most attractive blends contained the main compound E11-14OAc, 1% of the geometric isomer Z11-14OAc, and both of the other minor compounds. Addition of 4% of Z11-14OAc resulted in inhibition of attraction. The EAD-active compounds present in the gland of virgin females of *P. triquetra* were (*E*)-9-dodecenyl acetate (E9-12OAc), E11-14OAc, and Z11-14OAc in a relative ratio of 102.5100. Preliminary field tests showed that the presence of E9-12OAc, together with the main compound Z11-14OAc, is necessary for the attraction of males.

Based on our results, we suggest that the different composition of the sex pheromones is an important factor in the reproductive isolation of these sympatric and synchronic species.

Sex pheromone of the carrion beetle, *Oxelytrum discicolle*

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Carrion beetles are part of the great diversity of insects found on cadavers. In Brazil, the beetles of the genus *Oxelytrum* have great forensic importance in the post mortem interval estimation (PMI). So, the aim of this study was to determine the structure of the sex pheromone released by species. The volatiles from males and females were collected and the GC analysis revealed the presence of two male-specific compounds. The chemical structure of compounds was elucidated by mass and infrared spectra analyses, as well as by analysis of microderivatization products. Through the information collected, it was proposed as the major component (Z)-1,8-heptadecadiene and as minor 1-heptadecene. A seven steps synthetic route was developed to obtain the major component, which co-eluted with natural product in three different GC columns. Y-tube olfactometer assays showed that the mixture of synthetic standards were attractive to females, when combined with volatiles obtained from a rat carcass.

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Noneconomic Entomology Sex pheromones of giant silk moths

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The giant silk moths (Lepidoptera Saturniidae) are large and often very beautiful moths. Although they are not of economic importance, in North America and other parts of the world, they are highly prized by collectors. Unfortunately, populations of many saturniid moths appear to be in decline and several are now listed as endangered. The decreases in population are thought to be from the combined results of destruction of critical habitat, disruption of mating by street lights in urban areas, non-target effects of insecticides, and unintended parasitization by biological control agents introduced to control pest species. For example, the tachinid fly *Compsilura concinnata* which was originally introduced into North America to control gypsy moths is known to attack both luna moths and Promethea moths (Kellogg et al. 2003). Thus, the pheromones of endangered saturniid moths may be used as extremely sensitive and selective tools for monitoring their populations. Here, we describe the identifications of pheromones for three such species, the European Spanish moon moth, *Graellsia* (= *Actias*) *isabellae* (Millar et al. 2010), the North American Promethea moth *Callosamia promethea*, and ongoing work on the North American luna moth, *Actias luna*. The synthesis of the trienal and tetraenal pheromones of the first two species also will be described, along with results from field tests of the synthetic pheromones.

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SYMPOSIUM “Insect-Plant & Plant-Plant Interactions”
Wednesday 19/ 10:20-11:50 / Lleras Auditorium
Organized by Andrés González

**Chemically-mediated insect-plant interactions in native systems a
knowledge base for crop-pest systems**

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Economically important crops are often not native to the regions in which they are cultivated. Likewise, many insect pests follow their host plants to new habitats, and compete with native herbivorous insect species, which may in turn cause relevant damages. In their native range, insects must have coevolved with native host plants, establishing, among others, chemically-mediated interactions. However, the applied nature of many insect-plant studies frequently results in the characterization of such interactions in systems that have coevolved recently. While this vast knowledge base is extremely valuable concerning both fundamental and applied science, the study of native systems may also provide relevant insights albeit not being *per se* economically significant. Moreover, if these systems are closely related to crop-pest systems, information may be extrapolated to understand processes that may be relevant for pest management. Here we present partial results of two current studies on chemical interactions between plants and insect herbivores native to Uruguay *i*) the wild potato *Solanum commersonii* (Solanaceae) and one of its specialist herbivores, the sawfly *Tequus schrottkyi* (Hymenoptera Pergidae), and *ii*) the South American fruit fly *Anastrepha fraterculus* (Diptera Tephritidae) and its native host plants *Acca sellowiana* and *Psidium cattleianum* (Myrtaceae). In the first study, non-volatile plant defenses (glycoalkaloids) were studied for their effect in feeding preference by the specialist herbivore. Our results suggest that glycoalkaloids from *S. commersonii* act as host-plant chemical cues for the specialist sawfly, and may even serve as chemical defenses that the insect acquires for its own defense. In the second study, we analyzed the phenological change in volatile chemistry of host fruits of *A. fraterculus*, in search of potential chemical cues for fruit maturation state and oviposition suitability. We found that both fruits change dramatically their volatile profile after maturation, from a terpene-dominated mixture to one mostly composed of aliphatic and/or aromatic esters. Whether this chemical change is used by *A. fraterculus* to find or select suitable oviposition sites is now under investigation.

**Plant volatiles to manage herbivores and natural enemies in arable
crops**

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The actual agricultural system is based on intensive practices that were developed mainly during the 70s to 90s, which involves the establishment of great monocultures, high mechanization, and indiscriminate use of pesticides to control insects, diseases and weeds. However, the damage provoked to the environment by this production model and the search for safer and better-quality food have motivated the development of new tools to obtain a more sustainable agriculture. One of the great challenges to the Neotropical Agriculture is to reduce the indiscriminate use of pesticides. The semiochemicals is one tool that can be applied in the field to manage pests and its natural enemies, thus minimizing the use of pesticides. A roll of small lipophilic molecules from the plant secondary metabolism plays an important role in the chemical communication of different species that are present in arable crops. When plants are damaged by herbivores or are stressed using exogenous chemical application, their chemical profile of compounds change. As a result of this, a new chemical profile is used by natural enemies as cues to locate their hosts and also can act directly on the herbivores. In this conference we will present some laboratory and field studies showing that it is possible to use plant volatiles to manage natural enemies and herbivores. We will present practical examples of the influence of two compounds, *cis*-jasmone and (*E,E*)- α -farnesene, on the foraging behaviour of *Telenomus podisi*; and the influence of volatiles from cotton plants on the behaviour of *Anthonomus grandis*.

Financial support: CNPq, Embrapa, FAP-DF, CAPES.

Effects of root herbivory on the selection of tomato plants by a leaf herbivore, *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Plant-herbivores have been shown to interact indirectly through the plants' response. In the case of systemically induced plant responses, this interaction extends to herbivores inhabiting different parts of a plant, for example, above- and below-ground. Plant-induced responses elicited by root herbivores have been shown to affect feeding and development of above-ground herbivores. We assessed whether the presence of a below-ground nematode *Meloidogyne incognita* in the roots of *Solanum lycopersicon* could influence the oviposition decisions and development of an above-ground insect *Tuta absoluta*. Moreover, we also checked if the period of damage by the below-ground herbivore (i.e. 10 and 20 days after the infestation) affected the oviposition and development of the above-ground insect. In oviposition choice-tests females of *T. absoluta* deposited more eggs in healthy plants than plants with *M. incognita*. The result was the same for 10 and 20 days after infestation by *M. incognita* on roots. The developmental parameters were negative affected by the root feeder. Our results show that indirect interactions between below-ground and above-ground herbivores extend to behavioral avoidance, in terms of oviposition. Moreover, given the negative effect on development, *T. absoluta* seems to avoid this root infested plants to attempt to choose the "best" for their offspring.

The Pollination Syndrome Chemical Facts and Evolutionary Speculations

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Flowers use various cues such as color, shape, texture, and scent to attract pollinators and usually reward them with nectar and pollen. Co-evolutionary processes may lead to distinct and unmistakable odor bouquets that are attractive to specific pollinators. A further step in this process is the production and release of volatile signals providing wrong information for the receivers to deceitfully attract them into pollination. Such fraudulent pretencies may mimic the odor of food or prey and copy insect semiochemicals including alarm pheromones or sex pheromones etc. In such cases, no rewards will be necessary to the pollinators because they will inescapably follow the “unambiguous” volatile message.

The lecture will provide examples representing different scenarios

5. The role of spiroacetals in host-plant recognition between the flowers of *Campanula trachelium* and the pollinator bee, *Chelostoma rapunculi*, will be discussed.¹ Similarly, spiroacetals released by the deceptive trap flowers of *Ceropegia dolichophylla* are decisive in the attraction of cleptoparasitic fly pollinators.²
6. Pretending to be attacked by a herbivorous insect, *Epipactis* orchids attract carnivorous social wasps by releasing green-leaf volatiles (GLV). Cyclic 6-carbon compounds that may complement the present GLV-standard mix have been identified.³
7. The orchid *Dendrobium sinense* produces 11*Z*-eicosen-1-ol, a major component of the honey bee alarm pheromone to attract the hornet *Vespa bicolor* that preys on bees.⁴ Interestingly, this alcohol was also found in the labial gland secretion of the orchid bee *Euglossa viridissima*.⁵ Moreover, the corresponding acetate has been described as the aggregation pheromone of some *Drosophila* species,⁶ whereas the aldehyde was identified as a component of the trail pheromone of *Dolichoderus* ants.⁷
8. Australian deceptive *Chiloglottis* orchids produce 2,5-dialkylcyclohexan-1,3-diones, which are identical to the sex pheromones of their pollinators, thynnine wasps.⁸ At least one of these so called chiloglottones has also been identified in microorganisms.⁹

Striking structural relationships between semiochemicals and the role of microorganisms in the evolution in systems of chemical communication will be discussed.

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SYMPOSIUM “Marine Chemical Ecology”
Wednesday 19/ 13:00-14:30 / Lleras Auditorium
Organized by Mónica Puyana

**Variation in the metabolic profile of the octocoral *E caribaeorum*
collected at the Colombian Caribbean Sea**

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Erythropodium caribaeorum is an octocoral belonging to the family Anthothelidae. This soft coral grows on the surface of stony corals and rocks and is almost free of predators. Chemical studies of this soft coral showed a wide variation in the chemical composition (mainly diterpenes) related to the collection place. Thus, with the aim to contribute with the knowledge of this octocoral in our marine environments we conducted dereplication and metabolic profile analysis for eight samples of *E. caribaeorum* collected at locations of Santa Marta and Islas del Rosario in the continental coast of Colombia, and in Old Providence in the SW Caribbean.

The animal material was fractionated by RP-SPE cartridges and analyzed by HPLC-DAD and HPLC-HRMS for data collection. Data analysis follows a dereplication strategy using Antimarin™ data base for compound identification and a metabolic profiling approach based on chromatographic and HRMS data analysis using PCA and HCA methods for the evaluation of metabolic variations of the samples.

About the metabolic profile PCA plots as well as HCA plots showed how the samples from the same locations groups in a same cluster suggesting the effect of particular environmental conditions on the metabolic profile of the collected samples. Our dereplication results allowed identifying a different chemical profile for the samples collected at Islas del Rosario compared to other collection places. Some diterpenes could be identified from the Santa Marta samples, but more interesting was the number of unknown compounds from these samples, showing the metabolic potential of the samples for the search of new and useful chemical entities.

Dereplication and metabolomic analysis of the metabolic profile showed that samples from Santa Marta, Islas del Rosario and Old Providence have different metabolic profiles. This information could be the starting point for further ecological and bioprospecting studies on *E. caribaeorum*.

Microbial interactions of octocoral-associated bacteria

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Octocorals are sessile organisms that live in a close relationship with a complex microbial community. This coral-microbe consortium is known as the coral holobiont. Although the composition and structure of coral microbial communities have been described, the understanding of the function of these microbes is central to the emerging field of coral microbial ecology.

Some of the functions of mutualistic bacteria associated to corals include nitrogen fixation and the production of antimicrobial secondary metabolites. These antimicrobial-producing bacteria have been proposed as the first line of the coral's defense against pathogens. Experimental evidence regarding the ability of coral-associated bacteria to produce secondary metabolites with antimicrobial activity is abundant. However, the chemistry involved in these interactions remains mostly unknown.

In this talk we will present some of the results of our marine natural products program at INDICASAT which is focused in the study microbial interactions as a tool for drug discovery. Microorganisms were isolated from coral samples collected at different locations of Panama. Strains obtained were assessed using an antagonistic assay in agar plates against a panel of terrestrial and marine fungi. Strains that produced inhibition halos against bacteria or fungi were studied using different mass spectrometry techniques including ESI-QTOF MS and MALDI-TOF imaging MS. Secondary metabolites responsible for the antimicrobial activity were detected and identified using MS and NMR. Our results provide experimental evidence that support the coral probiotic hypothesis.

Ecological interactions of Caribbean benthic cyanobacteria

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Cyanobacteria are bacteria with photosynthetic capabilities that thrive in terrestrial, freshwater and marine ecosystems. Under the right environmental conditions, marine benthic cyanobacteria can form dense benthic mats that affect marine ecosystems, animals and humans. Cyanobacterial blooms are currently of major concern due to their recurrence and potential detrimental effects.

In order to address toxicity of cyanobacterial blooms from several locations, we evaluated their crude organic extracts against *Artemia salina*. Based on these results we selected a range of extracts in order to address their feeding deterrence against three different herbivores and allelopathic effects against corals.

We prepared artificial diets to address the feeding deterrence of cyanobacterial extracts against the specialist opisthobranch *Bursatella leachi*, the generalist sea urchin *Lytechinus variegatus* and the generalist fish *Stegastes planifrons*.

We evaluated allelopathic effects against the coral *Madracis mirabilis* by exposure of coral fragments to cyanobacterial extracts at different concentrations in enclosed water volumes. We performed toxicity assays against embryos of *Montastrea annularis*. We also evaluated coral bleaching as a sign of stress upon direct contact of *Madracis mirabilis* ramets with gels prepared with cyanobacterial extracts.

Most cyanobacterial extracts tested showed high feeding deterrence against fishes and urchins. Even opisthobranchs, that may consume cyanobacteria when other sources are scarce, were deterred by some extracts.

All tested extracts were toxic to coral embryos. Polyp retraction and bleaching were common responses of the coral *Madracis mirabilis* upon indirect and direct contact with cyanobacterial extracts.

Our data suggest that benthic cyanobacteria from the Colombian Caribbean are chemically defended, therefore not controlled by herbivores. Additionally, there appears to be a chemical mechanisms underlying interaction between cyanobacteria and coral larvae and adult colonies.

Coral undermining and death by encrusting excavating sponges Is allelopathy involved?

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The Caribbean encrusting and excavating sponges *Cliona tenuis* and *C. delitrix* successfully compete for space with reef corals, killing and displacing live coral tissue at rates of several cm per year. To evaluate whether there is allelopathy involved in coral death, an ecologically relevant bioassay was carried out, together with bioassay-guided fractionation of their extracts to find the compound(s) responsible; also, a series of histological observations and experiments with dissociated sponge and coral cells and coral larvae were carried out to understand the possible mechanism of coral death. Ground-and-polished histological sections of the zone of sponge-coral contact revealed that erosion of the supporting skeleton is not enough to produce coral polyp detachment, but showed accumulation of granular cells in both sponge and coral tissues when in contact, hinting of histocompatibility reactions. Healed sponge fragments placed directly over live coral for 1-2 days were killed by coral defenses for *C. tenuis* but not for *C. delitrix*; fragments of both sponges placed in close proximity to corals for 6 months did not have any effect on the adjacent coral tissue. The crude extract and the aqueous partition of both sponge species, and the *C. tenuis* pure compound [(-)-(5S)-2-imino-1-methylpyrrolidine-5-carboxylic acid], called clionapyrrolidin A], incorporated in gels at close to natural volumetric concentrations, killed coral tissue when brought into contact over live coral for 1-4 days. While clionapyrrolidine A dissolved in seawater was only mildly toxic against live coral and dissociated coral cells, and killed coral larvae only at high concentration, the aqueous fraction of *C. delitrix* was toxic against live coral tissue, larvae and dissociated coral cells. Mixtures of dissociated sponge and coral cells elicited greater coral cell death than unmixed controls, and were accompanied by a significant decrease in the pH of the mixture. Thus, although prolonged contact of clionapyrrolidine A (and responsible compound(s) in *C. delitrix*) in the enclosed spaces of the coral skeleton below the polyps may elicit polyp uplift or produce coral tissue death, other alternative mechanisms such as acid or enzyme secretion or histo-incompatibility cannot yet be ruled out.

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SYMPOSIUM “Chemical Ecology in Medical Entomology”
Thursday 20/ 10:20-11:50 / Lleras Auditorium
Organized by Pablo Guerenstein and Romina Barrozo

The bad taste governs feeding decisions in a blood feeder

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The ability to discriminate between nutrients from harmful substrates is essential for animal's survival. Though olfaction and vision contribute to find potential food, the taste sense works as a final control system driving food acceptance or rejection. Taste abilities vary in the animal kingdom, humans and most mammals can detect 5 taste modalities sweet, acid, umami, bitter and salt. Whereas sweet and umami have positive hedonic values, bitter and high salts doses are both related to aversive behaviors in most animals studied so far. In contrast to olfactory sense, the low spectrum of taste modalities that animals recognize is comparable to their low discrimination abilities. For example, humans can fairly distinguish between regular table salt (sodium chloride) and commercial salt substitutes (potassium chloride), though we have troubles to discriminate among bitter substances.

Triatomine insects (vectors of Chagas Disease in Latin America) feed on blood from small vessels of vertebrate hosts. As soon as they reach a potential host, they walk over their skin searching for an adequate site to pierce. Then, they insert their stylets and take a first sampling gorge to decide if food is acceptable or not. Our work shows that detection of certain substances such as bitter compounds and certain inorganic salts at high doses inhibits feeding behavior of these bugs.

Rejection of food is dependent not only on the chemical nature of tastants but also on the concentration. Aversive behaviors can be triggered during both the assessment of the surface to bite or within the first gorge of food. The sensory organs involved in taste detection are located in the antenna (i.e. external organs) and in the pharynx (i.e. internal organs) of the insects, as show our morphological inspections along with electrophysiological recordings. These two sensory stages work with different avoidance thresholds antennal input exerts a modulatory rejection signaling at higher doses than internal sensors. Additionally, we study whether bugs are able to discriminate among aversive stimuli, or alternatively they are simply indistinguishable negative input signals that induce feeding inhibition.

We use a multiapproach strategy that involves different levels of study. On one hand we target on the putative salt receptors and we study the transduction pathway possibly involved in the aversive detection; and on the other we use a cognitive approach to gain more insights on the ability of insects to discriminate

among aversive taste modalities (i.e. bitter vs. salts) and within a taste modality (i.e. amongst bitter compounds or salts). Our results highlight the relevance of taste perception of aversive and anti-appetitive compounds modulating the feeding behavior of a blood-sucking insect.

The role of epicuticular lipids in chemical communication in Chagas disease vectors

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The triatomine bugs are vectors of the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease. *Triatoma infestans* (Heteroptera Reduviidae) is a major vector of Chagas disease in South America, especially in the 1.3 million km² Gran Chaco geographic region, which has a mostly rural human population (1). Epicuticular lipids are contact cues in intraspecific chemical communication in insects, both for aggregation and sexual behaviour (2).

Aggregation behaviour plays an important role in their survival by facilitating the location of refuges and cohesion of aggregates, helping to keep them assembled into shelters during daylight time, when they are vulnerable to predators. There are evidences that aggregation is mediated by thigmotaxis, by volatile cues from their faeces (3), and by contact chemoreceptive signals from their cuticle surface (4). The epicuticular lipids of *Triatoma infestans* include a complex mixture of hydrocarbons, free and esterified fatty acids, alcohols, and sterols. We analyzed the response of *T. infestans* fifth instar larvae after exposure to different amounts either of total epicuticular lipid extracts or individual lipid fractions. Our work shows evidence that cuticular lipid extracts of *T. infestans*, and particularly the C180 and C260 fatty acids components of the free fatty acid fraction (FFA), promote insect aggregation (5). Octadecanoic acid (C180) showed a significant assembling effect in the concentration range tested (0.1 to 2 insect equivalents). The very long chain hexacosanoic acid (C260) was significantly attractant at low doses (≤ 1 equivalent), although a repellent effect was observed at higher doses.

Some hydrocarbon constituents serve as contact sexual pheromones in many types of insects. Using headspace solid phase microextraction, we found no sexual dimorphism in epicuticular hydrocarbons in *T. infestans*, but found female-specific fatty alcohols (eicosanol and docosanol). The role of epicuticular lipids in *T. infestans* copulation behavior was tested by observing male responses to live or various treatments of freeze-killed females. We report that hexane-soluble contact cues on females trigger copulation by males. Freeze-killed intact females were attractive to males, but no response was observed when males were exposed to hexane-washed females. Responses were partially recovered when epicuticular extract was applied to the dorsal surface of dead, hexane-washed females. One female equivalent of docosanol, evoked similar responses (6). Our results show that mating attempts by a *T. infestans* male requires contact of the male's antennae with the female, and that epicuticular lipids trigger this response. Female-specific docosanol seems to be an important chemical that mediates this behaviour.

Finally, the detection and description of aggregation and sexual contact pheromones has practical application in Chagas disease vector control. These data may be used to help design new tools against triatomine bugs.

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Chemical Ecology of phlebotomine sand flies, vectors of leishmaniasis

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Phlebotomine sand flies, vectors of leishmaniasis, are extensively collected in the field with light traps. There are approximately 900 sand flies species of sand flies around the world, 400 in Latin America⁽¹⁾.

Despite advances in our understanding of the chemical ecology of many important disease-transmitting hematophagous insects, and particularly Culicidae, very little is known about the chemicals responsible for attracting Phlebotomine sand flies⁽²⁾.

Most studies of chemical ecology in sandflies have focused on the American visceral leishmaniasis vector *Lutzomyia longipalpis*, in which attraction to both host odour components and male-produced pheromones have been demonstrated through laboratory olfactometer and field studies^(3,4). In comparison, almost nothing is known about the chemical ecology of related species, which transmit American cutaneous leishmaniasis (ACL), due in part to difficulties in raising these insects in laboratory.

At the beginning of our work we started raising some questions about the behavior of the ACL vector *Nyssomyia neivai* in wind tunnel. For instance 1) Can field-caught *N. neivai* be used to measure olfactory responses in the wind tunnel? 2) Does time of day influence *N. neivai* (nocturnal insects) responses in the wind tunnel?

Our wind tunnel is a transparent acrylic box (length 200 cm, width 20 cm, height 20 cm); air flow is obtained from a zero grade air cylinder, humidified using a water bath (36°C). Conditions within the wind tunnel were recorded at 24 ± 1°C and 70-75% relative humidity. Groups of three female *N. neivai* were placed inside a releasing chamber for 30 min to acclimatize prior to each test. The chamber was then placed inside the wind tunnel 50 cm downwind from the air intake and odour source, and the sandflies released by opening the chamber door. Each trial lasted 2 min, with activation (number of sandflies leaving the releasing chamber) and attraction (number of sandflies reaching the odour source) recorded for each group.

The results showed that it is possible to use field-caught sand flies to measure olfactory responses in the wind tunnel and the time of laboratory experiments with field collected insects need not be restricted to night and evening.

We also carried out experiments with host kairomones (lactic acid, 1-octen-3-ol and BG-lure) and phytokairomones (saturated primary alcohols 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 1-decanol). Out of the host kairomones evaluated, only 1-octen-3-ol presented strong attractive response.

For phytokairomones, except for propanol and butanol, which were not significantly different from the control for activation or attraction ($p > 0.05$), the other evaluated compounds presented some degree of activation or attraction for female *N. neivai* sand flies ($p < 0.05$). Octanol was even better than octenol at lower concentrations (50% and 10%).

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Formulations of oviposition attractants for *Aedes* mosquitoes: from laboratory to large scale production

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Dengue fever is an infectious disease caused by the virus that is transmitted by the bite of the female mosquito *Aedes aegypti* (Diptera: Culicidae). This disease is considered a major public health problem in Brazil and in Americas. According to the World Health Organization (who), annually, it is estimated that 80 million people are infected by the virus and an estimated 2.5 billion people live in areas with high risk of infection. In Brazil, every patient with dengue fever costs approximately USD 349,00 and hospitalization is about USD 2,130.70. Since there is no vaccine or specific treatment, the only way to prevent the disease is to control the vector. Monitoring adult *Aedes aegypti* abundance with fixed position traps has been considered as an alternative surveillance method that shows promise for directing vector control and predicting when and where dengue outbreaks will take place. Therefore, development of semiochemical to attract female *Ae. aegypti* mosquitoes to monitoring traps is crucial.

Olfactory cues from oviposition sites are now being used to lure gravid *Ae. aegypti* females into sticky traps for population assessments and indices of dengue risk. The development of the so-called MosquiTRAP, which incorporates a synthetic oviposition attractant (AtrAedes) is described.

Oviposition attractants for gravid *Ae. aegypti* were isolated and identified from *P. maximum* grass infusions (Sant'Ana *et al.* 2006) and these are considered an important tool for increasing the catch of gravid *Ae. aegypti* in the sticky trap MosquiTRAP (Eiras and Resende 2009, Eiras and Sant'Ana 2002). The chemical identification of volatiles produced by grass infusion of *P. maximum* that enhanced oviposition responses of gravid female *Ae. aegypti* was possible only by means of a combination of studies using electrophysiology (electroantennography), gas-chromatography coupled with mass-spectrometry (GC-MS), gas- chromatography coupled with electroantennography (GC-EAD) and behaviour using ovitraps (Eiras *et al.* 2010). Firstly, the grass infusions of different ages were extracted individually by solvent and by solid micro phase extraction (SMPE) and submitted individually to GC-MS, GC-EAD and EAG and compared with results from oviposition responses. The active compounds present in infusion extracts of high EAG and oviposition responses were submitted to GC-MS for identification. Seven compounds were identified as candidates for an oviposition stimulant or attractant of gravid *Ae. aegypti*: nonanal, benzothiazol, decanal, p-cresol, indole, 3-methyl indole and limonene) (Eiras and Sant'Ana 2002 - Patent).

Formulation of such compounds have been developed and evaluated in semi-field and field condition. Here in, examples of simple production of lures in small scales will be presented as an example of small scale in laboratory and a mass production by machinery. AtrAedes has been used in the field over 10 years in a successful monitoring dengue vector technology (MI-Dengue). Such technology has been used over 60 Brazilian cities, more than 25,000 traps in areas with about 6 million people.

