



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

2° Meeting of the Latin American Association of Chemical Ecology

December 2-5, 2012 | Huerta Grande | Argentina



2^o Meeting of ALAEQ Latin American Association of Chemical Ecology

*December 2-5, 2012
Huerta Grande-Córdoba,
Argentina*

Organized by

Latin American Association of Chemical Ecology

Centro de Investigaciones Científicas y
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Facultad de Agronomía, Universidad de Buenos
Aires, Argentina

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Organization



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Dear Colleagues,

Welcome to Huerta Grande, Argentina, and to the 2nd Meeting of the Latin American Association of Chemical Ecology (ALAEQ). After the success of our first meeting in Uruguay in 2010, which resulted in new intraregional collaborations, we were encouraged to organize this second edition.

Chemical Ecology is not new in our region and its growth was reflected in, and catalyzed by, the Brazilian EBEQ meetings. Our field continues to grow and is now being consolidated in more Latin American countries. Still, we need to strengthen our Association. We need all relevant players to work together towards the advancement of our field and, importantly, towards the application of our research in Latin America. Clearly, Chemical Ecology can make a contribution towards the development of the much needed sustainable, environmentally friendly, pest management strategies.

Fortunately, we again reached 150 participants from six Latin American countries (and Africa!), and the number of abstracts has clearly increased from our first meeting. Our Latin American community is hard at work and we have every reason to believe our scientific production will continue growing. Let us then meet once more, present our new work, get excited with the work of others, and discuss future collaborations and the future of our Association.

We want to thank our colleagues that helped organizing this event and, of course, all participants for their support. We also want to thank our sponsors, in particular Chemtica, and the speakers that came a long way from North America and Europe to present their work and discuss the work of others.

We wish you a productive and enjoyable meeting !

Jan Bergmann, Jorge Zavala, Pablo Guerenstein

PROGRAM

Sunday, December 2

- 14:00 - 17:30 **Registration and mounting of posters**
- 17:30 - 17:45 **OFFICIAL WELCOME AND OPENING REMARKS**
- Jan Bergmann**, President of the ALAEQ
- Pablo Guerenstein**, President of the Organizing Committee of ALAEQ 2012
- 17:50 - 18:40 **INAUGURAL CONFERENCE**
Chair: Pablo Guerenstein
- “Reverse Chemical Ecology”: Predictions for Chemical Ecology and Behavior of Moths from Olfactory Neurobiology*
John Hildebrand, University of Arizona, USA
- ORAL PRESENTATIONS I**
Chair: Jorge Molina
- 18:45 – 19:00 O1 - *Host plant-derived volatile blends: how do changing ratios of constituents influence female insect behavior and neurophysiological responses?*
Adriana J. Najar-Rodriguez, ETH Zurich, Institute of Agricultural Sciences, Switzerland
- 19:00 – 19:15 O2 - *Attraction of carrion beetles (Oxelytrum discicolle) to carcass volatiles and identification of the male sex pheromone*
Douglas H. Fockink, Universidade Federal do, Brasil.
- 19:30 - 21:30 **Welcome Reception**
-

Monday, December 3

- 08:15 - 08:50 **CONFERENCE**
Chair: Paulo Zarbin
- Cerambycid Beetles: A Gold Mine of New Semiochemistry*
Jocelyn Millar, University of California - Riverside, USA

COLLOQUIUM SESSION: “Chemistry of pheromones and other semiochemicals”

Chair: Paulo Zarbin

8:55 – 9:05

Introduction

9:05 – 9:35

The sex pheromone of the citrophilus mealybug: From identification to the development of applications in pest management

Jan Bergmann, Pontificia Universidad Católica de Valparaíso, Chile

9:35 – 10:05

The Absolute Configuration of Pheromones of Mealybugs and Triatomines

C. Rikard Unelius, Linnaeus University, Sweden & The New Zealand Institute for Plant & Food Research Ltd

10:05 – 10:35

Synthesis of Pheromone for Insect Control

Antônio Euzébio Goulart Santana, Universidade Federal de Alagoas, Brasil

10:35-10:55

Coffee Break

10:55 – 11:25

Highlights in Pheromone Chemistry at the Laboratório de Semioquímicos (UFPR)

Paulo H. G. Zarbin, Universidade Federal do Paraná, Brazil

SYMPOSIUM: “Marine Chemical Ecology”

Chair: Renato Crespo Pereira

11:30 – 11:40

Introduction

11:40 – 12:05

Marine Chemical Ecology: advancing into an unexplored area

Claudia Muniain, Universidad Nacional de San Martín, Argentina

12:05 – 12:30

Chemical studies on sponges of the genus Cliona

Leonardo Castellanos, Universidad Nacional de Colombia, Colombia

12:30 – 12:55

Marine Chemical Ecology in Brazil: origin, development and perspectives

Renato Crespo Pereira, Universidade Federal Fluminense, Brasil

12:55 - 14:10

Lunch break

- 14:10 - 14:40 **COLLOQUIUM**
How knowledge about the neurobiology and the behavior of the honeybee can contribute to improve pollination services and crop yield in sunflower fields
Walter M. Farina, Universidad de Buenos Aires, Argentina
- 14:45 - 14:55 **SYMPOSIUM: “Neurobiology of chemoreception”**
 Chairs: Fernando Locatelli and Marcelo Lorenzo
 Introduction
- 14:55 - 15:15 *The pleasure of eating in haematophagous insects*
Romina B. Barrozo, Universidad de Buenos Aires, Argentina
- 15:15 - 15:35 *Electrophysiological activity of ticks sensilla to phagostimulants, phytoecdysteroids and to host-related and tick pheromone-related synthetic compounds*
Lígia M. F. Borges, Universidade Federal de Goiás, Brazil
- 15:35 - 15:55 *Could pre-imaginal olfactory experiences modify the post-metamorphic odor-mediated responses in a social insect?*
Gabriela P. Ramírez, Universidad de Buenos Aires, Argentina.
- 15:55 - 16:15 *Plasticity in olfactory circuitry. Mating modulates olfactory tuning in Spodoptera littoralis*
Eduardo Hatano, Swedish University of Agricultural Sciences, Sweden
- 16:15 - 16:35 *Associative and non-associative plasticity improves perception of the relevant components in an odor mixture*
Fernando Locatelli, Universidad de Buenos Aires, Argentina
- 16:35-16:55 **Coffee Break**
- 17:00 - 17:30 **COLLOQUIUM**
Electrophysiological characterization of Grapholita molesta (Lepidoptera: Tortricidae) olfactory receptor neurons
César Gemeno. Universidad de Lleida, Spain
- ORAL PRESENTATIONS II**
 Chair: Walter Farina
- 17:35 - 17:50 *O3 - Activation and orientation of Rhodnius prolixus males in response to odors emitted by females*
Marcelo Lorenzo, René Rachou Institute/FIOCRUZ, Brazil