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Synthetic host odors could be used to manipulate both the host-seeking and oviposition behavior of triatomines

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Chagas disease is a serious health problem in Latin America. The vectors of this disease, which transmit the protozoan *Trypanosoma cruzi*, are triatomine insects. Vector control is the most effective method to prevent Chagas disease [1]. Vector management is mainly accomplished by insecticide spraying of infested houses. However, this is not ideal considering that the insects develop resistance to the insecticides, and that these chemicals could affect people living in sprayed houses. The use of lured trap devices is a sustainable and an environmentally-friendly method for vector control [2]. Thus, it would be possible to monitor and control the bugs without generating any resistance or toxic effect for humans. For this, powerful odor attractants have to be developed. For this, we carried out experiments on the olfactory basis of the host-seeking and oviposition behaviors of triatomines.

Using two of the most important Chagas vectors, *Rhodnius prolixus* and *Triatoma infestans*, we tested potential host odor attractants in a dual-choice trap olfactometer, which is a very challenging device. In this olfactometer, the insects have to be captured in a trap in order to score a response to an odor source. We tested several blends of synthetic odors that did not include CO₂, a compound that is either expensive or unpractical to use in the field. Synthetic blends, consisting of L-(+)-lactic acid, valeric acid and ammonium hydroxide (all odors detected by triatomines), were made using different volumes of the solutions of each of the three components in individual LDPS (low density polyethylene sheets), and were assayed using live mouse odor as positive control. The positive control, as well as the synthetic blends tested, evoked significant attraction (capture) both, in *R. prolixus* and *T. infestans*. The fact that the blends were able to significantly trap the insects would imply that they are strong attractants. The different blends tested varied in their capture performance. In the case of *R. prolixus* the best blend evoked 81% capture in a single night (this represents a significant capture respect to a negative control with no odor, G-test, $p < 0.05$, but not different from a mouse). For *T. infestans*, the same blend was also the best and also evoked 81% capture in a single night (a significant capture respect to the negative control, G-test, $p < 0.05$, but not different from a mouse). Adding of nonanal (a host odor detected by triatomines) did not improve capture, showing that the behavioral response to simple blends is complex.

The efficacy of odor emission-based trapping systems depends on emission of the right odor mixture (and this includes emission of the blend constituents at

appropriated ratios) and on how constant and prolonged that odor emission is [3, 4]. We analyzed such emission from our LDPS containers for all the mixtures assayed in behavioral experiments. That would let us characterize the blends tested in terms of the proportions of each odor constituent that was detected by the insect antenna. Using gas chromatography, in the case of valeric and lactic acid, and Nessler colorimetric measures with a spectrophotometer in the case of ammonia, we could estimate the odor mass emitted in a period of time and generate emission curves. Thus, we can now correlate the behavioral results with the blend that was actually detected by the insects so that it would be possible to reproduce the most attractive blend using other odor delivery systems in the lab or in the field.

Triatomine first-instar larvae need to feed within the first days after emergence from the egg in order to survive. Taking into account their small size and limited locomotion capacity, it seems reasonable that females should lay their eggs nearby a host. In fact, the triatomine *Rhodnius prolixus* lay (stick) their eggs on the feathers of avian hosts. We tested if the presence of a host has an effect on the spatial distribution and number of laid eggs.

We carried out tests in an experimental arena consisting of a PVC cylinder (0.2 m diameter, 1.70 m length). Two male and four female adult *R. prolixus* 1-2 weeks after feeding were placed inside a test and a control arena in rooms with controlled temperature ($27\pm 1^\circ\text{C}$) and 12/12 L/D light cycle, for 3 days. The stimulus, consisting of either a mouse or hen feathers, was placed below one end of the test cylinder while the other end remained empty; no stimulus was placed below control cylinders. Across the cylinder, little holes (behind which, a fine mesh was placed to avoid the escape of bugs or eggs) were made to allow air circulation from a host to enter the cylinder.

Both stimuli significantly stimulated oviposition in *R. prolixus* (Mouse total eggs test, 369; total eggs control =236, $N=16$, $p<0.05$; Feathers 332, 118, respectively, $N=16$, $p<0.05$, Mann-Whitney test). In addition, hen feathers evoked a non-homogeneous distribution of eggs within the cylinder (Kruskal-Wallis test $p<0.05$, $N=16$). The hen feathers (and the mouse) stimulated oviposition in *R. prolixus* even when the insects could not contact the source of stimulus, thus host odors alone can stimulate oviposition in *R. prolixus*. However, a role of other host cues cannot be excluded.

In summary, synthetic host odors could be used to manipulate both the host-seeking and oviposition behaviors of triatomines.

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SYMPOSIUM “Semochemicals & Pest Management”
Friday 21/ 10:20-11:50 / Lleras Auditorium
Organized by Mauricio Bento

**The basis for monitoring coffee leafminer *Leucopera coffeella*,
(Lepidoptera: Lyonetiidae) with pheromone traps**

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The use of pheromone traps was tested to monitor the males of *L. coffeella*, aiming to evaluate the components of the monitoring system of this coffee pest. By geostatistical analysis, we determined the ideal distance between traps catches considering when they behave independently. The traps were placed in groups of 12 using spacings of 2; 5; 10; 15 and 30 m within each group. Catches were recorded every 4 days for 16 days. Interference was found between traps with smaller distances of 10 m. The scope and magnitude of spatial dependence varied considerably between each assessment and wind direction. In the perpendicular and parallel to the lines of coffee planting directions, spaced traps 110 and 177 m respectively obtained a spatial independence in catches. In order to determine the density of traps per hectare, catches of males of *L. coffeella* in 190 traps in a area of 30 ha were evaluated. These traps were placed forming an irregular grid with minimum line spacing of 20 m between traps, were catches were recorded during 15 evaluations every 8 days. The data for all catches adjusted significantly to a negative binomial distribution and the dispersion parameter (common K). Based on common K equal to 2.16 was obtained 8 traps that are necessary to sample 30 ha, with accuracy level of 25%. The relationship between catches of males of *L. coffeella* and its population density was assessed in seven plantations at different agronomic conditions in Minas Gerais state, in varying periods 6-20 months. The location significantly affected the catches of males. The decrease in the average temperature favored the growth of the infestation of the coffee plantations and the capture of males. Triangulo Mineiro was found in a positive relationship between the capture of males and the proportion of mined leaves ($R^2 = 0.79$), and in the Zona da Mata this relationship was significant but weak ($R^2 = 0.06$). In the latter place also observed a positive relationship between captured males and eggs of the pest ($R^2 = 0.34$). These results have implications for monitoring *L. coffeella* when using pheromone traps, because it was observed a significant relationship between captures of males and the population density of the pest. These relationship was determined with one trap at every 4 ha and is appropriated to monitor *L. coffeella*.

Semiochemicals as key tools for pest management the role in the future codling moth management

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The choice of pest management tactics that growers adopt is an economic decision and has a number of associated risks with their implementation, such as poor spray timing and coverage, ineffective insecticide chemistry, disruption of natural enemies and resulting secondary pest outbreaks, selection for insecticide resistance and environmental contamination from insecticides. In the same way, the markets are imposing more restrictive pesticide residual limits and environmentally safe agricultural practices. Monitoring insect pests with sex pheromones, host plant volatiles and food baits have been developed and provide growers with low cost, effective tools to assess pest densities. A good model of this problematic is codling moth (*Cydia pomonella*), the main pest for pip fruits and walnut worldwide. Efforts to improve monitoring of codling moth include developing lures for female adults, such as more selective and effective non-sex pheromone lures; including components like acetic acid, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E*)- β -ocimene (beta ocimene). (*E,Z*)-2,4-decadienoate (pear ester), with or without (*E,E*)-8,10-dodecadien-1-ol (codlemone, the sex pheromone) and acetic acid, has been successfully implemented for female monitoring, and for more effective mating disruption or insecticide managements. New compounds like these are valuable tools for monitoring and pest management, but they will require the establishment of protocols that enhance the determination of action thresholds based on the trapping data obtained.

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Use of an off-ratio blend for mating disruption of a major citrus pest

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The leafminer *Phyllocnistis citrella* (Lepidoptera Gracillariidae) is a global pest of citrus and contributes to the incidence and severity of citrus bacterial canker disease. Results of response surface models generated by mixture-amount experiments suggested that an off-ratio blend consisting of the major pheromone component (*Z,Z,E*)-7,11,13-hexadecatrienal was the principal driver responsible for mating disruption in small plot field trials. Further field trials demonstrated that the single triene component was equal to or better than the “natural” 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal (*Z,Z*)-7,11 hexadecadienal. A commercial release device (DCEPT CLM™, ISCA Technologies Inc., Riverside, CA, USA) showed exceptional longevity in field trials in 2012 and 2013. Approximately 1,000 hectares (~400,000 trees) at each of three locations in southeastern and southwestern Florida were treated during the spring of 2014 with a subsidy for early adopters provided through an agreement with the Citrus Research and Development Foundation in collaboration with ISCA Technologies, USDA-ARS and the University of Florida. Contrasting sites of 350 to 500 ha were chosen to examine the effect of immigration of gravid females from outside of the pheromone-treated areas. Results will be presented to examine the biological and economic value of an area-wide approach to this environmentally benign biological approach to control of a major leafminer pest and associated canker disease.

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Economic and environmental benefits by using sex pheromone for citrus fruit borer management

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Citrus fruit borer, *Gymnandrosoma aurantianum* Lima (Lepidoptera, Tortricidae), is a pest of generalized occurrence in South-Central region of Brazil. In the decade of 1990, yield losses caused by this pest were estimated in up to 350 fruits/plant in the main citrus producing regions of São Paulo State, representing more than US\$ 50 million of losses per year. Since 2001, citrus fruit borer sex pheromone was identified and it has been used by 30 to 40% of citrus growers. Within this period (2001-2013), it was estimated that the use of sex pheromone in infested areas promoted a reduction of more than 50% of agrochemical applications and losses were reduced to 1 fruit per plant, on average. At the economic perspective, the use of pheromone for *G. aurantianum* management for a little more than 10 years allowed to avoid losses in citrus yield of more than US\$ 1.2 billion for Brazilian growers.

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**SYMPOSIUM “Importance, regulation and limitations on use of
pheromones in Colombia”**

Friday 21/ 13:30-15:00 / Lleras Auditorium

Organized by Nancy Barreto

Importance, regulation and limitations on use of pheromones

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This discussion space pursues to show the needs and constraints that exist in Colombia on the use of insect pheromones for research or commercial use. It will include the participation of the Colombian Agricultural Institute ICA, institution which governs their use and which also applies this technique for detection of quarantine pests. Considering that Brazil is one of Latin American countries with the highest pheromones registered products as a result of research from different private and public agencies in the country; a summary of progresses , applications and regulations from EMBRAPA experience will be presented . Finally , this space seeks to generate partnerships with international peers interested in cooperating with the developments on this subject in Colombia.

Limitations on the use of pheromones in Colombia

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Using pheromones to phytosanitary monitoring: Quarantine pests and low prevalence areas

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Regulations for import and use of pheromones in Colombia

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Investigation, use, regulations and trade of pheromones in Brazil

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ORAL PRESENTATIONS

Abstracts



1A-1 Volatile Organic Compounds (VOCs) Related to Chemical Signalization between Guava (*Psidium guajava* L.) and the Guava Weevil *Conotrachelus psidii* Marshall.

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Introduction

Conotrachelus psidii (Coleoptera Curculionidae) is one of the most important pests of guava, *Psidium guajava* (Myrtaceae). The guava weevil is mainly controlled through frequent application of insecticides; however, this management has not been successful in controlling this pest. The main goal of this research was to identify which host or conspecific VOCs can act as attractants to guava weevil. Semiochemicals that attract the guava weevil would allow developing new strategies for monitoring and controlling this pest.

Material and methods

The VOCs emission of guava reproductive tissues was collected *in situ* over during the day by headspace-solid phase microextraction (HS-SPME) in five reproductive tissues (flower bud, open flower, petal fall, fruit setting, and fruit growth), and by dynamic headspace (DHS) in the two guava stages where *C. psidii* commonly is found. The samples were analyzed by GCMS to identify the VOCs. After sexual differentiation of guava weevils, the volatile was comparatively analyzed by GCMS.

Results

Three guava VOCs were present in all of the guava reproductive tissues, being quantitatively important in flower bud and fruit setting, the two guava stages where *C. psidii* commonly is found. In contrast, VOCs of guava open flower were different from other tissues. Among identified compounds, four guava VOCs were detected in both, male and female insects, thus suggesting a possible role as allelochemicals for *C. psidii* host plant finding. Two male-specific guava weevil VOCs were identified as with potential to be pheromone candidates.

Conclusions

The results suggest that communication in *C. psidii* is mediated by semiochemicals, more specifically by kairomones in combination with insect produced aggregation pheromones. Behavioral and electrophysiological studies to evaluate the specific influence of VOCs identified, in the *C. psidii* are underway.

Financial support

Banco de la República and DIB - Universidad Nacional de Colombia and INCT Semioquímicos na Agricultura.

1A- 2 Methods and natural products applied in the development of pesticides to control of *Xylella fastidiosa* in citrus

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Introduction

Citrus is a target for several diseases that cause damages the production. Citrus Variegated Chlorosis is one of the main diseases which causes large losses. This disease is caused by *Xylella fastidiosa*. Unfortunately, it doesn't have specific pesticides in the market to combat this bacterium, so far.

Material and methods

Mg complexes were synthesized with natural products and evaluated in *in vitro*, and *in vivo* assays. The tested compounds as bactericides were azadirachtin, hesperidin, naringenin and their complexes. A method by microdilution in microplate was developed and applied for determining the MIC *in vitro*. The MIC technique was performed according to the methodology by the "National Committee for Clinical Laboratory Standards". Assays *in vivo* were carried out using orange healthy plants. Previously, the plants were artificially infected with *X. fastidiosa*. Afterward, the plants were treated with the tested compounds that showed positive results at the *in vitro* assays. Qualitative and quantitative Polymerase Chain Reaction were carried out to confirm the plants that were contaminated, and to evaluate which compounds were efficient in *X. fastidiosa* control, respectively. Furthermore, leaves were collected from plants in evaluation, and their xylems were visualized by Scanning Electron Microscopy.

Results

The developed methods showed reproducible results and could be successfully applied in the assays. The *in vitro* and *in vivo* results were similar. Naringenin (Mg-complex) and azadirachtin showed the best results in both *in vitro* and *in vivo* assays, in doses of 0.34; 0.8 μ M, and 0.63; 0.42 μ M, respectively. The vessels of the treated xylems with only water were colonized by bacteria; the plants treated with the compounds were clean.

Conclusions

The *in vitro* assays can be applied in previous steps in research works to identify new molecules which are able to control the *X. fastidiosa*. Natural products and derived compounds are an important source of biodegradable pesticides.

1A-3 An Oxidized Monoterpene Library Used to Probe Bark Beetle Chemoreception

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Introduction

Monoterpene hydrocarbons produced by trees play an important role in host recognition by bark beetles. Monoterpenes are toxic at certain concentrations, however bark beetles are capable of detoxifying monoterpenes via oxidative processes. Several well known oxidized terpene semiochemicals were initially isolated from live insects via volatile trapping, or dissection and extraction, both tedious processes. We developed a novel approach to facilitate discovery of new bioactive monoterpenoids.

Material and methods

Eight of the most commonly occurring conifer monoterpenes were used as oxidation substrates to prepare a synthetic library of several hundred oxidized monoterpene products. The library was screened using coupled gas chromatography electro antennogram detection (GC-EAD) with three species of *Dendroctonus* bark beetle pests. Antennally active compounds were bioassayed along naturally infested forest margins using attractive lures and multifunnel traps. Novel repellants were further characterized using a single tree protection assay.

Results

The terpenoid library simulates a wide array of stable metabolic products. Most of the library compounds displayed no antennal activity, but among the group of antenna stimulating compounds there are novel compounds in addition to well known compounds previously unknown as semiochemicals. Field trapping and tree protection bioassays have revealed several new compounds with behavioural activities.

Conclusions

A synthesized library of compounds can be used to map the specificity of bark beetle terpenoid receptors. Moreover, we have identified novel bark beetle inhibitors which are capable of protecting live trees from bark beetle mass attack. We have clearly demonstrated a practical application of our new method.

1A-4 Purification of metabolites from *Duguetia lanceolata* stem barks active for *Spodoptera frugiperda*

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Introduction

Duguetia lanceolata is an Annonaceae native to Brazil, phytochemical studies and an evaluation of the potential use of its metabolites are scarce. In previous studies, we reported a high insecticidal activity of this plant against *Spodoptera frugiperda*. Thus, this work aimed to perform the phytochemical study of *D. lanceolata* in order to isolate the bioactive molecule for *S. frugiperda*, an important pest of maize in Brazil and the Americas.

Material and methods

The fraction soluble in dichloromethane, derived from the methanol extract of *D. lanceolata* stem barks, was incorporated in an artificial diet and offered to *S. frugiperda* caterpillars at different concentrations for the determination of median lethal concentration (LC₅₀) and median lethal time (LT₅₀). This fraction was also subjected to silica gel column chromatography and high performance liquid chromatography (HPLC), in order to isolate the substances. The structural elucidation of molecules was possible through nuclear magnetic resonance experiments (NMR). The isolated compounds were also used in biological assays with *S. frugiperda*.

Results

After 72 h of feeding the insects with the fraction *D. lanceolata* stem barks, LC₅₀ was 294.6 ± 28.46 µg/mL of diet. The concentrations 1818.1, 909.0 and 454.5 µg/mL of diet caused LT₅₀ 23.4, 34.1 and 74.1 h, respectively. Three aporphinic alkaloids and a mixture formed of trans-asarone + 2,4,5-trimethoxystyrene were isolated and tested for *S. frugiperda*. The mixture of trans-asarone + 2,4,5-trimethoxystyrene was active for *S. frugiperda* (LC₅₀ 123.7 ± 7.80 µg/mL of diet). The mixture of trans-asarone + 2,4,5-trimethoxystyrene was subsequently isolated by HPLC. In a bioassay with the isolated substances, both trans-asarone and 2,4,5-trimethoxystyrene, were active. However, 2,4,5-trimethoxystyrene was more active (LT₅₀ 73.4 h) than asarone (LT₅₀ 170 h).

Conclusions

The molecules responsible for the insecticidal activity of *D. lanceolata* against *S. frugiperda* are trans-asarone and 2,4,5-trimethoxystyrene. Furthermore, 2,4,5-trimethoxystyrene is the most active molecule.

1B-1 Foliar volatile profiles of *Persea americana* var. Hass and pest incidence

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Introduction

Patterns of herbivory and pest incidence have been associated to the secondary metabolite profiles present in a plant population, also known as plant chemical phenotypes. These chemical phenotypes can be maintained in a population because some secondary compounds are under genetic control. We explored the relation between foliar chemical profiles in Hass avocado plants (*Persea americana* var. Hass) and herbivore incidence.

Material and methods

Sampling was conducted in trees from two orchards of Hass avocado with different management conditions. The foliar chemical profile was determined by gas chromatography. We also set up a common garden using propagated individuals with contrasting chemical phenotypes, and monitored the incidence of herbivores.

Results

The analysis of foliar volatiles in Hass avocado revealed 36 compounds. Eight chemical phenotypes were found; estragole, eugenol and β -caryophyllene were the major compounds; minor compounds were responsible for the differences among chemical phenotypes (p-cymene, camphene, caryophyllene oxide, limonene, β -myrcene, g-terpineol, β -cis-ocimene, α -farnesene). Incidence of miner worm (*Gracilaria perseae*), whitefly (*Tetraleurodes spp*) and red spider mite (*Olygonychus punicae*) was recorded. Only whitefly incidence was related to the chemical phenotype of Hass avocado. Herbivory showed to be different among chemical phenotypes.

Conclusions

Herbivore damage in *P. americana* var. Hass is associated to its foliar chemical phenotype. In particular, whitefly incidence is related to the chemical phenotype of its host. It is possible that herbivore susceptibility in *P. americana* var Hass has an inverse relation to tree growth and productivity, but further studies are necessary to confirm if such is the case.

1B-2 Flight attraction of *Spodoptera littoralis* (Lepidoptera, Noctuidae) males and females to cotton headspace and synthetic volatile blends

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Introduction

The insect olfactory system is capable of discriminating relevant odor cues against a complex odor background, which elicit innate behaviors. Identification of the stimuli which trigger upwind flight towards host plants or mates is a current challenge and is essential in developing new, sustainable plant protection methods, and for furthering our understanding of plant-insect interactions.

Material and methods

We used a combination of cotton headspace collection, chemical analysis through mass spectrometry coupled gas chromatography, electroantennographic detection and wind tunnel bioassays to find behaviorally active plant compounds and blends.

Results

We here show that both gravid females and virgin males of the Egyptian Cotton Leafworm, *Spodoptera littoralis*, use blends of volatiles compounds to locate one of their preferred host plants, cotton, *Gossypium hirsutum*. Gravid females flew upwind towards headspace at a dose of 1800 ng/h, based of the main compound, 4,8-dimethyl-1,3(E),7-nonatriene (DMNT), whereas males were not. Subsequent assays revealed that a synthetic blend consisting of nonanal, (Z)-3 hexenyl acetate, (E)- β -ocimene, and (R)-(+)-limonene was equally attractive as cotton headspace to females and males. Compounds such as DMNT and (R)-(-)-linalool seem to be antagonists which reduce upwind flight in both females and males.

Conclusions

Our findings provide an excellent platform for further investigations on the neural basis mediating innate behavior, and for the development of novel insects plant protection strategies against *S. littoralis*.

1B-3 Effects of the herbivore-induced maize volatiles in the host location behavior of three sympatrical species *Sesamia nonagrioides*, *Ostrinia nubilalis* and *Rhopalosiphum padi*.

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Introduction

Volatile organic compounds (VOCs) emitted by plants is known to play an important role in host location. However, the interactions between phytophagous insects and their host plants are partially mediated not only by VOCs, but also by other factors, such as the presence of other conspecific and heterospecific herbivores. The Mediterranean corn borer, *Sesamia nonagrioides*, and the European corn borer, *Ostrinia nubilalis*, are polyphagous moths that feed mainly on members of the Gramineae family, while *Rhopalosiphum padi*, is a polyphagous insect, that has recently become one of the most important pests of cereals in some European countries. These three species occur sympatrically in Spain, affecting maize crop yields. The chemical ecology of interactions between these three species was investigated in order to better understand the plant colonization process.

Material and methods

We collected VOCs by air entrainments, emitted from maize plants that were either undamaged or damaged by larvae of *S. nonagrioides*, *O. nubilalis* or adults of *R. padi*.

Repellency of conspecific and heterospecific damaged-VOCs to gravid females of *S. nonagrioides*, *O. nubilalis* and winged adults of *R. padi* were evaluated through behavioral experiments (two and four-arm olfactometer).

Electrophysiological and chemical analysis of the VOCs by GC-EAG and GC-MS was used in order to identify electrophysiologically active compounds.

Results

Damage from the three species resulted in three different volatile profiles, differing both qualitatively and quantitatively.

Behavioral test showed that gravid females of *S. nonagrioides*, *O. nubilalis* and alate *R. padi*, were both repelled or attracted to conspecific damaged-VOCs and/or heterospecific damage-VOCs

GC-EAG and GC-MS is leading to the identification of several electrophysiologically active compounds in the three studied species.

Conclusions

The host location behavior of gravid females of *S. nonagrioides*, *O. nubilalis* and winged adults of *R. padi*, were affected by conspecific damaged-VOCs and/or heterospecific damage-VOCs.

1B-4 Time for Outcrossing

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Introduction

Pollination interactions are vital for the survival of many Angiosperms. This can be mediated by different animals, such the large group involving insects. In other hand plants also developed traits to adjust to a particular pollination syndrome.

This study focus on the hawkmoth *Manduca sexta* and the solanaceous plant *Nicotiana attenuata*, which synchronizes its floral display with the hawkmoth night activity. Since most of its floral traits have a circadian pattern, we aimed on its time regulation and how its disruption affects the outcrossing.

Material and methods

Transgenic lines of *N. attenuata*, silenced on particular circadian clock genes were used to evaluate the association of the floral traits and the clock regulation. Flower movement, aperture, scent emission and nectar volume were measured at time intervals. Glasshouse and field pollination experiments were carried out to assess the outcrossing.

Results

The clock silencing caused several flower phenotypes, such as time shifts, reduced amplitude of traits even close to supression, such as scent emission. Under glasshouse conditions, a strong disruption in the floral traits reduced the pollination outcome, while early shifting was rather advantageous. Instead under field conditions, shifting had no particular effects and the floral trait disruption still caused a poor night pollination. Tested in a day pollination scenario, the trait disrupted flowers had an unexpected higher outcrossing.

Conclusions

Pollination syndromes have evolved in mutual selection between pollinator and flowering plants, while particular traits adapted to synchronize with its effective pollinators. This doesn't imply that a strict pollination interaction will be the best, but only that was selected while still other pollinator combinations exist and can be similarly effective.

2A-1 Isolation and identification of the sex pheromone components of the Argentine population of *Anastrepha fraterculus* (Diptera: Tephritidae)

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Introduction

Chemical communication plays an important role in the reproduction behavior of fruit flies. Mating is initiated by males that aggregate in small groups (leks) on vegetation and release sex pheromones that attract females.

Material, methods, results and conclusions

The volatile compounds emitted by sexually mature males of *Anastrepha fraterculus* population Tucuman have been collected on HayeSep polymer, eluted by hexane and identified using GC×GC-TOFMS. The key components involved in the sexual attraction of virgin female flies to males were pinpointed using GC-EAD and GC×GC-TOFMS analyzes of quantitative age-dependent changes in the content of pheromone components. Quantitative GC×GC-TOFMS analyses showed that in the period from hatching to adulthood the contents of some pheromone substances significantly increase depending on the maturity of the flies. GC-EAD experiments showed several areas of antennal activity. Both quantitative GC×GC-TOFMS analysis and GC-EAD data suggest that the male sex pheromone is rich multicomponent blend.

Acknowledgement

The MŠMT grant 7AMB13AR018 and IAEA grant No 16106 are acknowledged with a pleasure.