- 17:50- 18:05 *O4 - Bitter taste perception in the blood-sucking insect Rhodnius prolixus*
Isabel Ortega Insaurralde, Universidad de Buenos Aires, Argentina
- 18:05 - 18:20 *O5 - Sexual Behaviour in Rhodnius prolixus (Heteroptera, Reduviidae): the role of cuticular lipids*
Alicia Lorenzo-Figueiras, Universidad de Buenos Aires, Argentina
- 18:20 - 18:35 *O6 - Effects of biotic and environmental factors on the calling behaviour of virgin Pseudaletia adultera (Lepidoptera: Noctuidae) females*
Guillermo Rehermann, Universidad de la República, Uruguay
- 18:35 - 20:10 **POSTER SESSION 1**
- 20:30 – 22:00 **Dinner**
- 22:30 **Social Event: “Fogón”**
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Tuesday, December 4

- 08:15 - 08:50 **CONFERENCE**
Chair: Eraldo Lima
- Chemical Communication Among Plants and Insects*
Consuelo M. De Moraes, Penn State University, USA
- 08:55 - 09:05 **SYMPOSIUM: “Plant-insect and plant-plant interaction”**
Chair: Jorge Zavala
- 08:55 - 09:05 Introduction
- 09:05 - 09:30 *Contemporary evolution of Diabrotica in the Corn Belt*
Jorge A. Zavala, Universidad de Buenos Aires, Argentina
- 09:30 - 09:55 *Host selection, oviposition behavior and leaf traits of Salix spp. in a specialist willow sawfly*
Patricia C. Fernandez, EEA Delta del Paraná, INTA and Universidad de Buenos Aires, Argentina
- 09:55 - 10:20 *Tritrophic interaction Telenomus podisi – Euschistus heros – soybean cultivars*
Mirian F. F. Michereff, Embrapa Recursos Genéticos e Biotecnologia, Brazil

- 10:20 - 10:45 *Consumption of Pteridium spp and trophic transfer of allelochemicals to specialist herbivorous insects*
Maria Pia Calcagno, Universidad de Los Andes, Venezuela
- 10:45-11:05 **Coffee Break**
- 11:10 - 11:45 **CONFERENCE**
Chair: Jorge Zavala
- Light regulation of plant immunity*
Carlos Ballare, IFEVA-CONICET, Universidad de Buenos Aires, Argentina
- ORAL PRESENTATIONS III**
Chair: Maria Pia Calcagno
- 11:50 - 12:05 *O7 - Pyrrolizidine alkaloids (PAs) sequestered from both native and invasive Crotalaria host species provide chemical protection for the arctiid moth Utetheisa ornatrix*
Carlos H. Z. Martins, Unicamp, Brazil
- 12:05 - 12:20 *O8 - Characterization of phenylpropanoid compounds in soybean seeds induced by stinkbugs attack and methyl jasmonate application with HPLC- Mass Spectroscopy*
Lucía G. Barriga, Universidad de Buenos Aires, Argentina
- 12:20 - 12:35 *O9 - Role of volatile compounds in the interspecific interaction of Naupactus xanthographus (Coleoptera, Curculionidae) with two host plants*
Vera E. Waleska, Pontificia Universidad Católica de Valparaíso, Chile
- 12:35 - 14:05 **Lunch break** [40-min Meeting Executive Committee]
- 14:05 – 14:35 **COLLOQUIUM**
Manipulation of host-derived olfactory cues by vector-borne pathogens
Mark C. Mescher, Pennsylvania State University, USA
- 14:40 - 15:15 **CONFERENCE**
Chair: Cristian Villagra
- Chemical ecology of host-seeking in malaria mosquitoes: olfactory physiology, behaviour and applications*
Joop J.A. van Loon, Wageningen University, The Netherlands

SYMPOSIUM: “Chemical ecology of disease vectors”

Chairs: Carolina Spiegel and Pablo Guerenstein

- 15:20 - 15:30 Introduction
- 15:30 - 15:50 *Aedes aegypti* responses to co-specific mosquito extracts and a cuticular compound
Kelly S. Paixão, Universidade Federal de Minas Gerais, Brazil
- 15:50 - 16:10 *The complex reproductive behavior of Lutzomyia longipalpis complex (Diptera: Psychodidae)*
Carolina N. Spiegel, Instituto Oswaldo Cruz/Fiocruz, Brazil
- 16:10 - 16:30 **Coffee Break**
- 16:30 - 16:50 *Rhodnius prolixus* attraction to volatiles emitted by *in vitro* growing bacteria isolated from human faces
Jorge Molina, Universidad de los Andes, Colombia
- 16:50 - 17:10 *Development of methods for monitoring and control vectors of Chagas disease using host-odors*
Fabio Guidobaldi, CICYTTP-CONICET, Argentina
- 17:10 - 17:30 *Strategies to control triatomines based on sensory ecology principles and community participation*
Fernando Otálora Luna, Instituto Venezolano de Investigaciones Científicas, Venezuela

ORAL PRESENTATIONS IV

Chair: Jan Bergmann

- 17:35 - 17:50 O10 - *Differential expression of gut trypsin of Anticarsia gemmatalis larvae in response to soybean (Glycine max) protease inhibitors*
María Daniela Tejedor, INBA, CONICET, Universidad de Buenos Aires, Argentina.
- 17:50 - 18:05 O11 - *The sex pheromone of the Poplar moth Condylorrhiza vestigialis (Lepidoptera: Crambidae)*
Diogo M. Vidal, Federal University of Parana, Brazil
- 18:05 - 18:20 O12 - *Alarm pheromones of Pachycoris torridus*
Andréa M. V. Ferreira, Federal University of Sergipe
- 18:20 - 18:35 O13 - *Cuticular compounds recognition and mating behavior of the rice water weevil Oryzophagus oryzae (Coleoptera, Curculionidae)*
Camila B. C. Martins, Universidade Federal do Paraná, Brazil

- 18:35 - 20:10 **POSTER SESSION 2**
- 20:30 – 22:00 **Dinner**
- 22:30 **Social Event: “Karaoke”**
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Wednesday, December 5

- 08:15 - 08:50 **CONFERENCE**
Chair: Patricia Fernandez
- Semiochemical Based Attraction of Small Hive Beetle: a Window into Evolution and Invasive Biology*
Peter E. A. Teal, CMAVE-USDA-ARS, USA
- SYMPOSIUM: “Semiochemicals in Pest Management”**
Chair: Andrés González
- 08:55 - 09:05 Introduction
- 09:05 - 09:30 *Controlling Insect Pests with Specialized Pheromone & Lure Application Technology (SPLAT®)*
Agenor Mafra-Neto, ISCA Technologies, USA
- 09:30 - 09:55 *Current development and commercial potential of the pheromones in Argentina*
Enrique A. Lobos, Universidad Nacional de Santiago del Estero, Argentina
- 09:55 - 10:20 *Semiochemicals in the integrated pest management of temperate deciduous fruits in Latin America*
Eduardo Fuentes-Contreras, Universidad de Talca, Chile
- 10:20 - 10:45 *New application tactics to increase the use of semiochemicals in Latin American agriculture*
Raúl A. Laumann, Embrapa Recursos Genéticos e Biotecnologia, Brasil
- 10:45-11:05 **Coffee Break**
- 11:10 - 11:40 **COLLOQUIUM**
Chemical communication in the bronze bug, Thaumastocoris peregrinus (Hemiptera: Thaumastocoridae)
Andrés González, Universidad de la Republica, Uruguay

- 11:45 - 12:15 **COLLOQUIUM**
New Frontiers for Manipulation of Beneficial Insects in Crop Protection: from Chemical to Multimodal Communication
Miguel Borges, Embrapa Genetic Resources and Biotechnology, Brazil
- 12:20 - 12:50 **COLLOQUIUM**
Pheromones: Effect of social and political context on adoption of chemical ecology products in threshold countries
Alan Cork, University of Greenwich, UK
- 12:50 - 14:15 **Lunch break**
- ORAL PRESENTATIONS V**
Chair: Andrés González
- 14:15 – 14:30 O14 - *Identification and formulation of pheromones of the ambrosia beetle Megaplatypus mutatus and field management using reservoir and monolithic delivery systems*
Paola González Audino, Centro de Investigaciones de Plagas e Insecticidas. CIPEIN-CITEDEF-CONICET, Argentina.
- 14:30 – 14:45 O15 - *Olfactory Memories Promote Toxic Bait Ingestion*
Analía Mattiacci, Universidad de Buenos Aires, Argentina
- 14:45 – 15:00 O16 - *Reverse Chemical Ecology of Hylamorpha elegans Burmeister (Coleoptera: Scarabaeidae)*
Andrés E. Quiroz, Universidad de La Frontera, Chile
- MINI-SYMPOSIUM: “Functional Genomics of Chemoreception”**
Chair: Marcelo Lorenzo
- 15:05 - 15:15 Introduction
- 15:15 - 15:35 *Molecular bases of chemoreception in Rhodnius prolixus*
Jose M. Latorre-Estivalis, CPqRR-FIOCRUZ, Brazil
- 15:35 - 15:55 *Molecular and sensory correlates of host recognition in mosquitoes*
Omondi Bonaventure Aman, Swedish University of Agricultural Sciences, Sweden
- 15:55 - 16:15 **Coffee Break**
- ORAL PRESENTATIONS VI**
Chair: Andrés Quiroz

- 16:20 - 16:35 O17 - *Influence of UV-B radiation in chemical and photosynthetic response of the freshwater macrophyte Nymphoides indica (Menyanthaceae)*
Nathália Nocchi, Federal University of Rio de Janeiro, Brazil
- 16:35 - 16:50 O18 - *Interaction of Chemical and Structural Components Providing Defenses to Sea Pansies Renilla reniformis and Renilla muelleri*
Etiene E.G. Clavico, Fluminense Federal University, Brazil
- 16:50 - 17:05 O19 - *Intraspecific variation on herbivory defenses in freshwater plant Typha domingensis Pers.*
Luana Gonçalves, Federal University of Rio de Janeiro, Brazil
- 17:10 - 17:20 **CLOSING REMARKS**
- 17:25 - 18:25 **GENERAL ASSEMBLY**
- 20:30 ***Farewell Cocktail and Party***
-

Thursday, December 6

MORNING DEPARTURE

CONFERENCES
Abstracts

Sunday 2 / 17:50 - 18:40

**“Reverse Chemical Ecology”: Predictions for Chemical Ecology
and Behavior of Moths from Olfactory Neurobiology**

John G. Hildebrand

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*Department of Neuroscience, University of Arizona, Tucson, AZ 85721-0077
(USA)*

A principal goal of research in my laboratory is to understand neurobiological mechanisms through which information about behaviorally significant olfactory stimuli is encoded, processed, and integrated with inputs of other modalities in the insect brain. Specifically, we aim to learn how olfactory information ultimately initiates and controls the characteristic, natural behavioral responses of the giant sphinx moth *Manduca sexta*. We strive to advance fundamental knowledge about olfaction and at the same time to contribute to understanding of insect biology in the quest for improved methods for controlling agriculturally and medically harmful species and fostering beneficial insects.

Our studies have focused mainly on the antennal lobes (ALs), the primary olfactory centers in the moth's brain. As for such centers in the brains of most vertebrates and invertebrates, the ALs are characterized by glomeruli—condensed-neuropil structures in which primary-sensory and central neurons interact synaptically. We endeavor to understand how primary-sensory inputs from olfactory receptor cells are processed in glomeruli and represented in their outputs. In particular, our recent work asks how “meaningful olfactory objects”—natural, behaviorally significant mixtures of volatile organic compounds—are encoded in the neural outputs from the ALs.

We are especially interested in olfaction-dependent behaviors that are crucial for the survival of the moths: mate-seeking and interactions with host plants for feeding and oviposition. In some of our studies, olfactory neurobiology has led us to recognize naturally occurring volatiles that function in the chemical mediation of such interactions. This presentation will highlight examples of this approach, which we regard as “reverse chemical ecology,” or “chemical ecology by way of the brain.”

The research described in this presentation has been supported by research grants and contracts from NIH, NSF, DARPA, and Monsanto Company.

Monday 3 / 08:15 - 08:50

Cerambycid Beetles: A Gold Mine of New Semiochemistry

Jocelyn G. Millar¹, Lawrence M. Hanks², Ann M. Ray³

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²*Department of Entomology, University of Illinois, Urbana-Champaign, IL 61801, USA*

³*Department of Biology, Xavier University, Cincinnati, OH 45207 USA*

A number of species in the large coleopteran family Cerambycidae are pests of woody plants in their native countries, and they are of increasing importance as invasive pests when transported to new countries. Despite their importance, little is known about their use of semiochemicals; a decade ago, male-produced aggregation pheromones had been identified from <10 species. Since then, rapid progress has been made in a number of related areas. First, field tests with known cerambycid pheromones and analogs have shown that many species use pheromones. These trials have also shown that there is considerable biosynthetic parsimony within the family, with related species often using the same or very similar pheromone compounds. Second, a number of novel pheromone structures have been identified, including both male-produced aggregation pheromones and female-produced sex pheromones. Third, the first examples of antagonistic effects caused by pheromone components of congeners, to minimize cross attraction between sympatric species, have been documented. Fourth, effective traps and release devices for detection and monitoring cerambycids have been developed. To date, pheromones or likely pheromones have been identified from several hundred species.

Field trials also have shown that cerambycid pheromones are often synergized by host plant volatiles. However, as with other types of wood-boring insects such as bark beetles, species that feed on conifers may be repelled by volatiles from deciduous trees, and vice versa. Furthermore, cerambycids can be attracted to pheromones of other species that share the same hosts.