2A-2 Cis-jasmone induces a production of repellent compounds in cowpea, *Vigna unguiculata*

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Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae) is an important source of protein. Cowpeas are, however, attacked by a wide range of insect pests which can cause severe crop losses. The black cowpea aphid, *Aphis craccivora* Koch, is an important insect pest. *Cis*-jasmone The *Cis*-jasmone was reported in different studies as an organic compound that can repel insect and can increase the biosynthesis of VOCs and no volatiles secondary metabolites, and this attracted the natural enemies in field or in the laboratory in diverse crops (BIRKETT et al., 2000; BRUCE et al., 2003; BRUCE; PICKETT; SMART, 2003, PICKETT et al., 2005). We study the role of *cis*-jasmone in the activation of defense mechanism of cowpea, *Vigna unguiculata* to be resistant to black aphid, *Aphis craccivora*.

Material and methods

To take ahead this study some steps were conducted collection of VOCs, *Cis*-jasmone application, Collection of VOCs, Four-arm olfactometer bioassay and Gas Chromatography Analysis.

Results

The olfactory response of alate *A. craccivora* in four arm olfactometry wasn't statically significance when the volatiles organic compounds collected from untreated cowpea. However, the aphid spent significantly ($p < 0.05$) less time in arm containing volatiles organic compounds collected from *Cis*-Jasmone treated cowpea. Tentative identifications reveal that after *cis*-jasmone application the cowpea produced six new VOCs.

Conclusions

The *cis*-jasmone induces the production of VOCS in cowpea, and this VOCs were repellent to black aphid, *aphis craccivora*.

2A-3 Identification of absolute configuration of two components of the attractant pheromone of *Neomegalotomus parvus* (Hemiptera: Alydidae)

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Introduction

4-methylhexyl pentanoate and 4-methylhexyl butanoate were identified as components of the attractant pheromone of *Neomegalotomus parvus*, but their absolute configurations have not been established. The aim of this work was to synthesize the *R* and *S* isomers of these compounds in high enantiomeric purity from Kaffir lime essential oil.

Material and methods

Reduction of (*S*)-citronellal, steam distillation from Kaffir lime essential oil, using hydrazine furnished (*R*)-2,6-dimethyloct-2-ene, which was cleaved and converted into (*R*)-4-methylhexanoic acid using potassium dichromate. After that, it was reduced to (*R*)-4-methylhexan-1-ol with LiAlH₄; and finally esterified with butanoic or pentanoic acid chloride to obtaining (*S*)-4-methylhexyl butanoate and (*S*)-4-methylhexyl pentanoate, respectively. Gas Chromatography chiral analysis using a β-DEX™ 325 column and Y olfactometer bioassays using the synthetic compounds and natural extract were conducted to elucidate the absolute configuration of the esters produced by *N. parvus*.

Results

The GC chiral analysis using a β-DEX™ 325 column did not separate with a good resolution the *R* and *S* esters, but separate their respective alcohols. Therefore the natural extract was hydrolysed and the absolute configurations of the esters were obtained indirectly through the alcohols. The analysis showed that the configuration of the both esters produced by *N. parvus* was *S*. Preliminary Y olfactometer bioassays showed that adults of *N. parvus* did not distinguish between *R* and *S* isomers of both esters, however EAG studies and more bioassays are been conducted to confirm this previous result.

Conclusions

The sequence of reactions results in final products with a high enantiomeric purity of both esters of *N. parvus*. Kaffir lime showed to be a good alternative to obtain 100% of (*S*)-citronellal. The preliminary results on Y olfactometer bioassays indicate that the absolute configuration of both esters is not essential information to recognize the pheromone blend.

2B-1 Observations on *Heniartes stali* Wygodzinsky (Hemiptera: Reduviidae: Apiomerini), a resin bug harvesting the trichome secretion from an andean blackberry

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Introduction

Resin bugs collect viscous substances from plants with their forelegs and use them as an aid in prey capture and to protect eggs against predators. Previous observations revealed the occurrence of *Heniartes stali* on *R.cf. adenotrichos*, an andean blackberry with a cover of glandular trichomes. In this study we address the following issues Is *H. stali* and accidental or deliberate visitor on *R. cf. adenotrichos*?; Is *R.cf. adenotrichos* a sticky material source?; How does *H. stali* gather this exudate?

Material and methods

The presence of *H. stali* on *R.cf. adenotrichos* was recorded during a year in Cerro La Bandera, Mérida, Venezuela. Sticky material collected from the legs and eggs of the insect was compared to the trichome secretion of *R. cf. adenotrichos* by thin layer chromatography (TLC). The bugs were exposed to fresh segments of the plant and the gathering and relocation of the gummy secretion on body parts was monitored.

Results

H. stali nymphs and adults were regularly found on *R. cf. adenotrichos*. The sticky material collected from nymphs, females, and eggs showed a similar TLC pattern to that of the trichome secretion. The bugs gathered this material using the forelegs, transferred it to the midlegs, and subsequently transferred and stored it in the hindlegs. Portions of the sticky material on the hindlegs were smeared on the genital area during oviposition. Transference from the hind to the forelegs was also observed.

Conclusions

The sticky exudate of the trichomes appears to be the key element supporting the interaction *H. stali/R.cf. adenotrichos*. Its storage in the hindlegs and its transference to eggs and to the forelegs are novel findings for a *Heniartes* species. Additional research is currently being conducted to reveal the chemistry of the secretion and the advantages of its gathering for *H. stali*.

2B-2 The effect of plant domestication status over tomato herbivore-induced plant volatiles variation

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Introduction

Plant domestication has changed the concentration and composition of secondary metabolites like herbivore-induced plant volatiles (HIPVs). HIPVs can attract insects of the third trophic level, for instance predators and parasitoids, decreasing the negative impact of herbivores; therefore HIPVs are considered as an adaptive plastic response to damage. However, plant domestication status may be a factor that influence among-individual variation of HIPVs, thus modifying the chemical information emitted, possibly influencing the response of insects of the third trophic level. In this work we tested the hypothesis that plant domestication status is associated to HIPVs variation.

Material and methods

We used cultivated tomato (*Solanum lycopersicum* L.) and wild tomato (*S. lycopersicum* var. *cerasiforme*) accessions and nymphs of the psyllid *Bactericera cockerelli* (Hemiptera Psyllidae) as a model. Variation was studied using the variables fold-change in concentration and among-individual variation. Fold-change is a ratio calculated from the volatile emission of the same individual before and after damage. Among-individual variation was calculated from the values for absolute and relative volatile concentration (size and shape analysis) which indicates how similar the volatile emission among individuals is. We also explored the response to *B. cockerelli*-induced volatiles of females of its principal parasitoid, *Tamarixia triozae* (Hymenoptera Eulophidae) using two-way olfactometry.

Results

We found that cultivated tomato plants were more rich, diverse and variable in their volatile emission than their wild relatives. *B. cockerelli* nymphs induce high among-individual variation compared to that one induced by chewing-feeding insects or mechanical damage (*i. e.*, herbivore-specific among-individual variation). Finally, we found that females of *T. triozae* did not respond to *B. cockerelli*-induced-volatiles.

Conclusions

It is possible that, the among-individual variation induced by nymphs constrain the behavioral response of *T. triozae* to tomato induced-volatiles.

2B-3 Implications of the chemical and genetic variation of *Mikania micrantha* on its specialist herbivore *Stolas punicea*

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Introduction

The secondary metabolite content and genetic composition of plant populations have been frequently associated with the preference or performance of its herbivores. The study of chemical and genetic variation in native populations of invasive species can reveal geographic patterns relevant for understanding their relation with natural enemies.

We aimed to determine the preference of *Stolas punicea* (Coleoptera), a specialist herbivore, over *Mikania micrantha* (Asteraceae) plants from chemically and genetically different mosaics.

Material and methods

We determined the volatile terpenoid variation in 14 Mexican populations of *M. micrantha* from the Pacific and Atlantic tropical watersheds using GC-MS. The neutral genetic variation of 13 Mexican populations was assessed using six specific microsatellites.

We used cafeteria tests to assess the preference of the herbivore beetle *S. punicea* from two provenances (Michoacán and Veracruz) over plants grown from seeds of four distinct populations.

Results

No correlation was found between chemical differentiation and geographical distance, but Monmonier's algorithm identified four geographic barriers separating five chemically distinct mosaics.

A consistent genetic differentiation between Atlantic and Pacific plant populations was supported with a cluster analysis, a principal coordinates analysis, and a Bayesian analysis.

Beetles from Veracruz showed no preferences and fed equally on all the offered plants; however, those from Michoacán consumed completely the plants from Michoacán and avoided those from Tabasco.

Conclusions

The geographic origin of *S. punicea* plays an important role on host acceptance, and while chemical mosaics may not be relevant for some insect populations, others are not able to accept certain chemical phenotypes.

The genetic differentiation between Atlantic and Pacific plant populations suggests that different biogeographic processes have operated in the two regions, and could explain the apparent local adaptation of Michoacán beetles.

Our results underline the importance of testing potential control agents against the chemical and genetic variation of its host.

2B-4 Geographic structure of chemical variation in wild populations of *Jatropha curcas* L in Mexico

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Introduction

Jatropha curcas L (Euphorbiaceae) is a shrub that contains chemicals known to cause adverse effects to herbivores, but it is unknown whether the phytochemical variation is geographically structured and if in turn it is associated with the intensity of herbivory. This understanding would allow us to select individuals with appropriate chemical variation to decrease herbivory in *J. curcas* plantations in specific regions.

Material and methods

We sampled mature leaves from eight populations of *Jatropha curcas* of Michoacan, Veracruz and Chiapas to quantify the total content of saponins, trypsin inhibitors, phytic acid and phenols by spectrophotometry. To quantify herbivory 40 mature leaves were collected at random from each population were digitized. The leaf area consumed was used as an index of herbivory and was quantified using the Assess 2002 software.

Results

Cluster analysis, considering the type and total content of chemical compounds, grouped individuals of Michoacán and Veracruz with individuals of their own population, but the Chiapas individuals were scattered mainly within clusters with individuals from several Chiapanec populations. Principal component analysis showed that the content of phenols, saponins and trypsin inhibitors mainly determine the grouping of individuals. A multiple linear regression model considering the total content of chemical compounds, altitude and precipitation explains the intensity of herbivory (adjusted multiple $R^2 = 0.62$).

Conclusions

Chemical variation is structured at a regional and populational level for Michoacán and Veracruz individuals and at a regional, but not populational, for Chiapas individuals. Intensity of herbivory is associated with the total content of chemical compounds and the precipitation and altitude of the site of origin of populations. Thus herbivory and chemical composition are associated with geography.

3A-1 Monitoring *Hypothenemus hampei* population using alcohol baited traps as an early alert system to coffee farmers in Colombia

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Introduction

Coffee Berry Borer (CBB), *Hypothenemus hampei*, is a key pest in Colombia. It has been proved that a mix of methanol and ethanol acts as attractant to CBB adult flying females in field conditions. The extent of this finding has been limited to monitor flying populations as indicator for coffee berry protection in an IPM scheme; however, using these traps as a control strategy has been proposed.

Material and methods

Research was carried out in order to test (1) color, (2) height location in the tree, and (3) artisanal hand-made alcohol baited traps on the efficacy to capture adult flying CBB individuals. Furthermore, CBB infestation levels in the field were recorded during 12 months in a coffee plot containing 40 alcohol baited traps, which were compared to a control coffee crop. Moreover, we have placed 20 traps in several Colombian coffee Experimental Stations since 2002, recording weekly the total number of adults captured, as a monitoring strategy to early alert coffee farmers for IPM actions.

Results

(1) the color of the alcohol baited traps (transparent, red or white) to capture CBB adults in the field did not affect the number of females collected. (2) The recommended height to place the trap devices is 0.4 m above the ground, capturing twice the number of CBB individuals, compared to 1.5 m. (3) Artisanal hand-made alcohol baited traps captured similar number of CBB females in the field. These traps should not be recommended as a control tool, since their use did not help to decrease the infestation levels in the field.

Conclusions

Alcohol baited traps to capture CBB is currently been used successfully to monitor insect populations in Colombia, as an early alert system to recommend IPM strategies before surpassing infestation threshold levels.

3A-2 Impact of *S. commersoni* in the incidence of whitefly on tomato crop

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Introduction

Intercropping, the practice in which two or more crops grows in proximity, can offer advantages in terms of pest control.

Many insects, being the whitefly *Trialeurodes vaporariorum* one of the most important in our country, affect tomato crop.

Previous observations showed that plants of *Solanum commersoni* (Solanaceae), a wild type of potato, exhibit lower whitefly infestation rates than other genetically modified potato plants. This effect could be due to repellent volatiles emitted by *S. commersoni*.

The aim of this work was to test the hypothesis that *S. commersoni* could decrease whitefly populations in tomato plants when both plants are reared together.

Material and methods

Whitefly infestation rates were assessed on plants reared under different practices. Two experimental greenhouses were settled with tomato plants. In one of them plants were intercalated with *S. commersoni*; the other served as the control. After infestation with adults, the number of adults and nymphs was recorded weekly during 8 weeks (N=10 for each plant type). Results were compared by repeated measurements ANOVA tests.

Results

S. commersoni plants had fewer adults and nymphs (15 ± 9^a ; 11 ± 9^b respectively) than tomato plants intercropped with them (70 ± 13^a ; 100 ± 0^c) and than control tomato plants (71 ± 21^a ; 100 ± 0^c). Both kind of tomato plants (*S. commersoni*-intercropped and the control ones) exhibited no differences in their populations of adults or nymphs. ^aTotal number of adults, ^btotal number of nymphs, ^cnymphs from the bottom of the plant. Values given at final time.

Conclusions

Our study confirmed previous observations on *S. commersoni* plants having fewer whiteflies than other neighbor plants. If this observation is due to volatiles emitted from the plant, those compounds -when naturally produced- must have only a local effect, as tomato plants were not benefited by a decrease in whitefly incidence when they were grew next to *S. commersoni* plants.

3A-3 The effect of plant volatiles from sunflower, maize and pigeon pea with herbivory damaged made by stink bug *Euschistos heros* on the foraging behavior of the egg parasitoid *Telenomus podisi*

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Introduction

Plants that suffer damage caused by herbivory, release different kinds of volatiles that attract parasitoids (HIPVS). The egg parasitoid *Telenomus podisi* (Hymenoptera: Platygasteridae) is attracted by soybean HIPVS releases after the injured by the brown stink bug, *Euschistus heros* (Pentatomidae) and can discriminate between HIPVs elicited by this stink bug or the green stink bug, *Nezara viridula*. This work is aimed at the study of HIPVs induced by *E. heros* in different plant species as sunflower, pigeon pea and maize and the evaluation of the response of *T. podisi*.

Material and methods

Plants of each species were treated with three virgin females of *E. heros* leaving for 72 hours to herbivory (HIP - herbivory injured plants). Control plants (CP) were maintained without insects for the same period. To test the effect of HIPVs of each plants olfactometer bioassays were performed. The following combinations for the bioassays were attempted: CP x air, HIP x air, HIP x CP. In addition the volatile profile was identified using gas chromatography coupled with mass spectrometer.

Results

There were no differences on the parasitoid *T. podisi* responses to the combination of CP x air in the three species of plant tested, volatiles of HIP maize plants were attractive to the parasitoid females in relation to air, but volatiles of pigeon pea or sunflower HIP do not attract the parasitoid. When volatiles of HIP plants were contrasted with volatiles of CP plants the parasitoid had most initial choice for odor of maize and sunflower HIP plants. The chemical profile of these plants is being identified to show if there are differences between the released compounds.

Conclusions

Sunflower and maize plants when suffer the *E. heros* herbivory damage can release volatiles that attract the parasitoid *T. podisi* similarly to the observed previously for soybean plants.

3A-4 Are the wild and mass-reared fruit fly strains differentiable by their sexual behavior and male volatile compositions? The case of the Mexican fruit fly

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Introduction

Fruit flies are among the most important insect pests worldwide because of their direct economic impact and quarantine restrictions imposed by many countries. The Sterile Insect Technique (SIT) is a species-specific, non-polluting method of insect control. For SIT, insects are mass-reared, sterilized, and released into the field where males compete against wild males for matings with wild females. By the fact of continuous mass-rearing procedures, male fitness may result in a reduction of the field competitiveness. We have studied different alternatives for improvement of colony management systems.

Materials and methods

Sexual behavior and male volatile compounds related to sex pheromone were characterized through different chemical characterization techniques and bioassays from wild populations and four mass-reared strains of *Anastrepha ludens* (Loew) (Diptera: Tephritidae): 1) a standard mass-reared colony (control), 2) a genetic sexing strain (Tap-7), 3) a colony of males selected by desirable biological traits (selected), and 4) a colony started by crossing wild males with control females (hybrid).

Results

Higher male calling behavior from the mass-reared strains was observed than from wild males under laboratory and field cages. Calling behavior was almost a two-fold increase in wild males during the last half hour of observations. Selected and wild males achieved more successful matings with wild females than the other males in field cages. We found that mass-reared strains emitted significantly more amounts of (*E,E*)- α -farnesene, whereas wild males emitted significantly more amounts of (*E,E*)-suspensolide as they aged. By statistical analyses we inferred that male volatile changes are age-dependent.

Conclusions

We suggest that the differential behavior of the strains may be due to the environmental pressures and biological traits they have, which might be considered

as an indication of male quality. Characterization of these factors may be useful for optimizing the sterile insect technique in *A. ludens*.

3B-1 A symbiotic strategy for chemical egg defense in insects the *Lagriid-Burkholderia* case

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Introduction

As revealed by decades of research on chemical ecology, the biology of many insects relies strongly on chemical communication. While numerous descriptions of plant- and insect- derived chemical cues are available, there is a growing body of evidence showing that microbe-mediated chemical interactions can play a decisive role in the ecology of insects. Tenebrionid beetles within the Lagriinae subfamily live in association with *Burkholderia* (β -proteobacteria), a genus which exhibits extraordinary metabolic and ecological versatility including a number of plant-pathogenic traits. The potential role(s) of each partner in the lagriid-*Burkholderia* symbiosis were, however, not known until now.

Material and methods

In order to assess for potential functions of the bacteria, symbiont-free *Lagriid villosa* beetles were generated via egg-surface sterilization and after one generation the eggs were used for *in vivo* assays testing fungal inhibition. Additional *in vitro* tests were carried out against three different fungal species. Finally, the possibility of an insect-mediated infection of the food plant with *Burkholderia* bacteria was tested by exposing soybean plants to symbiotic beetles and quantifying *Burkholderia* in the leaves via qPCR.

Results

We could show that the presence of *Burkholderia gladioli* on the surface of *L.villosa* eggs significantly inhibits fungal growth as compared to symbiont-free eggs, suggesting a defensive function. Furthermore, *in vitro* bioassays confirmed growth inhibition caused by the symbiotic *B.gladioli*. First experiments also demonstrate the plausibility of host-mediated transmission of *Burkholderia* to soybean plants.

Conclusions

This egg defense strategy may reveal an unexplored component of symbiotic functions, in which metabolites produced by associated bacteria result in effective chemical protection at the nutrient-rich and immobile egg stage of insects. From the microbe's perspective, the advantage of dispersal to plant hosts via the insect might be a major factor promoting the symbiotic association.

3B-2 Identification by LC/ESI/MS/MS and LC/DAD of flavonol and isoflavonoid glycosides modulated by solar UV-B radiation and herbivory in soybean leaves at field: effects on *Anticarsia gemmatalis* larvae

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Introduction

Solar ultraviolet B (UV-B) radiation has been largely reported to enhance plant defenses against insects; however it is unclear what traits define insect resistance in field crop systems, such as soybean. Here we study the effects of solar UV-B radiation on: a) the modulation of phenolic compounds and trypsin inhibitors (TPI) in soybean leaves, and b) survival and mass gain of *Anticarsia gemmatalis* larvae in field conditions.

Material and methods

Cultivar (cv.) Williams and cv. Charata were planted in field conditions and two treatments were generated with plastic filters: ambient UV-B and attenuated UV-B. Survival of *A. gemmatalis* neonates was recorded daily and at day six weighted. Flavonoids glycosides from soybean leaves were identified by HPLC-DAD and HPLC-ESI(+)-Q-TOF.

Results

Exclusion of UV-B radiation increased mass gain and survivorship of larvae that fed on cv. Charata and no differences were found in cv. Williams. Although TPI activity, genistin, malonyl genistin and a benzoic acid derivate were induced by *A. gemmatalis* damage in both cultivars, induction of this compounds (except for the benzoic acid derivate) were higher in cv. Williams leaves grown under solar UV-B radiation. TPI activity and genistein derivatives did not explain the differences found in survivorship and mass gain of larvae that fed on cv. Charata under the two UV-B treatments. However, after identification and quantification of flavonols present in soybean leaves (tri and diglycosides of quercetin, isorhamnetin and kaempferol), we found an association between modulation of plant resistance against herbivory and two quercetin triglycosides, which are only present in cv.Charata.

Conclusions

We found that modulation of two quercetin triglycosides were more important in defining larvae performance than the modulation of other typical defenses such as TPI activity and genistin. We also identified a benzoic derivate which was induced by herbivory independently of UV-B radiation.

3B-3 Soil microbes influence the above-ground interactions of soybean plants with insects and pathogens

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Introduction

Soil-borne microorganisms can have significant effects on aboveground interactions between plants and other organisms, including pathogens and insect herbivores. For example, non-pathogenic soil microbes can stimulate Induced Systemic Resistance (ISR) by altering leaf metabolites and volatile emissions that enhance plant defense capabilities.

Material and methods

In a multi-factorial experiment, we characterized the emission of volatile organic compounds (VOC) by soybean plants in the presence/absence of two species of beneficial rhizobacteria and with or without infection by *Bean pod mottle virus* (BPMV) and herbivory by a beetle (*Epilachna varivestis*) that vectors this pathogen. In a separate experiment, we assessed the attraction of *Pediobius foveolatus*, a parasitic wasp that attacks *E. varivestis*, to soybean plants given similar treatments.

Results

We found that specific combinations of rhizobacteria enhanced herbivore-induced VOC emissions, partially compensating for the observed suppression of VOC emissions by BPMV. This pattern was reflected in our behavioral assays, where parasitoid attraction was reduced by BPMV infection but partially restored in the presence of some rhizobacteria combinations. Furthermore, parasitoids were most attracted to plants whose rhizobia combinations provided the greatest enhancement of herbivore-induced VOC emissions.

Using a multivariate analysis approach, we selected a list of the most important compounds driving the differences between one treatment and the others; these compounds can be considered as a volatile signature for each treatment and are likely to be causing the differential response of the wasps.

Conclusions

Our findings indicate that both virus infection and root colonization by rhizobacteria can alter the concentration and composition of VOC emissions in soybean, with potential implications for herbivore-natural enemy interactions and the spread of BPMV by *E. varivestis*.

3B-4 Effect of phenology on the volatile blends emitted by Uruguayan native host plants of tephritid fruit flies (Diptera: Tephritidae)

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Introduction

Developing alternative management strategies for controlling *Anastrepha fraterculus* (South-American fruit-fly) requires an understanding of the bioecology of tephritid flies and their host plants. Kairomonal chemical cues emitted by the ripen fruits may be important mediators of host finding. We present a comparative study of volatile profiles of immature and ripe fruits of *Acca sellowiana* and *Psidium cattleianum* (Myrtaceae), two native host plants of *A. fraterculus* in Uruguay, in search for possible chemical cues for ovipositing females.

Material and methods

Ripe and unripe fruits were collected from *A. sellowiana* and *P. cattleianum* trees in three localities from southern Uruguay. Fruits were immediately taken to the laboratory; they were counted, weighed, and VOCs were obtained during 24 h by air entrainment using Haysep-Q as adsorbent. Sampled volatiles were eluted with hexane (1 mL), concentrated under N₂ (100 mL) and analyzed by GC-MS.

Results

The main VOCs from unripe fruits of *A. sellowiana* and *P. cattleianum* were monoterpenes and sesquiterpenes. While ripe fruits also emitted these compounds, the main VOCs were ethyl esters of saturated and unsaturated short-chain aliphatic acids (*P. cattleianum*), or a mixture of aliphatic and aromatic ethyl esters (*A. sellowiana*). There were no differences in fruit VOC profiles from the same species in different localities.

Conclusions

Unripe and ripe fruits of native hosts of *A. fraterculus* showed clear quantitative and qualitative differences in their volatile profiles. These may be used by the fruit flies as kairomones, not only for host location, but also for determining host phenology. These may be relevant in the case of *A. sellowiana*, since no observable changes in color correlate with ripening. The constancy of VOC profiles among regions suggests that VOCs may be a reliable signal for the insect. Our results are discussed in terms of practical applications such as monitoring traps and variety resistance.

4A-1 Expression analysis of transcripts potentially related to olfaction by *Telenomus podisi* (Hymenoptera: Platygasteridae) and stimulated by sexual pheromonal compounds of its main hosts (Hemiptera: Pentatomidae)

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Introduction

A group of proteins related to the perception of odor molecules has been studied to help understand the behavior of insects (i.e. Odorant Binding Proteins – OBPs). The egg parasitoid *Telenomus podisi* has received significant attention as a biological control agent for the pentatomid stinkbug-soy complex (i.e. *Euschistus heros*, *Piezodorus guildinii* and *Nezara viridula*). The present work aimed to identify putative OBPs through transcriptome analysis of *T. podisi* and to evaluate the expression profile of the identified transcripts when wasps were exposed to different olfactory experiences.

Material and methods

The whole body of males and females (20 days-old) were used to sequence the transcriptome by Illumina GAIIX (1 lane, 100 pb x2). After quality control, the reads were assembled (Velvet and Oases) and the contigs annotated by molecular function (Blast2GO). Similarity analyses of the transcriptome and of the full-length putative OBPs (TpodOBPs) were conducted using BLASTx. The expression analysis of the mined, full-length putative OBPs was made by RT-qPCR using SYBR Green, comparing five different treatments. The treatments consisted of the sexual pheromone, or part of it, of their main hosts, and two controls (with and without n-hexane).

Results

Seven OBPs, 5 CSPs and 7 ORs were mined, in which, 3 of those OBPs were considered full-length. From these, similarity analysis showed that they were more similar to OBPs from their hosts (TpodOBP1 x EherOBP2 88.5% and TpodOBP2 x EherOBP1 82.4%) than to OBPs from any Hymenoptera (<35%). The sexual pheromone compounds did not alter the expression of the putative TpodOBPs.