Overall, we now know that use of volatile pheromones is widespread among cerambycids, with pheromones known from species in 5 subfamilies. Patterns of pheromone use within tribes and subfamilies are beginning to emerge. It is clear that there is much more to be discovered in terms of semiochemistry, and even more to be discovered about how semiochemicals mediate cerambycid behaviors.

Acknowledgments: This research was supported by grants from The Alphawood Foundation, Xavier University, USDA NRI grant #2009-35302-05047, and continuing support from USDA-APHIS.

Tuesday 4 / 08:15 - 08:50

Chemical Communication Among Plants and Insects

Consuelo M. De Moraes

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Center for Chemical Ecology, Department of Entomology, Penn State University, University Park, PA USA 16802

Plant volatiles are airborne chemicals emitted by plants as a by-product of normal physiological activity and also specifically in response to insect feeding, pathogen infection, and other environmental stimuli. These volatile emissions can provide information about the location, identity, and status of the emitting plant to other organisms that perceive them. Volatile-mediated interactions among plants and insects are widespread and well documented. In addition to serving as foraging cues for insect herbivores and pollinators, plant volatiles function as an indirect mode of plant defense by attracting predatory and parasitic insects that attack feeding herbivores. The possibility that volatiles play a similarly important role in plant-to-plant communication has long been debated, but only very recently has the prevalence and ecological significance of volatile signaling within and between plants become clear. A number of recent studies from my research program have contributed to an increased appreciation of the complexity and sophistication of volatile mediated plant-insect interactions and an enhanced understanding of the role of volatiles in plant-to-plant communication. In this presentation I will describe some of our recent and ongoing research and discuss its significance for broader conceptual issues in chemical ecology.

This talk could not be presented for reasons beyond the control of the speaker

Tuesday 4 / 11:10 - 11:45

Light regulation of plant immunity

Carlos L. Ballaré

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Plants have sophisticated defense systems to protect their tissues against the attack of herbivorous and pathogenic organisms. Many of these defense mechanisms are orchestrated by the plant hormone jasmonate (JA). Immune responses activated by JA are tightly controlled by the ecological context of the plant. Phytochrome B (phyB), a photoreceptor that plants use to detect the proximity of other plants, is emerging as a key regulator of JA signaling (1, 2). This photo-regulation of JA responses appears to play an important ecological role, modulating the allocation of limited resources to different physiological functions, including growth and defense (3). In this presentation, I will discuss recent advances in our understanding of the molecular mechanisms whereby phyB modulates defense responses, and address the functional implications of the cross-talk between phytochrome and JA signaling.

1. J. E. Moreno, Y. Tao, J. Chory, C. L. Ballaré, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4935 (2009).
2. I. Cerrudo *et al.*, *Plant Physiol.* **158**, 2042 (2012).
3. C. L. Ballaré, *Trends Plant Sci.* **16**, 249 (2011).

Acknowledgments : Supported by CONICET, ANPCyT and UBACyT

Tuesday 4 / 14:40 - 15:15

Chemical ecology of host-seeking in malaria mosquitoes: olfactory physiology, behaviour and applications

Joop J.A. van Loon¹, David Menger¹, Wolfgang R. Mukabana², Willem Takken¹

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²International Centre of Insect Physiology and Ecology, Nairobi, Kenya.

Malaria mosquitoes locate their blood hosts mainly by chemical cues present in human emanations. We have taken approaches integrating molecular, electrophysiological and behavioural paradigms to increase our understanding of the chemical ecology of host-seeking behaviour of *Anopheles gambiae*, the main vector of human malaria in Africa. Building upon a basic blend of carbon dioxide, ammonia, L-lactic acid and tetradecanoic acid we identified additional human-skin derived components to be used in baits to trap malaria vectors and other tropical mosquitoes. The aerial concentrations at the trap outlet at which some of these compounds are effective were 25-50 ppb [1]. Several of these synthetic odour blends were competitive with a human host with respect to their attractiveness. The focus in our work has subsequently shifted from volatile kairomones to spatial repellents. The most effective repellent compounds acted at ppb-levels [1]. We combined traps releasing attractants and dispensers emitting repellents to assess the potential of a push-pull system. Challenges to be addressed are to achieve a sustained release [2] and to establish a suitable spatial arrangement of traps and dispensers. A push-pull system was employed in a semi-field situation where it significantly reduced house entry of female *Anopheles gambiae*.

[1] Smallegange R.C., Bukovinszkyne-Kiss G., Otieno B., Mbadi P.A.; Takken W., Mukabana W.R. & van Loon J.J.A. (2012) *Physiological Entomology* 37: 60-71.

[2] Mukabana W.R., Mweresa C.K., Omusula P., Orindi O.B., Smallegange R.C., van Loon J. J.A., Takken W. (2012) *Parasites and Vectors* DOI: 10.1186/1756-3305-5-202.

This study was funded by a grant from the Foundation for the National Institutes of Health (FNIH) through the Grand Challenges in Global Health Initiative (CGGH#121).

Wednesday 5/ 08:15 - 08:50

Semiochemical Based Attraction of Small Hive Beetle: a Window into Evolution and Invasive Biology

Peter E. A. Teal¹, Adrian J. Duehl¹, Ayuka T. Fombong², Hans T. Alborn¹, Baldwin Torto².

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Insect behavioral preferences are tied to individual experience and evolutionary history. The Small hive beetle, *Aethina tumida*, is seemingly an anomaly among Nitidulids because members of the genus commonly feed on fruit and decaying material in association with fungi but the small hive beetle thrives in honey bee hives feeding on pollen and bee brood. However, we observed small hive beetle attraction to fruits in the field and compared this attraction with previous laboratory studies establishing attraction to chemical cues from bee hives and a symbiotic yeast fermenting pollen. Bioassays established that ripe fruits: Kei apples, cantaloupe and pears, were all more attractive than fermenting pollen dough, the attractant for small hive beetle attraction identified from honey bee colonies. The fruit produced compounds and ratios responsible for attraction of the beetle had many similarities and some differences to bee hive odors, associated with attraction. In Africa, fruit hosts are only seasonally available, while bee hives offer a more continuous and nutrient rich habitat particularly in seasonal times of drought when fruit are not available. The commonality among attractive volatiles between hosts, along with reproductive success on fruit indicate that the attractive preferences of the small hive beetle do indeed represent an evolutionary link in a poorly resolved clade. These overlapping preferences show a fruit feeding ancestry, but the beetle's physical and behavioral derived characteristics indicate how it has branched into a new habitat niche.

COLLOQUIA
Abstracts

COLLOQUIUM SESSION: “Chemistry of pheromones and other semiochemicals”

Monday 3 / 8:55 – 11:25

The sex pheromone of the citrophilus mealybug: From identification to the development of applications in pest management

Jan Bergmann¹, M. Fernanda Flores¹, Ashraf El-Sayed², Rikard Unelius^{2,3}, Tania Zaviezo⁴

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The citrophilus mealybug *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae) is a cosmopolitan species and is thought to be native to Australia. This polyphagous species feeds on a variety of host plants including citrus, avocado, berries, sugarcane, cocoa, grape, and apple and it has spread out of its native habitat and is currently geographically distributed worldwide. In New Zealand, it is a vector of grapevine leafroll-associated virus type 3 (GLRaV-3), which causes significant declines in quantitative and qualitative parameters of vine performance. Due to quarantine restrictions imposed by several countries, the presence of *P. calceolariae* and other mealybug species causes significant economic losses to Chilean fruit exporters.

We identified the sex pheromone of *P. calceolariae* to be chrysanthemyl 2-acetoxy-3-methylbutanoate by means of aeration of cohorts of virgin females, gas chromatography-mass spectrometry, derivatization reactions, and synthesis of model compounds. The synthetic pheromone was highly attractive to males in field tests [1, 2].

Applications for the pheromone of *P. calceolariae* and other mealybug species present in Chile are now being developed. The goal is to establish mealybug pheromones as a part of monitoring and/or control programs in Chilean agriculture. The first steps taken in this direction were the establishment of formal collaboration agreements between universities, growers, exporters associations, and the governmental Agricultural and Livestock Agency. Field experiments are now being carried out to evaluate the potential of the pheromones to be used in IPM programs in different crops.

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The authors thank Jocelyn Millar for constant support of their work. JB and TZ acknowledge funding by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT grant 11060527) and Fondo de Fomento al Desarrollo Científico y Tecnológico (FONDEF grant D10I1208). This work was supported by the Foundation for Research Science and Technology, FRST (Sustainable Integrated Pest Management in Horticulture, C06X0811).

The Absolute Configuration of Pheromones of Mealybugs and Triatomines

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At least twelve mealybug pheromones have been discovered until now and they all have unique structures involving unusual monoterpene esters. The determination of the absolute configuration of the Citrophilous mealybug sex pheromone was a real challenge as it contains three stereocenters and that air entrainments from thousands of mealybugs did not give enough material for an NMR sample.

GC-MS, hydrolysis and transesterification reactions in the microscale followed by synthesis, gave clues to the absolute configuration. But it was only at the final stage that the insects themselves gave us the unambiguous answer and we could finally deduce the structure of the three stereocenters in this evasive diester pheromone related to a pyrethroid.

New rather spherical structures released by the metasternal glands of at least two Triatomine vectors of the lethal parasite *Trypanosoma cruzi* (Chagas disease) has been identified. Here we report on their structure elucidation. In addition, a group of analogs has been identified from *Triatoma* spp, forming a new class of semiochemicals. The compounds seem to be involved in chemical communication between sexes as indicated by Vitta et al. 2009.¹

The synthesis involved a novel key step in the preparation of secondary alcohols; the reduction of a carboxylic ester to the corresponding aldehyde by means of DIBAL and *in situ* alkylation by a Grignard reaction at low temperature. All four stereoisomers of one of the new compounds were synthesized, and the absolute configuration of the natural product was determined by enantioselective gas chromatography and co-injection with the authentic reference samples on a modified cyclodextrin column.

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Synthesis of Pheromone for Insect Control

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There are several concerns about pesticides use in insect control. Since the seventies and eighties, the concept and practice of integrated pest management (IPM) strategies have evolved with the trend of reducing pesticide use. Indeed, pesticides were largely reported to induce human diseases and to be harmful for the environment. The success of pest control through pesticides is limited due to a series of reasons: (a) pesticides are very expensive compared to the crop losses avoided; (b) generally pesticides are non species-specific and can cause damages in beneficial insects; (c) many pests develop resistance towards chemical treatments; (d) pesticides are recognised to be unsafe for environment and human health. In general, synthetic pheromones are used in IPM, being selective and safe. Several improvements have been obtained in this area and slow-release devices have been developed, however, the bottleneck of the area continues to be the long and expensive syntheses, once only low quantities of compounds are needed, compromising prices. Hundreds of pheromonal compositions are now available, obtained through diverse synthetical methodologies. Pheromones and para-pheromones are multifunctional compounds: saturated and unsaturated, chiral and achiral, cyclic and acyclic. In their syntheses, the challenge is to obtain the products with the adequate chemical and stereochemical purities, especially concerning unsaturated compounds. Several methodologies are available for the double bond synthesis, including the Wittig reaction. In the present work, we will discuss synthetic approaches to the production of pheromones, in scale useful for pest management.

Highlights in Pheromone Chemistry at the Laboratório de Semioquímicos (UFPR)

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Laboratório de Semioquímicos (UFPR) was created in 2002 and it works with two lines of research. The first one aims the isolation and structural identification of semiochemicals, particularly insect pheromones. Thus, we study insect behavior to these chemicals. The second aims to synthesize the identified compounds. Our goals are to study insect behavior to various odor sources; to isolate and identify semiochemicals involved in insect communication; to perform bioassays evaluating the biological activity of semiochemicals; to determine plant-insect interaction mechanisms and synthesize the identified pheromones. In the last 10 years several species were studied among Lepidoptera (Tortricidae, Crambidae, Saturniidae, Pyralidae), Coleoptera (Curculionidae, Chrysomelidae, Silphidae, Tenebrionidae) and Hemiptera (Pentatomidae and Coreidae). In this presentation it will be discussed our latest studies on pheromone isolation, identification and synthesis.

Acknowledgements: INCT - Semioquímicos na Agricultura, CNPq, CAPES and Fundação Araucária.

Monday 3 / 14:10 - 14:40

How knowledge about the neurobiology and the behavior of the honeybee can contribute to improve pollination services and crop yield in sunflower fields

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The exponential increase of cultivated areas in the last century has promoted a sudden decline in biodiversity in agricultural ecosystems. Many of these crops need animal pollination as the main resource to improve yield. However, due to the decline of native pollinators, these ecosystems have become increasingly dependent on a single species as a pollen vector, the honeybee *Apis mellifera*. This situation forces to reevaluate the effect of these disturbed environments on this insect species, and also to rethink strategies to improve pollination efficiency within these agricultural settings by using honeybees. We focused on the seed-sunflower crops as an ecosystem with essential requirements of honeybees as the pollen vector. These crop fields require an intensive care, including the use of systemic insecticides and pollination services (allocation of healthy beehives around the cultivated area). In this environment, honeybees have to efficiently forage by using, among other abilities, their well-known individual and social learning. The associative learning gives them the possibility to revisit rewarding flowers, but also to acquire food-related information from other individuals within the colony, specifically the floral scent, which can be learned by hive bees of any age. With this in mind, on the one hand we analyzed whether associative learning might be impaired by non-target systemic insecticide traces such as those present in sunflower fields. On the other hand, we raised the question of whether a synthetic mix of floral volatiles that mimics the natural blend of sunflowers might improve the honeybees' pollination efficiency within the crop. Results at the levels of honeybee cognition and ecology together with landscape analysis by using GPS-yield mapping technology will be integrated to understand this tight honeybee-dependent crop.