Conclusions

Three putative OBPs were identified in *T. podisi* and they had low deduced amino acid similarity between them. Two of these OBPs (TpodOBP1 and TpodOBP2) had high similarity with OBPs from its preferred host (EherOBP1 and EherOBP2), suggesting that the parasitoid and host OBPs may be detecting similar olfactory compounds.

4A-2 Working model (combined strategy) to measure the potential of inhibitors in marine fouling process

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Introduction

In previous studies, sixteen cembranoids isolated and identified from the octocorals *Pseudoplexaura flagellosa* and *Eunicea knighti*, showed high antifouling activity established by quorum sensing inhibition test using *Chromobacterium violaceum*, as well as bacterial biofilm inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. According to the results, the cembranoids were important antifouling agents and based on the previous results we chose one of the most active cembranoid to evaluate its potential in a field experiment in natural conditions (the sea) as additive in an industrial coating.

Material and methods

White ceramic panels (12 cm × 12 cm × 5 mm) used as surface were coated with the test paints, a cembranoid-based paint (2.0% and 0.5%), a copper-based paint (30% of Cu₂O), and a kojic acid-based paint (0.5% of kojic acid). Field experiments were conducted at Rosario Islands, Caribbean coast of Colombia, between 25 April and 9 July of 2012.

Results

The results showed that the test panels treated with the cembranoid completely prevented settlement of macrofouling during the tested period, further, the panels were slightly fouled with microalgae but remained most free of fouling and showed a good antifouling performance after 75 days of exposure. The copper-based paint was cover with microfouling during the test period, otherwise showed good antifouling activity against macroorganisms and the panel control was completely fouled mainly by algae. Finally, barnacles and bivalves were observed alongside the pier wall, but were not found on the test panels.

Conclusions

The cembranoid completely prevented settlement of macrofouling and inhibited most of the microfouling during the period of testing. The above support the use of this cembranoids-type as antifoulant agents in a commercial paint, but due its structural complexity, the synthesis of more simple compounds and QSAR should be the next step in the search for potential antifoulant compounds.

4A-3 New compound identified as pheromone component of *Conotrachelus humeropictus* Fiedler (Coleoptera: Curculionidae)

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Introduction

The weevil *Conotrachelus humeropictus* is an important insect of cupuaçu (*Theobroma grandiflorum*) and cocoa (*T. cacao*) culture in Brazil. Damages are caused when larvae feeds decreasing yield and fruit quality. Currently, growers rely on chemical control to the pest. A sustainable pest management strategy is mating disruption using synthetic pheromones. The aim of this study is to identify the sex-pheromone compounds of *C. humeropictus*.

Material and methods

The insects were collected in Rondônia (Brazil) in June 2013. In our laboratory, they were separate by sex and provided with freshly sugar cane stems. Volatiles were collected daily in HayeSep polymer from groups of 6 males our 6 females, and then eluted with hexane. Diel periodicity of release was observed during 4 successive days. GC-MS analyses were carried out to identify and quantified the sex specific compounds. C14 was used as internal standard.

Results

GC-MS analyses reveled four males specific compounds: grandisol, grandisoic acid, an unidentified compound and a minor compound derived from the cyclization of grandisoic acid: 2,2,6-trimethyl-3-oxa-bicyclo[4.2.0]octan-4-one. Natural extracts were co-injected with authentic standards to confirmed the identities of the compounds as enantiopure 1R, 2S for both grandisol and grandisoic acid. The grasidoic acid was the major compound released mainly during the scotophase (76%). Amounts ranging from 179 to 55 ng per insect were quantified during the whole day.

Conclusions

Four male-specific compounds were released by *C. humeropictus*. From these, Grandisol and Grandisoic acid have been described as components from the aggregation pheromone of Curculionidae species in other studies. Behavioral studies are on the way to confirm the activity of these compounds in these species.

4A-4 Extraction, identification and behavioral activity of a male-produced aggregation pheromone in the Lesser Mealworm *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) in Brazil

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Introduction

The lesser mealworm, is an important pest of poultry industry. The presence of this insect can damage the insulation systems in poultry houses, negatively influence the indices of chicken growth performance and they also may transmit poultry diseases. The indiscriminate use of insecticides on poultry litter and the lack of a suitable insecticide application technology make this an inappropriate control. Therefore, it is necessary to develop new tools to control this insect in poultry houses and minimize the insecticide applications. The objectives of this study were to evaluate if the aggregation pheromone of a Brazilian population of *A. diaperinus* was the same of that identified for an American population and whether this aggregation pheromone has potential to be used to manage this insect.

Material and methods

Air-entrainment extracts were obtained from a Brazilian population (males and females) to identify the aggregation pheromone and "Y" tube olfactometer bioassay were conducted using the air-entrainment extracts and also solutions with the synthetic compounds to evaluate the influence of these compounds on the behavior of *A. diaperinus*. The extracts were analysed by GC-FID and GC-MS.

Results

Six male-specific compounds were identified (quantity/insect): (*R*)-limonene (49.0 ± 10.4 ng), (*E*)- β -ocimene (31.3 ± 6.9 ng), 2-nonanone (7.0 ± 1.4 ng), (*S*)-linalool (50.0 ± 12.9 ng), (*R*)-daucene (18.4 ± 1.2 ng), all described before in American population and a sesquiterpene (44.5 ± 10.8 ng), as new male specific compound in Brazilian population of *A. diaperinus*. The male extract and the total mixture of synthetic compounds were attractive to both sexes of the lesser mealworm.

Conclusions

A new component in the aggregation pheromone of Brazilian population of *A. diaperinus* was identified and the bioassay results suggest that it is essential to attract males and females. Olfactometer bioassays are being conducted with different mixtures of the pheromonal components.

4B-1 Phenalenone-type phytoalexins mediate resistance of banana plants (*Musa* spp.) to the burrowing nematode *Radopholus similis*

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Introduction

Bananas and plantains (*Musa* spp.) are among the world's most important crops and are a major staple food for more than 400 million people in the tropics. Banana yields are severely hampered by fungi, insects, and plant-parasitic nematodes. The burrowing nematode, *Radopholus similis*, is causing crop yield losses and extensive root lesions. Phenylphenalenones such as anigorufone are phytoalexins produced by *Musa* plants in response to nematode infestation.

Material and methods

The *Musa* cultivars Yangambi km5 and Grande Nain were inoculated with *R. similis*. Phenylphenalenones were identified from lesions of root tissues by HPLC, MS, and NMR spectroscopy. Laser desorption ionization-mass spectrometric imaging was used to localize phenylphenalenones in the root lesions and in the nematodes. In vitro bioassays determined the effect of anigorufone on the motility of the nematodes.

Results

Both the resistant “Yangambi km5” and the susceptible “Grande Naine” banana variety accumulate phenylphenalenones in infected regions of their roots, but not in healthy tissues. The concentration, however, was much higher in the immediate vicinity of root lesions of resistant bananas in comparison to infected root tissues of the nematode-susceptible banana plants. Thus, resistance is due to the high local concentration of phenylphenalenones in specific regions of the roots. While feeding on the roots or phenylphenalenone-enriched diet, nematodes ingested such compounds. Imaging techniques visualized anigorufone in lipid droplets within the body of the nematode. The lipid droplets extend, converge and finally kill the nematode, likely by displacing its inner organs and causing an eventual metabolic dysfunction.

Conclusions

Phenylphenalenones are natural products from *Musa* plants with potential application in nematicide control. Metabolic engineering to enhance the cellular concentration of inducible phenylphenalenones could be an appealing way to develop resistant banana cultivars.

4B-2 A Multi-species Bait for Chagas Disease Vectors

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Introduction

Triatomine bugs are the insect vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. These insects are known to aggregate inside shelters during daylight hours and it has been demonstrated that within shelters, the aggregation is induced by volatiles emitted from bug feces. These signals promote inter-species aggregation among most species studied, but the chemical composition is unknown.

Material and methods

In the present work, feces from larvae of *Triatoma infestans*, *Panstrongylus megistus* and *Triatoma brasiliensis* were obtained and volatile compounds were identified by solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). These substances were tested for attractivity and ability to recruit insects into shelters.

Results

We identified five compounds, all present in feces of *T. infestans*, *P. megistus* and *T. brasiliensis*. Behaviorally active doses of the five substances were obtained for all three triatomine species. The bugs were significantly attracted to shelters baited with blends of 160 ng or 1.6 µg of each substance.

Conclusions

Common compounds were found in the feces of vectors of Chagas disease that actively recruited insects into shelters, which suggests that this blend of compounds could be used for the development of baits for early detection of reinfestation with triatomine bugs.

4B-3 Linking herbivore feeding preference and performance on two *Solanum* host plants

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Introduction

Preference-performance studies focus on female oviposition preference and offspring performance. However, feeding preference and herbivore performance is also relevant for understanding insect-plant interactions. We correlated feeding preference and performance of herbivores of *Solanum* plants. Two generalist phloem-sucking herbivores (*Myzus persicae* and *Macrosiphum euphorbiae*, Hemiptera: Aphididae) and one specialist chewing herbivore (*Tequus schrottkyi*, Hymenoptera: Pergidae) were studied on two related host plants, the cultivated potato, *Solanum tuberosum*, and the wild congener *S. commersonii* (Solanaceae).

Material and methods

Feeding preference was studied in two-choice bioassays in petri dishes containing leaf discs of each plant species. We recorded the percentage of settled aphids on each leaf disk after 24h, and the percentage of leaf disk consumption (hourly for 3h) by *T. schrottkyi* larvae. Aphid performance was evaluated recording aphid survival on individual plants (10 adults/plant, N=10 plants/species). *T. schrottkyi* performance was assessed as larval weight, development time and adult emergence (one 1st-instar larva/plant, N=10 plants/species).

Results

M. persicae preferred to settle in the cultivated potato ($P < 0.01$), and survived better in the preferred host ($P < 0.001$). Meanwhile, *M. euphorbiae* did not show preference in choice bioassays ($P > 0.05$), and performed equally well in both plants ($P > 0.05$). The specialist *T. schrottkyi* preferred to feed on the wild species ($P < 0.001$), and showed a slight, non-significant difference in performance coincident with the preferred *Solanum* plant ($P = 0.07$).

Conclusions

Our results show that preference and performance clearly correlate in generalist herbivores (aphids), since they often select host plants to avoid plant toxins that decrease their performance. Such correlation in specialist herbivores (*T. schrottkyi*) may not be as tight, since they often select their preferred host plant cueing on secondary metabolites. The absence of these chemical cues, however, may not affect the nutritional quality of an alternative host. However, in the case of sequestering specialists, overall performance may be affected by the lack of defensive chemicals.

4B-4 Chemical phenotypes of *Persea americana* cv. Hass concur with differential incidence of the avocado branch weevil (*Copturus aguacatae*)

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Introduction

The Hass avocado (*Persea americana*) is a tree rich in volatile compounds with biological activity against insects as terpenoids, phenylpropanoids and acetogenins. The chemical profiles obtained with those volatiles (chemical phenotypes) have been used to distinguish avocado races and hybrids. In spite of the clonal propagation of Hass avocado and the genetic control of the chemical profiles, there are numerous chemical phenotypes which could be related to differential pest susceptibility.

In orchards, we observed differential incidence of avocado branch weevil (*Copturus aguacatae*), a major pest of avocado Hass. While some trees were severely infested, some neighboring trees were non-infested. We hypothesized that the differential susceptibility to avocado branch weevil is related to chemical profile.

Material and methods

We collected leaves and branches from 110 trees (54 infested and 56 non-infested) in six orchards in Michoacán, México. We obtained the chemical profiles of leaves and branches by GC-MS that were analyzed by multivariate statistical methods.

Results

We identified six clusters (or chemical phenotypes) of avocado trees with different incidence of branch weevil. Two clusters are composed of no-infested trees (resistant), two clusters have both no-infested and infested trees (middle susceptibility), and two clusters conformed mainly by infested trees (susceptible). A flexible discriminant analysis show that these phenotypes differ in concentration of some terpenoids as α - & β -pinene, β -phellandrene, o-cimene, β -trans-ocimene, α -caryophyllene, τ -elemene, cadinene, isolekene, α - & β -cubebene.

Conclusions

The differences in incidence of branch weevil are associated to different combinations of mono- and sesquiterpenes. Thus the chemical phenotype could be used as a tool for the identification of resistant trees because volatile chemical profiles are expressed constitutively. Further experiments will determine the nature (causal or correlative) of these associations of the branch weevil and the Hass avocado tree.

POSTERS
Abstracts



P-1 Ecological interactions between *Alternanthera brasiliana* and its endophytes Detection of Beauvericins

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Introduction

Alternanthera brasiliana is a Brazilian medicinal plant, widely used to treat infection and fever. It is understudied regarding its active metabolites and its association with endophytes.

During the present work we used bioguided assays in order to identify active compounds produced by endophytes isolated from *A. brasiliana*.

A strain of endophytic fungus produced beauvericins, which are typical fungal antibiotics and insecticides compounds. Such molecules were also detected in alcoholic and aqueous plant extracts suggesting that plant-microbe interactions have an influence in the presence of bioactive compounds in *A. brasiliana*.

Material and methods

Endophytes were isolated from stem and leaves according Petrini et. al. methodology. Five fungi strains were cultured in sterile rice. Extracts and fractions containing fungal metabolites were tested against five pathogenic bacteria (*E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *M. luteus*), and analysed by HPLC-MS in order to identify the active compounds. Plant extracts were also analysed by HPLC-MS to investigate whether the fungal metabolites were present in plants tissues.

Results

Bioassay-guided fractionation resulted in the identification of beauvericin A as major compound from *Fusarium* sp extract, along with four other minor beauvericins (beauvericin D, E, F), and a new cyclodepspeptide similar to beauvericin A, which were detected by HPLC-MS. Moreover, beauvericin A was also detected in *A. brasiliana* ethanolic extracts and water infusion.

Conclusions

Bioassay-guided fractionation is an efficient procedure to detect active compounds in complex matrices. In the present work this method provided identification of 5 beauvericins, being one of them reported for the first time. Moreover, these compounds seem to be involved in an ecological interaction between *A. brasiliana* and its endophytes.

In this way, these results arise two important considerations about interactions between *A. brasiliana* and its endophytes a) what is the role of beauvericins in plant-microorganisms interactions and b) what is the contribution of these compounds for *A. brasiliana* antibiotic properties.

P-2 Preliminar identification of potential sexual pheromone of *Copitarsia* spp. in ornamental crops by GC/MS analyses

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Introduction

In Colombia, moths of the genus *Copitarsia* (Lepidoptera Noctuidae) are pest of ornamental flowers. The leafworm causes serious economic losses to farmers due to the interception and subsequent destruction of flowers shipment at the detection sites. Unlike the chemical control, the integrated pest management includes the use of semiochemicals particularly sex pheromones, which have become an important strategy to complement the crop management by the prior identification and subsequent synthesis of the pheromone compounds. The aim of this study was to identify the chemotype of volatile components stored in the sexual gland of the most abundant females of *Copitarsia* spp. in Bogotá plateau, as part of a pest management strategy.

Material and methods

Eggs and larvae were collected on *Alstroemeria* and *Aster* commercial crops from eight municipalities of Bogota plateau. The specimens were reared on plastic cages under laboratory conditions at $19,72 \pm 0,45$ °C, $58,41 \pm 5,63$ % RH and photoperiod of 12:12 (LD). The pupae were sexed and separated by gender until adult emergence. Glands (25 to 30) were obtained from female adults by applying a little pressure on the tip of the abdomen. The female sex glands volatiles were extracted with hexane (500µl) and then analyzed by means of gas chromatography coupled to mass spectrometry.

Results

The compounds obtained have the typical structure of the female pheromones present on the Noctuidea superfamily. These pheromones have primary alcohols and their derivatives (mainly acetates and aldehydes), with a long straight chain (C10-C18). These compounds were found in lower abundance and they were consistent with the chemicals reported in the sex pheromone of *Copitarsia corruda* and *Copitarsia decolora*.

Conclusions

The compounds found in the study showed the typical pattern of chemicals, which are reported in the sex pheromone of Noctuids. The chemotype was then characterized by the presence of long-chain alcanols and their derivatives.

P-3 Pheromones as a strategy for the surveillance of pests under official control in Colombia

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Introduction

In Colombia, the Instituto Colombiano Agropecuario ICA, as the National Organization for Phytosanitary Protection, implements the specific surveillance of prioritized pests species through the Technical Direction of Epidemiology and Surveillance of Plant Health. This is done by the execution of the project for the Surveillance of Pests under Official Control and the Fruit Fly National Plan.

Material and methods

The use of pheromone traps is emerging as a fundamental tool for the monitoring of pests species, in the development of strategies for specific surveillance of pests under Official Control. Currently the ICA has established indirect surveillance through the use of pheromone traps in species under official control such as the cotton weevil *Anthonomus grandis* Boheman (Coleoptera Curculionidae), the fruit flies *Ceratitis capitata* Wiedemann and *Bactrocera* sp. (Diptera Tephritidae) and in the case of crop pests such as the Guatemalan potato moth *Tecia solanivora* Povolny (Lepidoptera Gelechiidae). Additionally, the institute is working on the mandatory implementation of trap networks, provided with an aggregation pheromone and kairomone, to monitor and control the populations of South American palm weevil, *Rhynchophorus palmarum* L. (Coleoptera Curculionidae) in all oil palm production zones of the country.

Results

One of the most important objectives of the project for the Surveillance of Pests Under Official Control is to implement experimental tests to validate the use of pheromone traps in other species under Official Surveillance (present or absent). This is the case of the avocado moth, *Stenoma catenifer* Walsingham (Lepidoptera Oecophoridae), a quarantine species present in the avocado production zones in Colombia.

Conclusions

In addition, the institute is currently implementing the necessary actions to import the specific aggregation pheromone for the palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera Curculionidae). This species is absent in Colombia, but surveillance actions have to be carried out in order to identify its presence.

P-4 *Apis mellifera* cuticular hydrocarbon analysis in a foraging context

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Introduction

When a honeybee (*Apis mellifera*) collects nectar with high sucrose concentration, it returns to the hive and dances vigorously to communicate the discovered food source. During this conspicuous display its body temperature rises, promoting the release of certain cuticular hydrocarbons (CHCs), which have been postulated as a relevant stimulus for inactive foragers. A rise in body temperature also occurs upon nectar collection and during food exchange (trophallaxis), and correlates with food profitability. We studied if CHC profiles are affected under different food quality contexts at three stages of the foraging cycle.

Material and Methods

We trained a group of forager honeybees to an artificial feeder offering high or low levels of sucrose concentration (2 and 0.5 M). Bees were captured at different stages at the feeding site, at the hive entrance, and during trophallaxis (inside the hive). Hive bees and foragers captured in empty feeders served as controls. The bees were sacrificed using CO₂ and the CHCs were extracted in dichloromethane and analyzed by GC-MS. We identified 48 CHCs that were quantified relative to an internal standard, and statistically analyzed by Principal Component Analysis.

Results

We found significant differences in CHCs of hive and forager bees, but not among forager bees in any of the phases of the foraging cycle or sucrose-concentration treatments. Our results are therefore not in line with previous studies reporting that four hydrocarbons, namely (*Z*)-9-tricosene, tricosane, (*Z*)-9-pentacosene and pentacosane, are produced in larger amounts by foraging bees advertising a profitable food source.

Conclusions

Our results show that while CHCs of honeybees vary between castes, no obvious qualitative or quantitative changes in CHCs can be correlated with food profitability.

P-5 Changing colours and odours exploring cues used by insect pollinators in two Brassicaceous plants

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Introduction

Flowering plants use different traits to attract pollinators, which indicate the flower location and the quality of the reward flowers offer. Visual and olfactory cues are among the most important floral traits exploited by pollinating insects. Pollination can alter physical and chemical cues of flowers, which can subsequently influence the behaviour of flower visitors. In this study, we investigated which are the main cues exploited by *Episyrphus balteatus* and *Pieris brassicae* when visiting flowers of *Brassica nigra* and *Raphanus sativus* plants. We investigated post-pollination changes and its effects on the behaviour of flower visitors and flower volatile emission.

Material and methods

We used two-choice bioassays to assess the use of the pollen as a cue by pollinating insects. Also, the preference between colour and odour as a main cue by pollinators were tested by offering visual and olfactory cues separately. In addition, we inferred whether behavioural responses could be correlated with changes in volatile emission, by collecting volatiles from flower headspace in greenhouse conditions.

Results

Pieris brassicae and *E. balteatus* did not use pollen as a cue in neither of the two species tested. Interestingly, pollinators showed a strong bias for the visual cues over the olfactory cues in *B. nigra*. Flower visits by pollinators were influenced by pollination in *B. nigra* and in contrast, plant responses to pollination did not influenced pollinator behaviour in *R. sativus*. These results correlate well with floral volatile emission of *B. nigra* and *R. sativus*.

Conclusions

Pollinators exploit different visual and olfactory traits when searching for nectar or pollen of flowers of two close related plant species.

P-6 Bioguided phytochemical study of *Annona mucosa* searching for natural insecticides against *Sitophilus zeamais*

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Introduction

The search for natural products with insecticidal potential to substitute synthetic insecticides in pest management has widely increased due the low toxicity and fast degradation of the biopesticides. In this work, *Annona mucosa* was studied searching for compounds to control maize weevil, a serious agricultural pest that causes large losses in the Brazilian crop production.

Material and methods

The phytochemical study of *A. mucosa* was performed based on the bioassays results against *S. zeamais*, where only the bioactive fractions were studied. For the bioassays, were evaluated the mortality of weevils submitted to the treatment with the samples and the lethal concentrations for the most active ones, that were studied through chromatographic techniques.

Results

Initially were evaluated extracts from leaves, branches and seeds from *A. mucosa*. The bioassay revealed being the seed extract the most active causing 100% of mortality of weevils at 1500 ppm and LC₅₀ of 288 ppm. After a liquid-liquid partition of this extract, the hidroalcoholic fraction, rich in acetogenins, showed 94% of mortality at 288 ppm. Among the subfractions, the most active one, tested at 58 ppm, presented mortality higher than 80% and was studied through HPLC, which lead to the isolation of 4 acetogenins, rolliniastatin-1 and 2, jimenezin and a novel acetogenin. Rolliniastatin-1, the majority compound from this fraction, was tested and resulted in 50% of mortality at 58 ppm, and was observed loss of motor coordination symptoms in all subjects tested.

Conclusions

The results showed the great insecticide potential of the species *A. mucosa* and of the acetogenins. The concentration used in rolliniastatin-1 bioassay is near of those values for commercial insecticides registered at Brazil's Ministry of Agriculture, highlighting the possibility of using this molecule as a bioinsecticide or as “lead structure” for the development of new insecticides that are less toxic and more specific.

P-7 Study of seasonal variation of epicuticular flavonoids and clerodane diterpenoids of *Baccharis macraei* Hook. et Arn.

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Introduction

This work provides a model for studying the variation of epicuticular secondary metabolites in *Baccharis macraei* (Asteraceae) associated to environmental season changes. This is a Chilean endemic species characterized by been an aggressive colonizing dominant plant that grows almost as a monoculture in some Chilean Central Coast Areas. Moreover, it is a perennial and dioecious plant. These characteristics make it a good candidate as a "sensor" of environmental changes, monitoring differences in epicuticular secondary metabolites throughout the year and also between male and female populations.

Material and methods

Aerial parts of *Baccharis macraei* were extracted by dipping the fresh plan material in cold CH₂Cl₂ for 15-20 s (twice). The extract was fractionated by CC (silica gel) using CH₂Cl₂-MeOH step gradient.

Crystallization of pure fractions allowed the isolation of three clerodane diterpenes. They were identified by IR, mono and bi-dimensional NMR (400 MHz) and MS (direct inlet).

A mixture of flavonoids, was isolated by precipitation in cold from the CH₂Cl₂ extract. The flavonoids were identified by HPLC-DAD-ESI-MS/MS.

To study variation of the isolated metabolites, HPLC-DAD was used.

The sampling model was conducted collecting random samples from five individuals of each sex in three sectors in the middle of each season. Total population 90 individuals.

Results

The clerodane diterpenes were identified as 2β-hydroxybacchamacranone, bacchamacranone and hautriwaic acid.

The flavonoids were identified as 5,7,4'-trihydroxy-6-methoxyflavone, 5,6,7,4'-tetrahydroxyflavone, 5,7,8,4'-tetrahydroxy-6-methoxyflavone, 3,5,7,3',4'-pentahydroxy-6-metoxiflavonona and 3,5,7,4'-tetrahydroxy-6,3'-dimethoxyflavone.

Conclusions

The compounds in the samples collected in atumm, winter and spring were evaluated. The results show seasonal variation and correlation between the level of UV radiation and the composition and structure of flavonoids.

Acknowledgments

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P-8 Chemical composition and insecticidal properties of essential oils of *Aristolochia chilensis* root and *Schinus areira* fruits against *Ulomoides dermestoides* (Coleoptera)

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Introduction

Aristolochia chilensis Bridges ex Lindl. (Aristolochiaceae) is a Chilean endemic plant. It grows from third to Metropolitan Regions. It has been commonly used as a medicinal plant, although, is known that contains carcinogenic aristolochic acids.

Schinus areira L. (Anacardiaceae) grows in Chile from the first to Metropolitan Regions. It has been used as ornamental tree and also associated to agricultural crops as insect repellent.

This study, reports the chemical composition of the essential oils (EO's) of *A. chilensis* root and *S. areira* fruits and the insecticidal activity against *Ulomoides dermestoides* (Chevrolat, 1878) (Coleoptera Tenebrionidae) a species that develops in stored food products, and has been used in laboratory experiments because it is easy to breed.

Material and methods

Essentials oils were obtained by hydrodistillation (Clevenger) and analyzed by TLC and GC-MS. The insecticidal activity was evaluated by fumigant method. Each of the samples was performed in triplicate. The mortality was determined after 24 hours and the dose-response mortality (LC₅₀) was analyzed by Probit analysis.

Results

From the essential oil of *A. chilensis* roots 32 compounds were identified corresponding to 86.79% of the total mixture. The main components were β -sesquiphellandrene (47.50%), β -bisabolene (20.71%) and γ -elemene (6.56%) (Sesquiterpenes). In addition, 28 compounds were identified from essential oil of *S. areira* fruits, which accounted for the 97.87% of the total extract. The main components were α -phellandrene (37.38%), limonene (19.80%) and β -myrcene (16.81%) (Monoterpenes). The essential oils showed different insecticidal activities (LC₅₀ values). The essential oil of *S. areira* fruits showed a higher activity than essential oil of *A. chilensis* root, with 23.70 mg·L⁻¹ and 666.14 mg·L⁻¹ LC₅₀ values, respectively.

Conclusions

We conclude that the different insecticidal activity of the essential oils is due, at least in part, to differences in the volatility among mono and sesquiterpenoids.

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P-9 Identification of volatile organic compounds in different breeds of sheep, preliminary results

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Introduction

Estrous synchronization in production animals is fundamental for the use of biotechnologies and improvement in the reproductive efficiency of herds. Using Volatile Organic Compounds (VOCs) may be effective for performing this procedure without synthetic hormones, obtaining offsprings next to organic production, gaining space on market. This work aims to identify VOCs released by sheep, and analyze if there are differences between the several breeds studied and the places where these compounds were collected. VOCs will be used in future studies on the synchronization of estrus in anestrus females.

Material and methods

The study was conducted in male sheep of different breeds Morada Nova, Bergamácia White Dopper, Doper and Santa Inês, all adults in reproductive age, raised in private properties in the state of Alagoas, Brazil. Samples were extracted from the head and dorsal region of them in an attempt to get the VOCs released to discover if there would be differences between the compounds according to the region. The analyzes were performed at the Laboratory of Natural Resources of the Institute of Chemistry and Biotechnology, in the Federal University of Alagoas, with the aid of a Gas Chromatograph with FID detector (GC / FID), QP2010 plus RTX-5 columns (30 m, 0, 25 mm internal diameter, 0.25 µm diameter of the film; Restek) nitrogen as carrier gas, initial oven temperature 30° C and heating ramp 10 ° C.

Results

Preliminary results showed a variety of compounds between the breeds studied, noting that among the regions where VOCs were collected there was no significant variation.

Conclusions

Identifications of VOCs originated from sheep by using chromatographic techniques is being released as a novelty in sheep production in Brazil, which will facilitate and ease the time spent and costs in the process of estrus synchronization in small ruminants for artificial insemination or controlled breeding.

P-10 Detection of Conspecific and Heterospecific Semiochemicals by *Sesamia nonagrioides* (L.) (Lepidoptera: Noctuidae) Gravid Females

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Introduction

In Spain, different lepidopteran caterpillars occur sympatrically, feeding on different parts of the maize plant *Mythimna unipuncta* and *Spodoptera exigua* feed on the leaves; and *Sesamia nonagrioides* and *Ostrinia nubilalis* are stem borers. Except for *O. nubilalis*, the moths selected for this study belong to the Noctuidae family and share some pheromone components. The study of the effect of pheromone detection between different species has generated increasing interest because some pheromone compounds can act as a behavioral antagonist of other sympatric species.

Material and methods

We evaluated the electrophysiological and behavioral response (Dual-choice olfactometer and wind tunnel) of mated and unmated females and males of *S. nonagrioides* to their own complete pheromone blend, to its own four components separately, and to the pheromone components of the sympatric species *O. nubilalis* and *M. unipuncta*.

Results

Electroantennogram recordings revealed that females of *S. nonagrioides* can detect their own pheromone blend and its individual components. Moreover, our results show that unmated females and males of *S. nonagrioides* are more sensitive to the female pheromone, showing higher electrophysiological response than the mated females and males. Electroantennogram recordings showed that males and females can detect the major sexual pheromone component of *O. nubilalis* (Z)-11-tetradecenyl acetate and the minor component of the pheromone of *M. unipuncta* (Z)-9-hexadecenyl acetate. When the sex pheromone stimulus was presented in the dual-choice assays, gravid females of *S. nonagrioides* were attracted to both their own complete pheromone blend and one of their own minor pheromone components (Z)-11-hexadecenal, but repelled by the major sexual pheromone component of *O. nubilalis*.

Conclusions

S. nonagrioides females can detect their own pheromone components and the pheromone components of other sympatric species and can modulate their behavior according to the odors detected; gravid females are attracted to the own pheromone components but repelled for some pheromone components of other sympatric species.

P-11 Investigation of host kairomones from soybeans (*Glycine max*) for the cigarette beetle (*Lasioderma serricornes*)

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Introduction

The cigarette beetle, *Lasioderma serricornes*, is a cosmopolitan pest that attacks a wide range of stored products. The presence of this insect in stored soybeans (*Glycine max*) has become a problem for Brazilian farmers because it is the most important exportation crop of Brazil. In this study, four-arm olfactometer bioassays were conducted to evaluate the responses of *L. serricornes* to volatiles from soy seeds.

Material and methods

The *L. serricornes* cultures were maintained in soybeans in an incubator at constant temperature of $27^{\circ}\text{C} \pm 2$ under a 12:12 LD photoperiod. The extracts were prepared using organic soybeans available in large market chains in Brazil. The seeds were ground and extracted with ethyl ether. Volatile Organic Compounds (VOCs) released directly from crude seed extracts were assayed using a four-arm olfactometer with one treated arm (extract), while the other three arms served as controls (ethyl ether). Data was collected using the Olfa software (mean time spent in each arm and number of entries) and then statistically analysed. The insects were sexed after the bioassays by external observation of the V-shaped apodeme in the abdomen of females.

Results

The *L. serricornes* adults spent significantly more time in the region of the treated arm of the olfactometer where the VOCs released by the soy crude extracts were present

Conclusions

Soybeans contains semiochemicals attractants for *L. serricornes*, probably used in host location, which can provide new tools for the integrated management of this pest in and around storage granaries.

P-12 Chemical characterization of two host plants (*Cucurbita* spp.) of the oligophagous coccinellid *Epilachna paenulata*

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Introduction

Oligophagous insects usually find and recognize their suitable hosts by the semiochemicals produced by the plants. For specialist insects, although their host plants may be similar in their chemical profiles, small differences may contribute to differences in their performances. *Epilachna paenulata* (Coleoptera Coccinellidae), a specialist on Cucurbitaceae, is able to complete its life cycle in different *Cucurbita* species. The aim of this study was to chemically characterize *Cucurbita maxima* and *C. moschata* and to analyze the effect of *Cucurbita* spp. secondary metabolites in the behavior and performance of *E. paenulata*.

Material and methods

The foliar VOCs and epicuticular waxes from both cucurbits were obtained from undamaged plants. Both kind of extracts were analyzed by GCMS. The behavior of *E. paenulata vis a vis* to the VOCs was studied by olfactometry. Besides, ethanolic extracts were obtained from individual leaves and analyzed by NMR. Adult feeding preference was studied offering leaf pieces from both plants. Finally, the effect of the diet on the insect life cycle was studied rearing larvae on both plant species and evaluating their mass gain and development time.

Results

VOC profiles from both plants greatly differed. The wax and ethanolic extract profiles showed minor differences in the fatty acids and aromatic compounds. However, olfactometer tests showed no significant differences in the adult preference by VOCS of any of the plants; and no differences were neither detected in their feeding preference. Performance (gained mass and development time) showed no significant differences correlated to diet or previous food experience of the larvae.

Conclusions

Despite the chemical differences between both cucurbits, *E. paenulata* does not use that information as a token stimuli to choose any of the plants. This observation is consistent with the observation that the life cycle is completed equally in both plants.

P-13 Chemical composition of extracts obtained from the body of *Eupalamides cyparissias* Fab. (Lepidoptera: Castiniidae)

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Introduction

Eupalamides cyparissias is a native South American butterfly that infests the coconut tree (*Cocos nucifera* L.) and oil palms (*Elaeis guineensis*). This agricultural pest decreases the tree branches efficiency by 20% making it second to coleoborers, which are considered the most important pest due to their voracity.

Material and methods

Pupas were obtained from an oil palm plantation located in Moju-PA, Brazil. They were separated according to sex and maintained in the laboratory at $27^{\circ}\text{C} \pm 1$ and 70% humidity until emergence. Female and male couples were individually released into a mesh cage (3.0 x 3.5 x 2.0m). Extracts were prepared with hexane between 500 and 600am and 1800 and 2000pm. Extractions were obtained from the following parts female ovipositor, posterior and anterior wings, male legs, and virgin females during calling. Each extract was analyzed using GC and GC-MS with RTX-1 and RTX-5 columns, initial temperature of 50°C (5min), temperature was increased at a rate of $8^{\circ}\text{C}/\text{min}$ until reaching 300°C (10min).

Results

GC and GC-MS sample analyses identified compounds from different structural classifications such as alcohol, ketones, esters, aldehydes, carboxylic acids, and hydrocarbons. Hexadecanal was present in the extracts of the ovipositor from virgin females, a possible pheromone candidate. Farnesol was found in the extracts from male wings.

Conclusions

Extracts and compound standards will be furthered analyzed using electroantennogram to determine their electrophysiological role.

P-14 Electrophysiological responses of gustatory sensilla on the ovipositor of the parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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Introduction

Diachasmimorpha longicaudata is a solitary endoparasitoid that attacks Tephritidae fruit fly larvae whilst they are feeding inside the fruit. Therefore, it is likely that assessment of host parasitization status is based on sensory input from the ovipositor. Host discrimination ability has been demonstrated through behavioral studies. Nonetheless, superparasitism (oviposition in a parasitized larva) is frequently observed. Since supernumerary larvae do not develop into adult parasitoids, this phenomenon is considered a limitation for mass rearing. Here, we evaluated electrophysiological responses of gustatory sensilla on the tip of the paired ovipositor valves.

Material and methods

Haemolymph of parasitized or unparasitized *Ceratitis capitata* larvae was used as stimulus and physiological saline (PBS) was used as control. The female parasitoid abdomen was connected to a DC amplifier and a glass capillary containing the stimulus or PBS was connected to ground. Neuronal activity recording started automatically when the capillary contacted a single gustatory sensillum. Spike frequencies were compared between treatments by means of ANOVA. Scanning electron microscopy (SEM) was performed to corroborate the presence of these gustatory sensilla.

Results

No differences were found in the frequency of spikes among the stimuli. PBS elicited almost no response. Adaptation was observed, given that 100 ms after stimulus onset an abrupt decrease in activity was recorded, followed by an almost constant firing rate. SEM pictures showed a low density of gustatory sensilla on the tip of each paired valve allowing single sensillum recordings. The firing pattern and previous ultrastructural studies indicated that the sensilla under study are innervated by only one chemosensory neuron.

Conclusions

The electrophysiological recordings showed that the stimulated neurons did not detect chemical differences associated to host parasitization status. Further studies on sensilla of different valves of the ovipositor are needed to evaluate the role of dissolved chemical cues in host status assessment.

P-15 Bioactivity of extracts and fractions from *Duguetia lanceolata* against *Leucoagaricus gongylophorus*

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Introduction

Leaf-cutting ants, *Atta sexdens rubropilosa* (Hymenoptera), dominant herbivores in the tropics, are considered a serious pest for agriculture. They cut high amounts of vegetal matter to feed their symbiotic fungus, *Leucoagaricus gongylophorus* that is responsible to produce the enzymes necessary to metabolize polysaccharides to mono and disaccharides, that supplies the major part of the energy needs of adult workers. This relationship between the leaf-cutting ants and its symbiotic fungus is essential to their survival.

Different methods and new strategies to control these pests have been studied, aiming to replace the synthetic insecticides and fungicides for natural products because the synthetic ones have often temporary effects and affect non-target species.

The genus *Duguetia* consists of approximately 70 species, of which 50 are found in Brazil. The interest in studying plants from this family is related to the presence of several secondary metabolites with diverse biological and pharmacological activities. This work investigates the biological action of *Duguetia lanceolata* against the *L. gongylophorus*.

Material and methods

Ethanollic extracts of leaves (LE), branches (BE), fruit (FE), and seeds (SE) were prepared and each of them was submitted to a liquid-liquid partition that resulted in hexanic, dichloromethanic, ethyl acetate and hidroalcoholic fractions. All these fractions were analyzed by ¹H NMR and TLC and tested against the fungus *L. gongylophorus*.

Results

At a concentration of 1000 mg.mL⁻¹ the extracts inhibited the symbiotic fungus growth in 5% (LE), 1% (BE), 86% (FE) and 14% (SE), while the most active partition fractions presented inhibition of 70% (FE-hex), 90% (FE-dichlo), 77% (LE-hex) and 75% (BE-dichlo).

Conclusions

The ¹H NMR and TLC analysis indicated the presence of steroids and alkaloids. The fractions with higher activity are been subjected to chromatographic processes followed by new tests in the search for substances with fungicide properties. FAPESP, CNPq, CAPES, INCT-CONTROLE BIORRACIONAL DE INSETOS PRAGAS

P-16 Histopathological effect of soybean genotypes in the midgut epithelium of *Anticarsia gemmatalis* (HÜBNER, 1818) resistant to the virus AgMNPV in different larval generations

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Introduction

The velvetbean caterpillar *Anticarsia gemmatalis* is been controlled through the use of nucleopolyhedrovirus (*AgMNPV*) or plants that presents compounds like rutin and genistin that promote insect resistance. The midgut (MI), the portion of digestive tube responsible to food absorption and principal via to access of biopesticides, is constituted by the peritrophic membrane, the epithelial layer and muscle layer. The different types of epithelial cells vary in their chemical composition according to their specific function. The aim of this study was analyze the effects of rutin and genistin on the morphology and chemical composition of the epithelial cells of MI in *A. gemmatalis* larvae resistant to *AgMNPV* in four generations with selection pressure.

Material and methods

Larvae were fed with soybean leaf "in natura" BRS 257 (control); BR 16 (susceptible to insects), Dowling, PI 229358 and PI 227687 100 IAC (insect resistant). The MI larvae 3th/4th instar was collected, fixed in Karnovsky, processed for morphological and staining for neutral polysaccharides (PAS) analysis.

Results

The columnar cells were the most altered in every generation with intense vacuolization and release of cytoplasmic protrusions. Goblet cells showed a cavity voluminous and the regenerative cells an intense proliferation in some treatments and generations.

The histochemical analysis revealed qualitative changes of neutral polysaccharides in epithelial cells that varied in the location and intensity of reaction, being the treatments Dowling, IAC 100 and PI 227687 the more expressive in all generations.

Conclusions

Our results showed that the flavonoids rutin and genistin present in commercial resistant cultivars (Dowling and IAC 100) and in genotypes resistant to insects (PI 227687 and PI 229358) are considered ideal to decrease the resistance of the larvae of *A. gemmatalis* to the *AgMNPV*.

P-17 Stable Isotope Labeled Salicinoids Generated by Incubating Poplar (*Populus trichocarpa x deltoides*) Plants under an Atmosphere of $^{13}\text{CO}_2$

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Introduction

Isotopically labeled compounds have been successfully used for biosynthetic studies since decades. Labeled substances are generally quite expensive and in special cases not even available. Then, the required compounds need to be prepared by chemical synthesis.

In case of the salicinoids, a group of secondary phenolic metabolites occurring in the *Salicaceae* family, the labeled compounds are not commercially available and there is no synthesis known so far. However, they are of great interest for biochemical investigations like detoxification and biosynthetic studies. An elegant way to get excess to those substances is the *in vivo* generation using living plants. By administering them with labeled precursors they serve as kind of bioreactors producing labeled metabolites that then can be isolated, characterized and finally used for further experiments.

Material and methods

We designed and constructed a device for incubating plants under a $^{13}\text{CO}_2$ atmosphere and used it for labeling experiments. The incubation chamber is of flexible size, equipped with an automatic cooling unit and sensors to monitor CO_2 concentration, temperature, and humidity.

Results

In order to generate labeled salicinoids, a 14-day labeling experiment with $^{13}\text{CO}_2$ was carried out by using a poplar plant (*Populus trichocarpa x deltoides*) as model organism. An isolation method based on various solid phase extraction steps was developed. HR-MS and NMR techniques were used to characterize the total and position-specific ^{13}C -enrichment of the isolated salicinoids. ^{13}C -Enrichment of up to 70% was achieved in the desired molecules.

Conclusion

In conclusion the experimental system is suitable for retrobiosynthetic and metabolomic experiments, source-sink studies, raising fully ^{13}C -labeled plants as a source of uniformly labeled compounds, etc., and as a platform which is universally applicable for other biological systems.

P-18 Synthetic inhibitors of fatty acid synthase of leaf-cutting ants.

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Introduction

Leaf cutting ants constitute one of the main forest plagues in the Americas. The fungus garden of these eusocial insects can be affected by a large number of microbial parasites and pathogens. Secretions of the metapleural glands have special significance as an ant-specific immunity component. Chemically diverse substances, mainly fatty acids, from these glands are known to have antimicrobial properties and hence to play an important part in ant life, such as *cerulenin* [(2*R*, 3*S*)-3-(4*E*,7*E*)-nona-4,7-dienoyl]oxirane-2-carboxamide]. In this communication is presented synthesis of analogues of *cerulenin*, which through inhibition of the synthesis of fatty acids [fatty acid synthase (FAS) enzyme], the colony becomes more susceptible to attack from natural parasites, thereby being one of the less harmful to the environment control.

Material and methods

Synthetic analogues that showed greatest inhibition of FAS were ethyl 3-nonyloxirane-2-carboxylate (**1**), 3-hexanoyloxirane-2-carboxamide (**2**), 3-decanoyloxirane-2-carboxamide (**3**), 3-(2-phenylacetyl)oxirane-2-carboxamide (**4**). The methodology for the synthesis (**1**) consists of 3 steps oxidation from dodecanal, after reaction of Horner-Wadsworth-Emmons between the aldehyde and the diethylphosphonate provided the olefin. Subsequently esters were subjected to epoxidation reactions with nucleophilic *m*-chloroperbenzoic acid providing (**1**); for **2**, **3** e **4** will be presented in the poster. Tests with synthetic derivatives were performed with FAS enzyme reading in Elisa plates and it was possible to monitor the rate of oxidation of NAPH in the presence of acetyl-CoA and malonyl-CoA, and thus calculated the rate of inhibition.

Results

The synthetic analogues showed inhibitions of 51% (**1**), 56% (**2**), 79,9% (**3**) e 50,7% (**4**) of the fatty acid synthase (FAS).

Conclusions

The results indicate that synthetic analogues may be an efficient control of leaf cutting-ants by enzyme inhibition while reducing the environmental impact of applying chemical products.

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P-19 Subchronic exposure to diflubenzuron causes health disorders in neotropical freshwater fish, *Prochilodus lineatus*

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Introduction

Diflubenzuron (DFB) [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] is a selective insecticide that inhibits chitin synthesis and interferes with cuticle formation at the time of molting, causing insect death. The insecticide has been intensively applied in water or fish feed, in the fish farms to control the ectoparasites and insect larvae during fish larviculture.

Material and methods

The action of diflubenzuron (DFB) was evaluated in a freshwater fish, *Prochilodus lineatus*, exposed to 0.06, 0.12, 0.25 and 0.50 mg L⁻¹ DFB for 14 days; thereafter, the erythrocyte nuclear abnormalities (ENA), the activity of Na⁺/K⁺-ATPase, H⁺-ATPase and carbonic anhydrase in the gills and the lipid peroxidation (LPO) and histopathological changes in the gills and liver were determined.

Results

Micronuclei increased in fish exposed to 0.25 and 0.50 mg L⁻¹ DFB. Plasma Cl⁻ and the carbonic anhydrase activity decreased, and the activities of Na⁺/K⁺-ATPase and H⁺-ATPase increased in fish exposed to 0.25 and 0.50 mg L⁻¹ DFB. The LPO did not change in the gills but increased in the liver of fish exposed to 0.25 and 0.50 mg L⁻¹ DFB. In the gills, the histopathological changes indicated disperse lesions and slight to moderate damage in fish exposed to 0.50 mg L⁻¹ DFB, while in the liver, the changes were significantly greater in fish exposed to 0.25 and 0.50 mg L⁻¹ DFB, indicating moderate to severe damage.

Conclusions

DFB presents toxic potential for *P. lineatus* under continuous exposure causing health disorders when the fish is exposed to the two highest DFB concentrations applied for control parasites in aquaculture and mosquito population in the environment.

P-20 Chemical composition of the female abdominal gland of *Hyponeuma taltula* (Schaus, 1904) (Lepidoptera, Noctuidae)

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Introduction

Sex pheromone blends are produced and released by the pheromone gland that is located between the 8 and 9th abdominal segments of females of moth. Type I pheromone components are of straight-chain C₁₀–C₁₈ (un)saturated aliphatic compounds with oxygenated functional groups like aldehyde, alcohol and acetate ester. They are synthesized from acetyl-CoA using modified fatty acids in biosynthetic pathways. *Hyponeuma taltula* (Lepidoptera; Noctuidae) is a pest of sugarcane in Brazil that leads to death of the apical bud in adult plants.

Material and methods

Abdominal glands were excised from 2 to 3 days old virgin females in calling behavior, and were immediately immersed in 50 μ L of hexane. The hexane extract was then transferred into a glass vial and stored at -10°C until use. A total of 30 female glands were analyzed. Extracts were analyzed by CG-MS and CG-FID, which were equipped with DB-5 capillary column. Identification of compounds was conducted by analyses individual of the mass spectra and compared with KI's from reported compounds and with entries from the NIST08 database using the data acquisition software CGMS-solution.

Results

Five extracts of glands were analyzed and 11 compounds were found to be present in every repetition including four linear alkanes which were identified as tridecane, henecosane, tricosane and pentacosane by comparison with standards. Furthermore, saturated aldehydes like dodecanal and hexanal, as well as an unsaturated aldehyde and an unsaturated alcohol.

Conclusions

Preliminary analysis of constituents of the hexane extracts from abdominal gland has led to the identification of linear alkanes between C₁₄ and C₂₅, (un)saturated aldehydes between C₁₂ and C₁₆ and an unsaturated alcohol.

**P-21 Study the pattern of adult behavior and evidence of pheromone
Hyponeuma taltula (Schaus, 1904) (Lepidoptera, Noctuidae)**

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Introduction

Investigations on the courtship and mating behavior of nocturnal moths have described behavioral patterns regarding copulation and have led to the identification of pheromones produced by females that act over long distances to attract males. These compounds have received attention over the past decades, as they are at the core of efficient methods for pest population monitoring. *Hyponeuma taltula* (Lepidoptera; Noctuidae) is a pest of sugarcane in Brazil that leads to death of the apical bud in adult plants.

Material and methods

Laboratory population was established using larvae collected in Boca da Mata, Brazil, and kept at ~27,2°C and 81%RH with a photoperiod of 14:10h (LD). A couple of insects (♀/♂) of known age were placed in a glass chamber and their behavioral patterns were scored and ranked during different periods in the scotophase. In order to determine the sexual source that attracts the male, both the female moth and an abdominal extract were tested.

Results

Sexual activity was observed around the 3rd hour of the scotophase when the females began calling and expose their glands. An increase in the frequency of male habitual behaviors and the presence movements associated with calling, cutting and mating behavior was noticed. If clasping is successful, copulation takes place and the male turns 180 degrees. Copula lasts for 75 ±15 min. Males were attracted by abdominal extracts and females.

Conclusions

Under the laboratory conditions used for *H.taltula*, peak in sexual activity was noted between the third and sixth hour after initiating scotophase. The males are sexually active throughout adult life, whereas virgin females are only active between the second and seventh day of life. Based on these observations the ethogram of the sexual behavior was established. The male sexual behavior is triggered by the release of sex pheromones from the female.

P-22 Influence of the sex pheromone of sympatric hosts on the behavior of *Hexacladia* sp. (Hymenoptera: Encyrtidae)

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Introduction

Females of parasitoids need to find suitable hosts for the development of their offspring. These insects have developed sophisticated mechanisms to find their hosts, using different cues, such as the semiochemicals from their hosts. Although release in very tiny amounts, host pheromones are a reliable identity cue and directly related to the presence of the host on the habitat. The objective of this study was to analyze the response of *Hexacladia* sp., a parasitoid of stink bugs adults, to the sexual pheromones of two Pentatomidae species *Euschistus heros* and *Nezara viridula*.

Material and methods

To evaluate the influence of the sex pheromone of each species of stink bug on the foraging behavior of *Hexacladia* sp, Y-olfactometer bioassays were performed using synthetic solutions of each sex pheromone at 0.01 mg/mL in n-hexane, 5 µL of each solution were placed in a small piece of filter paper and were contrasted with n-hexane (control). The behavior of females of *Hexacladia* sp was observed during ten minutes (n = 40 for each host pheromone). The filter paper was replaced at each bioassays. The initial choice (first arm of the olfactometer choosed by the parasitoid) and residence time (time in each arm of the olfactometer) in each arm of the olfactometer were analyzed.

Results

Females of *Hexacladia* sp. were attracted to the pheromone of *E. heros* and stayed for a longer time in the arm containing the sex pheromone, compared to control arm. In contrast, *Hexacladia* sp do not show significant response toward the sex pheromone of *N. viridula* in relation to control.

Conclusions

The parasitoid appears to use the sex pheromone of *Euschistus heros* in their foraging behaviour. This result is in concordance with the preference for this host observed from field surveys. Further studies are been conducted to evaluate this hypotheses.

P-23 Effects of volatile organic compounds emitted by soil bacteria on growth promotion of *Lactuca sativa* L.

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Introduction

Volatile organic compounds (VOCs) emitted by bacteria have showed that induce plant growth promotion, emerging as a sustainable alternative in crop production. In the recent years studies have showed that VOCs can modify root architecture and increase both fresh and dry weight. Because *Lactuca sativa* is a species with high human consumption and characterized by a high nutrient demands, arise as a model for studying the use bacterial VOCs in horticulture.

Material and methods

Four bacteria strains were selected through acetoin production (bct4, bct29, bct9 and bct53). Bacterial VOCs experiments were performed in bi-compartment petri dishes with 2-days-old *L. sativa* seedlings placed on one compartment with Murashige & Skoog medium (MS). In the other compartment, 15 µL of each bacterial suspension were inoculated at 10⁹ cfu/mL. Different media, such as MS, methyl red Voges Proskauer (MRVP-A) and nutrient agar (NA) were tested. Seven days after inoculation the number of lateral root (NLR), primary root length (PRL), shoot length (SL) and dry weight (DW) were measured.