Monday 3 / 17:00 - 17:30

**Electrophysiological characterization of *Grapholita molesta*
(Lepidoptera: Tortricidae) olfactory receptor neurons**

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We are investigating the olfactory neuroethology of *Grapholita molesta*, a small tortricid moth that affects peach and apple fruit production worldwide. Our aim is to understand the mechanisms involved in the pheromone-plant synergism reported in this species [1]. At the CNS level the antennal lobes of males and females have been reconstructed and the location and physiological response of projection neurons to pheromone odors has been determined [2], however little is known about the peripheral olfactory system. Males are tuned to a narrow ratio of the main sex pheromone components, Z8-12:Ac and E8-12:Ac, with no evidence for variation among populations from around the world. In males, ORNs for Z8-12:Ac and E8-12:Ac are housed in different sensilla trichoidea. A large proportion of male sensilla trichoidea do not respond to pheromone compounds. ORNs for the third pheromone compound, Z8-12:OH have not been found. Sensilla auriculica contain ORNs that respond to plant odors with varying degrees of specificity and sensitivity. Sensilla type, abundance and ORN specificity vary between the scaled and bare regions of the antenna.

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Tuesday 4 / 14:05 – 14:35

Manipulation of host-derived olfactory cues by vector-borne pathogens

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An increasing number of studies indicate that vector borne pathogens can alter the phenotypes of their primary hosts in ways that influence the frequency and nature of interactions between hosts and vectors. We have been exploring the effects of pathogens on host volatile emissions in a number of plant disease systems. This work suggests that pathogens frequently alter host odors (as well as other aspects of host chemistry) in ways that influence the behavior of insect vectors and of non-vector insects. Moreover, pathogen transmission mechanisms appear to be an important factor shaping the evolution of effects on host-vector interactions. I will present recent results from work on viral and bacterial plant pathogens and discuss implications for disease transmission, ecology, and diagnosis.

This talk could not be presented for reasons beyond the control of the speaker

Wednesday 5 / 11:10 - 11:40

Chemical communication in the bronze bug, *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae)

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Forest plantations in Uruguay have nearly doubled in the past decade, with fast-growing *Eucalyptus* spp. occupying nearly 70% of planted forests. The bronze bug, *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), originally restricted to Australia, has become one of the most important emerging pests of eucalyptus tree plantations in the Southern hemisphere. *T. peregrinus* is a small flattened bug that feeds on mature eucalyptus leaves, causing them turn brown and often fall from the tree. Although population dynamics and behavioural patterns in the field are not yet clearly understood, circumstantial observations suggest that males and nymphs aggregate, possibly by means of semiochemicals. We used gas chromatography coupled to mass spectrometry to analyze *in vivo* volatiles and cuticular lipids from virgin adults. Volatile extracts contained a male-specific hemiterpene derivative, 3-methylbut-2-enyl butanoate, which was identified from its mass spectral data and chromatographic comparison with a synthetic standard. Cuticular lipids from males also contained large amounts of 3-methylbut-2-enyl butanoate, along with smaller amounts of the isomer 3-methylbut-3-enyl butanoate. Both terpene derivatives were also found in female cuticular lipid extracts, though 3-methylbut-2-enyl butanoate in much smaller amounts. Interestingly, the acetate and propionate esters of 3-methylbut-3-en-1-ol were also present in female cuticular extracts and absent in the males. Y-tube olfactometer bioassays showed that virgin males are attracted to live virgin males, male volatile extracts, and 3-methylbut-2-enyl butanoate. Virgin females showed no such preference, suggesting that male volatiles do not play a role in sexual communication. Furthermore, male nymphs were attracted to 3-methylbut-2-enyl butanoate, reinforcing the notion of a male aggregation pheromone. The ecological significance of these compounds, and their potential use for the management of *T. peregrinus* in eucalyptus forests, will be further investigated.

Funding: ANII/Innovagro

Wednesday 5 / 11:45 - 12:15

New Frontiers for Manipulation of Beneficial Insects in Crop Protection: from Chemical to Multimodal Communication

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Achieving Food and Environmental security is recognized as one of the main directions of research in biological and agronomical sciences over the next few decades. New approaches to minimize the use of pesticides and improve the quality and productivity of food is crucial. Two tools have been proposed to minimize insecticide application on arable crops: (i) the use of semiochemicals from plants and insects and (ii) natural enemies. Soybean is one of the most important cultures in Brazil and stink bugs are the principal pests all around the country. They have been partially controlled by egg parasitoids (Platygastridae) through inundative release. However, this approach has limitations because it is laborious and costly. An alternative approach could be the use of semiochemicals to behavioural manipulation of natural enemies for conservative biological control strategies. Platygastridae wasps to locate their host use either herbivore-induced plant volatiles, herbivore-defensive compounds, sex pheromones and other host producer cues, vibratory signals for instance, to find their host. Another alternative is the manipulation of plant volatiles naturally as in push-pull systems or by breeding, metabolic engineering or exogenous application of naturally-occurring elicitors of plant defence to manage parasitoids. Notwithstanding, the dosage, time of application, and other aspects need to be improved before extensive use of this strategy in soybean crops. The integrated use of semiochemicals with other cues could help to develop more efficient tools for this behavioural manipulation. We intend to present results about the multimodal communication of Platygastridae egg parasitoid wasps, and discuss future steps required prior to application in agriculture.

Acknowledgments: The research was supported by FAP-DF, CNPq and Embrapa

Wednesday 5 / 12:20 - 12:50

Pheromones: Effect of social and political context on adoption of chemical ecology products in threshold countries

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In common with other IPM component technologies, semiochemical products have to be manufactured, marketed and promoted to achieve impact at the farmer level. This process is influenced by a wide range of stakeholders. For most researchers demonstrating efficacy of a product is the conclusion of a project but, in fact, it is just the beginning of a process that leads to sustainable adoption. Importantly, farmers require complete crop production solutions and a pheromone-based control method for one pest species can often be of insufficient value to warrant changing their cultivation practices to adopt. Ideally pheromone products should be compatible with and incorporated into packages of IPM technologies. This process is not helped by the fact that the concept of IPM is poorly defined; embracing a continuum from bio-intensive to chemically intensive systems. Pheromones and related semiochemicals are usually classified as biopesticides, but frequently come under the Pesticide Act of threshold countries because they are synthetic compounds, unlike related microbials which are not considered to be pesticides, despite their inherent toxicity! Classifying pheromones as pesticides has tax implications for importation of products and that will affect pricing and ultimately availability. Government initiatives to promote semiochemicals to farmers can have a profound influence on adoption but in some countries these activities have had a detrimental effect. Ultimately conditions have to be put in place that support the entrepreneurial skills of the commercial sector in order to fast-track IPM component technologies and, in particular pheromone products, into the market-place. The lecture will consider an example of a process that has ultimately led to sustainable farmer adoption on a large scale.