Results Main results indicated that the effect of bacterial VOCs was dependent on both culture media and bacteria strain. For instance VOCs emitted from bct4 elicited the highest PRL value when it was cultured in MS (153.0%), and bct9 cultured on NA released VOCs eliciting 82.65% of DW increasing. The lowest value of PRL and DW were obtained when bct53 was cultured either on MRVP-A, MS or NA.

Conclusions

Bacterial culture medium is essential for promoting growth on *L. sativa* by bacterial VOCs.

P-24 Sex Pheromone Of *Pseudococcus meridionalis* First Steps In Its Identification

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Introduction

The mealybug *Pseudococcus meridionalis* (Prado) is an agricultural pest in Chile that affects crops with an economical importance, mainly vineyards. Due to quarantine restrictions and difficulties in the correct identification of mealybug species, we have been studying the chemical communication of these species and their relationship with others mealybug pheromones.

Material and methods

Adult *P. meridionalis* were collected from vineyards located in Santiago, Chile and reared in the laboratory on sprouted seed potatoes. After 20-25 days, female nymphs were separated from males to ensure virginity after emergence. Volatiles from matured virgin females were collected on activated charcoal, eluted with hexane and tested by laboratory biosays. Additionally, derivatization reaction with NaOH and H₂ cat were done.

Results

Analysis by gas chromatography-mass spectrometry and comparison with control samples revealed the presence of a female-specific compound with a retention index of 1358 on a non-polar Rtx-5 column and 1610 on a polar Stabilwax column and molecular formula C₁₃H₂₂O₂. Derivatization reactions such as catalytic hydrogenation and basic hydrolysis with NaOH, showed disappearance of peak interest.

Conclusions

Chemical analysis and laboratory bioassays evidence that exist a chemical communication between male and female of *Pseudococcus meridionalis*. New analysis and synthesis of model compounds are being carried out in order to elucidate the structure of this pheromone candidate.

P-25 Synthesis of the (*E*)- β -ocimene, component of the aggregation pheromone of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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Introduction

(*E*)- β -ocimene is a component of the aggregation pheromone of *A. diaperinus* identified from a Brazilian population, which is composed by six compounds, (*R*)-limonene (*E*)- β -ocimene, 2-nonanone, (*S*)-linalool, (*R*)-daucene, and (*E,E*)- α -farnesene. The (*E*)- β -ocimene is not available commercially; therefore the objective of this work was to synthesize this compound in high purity and quantity to be used in behavioral bioassays in laboratory and under field conditions.

Material and methods

The (*E*)-3,7-dimethylocta-1,3,6-triene ((*E*)- β -ocimene) was synthesized using a fast and simple three steps known route. The Wittig reaction using methyltriphenylphosphoniumbromide and (*E*)-ethyl-3-methyl-4-oxo-2-butanoate furnished (*E*)-ethyl-3-methylpenta-2,4-dienoate, which was reduced with LiAlH₄ and converted into bromide using CBr₄ and PPh₃. Finally, through cross-coupling reaction using 2-methyl-propenylmagnesium bromide under catalysis of Li₂CuCl₄ the required compound (*E*)- β -ocimene was obtained. To test the biological activity of the compound "Y" tube olfactometre bioassays were performed using adults of *A. diaperinus*.

Results

The GC-FID analysis indicates 56%yield and an isomeric purity of 96% of the (*E*)-isomer. Olfactometer bioassays showed that adults of *A. diaperinus* responded to a synthetic blend containing the compound (*E*)- β -ocimene 96% and when this compound was removed from the blend the insect was not attracted.

Conclusions

The sequence of reactions results in a final product with 96% of enantiomeric purity to the isomer (*E*), which was used in some bioassays with adults *A. diaperinus* insects. We obtained response to the blend with this compound, but further bioassays are being conducted to evaluate whether *A. diaperinus* can distinguish the (*Z*) and (*E*)- β -ocimene isomers in the aggregation pheromone blend.

P-26 Stink bugs (*Nezara viridula*) induce soybean MAPKs activation and expression of genes related with cell wall proteins in developing seeds modulating chemical defenses

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Introduction

Stink bugs (*Nezara viridula*) are a key pest in soybean (*Glycine max*), which attack decreases yield crop. Plant response begins with the recognition of cell injury and oral secretions, triggering mitogen activated protein kinases (MAPK) pathway and inducing defenses against herbivores. Stink bugs preferentially feed on young developing seeds and inject toxic saliva that causes further tissue damage. Soybean MAPK's role in mediating responses to insects attack remains largely unexplored.

Material and methods

In this work we examined in developing seeds under attack of stink bugs the transcription of genes involved in cell wall modification, as well as early and late MAPKs involvement in defense modulation.

Results

We found that MPK3, MPK4 and MPK6 as well as a MPKK are differentially transcribed in time after insect perception. We analyzed the phosphorylation status and identified soybean MPK6 as an herbivore and wound-induced kinase, while MPK3 and MPK4 were found to be activated after SA and JA treatments. Although two critical genes involved in flavonoid synthesis, Phe-ammonia lyase (*PAL2*) and chalcone synthase (*CHS7*) were up-regulated under all treatments, only stink bug attack induced isoflavone synthase (*IFS*), an enzyme responsible for the synthesis of daidzein and genistein, which are the main chemical defenses against these insects. We also analyzed the cell wall transcriptional response and found that 72h after stink bugs attack increased the expression of expansine (*EXP*), xyloglucan endo-transferase (*EXT*), pectate lyase (*PL*) y polygalacturonase (*PG*).

Conclusions

Our results suggest that stink bug attack not only increases the expression of cell wall genes causing a re-arrangement of the wall, but also triggers JA and SA-mediated defenses through a tightly regulated induction of MAPKs transcription and phosphorylation of particular MAPKs in a time-dependent fashion.

**P-27 Searching for new compounds with insecticidal activity against
Atta sexdens rubropilosa from *Picramnia glazioviana*
(Picramniaceae)**

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Introduction

Environmental problems such as elimination of pollinating insects and contamination of water sources are directly related to inadequate agricultural practices with indiscriminate use of synthetic pesticides, which was and still is the main tool in the control of pest insects. Among these pests, leaf-cutting ants, *Atta sexdens rubropilosa*, are known as serious pest for agriculture by attacking and damaging several cultures, resulting in economic and environmental losses [1]. In this context, allelochemicals produced by plants has been widely investigated to obtain new insecticide compounds that can be used in integrated pest management (IPM).

The genus *Picramnia* shows high diversity of secondary metabolites accumulated in their structures, such as anthrones, oxantrones, anthraquinones, coumarins, and triterpenes, classes of compounds with insecticidal activities [2].

Material and methods

This work investigated the toxic effect of *P. glazioviana* against the *Atta sexdens rubropilosa* using ethanolic extract of leaves (PGL) and fruit (PGF) tested in incorporation into artificial diet (2 mg mL⁻¹).

Results

According to median survival (Md) of *A. sexdens rubropilosa* submitted to bioassay, significant differences in relation to the control according to the statistical test "log rank" (p < 0.05, Md-control = 14 days) were observed for PGL, Md = 9 days, and no activity for PGF, Md = 16 days.

The active extract (PGL) was analyzed by ¹H NMR showing signals characteristic of anthrones. This extract was subjected to liquid-liquid partition in hexane, dichloromethane, ethyl acetate and hydroalcoholic in order to obtain the fraction rich in anthrones, an active class as insecticide.

Conclusions We concluded that extracts are promising an active class as insecticide, as well as studies are in progress to isolate and identify their structures and activity of pure compounds.

P-28 Responses of the aphid *Aphis gossypii* to volatiles emitted after application of *cis*-jasmone to bell peppers (*Capsicum annuum*)

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Introduction

Cis-jasmone is one of the promising compounds to induce resistance in plants by activating a defense based on chemical signals without unduly influencing other important plant physiological processes. Thus, this study aimed to investigate the effect of *cis*-jasmone on the bell pepper– aphid interaction.

Material and methods

Behavioral tests with aphids in olfactometers and eletroantenografia (EAG) were conducted using the volatile of the Sweet Pepper All Big cultivar (susceptible to the aphid *Aphis gossypii*) with and without *cis*-jasmone and Sweet Pepper Hybrid Green Belt cultivar (resistant).

Results

The results obtained from olfactometer bioassays showed that the odors emitted by resistant cultivar and susceptible cultivar after application of *cis*-jasmone provided a repellent effect to the aphids. The results obtained from EAG showed that the aphid responded to eight volatile compounds. Among them, 6-Methyl-5-hepten-2-one (MHO) and 3- Methoxycinnamaldehyde (MCA) were common to the resistant cultivar volatile and volatile from the susceptible cultivar after application of *cis*-jasmone evidencing is responsible for repellency to aphids. The results obtained from olfactometer bioassays showed that the MHO and MCA at 1% and 0,01% respectively provided a repellent effect to the *A. gossypii*.

Conclusions

These results indicate that *cis*-jasmone has the potential to control aphids on bell pepper plants; MHO and MCA at 1% and 0,01% respectively are repellents to *A. gossypii*.

P-29 Diel periodicity in the volatile emissions of the bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), a pest of eucalyptus

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Introduction

Thaumastocoris peregrinus is an emerging pest of eucalyptus production in the Southern hemisphere. Research on the chemical communication system of *T. peregrinus* can lead to sustainable management strategies, thus far unavailable. Recent studies reported that *T. peregrinus* males emit volatiles that attract conspecific males. However, no ecological role has been determined for this interaction. Daily temporal patterns of emission can provide clues for the behavioural function of these semiochemicals. We determined the diel periodicity of these emissions, by analysing volatile profiles from males, females and couples in 4-hour periods.

Material and methods

Insects were obtained from a laboratory colony and used within one-week after adult emergence. Volatile compounds were sampled by air entrainment using HaySep-Q as adsorbent, in glass/teflon chambers enclosing either males, females or couples with shoots of *Eucalyptus tereticornis*. A fourth chamber with shoots was used as control. Volatile collections were done simultaneously, in 4-hour periods during 24 hours. Volatiles were desorbed and analysed with GC-MS.

Results

The main volatile compound from males, 3-methylbut-2-enyl butanoate, showed a clear diel pattern, with maximum amounts in the two afternoon collection periods. This diel pattern was not observed when males were enclosed with females. Isolated females did not show specific compounds or a periodicity of volatile emissions.

Conclusions

Our results show that 3-methylbut-2-enyl butanoate, a putative male aggregation pheromone, is emitted in larger amounts within a specific time window, and that the presence of females results in a decreased emission. These results suggest that the compound bears a function in intraspecific chemical communication, and that such function may relate to male mating strategies that need to be characterized. These studies may provide insights in the role of semiochemicals in the mating behaviour of *T. peregrinus*, which may result in new strategies for the management of this forest pest.

P-30 Physical characterizations of pheromonal organic dispenser

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Introduction

Semiochemicals are increasingly embedded in the strategies of Integrated Pest Management. Concomitantly, new technologies are developed to increase the efficiency of controlled release of semiochemicals, following a zero order kinetics. Beyond efficient, releasing systems must also be biodegradable. The objective of present work was to produce spheres of chitosan and characterize them by diameter, density, porosity, viscosity.

Material and methods

The spheres were produced with 1.2% chitosan gel and precipitated in 2 M NaOH. The crosslinked spheres with 0.5% glutaraldehyde were also synthesized. The characterizations were done after their preparation according to Gonçalves (1996).

Results

The pores of chitosan spheres are smaller due to blockage of pores on the support surface by steric hindrance of glutaraldehyde molecules. No marked differences in the density and diameter parameters of microspheres are observed. As the glutaraldehyde concentration used is low, there are not enough amount of aldehyde groups on the polymer surface, resulting in the formation of the imino bonds between the amine groups of the polymer (intercrossed links) to enhance the density and decrease the diameter of the microspheres. Viscosity of the chitosan sphere was 2857,686 cSt/s and spheres incorporated with pheromone was much less viscous, 1526.407 cSt/s. Surface scanning of chitosan microspheres with and without glutaraldehyde confirms their porosity, as substances are being added to chitosan spheres, the pores are being filled. Micrographs of the chitosan spheres showed a surface full of protuberances caused by package distribution of substances within the spheres.

Conclusions

The experiments were sufficient to characterize the chitosan spheres and establish parameters to choose the formulation of these spheres to be used as pheromonal releasing system in future assays.

P-31 Optimization of a CO₂-free synthetic host-odor blend to attract triatomines

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Introduction

Triatomine (vector) control is the most effective method to prevent Chagas disease. Currently, vector control is mainly accomplished by insecticide spraying. However, this is not ideal considering that the insects develop resistance to the insecticides, and that these chemicals could affect people living in sprayed houses. The use of lured traps is a sound alternative method for vector control.

Material and methods

Using two of the most important Chagas vectors, *Rhodnius prolixus* and *Triatoma infestans*, we tested potential odor attractants in a dual-choice trap olfactometer. The synthetic blends tested consisted of L-(+)-lactic acid, valeric acid and ammonium hydroxide. These compounds were placed in individual LDPS (low density polyethylene sheets), and were assayed using a live mouse as positive control. Different blends tested were the result of using different volumes of the solutions of each of the three components.

Results

The different blends tested varied in their capture performance. In the case of *R. prolixus* the best blend evoked 81% capture in a single night (this represents a significant capture respect to the negative control, G-test, $p < 0.05$, but not different from a mouse). For *T. infestans*, that blend was also the best and also evoked 81% capture in a single night (a significant capture respect to the negative control, G-test, $p < 0.05$, but not different from a mouse). Adding of nonanal did not improve capture.

Conclusions

Different blends of the host-odor constituents used evoke capture. We are currently measuring the odor emission from our lures in order to know what air concentration of each odor evoke capture in triatomines, and at what level. We aim at optimizing a lure for field use.

P-32 Oviposition in the blood-sucking insect *Rhodnius prolixus* is modulated by host odors

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Introduction

First-instar larvae of triatomines need to feed within the first days after emergence from the egg in order to survive. Taking into account their small size and limited locomotion capacity, it seems reasonable that females should lay their eggs near a host. In fact, the triatomine *Rhodnius prolixus* lays (sticks) their eggs on the feathers of their avian hosts. In order to understand the sensory bases of this behavior, we tested if the presence of a host has an effect on the spatial distribution and number of the stuck eggs.

Material and methods

We carried out tests in an experimental arena consisting of a PVC tube (0.2 m diam, 1.70 m length). Two male and four female adult *R. prolixus* 1-2 weeks after feeding were placed inside a test or a control tube under controlled ambient conditions, for 3 days. The stimulus, consisting of either a mouse or hen feathers, was placed below one end of the test tube while the other end remained empty; no stimulus was placed below the control tube. Little holes allowed sensory cues from the stimuli to enter the tube.

Results

Both stimuli significantly stimulated oviposition in *R. prolixus* (Mouse total eggs test 369, total eggs control 236, N=16, $p < 0.05$; Feathers 332, 118, respectively, N=16, $p < 0.05$, Mann-Whitney test). In addition, hen feathers evoked a non-homogeneous distribution of eggs within the tube (Kruskal-Wallis test $p < 0.05$, N=16).

Conclusions

A live mouse and hen feathers stimulate oviposition in *R. prolixus* even when the insects could not contact the source of stimulus, thus host odors alone can stimulate oviposition in *R. prolixus*. However, a role of other host cues cannot be excluded.

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P-33 Synthesis of jasmonic acid and derivatives for Integrated Pest Management

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Introduction

The use of semiochemicals is an ecologic alternative for the development of Integrated Pest Management technologies. Jasmonic acid and its derivatives have the potential to induce pest resistance. These compounds found in the plant when harmed, lead to the release of volatile compound mixtures that attract natural predators of the harming pests. Existent studies of tritrophic interactions in soy crops indicate that the plant VOCs are the main communication strategy used by parasitoids to localize stinkbug eggs as a host. Also, several similar reports for other crops have been published in the last time. Our work involves the design and synthesis of different jasmonoids to use them in biological control plans in soy, pursuing a technology by the development of jasmonate derivatives eliciting a stronger response than the natural semiochemicals, and thus achieve better control of the soy pest without the use of environmentally harmful agrochemicals.

Material and methods

Jasmonic acid derivatives were synthesized through Palladium cross-coupling reaction and Michael addition as key steps. Field test were done by spraying jasmonoid solutions in soy, alfalfa, and wheat crops, and using sticky traps to evaluate parasitoid presence.

Results

Molecular simplified analogs of jasmonic acid were prepared by a versatile and efficient methodology involving the functionalization of cyclopentenone cores. By varying the side chain moiety, a wide variety of analogs can be prepared. Also, this strategy was used in the synthesis of jasmonic acid and *cis*-jasmonone achieving promissory results. Preliminary field test in soy, alfalfa and wheat crops showed parasitoid attraction.

Conclusions

A versatile and efficient methodology was developed for the synthesis of jasmonoid derivatives. Promising results were achieved in field tests, to evaluate efficacy as parasitoid attractors. Through this work we pretend to develop a technology based in semiochemicals for assisting crop biological control.

P-34 Preliminary study of the identification and synthesis of pheromone Tebo Worm (*Chilecomadia valdiviana*, Lepidoptera: Cossidae)

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Introduction

Chilecomadia valdiviana, also known as "tebo worm", is an insect native to Chile, which primarily feeds on the wood of shrubs and trees, among them economically important species such as apple and eucalyptus trees. The feeding of the larvae causes weakening and, in case of severe infestation, death of the tree. Apart from the direct damage, economic consequences result from quarantine restrictions on exports.

The lack of effective control methods, led us to consider the aim of studying and analyzing the adult individual intraspecific chemical interaction, to clarify the structures of the chemicals involved, to be incorporated into programs of integrated pest management.

Material and methods

Larvae and pupae were collected from agricultural areas in the region of Maule, Chile, and kept in the laboratory until the emergence of adults. Virgin females were observed to characterize their calling behavior. The abdominal glands of virgin females were removed and extracted with hexane, and subsequently analyzed by GC-MS and GC-EAD.

Results

Females perform calling behaviour between 300 and 400 AM. The analysis of the extracts by GC-MS and GC-EAD revealed the presence of a major EAD-active compound with a Kovats index 2103 in RTX-5 column, and the presence of several minor structurally related compounds.

Conclusions

The calling behavior of adult females has been established, and a major compound has been tentatively identified as the main pheromone candidate.

P-35 Unraveling the role of phenylphenalenones as key metabolites in Musa plants against the attack by the pathogen *Mycosphaerella fijiensis*

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Introduction

Bananas are among the most important crops worldwide since they not only represent a staple food but also the major economical income for many producing countries. However, the production of commercial cultivars of *Musa* (banana) belonging to the Cavendish subgroup is enormously affected by insects, nematodes and microbial infections. The Black Sigatoka Disease, caused by the ascomycete fungus *Mycosphaerella fijiensis*, is considered the most detrimental disease of this crop. Despite the attempts of rearing genetically modified *Musa* plants resistant to this pathogen, the use of fungicides still remains the only available method to control this disease. Previous studies have shown that the expression of pathogenesis related proteins, morphological modifications and the production of a class of secondary metabolites named phenylphenalenones (PP's) constitute the main defense mechanisms in the *Musa* genus. Indeed, due to the antimicrobial and nematicidal properties of PP'-type- metabolites, they have been considered for playing an important role in the resistance displayed by some *Musa* plants against pathogens and herbivores.

Material and methods

A chemical phenotyping approach based on NMR and HPLC analysis was conducted in two *Musa* varieties, Khai Thong Ruang (resistant) and Williams (susceptible), in order to explore the differential, spatial and temporal expression of the major phenylphenalenones triggered during such plant-pathogen interaction.

Results

The results show clearly that the resistant variety is able to recognize the pathogen in an early stage of the interaction whose chemical response involves a large pool of PP's able to inhibit the growing of the microorganism; instead, the susceptible variety had a late response and its weak chemical profile was not enough for avoiding the progress of the disease.

Conclusions

Hereby, the early recognition of the microorganism followed by a local response with accumulation of a diverse PP's-type metabolites were the most relevant characteristic displayed by a successful incompatible *Musa-M.fijiensis* interaction explored in this study.

P-36 Behavioural responses of *Stomoxys calcitrans* to possible oviposition sites found in sugarcane mills

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Introduction

Brazil, a leading country in meat export and sugarcane production has stated that yearly outbreaks of *S.calcitrans* are responsible for enormous economical losses. In recent years livestock farms have associated the occurrence of *S.calcitrans* to the proximity of sugarcane mills leading to a dispute of who is responsible for the economical losses that meat producers suffer. Vinasse and filter cake are residues commonly used as fertilizer in sugarcane crops that can extent hundreds of hectares. *S.calcitrans* at all life-stages are commonly found in areas where these fertilizers have been used suggesting that these products do not only provide a suitable oviposition site but might be responsible for attracting these flies. The main objective of this study was to determine attraction of *S.calcitrans* to possible oviposition sites found in sugarcane mills.

Material and methods

Volatiles were collected from 5 different samples vinasse, vinasse + dried straw at two different fermentation stages, filter cake at two different fermentation stages, using headspace collection.

Insect bioassays were conducted using a Y-tube olfactometer. Sample size was 50 female flies 25 with 2-3 days post emergence and 25 with 7-8 days after emergence. Each sample was analysed using gas chromatography (GC) and gas chromatography coupled mass-spectrometry (GC-MS).

Results

Samples that caused a significant attraction were vinasse ($p=0.001$), vinasse + dried straw both 2 ($p=0.001$) and 5 days of fermentation ($p=0.001$). A higher attraction was noted in flies with 8-9 days of life. GC and GC analysis showed a slight difference in compound profile of each sample.

Conclusions

One can conclude that these fertilizers used in sugarcane mills causes an attraction of female *S. calcitrans* in particularly with 7-8 days of life, suggesting that these sources may serve as oviposition sites.

**P-37 Chemical ecology of *Ozopherus muricatus* Pascoe, 1872
(Coleoptera: Curculionidae)**

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Introduction

Ozopherus muricatus Pascoe 1872 (Coleoptera Curculionidae) is endemic in the amazon region, current knowledge of this insect is limited, however, it has caused great economical concerns due to its infestation of the assai palm, in particularly the state of Rondonia. The aim of this study was to investigate the chemical ecology of this insect in order to obtain knowledge for the development of control methods against this pest.

Material and methods

Volatiles were collected from both male insects and female insects separately through air entrainment for 48 hours. Volatile were also collected from the following parts of the host plant branch with flowers, branch with immature fruits, branch with ripe fruits, and pieces of branch for 8 hours. Each obtained sample was analysed using gas chromatography (GC), gas chromatography coupled mass spectrometry (GC-MS), and electroantennogram coupled gas chromatography (GC-EAG). Behavioural assays were conducted using a Y-tube olfactometer in order to determine attraction or repellence from all host plant volatile samples.

Results

Results showed that males were significantly attracted to branch with flowers and pieces of branch samples. Both sexes were significantly attracted to the volatiles of each other thus suggesting the presence of aggregation pheromone emitted by both sexes. A total of 14 electrophysiological responses were observed for the following samples branch with assai flower, pieces of branch, male and female samples. 12 out these 14 electrophysiological active compounds were identified benzyl alcohol, oxide *cis*-linalool, 2,2,6-trimethyl-3-keto-6-vinyltetrahydropyran, oxide *cis*-linalool (furanoid), oxide *cis*-linalool (pyranoid), α -neo-iso-menthol, 2,9-dimethylundecano, Cupareno, p-ethylanisol, 2,3,5-trimethyl-decane, 2,6,10-trimethyl-dodecane (Farnesene), 3,7,11,15-tetramethyl-1-hexadecanol.

Conclusions

One can conclude that volatile sample of both sexes could have an aggregation pheromone and that the assai plant emits kairomones that attracts the insects.

P-38 Host seeking behaviour of *Stomoxys calcitrans* in privately owned farms

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Introduction

Stomoxys calcitrans commonly known as “stable fly” are commonly found in close proximity of large mammals, causing great distress as both sexes are hematophagous. Its painful bite causes great distress in livestock and has shown to be responsible for huge economical losses due to reduced milk production and weight loss. Females oviposit in a decaying matter such as rotting hay and manure, which is commonly found in close proximity to livestock. The main objective of this study was to investigate the host-seeking behaviour of *S.calcitrans*.

Material and methods

Volatiles were collected from the skin of cattle, horses, cattle faeces, horse faeces and fermented grass using headspace collection. Collection of skin volatiles was conducted for 2 hours, and 24 hours from faeces and fermented grass.

Behavioural assays were conducted using a Y-tube olfactometer in order to determine attraction to the volatile samples. Sample size was 25 femalesmales for the host volatile samples; 25 females with 2-3 days of life and 25 females with 7-8 days of life for the other samples. Each sample was analysed using gas chromatography (GC) and gas chromatography coupled mass spectrometry (GC-MS).

Results

Both females and males showed a significant attraction to horse volatiles ($p= 4 \times 10^{-4}$, $p= 14 \times 10^{-2}$), however, no significant attraction was notices for cow volatiles ($p= 0.39$). Horse faeces and fermented grass was attractive for both ages of female flies. GC comparison between horse and cow volatiles showed a difference in compound profile.

Conclusion

Results obtained agree with current host-seeking behaviour literature and preference between horses and cattle. Grass infusion is known to serve as an oviposition site for many vectors, and concluding from the obtained results it is a probable oviposition site for the stable flies.

P-39 Cuticular hidrocarbons and their influence in reproductive behavior of stink bugs

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Introduction

In the last stage of the reproductive behaviour of stink bugs (Hemiptera Pentatomidae) the insects develop stereotyped movements and intensive exploration of its partner using antennae. This behaviour has been associated with the presence of chemical signals in the cuticle of insects that could provide information about species identity and sex of the partner. We investigate the influence of hydrocarbons of the cuticle to species recognition in two sympatric species of *Chinavia*, *C. ubica* and *C. impicticornis*.