SYMPOSIA
Abstracts

SYMPOSIUM: “Marine Chemical Ecology”
Monday 3 / 11:30 – 12:55

Marine Chemical Ecology: advancing into an unexplored area

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In Argentina, Marine Chemical Ecology (MCE) is practically a vacant field; interpreting the role of secondary metabolites present in different marine organisms and their chemical and biological interactions is still a challenge to be met. Interdisciplinary research on natural marine products conducted in the last years has allowed us to elucidate the very often novel chemical structure and to recognize the origin of metabolites, which may be dietary or biosynthesized by the organisms. In several of these research works, interdisciplinary approaches involving MCE have sometimes remained inconclusive.

The present work provides a review of the principal results in the field of MCE, many of which have still not been published. Those findings have been reported for invertebrates and their prey, mainly mollusks and echinoderms from the Magellanic region. Most of these species are endemic to South America and inhabit from the intertidal to great depths^{1,2}. The chemical interactions evaluated with bioassays have been related to feeding and defense by means of *in situ* experimental studies, and in the marine laboratories set up for that purpose. Because of the great potential of marine biodiversity for global-scale interdisciplinary studies, we consider that these advances in MCE are the first step towards a promising research line.

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Chemical studies on sponges of the genus *Cliona*

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Excavating sponges of the genus *Cliona* compete for space with hard corals by undermining, killing, and displacing their live coral tissue [1]. Besides, these sponges have their own surfaces clean and free of epibiont organisms regardless of the strong fouling pressure. Dead coral tissue commonly present in the boundary between corals and *Cliona* sponges let us considering that some allelopathic compounds could be involved in the competitive interaction. Initial studies showed that crude extracts from these sponges kill coral tissue, have antifouling properties [1b] and decrease the photosynthetic potential of coral zooxanthellae [3]. In this study we determined which specific chemical compounds are responsible for such activity. The caribbean sponges *Cliona delitrix* and *C. tenuis* were chosen as study models. In order to establish the allelopathic activity, extracts, fractions and pure compounds were incorporated at approximately natural volumetric concentrations into Phytigel™ gels, and placed in contact with live coral [2]. After four days the health of coral tissue under the gels was evaluated. The most polar fraction of the raw extract of *C. tenuis* yielded clionapyrrolidine A as responsible of the allelopathic activity observed in the aqueous fraction [3]. This is the first report of a pure chemical produced by a sponge that kills coral tissue upon direct contact [4]. However, healed *C. tenuis* fragments placed directly onto live coral were killed readily by coral defenses, and fragments placed in close proximity to coral did not have any effect on the adjacent coral tissue [4], so the mechanism of action of this compound remains unknown. To test for antifouling activity, chemical fractions from sponges were also included on Phytigel™ gels and placed underwater as an artificial substratum. The amount of organisms attached on different gel treatments and controls was compared. The organic fraction of both sponges was characterized as responsible of the antifouling activity showed by the raw extracts. These fractions were identified as a complex mixture of methyl esters, and enriched fractions of glycerides, glycolipids, phospholipids and free fatty acids.

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Acknowledgements: Colciencias, DIB

Marine Chemical Ecology in Brazil: origin, development and perspectives

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This talk will explore the development of marine chemical ecology in Brazil since its origin to recent periods. Basic seaweed studies are predominant, mainly those approaches focused on the effect of crude extracts and some pure compounds evaluated as chemical defenses against consumers. These studies also constitute important contributions related to structure and function, intra-population variation, storage and transport of chemical defenses; recent studies are focused on *Laurencia* species (red seaweeds) as model to understand the “surface ecology” of defensive chemicals. The number of invertebrate explored in these chemical ecology studies are few, but they verified the presence and activity of secondary metabolites as chemical cues for hosts to associated organisms and the chemical defenses in exotic species. Studies on seaweeds and marine invertebrates were performed considering both ecological and evolutionary aspects of the biological interactions chemically mediated. Recent contributions explored the contribution of environmental and genetic factors for the production of chemical cues to understand the pattern of abundance and action of secondary metabolites and their roles in marine community structure. In addition, there are several evidences of chemical ecology studies as support to sustainable development and conservation of biodiversity, as well as to assert the economic development of Brazil.

Acknowledgements: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Financiadora de Estudos e Projetos (FINEP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), who have long been sponsors of our work on marine chemical ecology and bioactivity of marine natural products.

SYMPOSIUM: “Neurobiology of chemoreception”
Monday 3 / 14:45 - 16:35

The pleasure of eating in haematophagous insects

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Food taste delivers reliable information about the quality of feeding sources. In phytophagous animals, for example, bitter substances are generally toxic and poorly sweetened sources indicate poor nutritious diets. So, food quality recognition followed by an associated decision making will have important physiological consequences for animals. Triatomine insects feed on blood from vertebrates. Once they find a potentially suitable host (followed by olfactory and thermal cues principally), they pose over the host skin (*skin recognition phase*) and pierce it by moving their mouthparts until a venule or arteriole is found. Next, they pump a small quantity of blood initiating the *sampling phase* of food. If the sampled diet fulfils bug's feeding requirements, the insect will continue with the ingestion of food.

Up to now, no information is available in literature about fine discrimination of gustatory preferences in triatomines and how different cues might be modulating the decision making during the *skin recognition phase* and the *sampling phase*. We started filling a gap of knowledge on the ultrastructure of taste organs and their physiological and behavioural responses to relevant substances of bugs' diet. Taste receptors located in the antenna and in the epipharyngeal organ (internal receptors placed in the alimentary canal) seems to become crucial in terms of recognition and assessment of food quality. Taste perception in triatomine bugs leading to aversiveness or acceptance of a food source is dependent on chemical identity (salts, alkaloids “bitter” compounds, ATP) and other chemical qualities like concentration, osmolarity, pH, ionic composition.