Material and methods

20 males and 20 females of *C. ubica* and *C. impicticornis* were anesthetized on a CO₂ stream and maintained in freezer for 10 minutes, after this each insect was lightly rubbed with hospital sterile cotton swab. The swab was washed between one insect and another in a vial containing 1 mL of *n*-hexane, a new swab for each species and each sex was used. The extracts were purified using a silica gel (500 mg) column and 3 mL of *n*-hexane to separate the apolar from the polar compounds. The hexanic extracts were concentrated under nitrogen flow to 100 µL. Five samples were prepared for each sex of each species. These extracts were analysed by GC-FID and GC-MS. Arena bioassays were performed with dead females, which were offered to live males. After observation of the behaviour the same females were washed with hexane in order to extract the hydrocarbons and again offered to males and their behaviour was observed. Bioassays were performed with both species and each bioassay lasted 15 minutes.

Results

Preliminary results of the arena bioassays suggest that cuticular hydrocarbons are also important species recognition for stink bugs. The GC and GC-MS analyses of the extracts obtained suggest inter-specific and inter-sex differences in cuticular hydrocarbons.

Conclusions

Cuticular hydrocarbons may be important clues in interspecific recognition in last steps of reproductive behavior of Pentatomidae bugs.

P-40 Interaction among stinkbugs (Heteroptera: Pentatomidae) and the egg parasitoids *Telenomus podisi* and *Trissolcus basal*s (Hymenoptera: Platygasteridae) mediated by chemical footprints

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Introduction

Egg parasitoids from the family Platygasteridae (Hymenoptera) are major biological control agents of stinkbugs on soybean crops. Chemical and visual stimuli are involved in host searching behavior and chemical trails from the host footprints are important to host finding. The objective of this work was verify whether the egg parasitoids *Trissolcus basal*s and *Telenomus podisi* are able to recognize chemical trails from different species of stinkbugs and also show preference for traces of their preferential hosts.

Material and methods

Females of the bugs *Nezara viridula*, *Euschistus heros* (preferred hosts) and *Dichelops melacanthus* (a non-preferred host) were allowed to walk during two hours over glass surfaces (experimental arenas), which was physically divided into two areas to deposit their chemical footprints in these different areas. For each bioassay females of two species were released in one of the areas of the glass plate (n =2 insects/area) at the same time, thus, three different combinations of treatments were made *E. heros* vs. *N. viridula*, *E. heros* vs. *D. melacanthus* and *N. viridula* vs. *D. melacanthus*. After these two hours the stink bugs and the physical division were removed and one parasitoid females (n = 40/parasitoid species and treatment) was released inside the glass plate and its behavior monitored during 10 minutes using the software SACAM.

Results

Both parasitoids responded selectively toward footprints of their preferential hosts *Tr. basal*s preferred *N. viridula* footprints and *Te. podisi* preferred *E. heros* footprints. The parasitoids spent significantly more time on the traces of its preferred host and their walking trails showed higher tortuosity and turning rate than in non-preferred host footprints.

Conclusions

Parasitoids are able to recognize and differentiate chemical footprints from different species of stinkbugs showing preferences by those of their favorite host. Chemical characterization of footprints of stink bugs species are being conducted to test the hypothesis that different chemical composition of footprints are involved in the recognition and discrimination by the parasitoids.

P-41 A New Gall-Midge (Diptera: Cecidomyiidae) associated to *Haplopappus foliosus* (Asteraceae) Taxonomic and Chemoecological Characterization.

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Introduction

Gall-Inducing insects are among the most exquisite examples of plant-arthropod coevolution. This association is often highly specialized as it involves differential growth of plant tissues, symbionts and deformation of its organs in order to host inquiline insects.

Material and methods

In this work we performed a taxonomic and chemoecological characterization of a previously not studied association between an unidentified gall-inducing fly (Diptera Cecidomyiidae) and its host plant, the asteraceous shrub *Haplopappus foliosus*. We extracted apical compounds by dipping fresh plant material in cold CH₂CL₂ for 30s. Following we performed HPLC, C18Column, UV-VIS and 240 waveleght detector.

Results

We discovered significant differences in diameter of induced tissue versus normal apical branch. Moreover, we discovered several insects inhabiting induced tissue. Among these we suggest a small cecidomid fly *Asphondylia* sp. as the inductor of this structure, and two unknown Hymenoptera parasitoid. Together with we found differences in the chemical composition of secondary metabolites extracted from gall versus healty apical branches.

Conclusions

Based on our results, we suggest gall forming is related with induced metabolical changes due to fly parasitism, these changes may trigger the secondary colonization by parasitic hymenoptera.

P-42 Olfactory cues involved in hygienic behavior in *Apis mellifera* to *Varroa destructor*.

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Introduction

The infestation caused by the parasitic mite *Varroa destructor* (Acari Varroidae) is one of the major problems facing the apicultural sector. Honeybees have a resistance mechanism against brood diseases (e.g, varroosis) that consists in detect, uncap and remove dead or diseased brood from the hive (hygienic behavior). Previous studies showed that the detection of diseased brood by worker bees would be triggered by olfactory cues. The aim of this study was to collect and identify volatile compounds associated with infested bee brood with *V. destructor* at different times of mite reproductive cycle.

Material and methods

Newly capped bee brood was artificially infested with a mite (foundation female). At different times (62 hs, 5 days and 10 days post-infestation) 25 individuals were removed and placed in glass aeration chambers. Volatiles were collected for 24 h on filters containing adsorbent material (Hayesep Q). As a control, in one of the chambers non-infested brood was placed. Volatiles were eluted with metilene chloride, afterwards adsorbed on an SPME fiber before analyzing by means of GC-MS. Differences between infested and non-infested brood have been evaluated by comparing chromatographic profiles.

Results

We found differences between the profiles obtained from infested and control brood as well as among different infestation times. Results also suggest differences in the area of the peaks between infested and control brood, and along advancing infestation. The identification of characteristic peaks in infested brood is being undergoing.

Conclusions

The results of this study will allow to propose possible candidate compounds or a mixture of compounds that trigger the hygienic behavior in honeybees through the infestation with *V. destructor*. This information will contribute to understand the

mechanism by which bees defend against the parasite, providing information on varroa control strategies and genetic selection of *Apis mellifera* tolerant colonies.

P- 43 Investigating the pheromone composition of *Cydia araucariae* (Lepidoptera, Tortricidae), a seed borer of the endangered tree species *Araucaria angustifolia*

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Introduction

Every year, thousands of Araucaria trees seeds are used by *C. araucariae* larvae as food source impeding their development into new trees. Thus, these seeds are sold in markets in the south of Brazil (April-July) for human consumption. Diminishing the seed attack by these insects is the first step into an improved seed production collaborating with reforestation programs and expanding its commercialization. Because there are no efficient methods to deter the seeds' attack, producers wait for the development of alternative methods to monitor and control this pest, such as with pheromone traps.

Material and methods

Infested seeds were collected in Araucaria plantations or purchased by collectors, and maintained under laboratory controlled conditions (20 ± 2°C, 50 ± 10% RH and 12L:12D). Pupae were sexed and individualized in small plastic containers until adults' emergence. Couples were rapidly observed to determine the period of sexual activity. Virgin female pheromone glands were extracted during the period of sexual activity with tweezers using hexane as solvent. Extract analyses are underway on GC, GC-MS and GC-EAD. Because pheromone components of *Cydia* spp. are shared by various species, some reference compounds, such as acetates and alcohols were also tested against males' antenna in GC-EAD tests. Kovats indexes values (KI) were calculated for all antennal responses.

Results

Six consistent male antennal responses were observed against female extracts. Comparing their KI values with reference compounds' KI values, five of them matched for *E8*, *E10-12Ac*, *E8-12Ac*, *12Ac*, *14Ac* and *16Ald*. Thus, all of them were active on male's antenna.

Conclusions

Couples are sexually active on photophase. Female gland extracts were active on EAG tests against males' antenna which were sensitive to five female extract compounds. Two of these compounds matched with acetates and an aldehyde already described as pheromone components of other *Cydia* species.

P-44 Varietal Differences in Constitutive and Herbivore-induced Volatile Organic Compounds Emitted by Strawberry (*Fragaria x ananassa*)

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Introduction

Plant volatile organic compounds (VOCs) drive the location of oviposition sites and food sources by herbivores. In addition, herbivore-induced VOCs allow carnivores to locate prey and/or repel herbivores. Varietal differences in VOCs within one species can influence herbivores' oviposition preference and natural enemies attraction, what results in a resistant trait. Little is known about the varietal differences and induction of VOCs in strawberry plants. We characterized volatiles from healthy and *Duponchelia fovealis*-damaged plants in two strawberry varieties and determined compounds that are perceived by adults. *D. fovealis* was reported for the first time in Brazil in 2010 on strawberry. It feeds on leaves and bores the crown leading to plant's death.

Material and methods

VOCs from control and herbivore-damaged plants from strawberry var. Sao Andreas and Albion were collected for 24 hours, eluted with double-distilled HPLC-grade hexane and analyzed by GC-MS. Antennal responses of gravid females to VOCs was accomplished by GC-EAG technique using plant extracts from herbivore-damaged plants. Complementary non-choice oviposition tests were conducted.

Results

Quantitative and qualitative variations were found in the emission of both varieties. Whereas β -pinene and limonene were not detected in control plants var. São Andreas, (E)-3-hexen-1-ol was detected only in this variety. Larger quantities of α -pinene and indole were emitted by Albion, independently of herbivory. Varietal differences in the emissions of germancre D as well as other compounds such as β -caryophyllene were affected by herbivory. The antennae of gravid females responded consistently to limonene, (E)- β -ocimene, (Z)- β -ocimene, β -caryophyllene, Germacrene D, (E,E)- α -farnesene. In no-choice bioassays, however, no difference in the number of eggs laid by females was observed.

Conclusions

D. fovealis responds to compounds that vary greatly in their emissions in relation to plant genotype. Further studies are needed to understand how such variations affect oviposition preference in dual-choice situation.

P-45 Behavioural response of the horn fly (*Haematobia irritans*) towards volatiles collected from Holstein-Friesian cattle

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Introduction

The horn fly (*Haematobia irritans*) is an ectoparasite of economical importance due to the damage it causes livestock through its painful bites that results in reduced milk and meat production. In addition to their nuisance they also have the ability to carry eggs of the human botfly (*Dermatobia hominis*) and infest the livestock. This study aimed to evaluate the behavioural effect that volatiles emitted by the Holstein-Friesian cattle have on the horn fly.

Material and methods

A method was developed to collect volatiles from females and male Holstein-Friesian cattle. The collected samples were analysed using gas chromatography coupled mass spectrometry (GC-MS). Behavioural assays using a Y-tube olfactometer were conducted. Sample size was 30 flies/test sample. Flies were obtained from a laboratory colony and used after 24 hours of age. They were individually observed for 10 minutes. Response was considered positive when the fly reached the end of the arm containing the test sample. The filter paper containing the test sample was alternated after 10 repetitions. The olfactometer was cleaned every time a new test sample was tested.

Results

The method used to collect volatiles showed to be efficient as the peaks were repeated in each sample and only varied in quantity. Results were statistically analysed using Chi-squared. The behavioural assay showed that the flies were attracted by the volatile samples both from female and male cattle ($p < 0.05$ and $p < 0.01$, respectively), not showing any preference of sex.

Conclusions

The methodology used to collect volatiles was efficient as all compound profiles showed minimal difference from the chemical composition of the analysed samples. Behavioural assays and compound identification are good approaches to investigate novel compounds that can be used as control methods. One can conclude that volatiles emitted by the Holstein-Friesian cattle have an attractive effect on the horn fly.

P-46 Preliminary study of some weeds from banana cultures as ants control agents

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Introduction

The main goal of this work is to introduce a student's group into the basic concepts of plant-insect interactions. Frequently, in order to control several fruits pests, the food producers apply high doses of synthetic pesticides, like the blue-bags with Chlorpyrifos, used for banana and soursop cultures, searching to control the ants and other pest transported by them (cochineal).

Material and methods

As a basic part of this project, it was selected an organic banana orchard located near the town of Anolaima (Cundinamarca), where firstly, the most abundant weeds growing there were collected and selected. These samples were classified and from them the essential oils were extracted by hydrodistillation, the respective hydrolates preserved and with the residue, the alcoholic extracts were obtained. With the oils, hydrolates (aqueous extract) and the raw polar extracts, different treatments were designed to be applied to a group of banana plants affected for at least three different type of ants. Actually the ant species are in taxonomical classification. The weed species selected included *Hypoestes phyllostachya* (Acanthaceae) that come from Madagascar, *Ageratum conyzoides* (Asteraceae) of wide distribution in Latin America *Cimnapogon citratus* (Poaceae) ("asiangrass") and other two species from Fabaceae and Labiatae families actually in botanical classification. Binary and ternary treatments in composition (0.5-2.5%) were applied weekly for 6 times and the results were evaluated and will be discussed.

Results

The ants population was reduces or deterred and utilization of residual products such as weeds essential's oils and extracts from a specific orchard, could give good results to control some pests from fruits and can reduce the application of herbicides and wide spectrum pesticides like Glyphosate and Chlorpyrifos.

Conclusions

Use of agricultural or agro-industrial residues as part of preliminary formulations as pest control agents are an interesting and friendly strategy to protect the environment and the welfare of workers.

P-47 Analysis of antennal phenotype of synanthropic populations of *Panstrongylus chinai* from Loja province-Ecuador

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Introduction

Chagas disease (ChD) is caused by the protozoan *Trypanosoma cruzi*. *Panstrongylus chinai* is one of the main vectors of ChD that has been found naturally infected with *T. cruzi*. This species has been reported inhabiting synanthropic environments (domestic and peridomestic). The adaptation of triatomines to different habitats including a series of morphological and behavioral changes, its understanding could provide a suitable design of vector control campaigns. The use of geometric morphometric and antennal phenotype allow the interpretation of the functional and phenotypic adaptations of certain species to different habitats, hosts and environmental factors. The aim of this study was to characterize the antennal phenotype of synanthropic populations of *Panstrongylus chinai* from Loja province-Ecuador.

Material and methods

58 antennae of *P. chinai* were analyzed under an optical microscopy and a drawing chamber, three types of chemoreceptors and one mechanoreceptor were mapped and counted along the entire length of pedicel and the two flagellar segments. Multivariate discriminant analysis of the data was performed in the PAD software version 84.

Results

The results showed the existence of differences between habitats (intradomicile vs. peridomicile) in males, but in females differences attributed to the habitat were not detected. Additionally, the existence of sexual dimorphism in this species was evidenced.

Conclusions

We can mention that the study of the antennal phenotype is a simple and efficient indicator for the recognition of the origin of triatomines that attempt to colonize new habitats. Finally, *P. chinai* should be categorized as a potential vector of ChD, and should be monitored continuously in the Andean valleys of Loja (Ecuador) and sectors of Peru.

P-48 Synthesis of 3,7- dihydroxy- 4,6- dimethoxy aristolactam from boldine. A model compound to study their uptake and biotransformation by larvae of *Battus polydamas archidamas*

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Introduction

In order to perform a sequestration and biotransformation study of ALs in *Battus polydamas archidamas* is necessary to add to the natural diet (*Aristolochia chilensis*) an AL with a different substitution pattern of the ALs naturally found in *A. chilensis*.

The *de novo* synthesis of ALs is particularly challenging and occurs with very low yield. This research presents as an alternative, the chemical modification of the aporphine alkaloid boldine, to produce 3,7-dihydroxy-4,6-dimethoxy aristolactam

Material and methods

Boldine (Sigma-Adrich) was modified according to reaction sequence of Figure 1

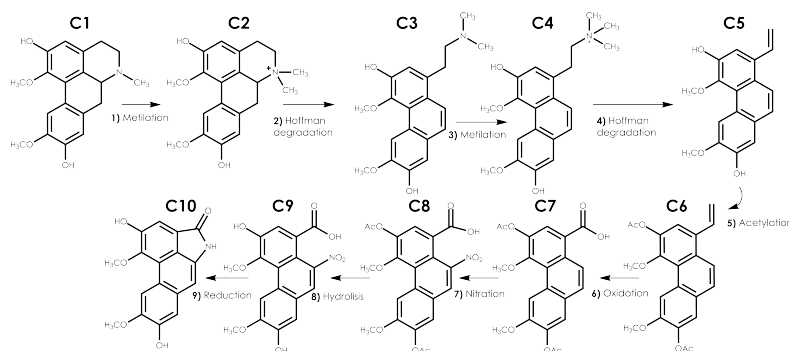


Figure 1 Scheme synthesis of 3,7 Dihydroxy-4,6-dimethoxy aristolactam

To determine the structure and purity of synthetic compounds, a combination of HPLC/DAD, NMR and LC/ESI/MS was used.

Results

Following the proposed method, the synthesis of 3,7-Dihydroxy-4,6-dimethoxy aristolactam was achieved with 20% yield.

Conclusions

The chemical modification of aporphine alkaloids, with a convenient pattern of substitution, is a simple alternative to produce aristolactam in good yield.

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P-49 Antennal responses to plant and animal-derived volatiles in *Lucilia sericata* females during different phases of the reproductive cycle

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Introduction

Lucilia sericata females use chemical information to localize decaying bodies only during certain phases of their reproductive cycle where high amounts of proteins are needed for ovarian development and for ovoposition. The aim of this work was to test age-dependent regulation of the antennal responses to volatiles derived from animal and plants in *Lucilia sericata* females during different phases of their reproductive cycle.

Material and methods

We compared the electrophysiological responses of *Lucilia sericata* induced by an animal-derived volatile (Butanol) and a plant-derived volatile (Citral), on young (2 days old) and gravid (7 days old) females using extracellular recordings.

The age of the flies was determined by comparing the ovary size and the size of the posterior cross vein on the left wing.

Results

No evidence of age related regulation was observed in the electrophysiological responses of females of *Lucilia sericata* exposed either to butanol or citral. However, adaptation of the electrophysiological responses were highly different between the two volatile compounds tested. Butanol produces a phasic response while citral produces a tonic response. We suggest that the differences in adaptation observed may be due to intrinsic specialization of the chemical receptors located in the cuticular sensilla on the antennae of *Lucilia sericata*. Such differences in adaptation could reflect the differences in chemical structure between volatile compounds with specific ecological importance to *Lucilia sericata*.

Conclusions

No evidence of age-dependent regulation was observed in the olfactory system of *Lucilia sericata*, but further investigation is needed to explain the differences in adaptation of the antennal responses observed and their possible relation with the chemical structure and ecological relevance of the volatile compounds tested.

P-50 Potential allelopathic effect of *Cecropia pachystachya* Trec on *Lactuca sativa*

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Introduction

Cecropia pachystachya Trec, also known as embauba is distributed from Mexico to Argentina. Due to the abundance of this plant in native and restored forested areas it is believed that they have an advantage over other plants including weed species, either through direct interference or allelopathic effects. The aim of this study was to evaluate the allelopathic activity of ethanol extracts from the root and the stalk of *C.pachystachya* upon seed germination of *Lactuca sativa*.

Material and methods

The ethanol extracts were prepared from crushed and dried plants, obtaining extracts from the root, the stalk, root bark and stalk bark. For the bioassays, each sample was solubilized in water and DMSO (0.1%) at different concentrations. Six samples were used each extract sample, DMSO as negative control, and distilled water as the blank, each one with four replicates. Throughout the bioassays, Petri dish were covered with 4 layers of filter cake and humidified with 7.5mL of test sample. 30 *L.sativa* seeds were added onto each petri dish and maintained at 25°C in a sterile environment with a 12h12h photoperiod throughout 14 days.

Results

Obtained results were analyzed using variability test and the averages were compared using the Scott-Knott test with a 5% of probability using the SAEG software. Results showed a significant difference between the various extracts and control. The extract that showed greatest activity were the root extract (8.94mg/mL) and from the stalk (9.58 mg/mL). They also demonstrated effect upon germination and development of the root and hypocotyl.

Conclusions

One can conclude that this plant showed allelopathic effects with potential for agricultural usage.

P-51 Preliminary Study on the Identification of the Sex Pheromone of *Nomophila indistinctalis* (Lepidoptera: Crambidae)

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Introduction

Nomophila indistinctalis is a polyphagous species, with a distribution in Chile from I - IX Region and Juan Fernandez Island. Its main hosts are beet and broadleaf weeds, so it is considered to be of economic and quarantine importance. A large number of pheromones have been identified from Crambidae; however in this species no studies regarding the identification of the sexual pheromone exist. Considering this, it is important to identify the pheromone in order to develop applications for the control of this pest based on the pheromone.

Material and methods

Nomophila indistinctalis adults were collected in Tierruca field, San Clemente (Chile) during March 2014. Moths were taken to the laboratory and were placed in vials in groups and fed with honey and corn until egg laying. The larvae were supplied with an artificial diet Stonefly *Heliothis* until the emergence of adults. Female moths were observed over a period of 72 hours to characterize the calling behaviour. Subsequently, female abdominal glands were extracted in hexane at -10 °C for 2 hours and the extracts were analyzed by GC/MS and GC/EAD.

Results

Our preliminary studies indicate that 2-3 day old females are calling at the end of the scotophase, between 4 and 6 am.

GC/MS analysis of the female gland extracts showed the presence of candidate compounds, which could not be identified yet.

Conclusions

The calling behavior of adult females has been established, however, more work is needed to identify the pheromone candidates.

P-52 *Pseudomyrmex mordax* response to volatiles emissions of Neotropical *Triplaris cumingiana*

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Introduction

Plant volatiles show a varied composition respect their functional group. Some organisms are attracted favoring pollination; in other cases generate allelopathic effects as well as plant-insect associations occur; in this case the ants hosted in the tree represent an indirect strategy for the tree to defend against herbivores. The absence of ant colonies represents an increase in herbivory that affects significantly the growth and reproduction of plants. Insects show a specific behavior when exposed to volatile plant compounds. The objective of this study is to identify the volatiles compounds of *Triplaris cumingiana* leaves and test the response of *Pseudomyrmex mordax* ants to certain constituents present in the plant.

Material and methods

The volatiles compounds of the leaves of *Triplaris cumingiana* were obtained by Solid Phase Microextraction (SPME), the fiber used in the experiment was a 50/30 μm SPME fiber DVB/Carboxen/PDMS Stable Flex (Supelco, USA), The fibers were saturated during 15 min at 25 °C and analyzed using GC/MS and GC/FID, with a HP-5MS column. Bray–Curtis analysis was done to compare significant differences of compounds between undamaged and damaged leaves. After the identification of the volatiles compounds, the response of the ants was studied against factors such as foliar damage, vibration signals, visual cues, plant extracts and green leaves volatiles.

Results

A total of 39 compounds were identified, with different functional groups. The major components identified were (*E*)-2-hexenal, (*E*)-3-hexen-1-ol and hexanal. The ants responded to all treatments; with respect to the substances used, (*E*)-2-hexenal which result in the faster treatment to induce ant recruitment in high magnitudes.

Conclusions

Of the 39 compounds identified, were founded two were aldehydes, three ketones, seven esters, eight alcohols, fourteen terpenes and five of varied composition. The major volatiles compounds were mainly aldehydes and alcohols of six carbons, commonly known as green leaf volatiles. Ants responded positively to all the treatments, but the (*E*)-2-hexenal was the best signal in produce and indirect defense response.

P-53 Chemical profile of *Memnoniella levispora* fungus in different medium crop

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Introduction

The Brazilian citriculture represents one of the most important segments of Brazil agribusiness, however several pests and diseases, for example the Gomose, affecting the citrus crop, causing considerable economic and environmental losses. The Gomose has species of genus *Phytophthora* as the causal agent. Due to the importance of citrus and indiscriminate use of pesticides, alternative methods of gomose's control are necessary. By this reason the *Memnoniella Levispora* fungus, shown as resistance inductor potential against this pathogen. The main purpose of this work is analyze the chemical profiles in different culture medium, using spectroscopy techniques to optimize the growth conditions of the fungus.

Material and methods The culture media tested was Potato dextrose (PD), PD supplemented with 1% yeast extract, PD supplemented with 1% malt extract, Czapeck and Carrot-corn (30gL⁻¹ of carrot, 30gL⁻¹ of corn). Two discs of 5 mm diameter of isolated's pure culture were transferred to Erlenmeyer flasks of 500 ml, containing 200 ml of distilled water and the culture media. After 21 days of incubation, the mycelium and liquid part were separated by vacuum system. The mycelium was dried and the liquid were partitioned with ethyl acetate and butanol. The respective extract were concentrated and analyzed by HPLC-UV and NMR.

Results The chromatography screening was made comparing the blank (culture medium without fungus) and their respective medium, additional of this, comparing other medium too. The Carrot-corn medium culture, showed the best growth mycelia and major productions of substances.

Conclusions

We concluded that the best medium culture for the large scale growth to further elucidate and isolate these substances is the carrot-corn.

P-54 Studying the metabolomics of sugarcane during herbivory by *Diatraea saccharalis*

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Introduction

It has been estimated that about 10% of sugarcane crop loss is caused by insects, and sugarcane borer, *Diatraea saccharalis*, is the most important sugarcane pest in Brazil. It causes damage by tunneling within the stalk, which reduces stalk weight and sucrose yield.

Material and methods

Seventeen sugarcane plants, 40 days after planting, were subjected to herbivory by second-instar larvae of *D. saccharalis*, analyzed over a 72 hours period, and harvested every 24 hours. Sixteen plants were not submitted to herbivory and used as a control group. Aiming at metabolomics analysis, leaf extracts were prepared in a system of deuterated solvents (water / methanol), and subjected to ¹H- and J-resolved- NMR experiments. The ¹H NMR data were submitted to orthogonal projection on latent structure-discriminant analysis (OPLS-DA).

Results

Several metabolites could be identified by analysis of J-resolved spectra alanine, isoleucine, threonine, valine, aspartate, asparagine, succinic acid, lactate, malic acid, choline, inositol, glycine, serine, glucose, sucrose, shikimic acid, fumarate, caffeoyl quinic acid, acotinic acid, syringic acid and trigonelline. The OPLS-DA of all samples showed a clear separation between samples of control and herbivory groups. Furthermore, a notable variation within each group could be observed for samples harvested at different times of herbivory (24, 48 and 72 hours). Therefore, OPLS-DA was also applied to assess the metabolic changes occurring in different times of herbivory (C24h versus H24h; C48 versus H48h and C72h versus H72h), resulting in a good distinction between the subgroups.

Conclusions

The use of NMR coupled with OPLS-DA allowed differentiation of control samples of sugarcane from those subjected to *D. saccharalis* herbivory, at 24, 48, and 72 hour points. The OPLS-DA loadings plot indicates in all times a depletion of sugars and also an elevation of acaffeoyl, probably chlorogenic acid, and ferruloyl derivatives (probably chlorogenic acid) in plants after herbivory.

P-55 Chemical defense, nutritional quality and herbivory on the mistletoe *Phoradendron carneum* (Loranthaceae) and its host plant *Ipomoea murucoides* (Convolvulaceae) in a fragmented landscape in Mexico

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Introduction

Forest fragmentation can have negative effects on the performance and functioning of plants and modify biotic interactions. Parasitic plants (mistletoes) can increase their establishment success in fragmented forests due to a combination of changes in biotic and abiotic conditions. Similarly, chemical defense and plant nutritional quality are factors affecting the preference of herbivores that may change in fragmented environments. The objectives of this study were to determine the patterns of herbivory, nutritional quality and chemical defense in the mistletoe *Phoradendron carneum* and its host-plant *Ipomoea murucoides* in a gradient of fragmentation in the Cuitzeo Basin, Mexico.

Material and methods

The study was conducted in three site with different degrees of fragmentation. In each site, 10 *I. murucoides* individuals infected with *P. carneum* and 10 uninfected individuals were selected. For each host individual diameter at breast height (DBH) and abundance of mistletoes were recorded. Leaves were collected from infected and uninfected *I. murucoides* individuals and from a mistletoe from each infected host, and used to quantify levels of herbivory damage (using the Assess Image Analysis Software), water content and the concentration of total nonstructural carbohydrates (determining phenol and H_2SO_4), phenols (Folin-Cicalteau) and flavonoids (determination $NaNO_2$ and $AlCl_3$).

Results

The mistletoe infection level was higher in individuals of *I. murucoides* that had higher DBH and at the site that had a higher degree of fragmentation. Also, individuals of *I. murucoides* infected with *P. carneum* were those that had greater herbivore damage. A negative relationship between the number of established mistletoes per host and phenol concentration was found in the host. The number of mistletoes and herbivory levels were negatively correlated with carbohydrate concentration in individuals of *I. murucoides*.

Conclusions

The results suggest that mistletoe infection significantly alters the production of secondary compounds in their hosts, increasing the levels of herbivory by insects in fragmented environments.

P-56 Identification of Male-Produced Aggregation Pheromone of the Curculionid Beetle *Acrotomopus atropunctellus*

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Introduction

The sugarcane stem weevil, *Acrotomopus atropunctellus* (Boheman) (Curculionidae Molytinae Cholini) is an endemic species to provinces of Tucumán, Salta and Jujuy, northwestern region of Argentina. Since the last decade, the species has become a serious pest due to the increased sugar cane production (*Saccharum officinarum*), over 250,000 hectares. The adult weevils are small, hard to found in the field, and difficult to manipulate in the laboratory. They produce small holes with irregular borders in buds, stems and central veins of leaves. Females lay eggs in the basal and middle portions of young plants. Larvae feed inside the stems and reach the stumps by winter, reducing the reshooting capacity of cane in subsequent growth seasons. Until now, there are not reports about the chemical ecology of this weevil.

Material and methods

The colony was established from *Acrotomopus atropunctellus* adults collected from sugarcane fields located in Cruz Alta, Tucumán, Argentina, in January 2012 and 2013. Groups of 20 males and 20 females were kept separately in all-glass aeration chambers with two pieces of sugarcane. Volatile emissions were collected every 48 h and trapped with TENAX TA polymer. Charcoal-filtered, humidified air was pushed through the aeration system (1.0 L/ min). Adsorbed volatiles were eluted with 1 ml of hexane HPLC grade. The extracts were analyzed by CG-FID and CG-MS.

Results

In chromatograms of male emissions was possible to observe a male-specific compound with a molecular mass of 126 g.mol⁻¹. The NIST spectrum library proposed 6-methyl-5-hepten-2-one. The identity was confirmed by co-injection with a commercial standard. Bioassays were carried out to study the attractiveness activity of this compound.

Conclusions

It was possible to confirm the presence of a male-specific compound in *A. atropunctellus*, its identity and action as an aggregation pheromone because attracted both sexes.

P-57 Effect of acaricides in semiochemicals of healthy and *Nosema* parasitized honeybees

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Introduction

Semiochemicals are important clues that regulate colony homeostasis in *Apis mellifera*. Cuticular hydrocarbons (CHCs) are involved in social recognition and ethyl oleate (EO) plays a role as primer pheromone in honeybees. Different pathogens can alter these semiochemicals. We here present a study on the effect of 3 widely used acaricides on CHCs and EO when applied to *Nosema ceranae*-infected and uninfected bees.

Material and methods

To test the effect of *Nosema* infection and amitraz, coumaphos and fluvalinate on CHCs and EO we follow a procedure previously described (J Chem Ecology 36 522, 2010). Hexane extracts from healthy and infested bees were partitioned by SPME. Two of the fractions obtained were analyzed by GCMS. Quantification was done using arachidonic acid methyl ester as internal standard for EO and normalizing the total area for CHCs.

Results

CHCs showed no overall differences among the 8 experimental groups (ANOVA tests, $P > 0.05$). The only difference found was between methylalkanes in healthy bees (5.5 ± 0.5) and *Nosema*-infected bees $8.4 \pm 1.4 \mu\text{g}$ (t-test, $P < 0.05$). On the other hand, in this study EO was not higher in *Nosema*-infected bees compared to healthy ones (5 ± 1 and $10 \pm 4 \mu\text{g}$ respectively), as it was previously reported. However, when the 3 chemicals were applied separately, EO did increase compared to untreated healthy bees. Further, in the case of coumaphos, EO increased in both, healthy and infected bees (34 ± 9 and $42 \pm 13 \mu\text{g}$ respectively). Besides, the levels of two other ethyl esters (linolenic and oleic) were also affected by the treatments.

Conclusions

While we did not find an effect of *Nosema* infection on the bee pheromones, the use of chemical treatments to prevent or treat comb diseases may alter the normal colony homeostasis, irrespective of the colony being ill or not.

P-58 Chemometrics in the analysis of complex volatile chemical mixtures

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Introduction

GC-MS analysis of complex mixtures with underlying biological questions involves the identification and peak-integration of individual compounds for subsequent multivariate statistical analysis. This strategy is time consuming, and often implies the subjective exclusion of minor components. Raw GC-MS data can also be analyzed by data-driven information extraction, an approach known as chemometrics. Here we present a comparative analysis of both methods, using cuticular hydrocarbons (CHCs) from honeybees.

Material and methods

CHCs were extracted from hive and forager bees after visiting artificial feeders with different sucrose-solution treatments. GC-MS data (19 samples/treatment) were first analyzed by manual integration of 48 peaks, with subsequent Principal Component Analysis (PCA) of net CHC amounts. For the chemometric approach, GC-MS files were converted to Network Common Data Form (NetCDF) and imported into MZmine2.10, defining parameters for mass detection, chromatogram building and peak deconvolution. Chromatograms normalized for retention times and peak areas were then aligned (RANSAC tool), generating a matrix of 1235x76 that was subjected to PCA.

Results

Both methods produced identical results, with a clear separation of hive bees from forager bees with respect to their CHCs, and no separation of forager bees according to feeding treatment. Furthermore, eight out of ten most relevant CHCs singled out by their PCA loadings were the same in both methods. While the conventional method took approximately one week for processing the experimental data set, the chemometric approach took approximately 30 min for file conversion and additional 30 min to produce the first PCA results.

Conclusions

While expertise is the key factor for identifying biologically relevant compounds, we here show that data-driven methods are efficient and reliable tools when complex volatile profiles are under investigation. A quantitative overview of such data sets can be achieved within one day, and the main compounds to focus on for identification can be efficiently selected.

P-59 Identification, Characterization and Synthesis of the Sex Pheromone Components of *Thelosia camina*

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Introduction

The production of yerba mate (*Ilex paraguariensis*) is an important economic and social activity in Brazil. There are countless applications of this species, such as drinks, natural coloring, medicines, toilet articles and more. Within the production system of mate some pests can reduce the productivity such as *Thelosia camina* (LEPIDOPTERA EUPTEROTIDAE). The use of synthetic sexual pheromones is one strategy that can be used to disrupt mating and reduce pest populations. Identification and characterization of chemical compounds with biological activity is the first step towards developing these synthetic compounds.

Material and methods

The natural pheromone of this insect was obtained by solvent extraction of the dissected pheromone glands from the female abdomen. The chemical structures of the identified compounds were characterized by spectroscopic information obtained by GC-EAD, GC-MS and GC-FTIR

Results

The electroantennographic activity of the natural compounds tested in GC-EAD, showed responses to three major compounds. The structural elucidation of these compounds was based on comparison of the mass fragmentation of the compounds in the literature and their Kovats index and spectroscopic data obtained by GC-FTIR profile. The position of the conjugated double bonds seen in the infrared spectra of analyzed compounds were determined by the addition of 4-methyl-1,2,4-triazoline-3,5-dione (MTAD). In the mass spectra of the female extract derivatized with MTAD, the presence of peaks related to fragmentations at carbons bonded directly to the cycloadduct was observed. Based on these results, three structures were proposed to the most abundant compounds 13Z, 15Z-18Ald (Kovats 2081); 13Z, 15Z-18OH (Kovats 2143) and 13Z, 15Z-18Ac (Kovats 2269). A synthetic route was proposed in order to obtain standards of the major components of the extracts from glands of female *Thelosia camina*

Conclusions

It was possible to identify and synthesize three major compounds from *Thelosia camina* using GC-MS, GC-FTIR, KI and microderivatizations. The attractiveness of males to the reference compounds is ongoing.

P-60 Acaricide activity of Annonaceae fractions against *Tetranychus urticae* and metabolic profile of *Duguetia lanceolata* by GC-MS analysis

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Introduction

Two-spotted spider mite *Tetranychus urticae* is a polyphagous and cosmopolitan species, that causes damage to various crops of economic importance. Thus, in order to contribute to the development of new products for the control of *T. urticae*, the objective of this study was to evaluate the bioactivity of Annonaceae fractions for *T. urticae* and perform an exploratory analysis of the selected fraction through gas chromatography-mass spectrometry (GC-MS).

Material and methods

In this study, the leaves, stem barks and berry fruits of *Duguetia lanceolata* were employed for the preparation of methanol extracts. The methanol extracts were subjected to liquid-liquid partition using water and dichloromethane. After removal of the solvent in a rotary evaporator, the soluble fractions in dichloromethane were employed in the test with *T. urticae* adult females. The method employed was a microimmersion bioassay. The most active fraction, *D. lanceolata* stem barks, was employed to study the metabolic profile analysis by GC-MS.

Results

The fractions from leaves, stem barks and berry fruits of *D. lanceolata* affected the survival of *T. urticae* females. However, the best results were found when the fraction *D. lanceolata* stem barks were used. This fraction caused 94.1% mortality in *T. urticae* females after 24 h from the beginning of the bioassay. The metabolic profile analysis of the fraction *D. lanceolata* stem barks by GC-MS, suggested that the main constituents are 2,4,5-trimethoxystyrene and trans-asarone.

Conclusions

The fraction from *D. lanceolata* stem barks reduced *T. urticae* survival. According to GC-MS analysis, the major constituents of this fraction are 2,4,5-trimethoxystyrene and trans-asarone. The results of this study show the potential of the fraction *D. lanceolata* stem barks in the development of new products for the control of *T. urticae*.

P-61 Effect of acetylsalicylic acid on the resistance dynamics of several species of horticultural crops against foliar mycosis

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Introduction

Pathogens are responsible of a great part of the decrease in the agricultural crop production. Facing these stresses, plants present a varied system of resistance going from physical barriers up to molecular signaling. In the last ten years the interest of using plants resistance induction as a method for pests and pathogens control has increased dramatically. However, often studies in this field do not pay attention on the possible different outcome related to plant species response diversity. Despite the large volume of research regarding this topic most of it has been performed in a small number of “model” species, such as *Arabidopsis thaliana*.

Material and methods

In a group of four horticultural crops species we applied acetylsalicylic acid (AAS) as resistance inductor before and after a fungal infection. Followed we evaluated plant response in terms of foliar associated symptomatology (wilting, chlorosis and necrosis).

Results

We found every plant species used respond in a different fashion to the treatments.

Conclusions

Our result stresses the relevance of using different species as models in the study of plant resistance dynamics. In relation our proposal of using plant resistance in applied endeavors this requires local refocusing both theoretical and applied efforts placing emphasis on fieldwork testing.

**P-62 Olfactory response of *Telenomus podisi* and *Trissolcus basal*
(Hymenoptera: Platygasteridae) to the sexual pheromone of different
hosts (Hemiptera: Pentatomidae)**

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Introduction

The egg parasitoids *Telenomus podisi* and *Trissolcus basal* have received significant attention as biological control agents of the pentatomid stinkbug-soybean complex, such as *Euschistus heros*, *Piezodorus guildinii* and *Nezara viridula*. To locate its hosts these parasitoids use mainly chemical signals and cues originated by plants or the host itself (e.g. alarm pheromones, host tracks, and vibratory signals). The present work aimed to evaluate the behavior of *T. podisi* and *Tr. basal* towards the isolated sexual pheromones of possible hosts (*E. heros*, *P. guildinii*, and *N. viridula*) from solutions in different concentrations, considering that each parasitoid has a host preference that maximizes their fitness.

Material and methods

The attraction of both parasitoids to the sexual pheromone of its hosts were evaluated using a dual-choice olfactometer ('Y'-type) where 24 hours-old naive females were put and had their first choice (χ^2) and residence time (Wilcoxon test) observed. For that analysis, five solutions were made (Eher1, Eher2, Eher3, Pgui, and Nvir), each one in three different concentrations (1.0 mg/mL, 0.1 mg/mL e 0.01 mg/mL), totaling 15 treatments. For each set of bioassay (n=5) 5 μ L of solution were used.

Results

Both the parasitoids showed a selective response towards the treatments in different concentrations (*T. podisi* - Eher3 (0.01 mg/mL), Pgui (0.01 mg/mL) e Nvir (0.1 mg/mL); *Tr. basal* - Eher1 (1.0 mg/mL e 0.1 mg/mL) Eher3 (1.0 mg/mL) e Pgui (1.0 mg/mL).

Conclusions

Telenomus podisi and *Tr. Basalis* were attracted by the sexual pheromones of possible hosts and there was a dose-dependent influence on its attraction. However, the results differed from the initial hypothesis related to a different host preference between the two parasitoids.

P-63 Herbivore-induced Volatile Organic Compounds emitted by Maize elicit Electrophysiological Responses in *Spodoptera frugiperda* females

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Introduction

Herbivore-induced volatile organic compounds (VOCs) are known to act as an indirect defense by attracting natural enemies of herbivores. In addition to natural enemies, induced VOCs are perceived by other organisms such as herbivores. In nature, herbivores exploit plant VOCs to find suitable oviposition sites and/or food sources. According to the “preference-performance hypothesis” mothers prefer to lay eggs on host plants that will allow their offspring develop better. A few studies have shown that herbivores can be repelled by herbivore-induced VOCs, but aggregation behavior has also been observed in response to previous infestation. In comparison to the vast amount of studies that addressed natural enemies’ responses to herbivore-induced volatiles, few studies have focused on how herbivores respond to induced VOC blends.

Material and methods

VOCs from control and *Spodoptera frugiperda*-damaged plants were collected for 24 hours, eluted with double-distilled HPLC-grade hexane and analyzed by GC-MS. Compounds were identified on the basis on Kovats Indexes and mass spectra. The response of the antennae of virgin and gravid females to VOCs was accomplished by GC-EAG technique using plant extracts from herbivore-damaged plants. Dual-choice oviposition tests (healthy vs damaged plants) were also conducted.

Results

GLVs, terpenoids, and indole were induced upon herbivory. From these, (Z)-3-hexenyl acetate, β -linalool and 1-H indole elicited response in all the antennae tested. In addition Eight out of 11 antennae or more responded to ylangene/(+)-cyloisativene, (E)- β -farnesene and TMTT. Other compounds that elicited response in a few of the antennae were DMNT, geranyl acetate, (E)- α -bergamotene and β -sesquiphellandrene. Larger numbers of eggs and eggs per cluster per plant in control plants.

Conclusions

As in other *Spodoptera* species, adult females of *S. frugiperda* can perceive several compounds induced by conspecifics. These compounds may “warn” mother about possible competition and predation, preventing them from ovipositing.

P-64 Repellent effect of the *Nothofagus dombeyi* (Mirb.) Oerst. essential oil on *Aegorhinus superciliosus* (Coleoptera: Curculionidae)

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Introduction

Aegorhinus superciliosus (Guérin) (Coleoptera Curculionidae) is an important native pest in fruit crops, mainly in blueberry fields in the south of Chile. This insect cause significant damage during its adult stage defoliating leaves. However, the most significant damage is produced by the larval stage, when feeding on roots and rootles, drilling holes and forming galleries. At present, traditional control methods (mainly chemical and biological) have not been fully satisfactory. Therefore, the searching of new control alternatives is imperative. The aims of this study were to evaluate the biological activity of *N. dombeyi* essential oil, a native tree from Chile, on *A. superciliosus* and to identify the volatile compounds present in its composition.

Material and methods

Olfactometric bioassays were used for determinate the behavioral response of *A. superciliosus*. The extract was obtained from leaves of *N. dombeyi* for steam distillation and the resultant emulsion was extracted with dichloromethane. For the identification of volatile metabolites, Gas chromatographic and mass spectral was used.

Results

Main results showed which *N. dombeyi* extract elicited a repellent effect in both sexes of *A. superciliosus*. The main compounds indentified were α -santalene, nerolidol and α -terpineol.

Conclusions

The bioactivity of the *N. dombeyi* essential oil on *A. superciliosus* could be attributed to the presence of terpenes in the chemical composition of the extract.

P-65 Chemical phenotypes of *Persea americana* var. *drymifolia* and its relation to the attack *Trioza anceps* (Trioziidae)

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Introduction

Persea americana var. *drymifolia* (creole avocado) wild forests are in a precarious condition. One way to rescue their richness is through the establishment of germplasm banks; several Mexican institutions have been given the task of preserving, evaluating and documenting genetic resources of avocado in these banks. We analyzed the foliar chemistry of creole avocado populations located at the 1) germplasm bank of the National Institute of Forestry, Agriculture and Livestock in Celaya, Guanajuato, Mexico; and 2) Facility and Advanced Research Unit for Agricultural Biotechnology, Faculty of Agrobiology "Presidente Juárez" of the Universidad Michoacana de San Nicolás de Hidalgo in Uruapan, Michoacan, Mexico. The purpose was to compare the chemical profiles of these two germplasm banks and detect their relation to gall-forming insects.

Material and methods

We used gas chromatography and mass spectrometry to determine the foliar chemical profiles of avocado trees in two germplasm banks.

Results

For the germplasm bank at Celaya the predominant compound was estragole with 38% followed by 11% caryophyllene; *p*-cymene, sabinene, *b*-pinene, anethole, eugenol-methyl-ether, germacrene D, caryophyllene oxide and hexadecane were between 2% and 4%; and some trees were heavily attacked by galls. For the germplasm bank at Uruapan, major compounds were as follow estragole 27%, caryophyllene 6%, β -pinene 5%, hexadecanoic acid 5%, heptacosane 4% and α -tocopherol 6%; this bank showed no attack of galls-forming insects.

Conclusions

We found specific chemical profiles that are related to the incidence of gall-forming insects. With this work we can recommend resistant avocado individuals that may be established in places where there is a high incidence of gall-forming insects.

P-66 Testing the host and qualitative manipulation hypothesis with the system

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Introduction

Evidence suggests that the parasites can affect characters in hosts and vectors to increase transmission. The host manipulation hypothesis suggests that parasites modify host's phenotypic character that benefits the transmission. On vectors, the qualitative manipulation hypothesis states that infected vectors show attraction to suitable hosts for the parasite. In Chagas disease transmission is unknown the effect of *Trypanosoma cruzi* infection on the odor profile of vertebrate host and the attraction of vectors infected or non-infected. To answer these questions, we carried out behavioral experiments to test the interaction *Rhodnius prolixus* - *T. cruzi* - *Mus musculus*.

Material and methods

A modified T-shape olfactometer was used to evaluate the attraction response of fifth instar nymphs of *R. prolixus*, against the odor profile emitted by *Mus musculus* (swiss strain) infected and non-infected with *Trypanosoma cruzi* strain Dm28 (host manipulation hypothesis). To test the qualitative manipulation hypothesis, infected *R. prolixus* nymphs and infected or non-infected *Mus musculus* were used in the experiments. Binomial tests were used to analyze the experimental data.

Results

Mus musculus non-infected attracted significantly infected and non-infected *R. prolixus* when tested against the air. However no statistically significant differences were found in attraction of infected or non-infected *R. prolixus* to *M. musculus* infected tested against *M. musculus non-infected*.

Conclusions

No behavioral evidence was found in the *R. prolixus* - *T. cruzi* - *M. musculus* system supporting the host or the qualitative manipulation hypothesis mediated by odor profile.

P-67 Sequestration of aristolactams from *Aristolochia chilensis* in *Battus polydamas archidamas* (Lepidoptera, Papilionidae)

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Introduction

The Aristolochiaceae contains aristolactams (ALs), a group of aporphinoids with a phenanthrene backbone in their structure.

Recently, sequestration study of ALs from *A. chilensis* in *B. polydamas archidamas* shows that AL-I and AL-II present in *A. chilensis* were sequestered by fifth instar larvae of *B. polydamas archidamas*. The relation AL-II/AL-I in *A. chilensis* was much lower than in *B. polydamas archidamas*, suggesting preferential biotransformation of AL-I (demethylation and glycosylation) and / or bioaccumulation of AL-II.

In order to study the possible biotransformation of AL-I to more polar ALs comparative study of the ALs fraction of *B. polydamas archidamas* and *A. chilensis* was preformed.

Material and methods

Fifth instar larvae of *B. polydamas archidamas*, and leaves of *A. chilensis* were collected (33 ° 28 'S and 70 ° 56' W) and were processed to give fractions of neutral compounds. The PTLC separation of both extracts yielded the ALs fractions. Those, were analyzed by HPLC/DAD and LC/ESI/MSⁿ using ALs standards and interpretation of mass spectra.

Results

From leaves of *A. chilensis* the following ALs were identified AL-I, AL-II, 2-OMe-AL-I, AL-AII, piperolactam and velutinam.

From larvae of *B. archidamas polydamas* the following ALs were identified AL-I, AL-II, 2-OMe-AL-I, AL-BII, AL-Ia, AL-I-N-glucoside, AL-Ia-N-glucoside and AL-IVa-N-glucoside.

Conclusions

The sequestration of ALs by larvae of *B. polydamas archidamas* is selective; the phenolic ALs identified in the leaves of *A. chilensis* were not detected in larvae. Also AL-BII was identified in the larvae but not in the plant suggesting bioaccumulation.

The presence of AL-Ia, AL-I- N-glucoside AL-Ia-N-glucoside and AL-IVa-N-glucoside in the larvae is in agreement with biotransformation of the AL-I and AL-II.

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P-68 Chemical cues for host plant location by the grape weevil, *Naupactus xanthographus* (Coleoptera, Curculionidae)

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Introduction

The grape weevil, *Naupactus xanthographus*, is an insect present in southern South America, with polyphagous habit and high reproduction rate. In Chile, it is considered a primary pest due the damage caused by larvae and adults. Also, it has quarantine status in the United States and Japan. Its main host species are economically important species, such as grape, avocado, or apple. In this work, we studied the volatile compounds involved in behavioral responses of males and females to two host plants, grape (*Vitis vinífera*), and avocado (*Persea americana*).

Material and methods

Constitutive and herbivore-induced volatiles were collected in a system “Headspace Entrainment Equipment”. The behavioral responses to different stimuli were determined using a Y-tube olfactometer. Finally, the identification of compounds emitted by both host plants was performed by GC/MS. Significant differences in the constitutive and herbivore-induced profiles were analyzed by PCA (Principal Component Analysis).

Results

Our results indicate that males are attracted to i) constitutive volatiles over hexane, ii) herbivore-induced volatiles over hexane, and iii) herbivore-induced over constitutive volatiles. Also, males showed no attraction when presented to volatiles of both host plants simultaneously. Females showed no significant preference to constitutive or herbivore-induced volatiles.

GC/MS analysis allowed identifying volatile compounds emitted by these hosts, mainly GLVs, terpenes and aromatic compounds.

The model PCA allowed discriminating compounds involved in the different behavioral responses in Y- bioassays. For both hosts, the preference of males to herbivore-induced volatiles is associated with a low concentration of some terpenes (e.g. (E,E)-cosmene) and aromatic compounds (e.g. 3-ethylbenzaldehyde) in grape, and the high concentration of b-ocimene in avocado.

Conclusions

We conclude that volatiles compounds of the plants are importante for *N. xanthographus* to locate their hosts. The differences in the responses are associated with variability in the emission profiles for each host.

P-69 Microbiological Associations in a Cecidogenic Interaction between *Haplopappus foliosus* (Asteraceae) and its Gall Midget Parasite (Diptera: Cecidomyiidae: Asphondylia).

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Introduction

Most plant-insect associations relies on the contribution of interacting microorganisms, in the case of gall-making insect fungi and bacteria are related in plant tissue growth induction as well as insect protection and nurturing. In this work we explored the biodiversity and composition of the microbiological community associated to the formation of an apical gall in the plant *Haplopappus foliosus* (Asteraceae) by a gall midget fly from the genus *Asphondylia* sp. (Diptera Cecidomyiidae).

Material and methods

Gall-induced and healthy apical branches were studied to determine microbial diversity through microbiological culture. Samples were inoculated in Sabouraud, PA and M1 medium (agar and broth). Microbial isolates were characterized for morphology, pigment production and bioactives compounds. Some fungal isolates secreted substances in agar surface which were collected by capillarity. Microbial composition was determined by Margalef diversity index, resulting in values of 10,25 and 1,95 for galls and apical branches respectively (N=60).

Results

Based on our preliminary exploration we found significant differences in the biodiversity index when comparing gall vs apical branch. Furthermore, when we cultured some of the fungi found inside the gall we found out that there was a given strain whom profusely secreted a dense substance.

Conclusions

We are currently using analytical techniques in order to disentangle the composition and potential function of these secretions in the formation of the gall association.

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