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Electrophysiological activity of ticks sensilla to phagostimulants, phytoecdysteroids and to host-related and tick pheromone-related synthetic compounds

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Phagostimulants are chemical compounds that stimulate feeding. Phytoecdysteroids (PEs) are ecdysteroid analogues (arthropods moulting hormones) produced by some plants that disrupt the growth and development of insects, and can be perceived by the taste receptors of insects [1]. Some tick species have pores in their inner cheliceral digits, which are involved in taste perception [2]. This study investigated the involvement of the those pores of the dog tick *Rhipicephalus sanguineus* in the perception of seven phagostimulants and six PEs, using the single-sensillum recording technique. Strong activity to glucose, ATP, GSH and high concentrations of salts was recorded. The responses to ATP and to KCl at 1 M were multicellular, while the responses to the other stimulant compounds were monocellular. Glucose and GSH stimulated different neurons. The taste response of *R. sanguineus* chelicerae seemed to be selective, given that substances that were not expected to participate in this tick's biology were not perceived. In unfed ticks, makisterone A and pterosterone elicited frequencies of neural impulses higher than in a negative control. In fed ticks, only pterosterone remained active. In behavioural attachment assays, no difference was observed between electrophysiologically active compounds and the negative control. These results show the capability of *R. sanguineus* ticks to detect PEs, although they do not clarify the role of them in tick biology. Using the same technique host-related and tick pheromone-related compounds were tested in two olfactory sensilla of non-fed *Amblyomma cajennense* ticks. 2,6-DCP was active on both sensilla. The olfactory neurons of this sensillum also responded to nonanal, while those of DII.1 responded not only to 2.6 DCP but also to 2-nitrophenol and to 1-octen-3-ol. These results confirm the importance of 2,6-DCP in the chemical ecology of *A. cajennense* and indicate other compounds that may interfere with the behavior of this tick and which should be investigated.

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Could pre-imaginal olfactory experiences modify the post-metamorphic odor-mediated responses in a social insect?

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In honeybee colonies the information of exploited resources is transferred among nest mates through mouth-to-mouth trophallaxis events. These social interactions also involve larvae as food recipients. We wonder if odor-rewarded experiences that occur during pre-imaginal stages influence post-metamorphic odor-mediated responses. To address this issue we tested learning performance and memory retention in adults of 3/5 days old that underwent a pre-imaginal experience. Such experience was done by means of a scented-sucrose solution offered inside the hive and tested under the proboscis extension response (PER) paradigm in the lab. Results showed that precocious experience increased the PER-levels toward the pre-exposed odor suggesting retention of information gained prior to the emergence. Interestingly, high PER-levels to novel odors were also found accordingly to perceptual similarities between pre-exposed and different novel odors, resembling generalization in tested adults. Lastly, we found that even those bees that did not respond to the pre-exposed odor improved their learning performance in a PER-conditioning. Moreover, this pre-exposure modified olfactory-receptor responses of the pre-imaginal trained bees' antennae, quantified by means of electroantennograms. Then, odor-rewarded experiences during pre-imaginal stages led to behavioral and physiological changes in recently emerged adult bees. This kind of pre-imaginal experiences may allow bees to assess food information very early in life with consequences in their odor-mediated responses like the learning ability during adult stage.

This study was partly supported by the University of Buenos Aires, CONICET and ANPCyT.

**Plasticity in olfactory circuitry.
Mating modulates olfactory tuning in *Spodoptera littoralis***

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As long-lived animals, we may not as much appreciate the dramatic dynamics of ecosystems. Resources vary rapidly along with seasonal and diel patterns and animals are therefore constantly in need of syncing their behaviour with the environment, as well as with their own physiological status. Recently, our group found that female *Spodoptera littoralis* moths swap their olfactory preference after mating. Whereas unmated females prefer odours from lilac flowers (*Syringa vulgaris*) over cotton plants (*Gossypium hirsutum*), mated females clearly prefer odours from cotton plants. This is paralleled by modulation in antennal sensitivity to odours. The antennae of virgin females are, for example, more sensitive to the floral odour (S)-(+)-linalool than mated females, whereas the opposite is true for (R)-(-)-linalool. In total, 15 VOCs were found to be active at the antennal level among the volatile blends from cotton leaves and lilac flowers. Calcium imaging shows that the ensemble of AL glomeruli dedicated to either lilac or cotton odour is selectively up- and downregulated in response to mating. Therefore, mating triggers a behavioural switch of *S. littoralis* females by inducing changes in the neural circuits of the antenna and AL. Tuning of VOC sensing according to the internal state provides highly effective means of directing insects to the right resources at the right time. However, the neurophysiological mechanisms underpinning this switch in olfactory sensitivity and behaviour remain unknown. How precisely these rapid and finescale adjustments of the olfactory circuitry are achieved, and which are the biochemical players in these cascades, is now the focus of this project.

[1] Saveer A., Kromann S. et al (2012) Proc. Roy. Soc. B 279, 2314-2322.

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Associative and non-associative plasticity improves perception of the relevant components in an odor mixture

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Animals live immersed in a world of countless stimuli. To deal with the excess of information they have systems that process sensory afference and optimize detection of relevant information. In the present work we ask how this detection is optimized when the classification “relevant or irrelevant” is not fixed but depends on the experience of each individual. Is sensory processing adjusted in a dynamic way that helps distinguishing the relevant information from the noise in which it can be embedded?

The olfactory system of insects provides a good model for this study. Olfactory stimuli to which insects are normally exposed are complex mixtures, in which irrelevant components may hide the presence of relevant ones. We performed calcium imaging in the antennal lobe of honey bees (the first processing center for olfactory information) and measured the neural representation of mixtures and pure components after associative and non-associative learning. We found that the relative weight of the components in the neural representation of the mixture depends on the previous experience of the animals with the components. The change in the representation of the mixture seems to be consequence of changes in the competitive interactions among the components. The results are consistent with a model in which the antennal lobe acts to filter olfactory information according to its relevance.

**SYMPOSIUM: “Plant-insect and plant-plant interaction”
Tuesday 4 / 08:55 - 10:45**

Contemporary evolution of *Diabrotica* in the Corn Belt

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Western corn rootworm (*Diabrotica virgifera*) (WCR) is the most important pest of corn in USA. Larvae of this insect species feed exclusively on roots of corn. However, broad adoption of annual crop rotation between corn (*Zea mays*) and nonhost soybean (*Glycine max*) exploited WCR biology to provide excellent WCR control, but this practice dramatically reduced landscape heterogeneity in East-central Illinois and imposed intense selection pressure. This selection resulted in behavioral and physiological changes in a new biotype “rotation-resistant” (RR) WCR adult. Although soybeans are well defended against Coleopteran insects by cysteine protease inhibitors, RR-WCR feed on soybean foliage and remain long enough to deposit eggs that will hatch the following spring and larvae will feed on roots of planted corn. Fifteen years of research have failed to identify any diagnostic differences between wild-type (WT)- and RR-WCR or a mechanism that allows for prolonged RR-WCR feeding and survival in soybean fields. In our study, we were able to identify differences in behavior, physiology, digestive protease activity (threefold to fourfold increases), and protease gene expression in the gut of RR-WCR adults. Our data suggest that higher constitutive activity levels of cathepsin L are part of the mechanism that enables populations of WCR to circumvent soybean defenses, and thus, crop rotation. Our findings provide evidence of the possible cause and consequences of the contemporary evolution of the WCR in response to selection imposed by the modification of the agroecosystem. The RR-WCR illustrates how agroecological factors can affect the evolution of insects in human-altered ecosystems.

Host selection, oviposition behavior and leaf traits of *Salix* spp. in a specialist willow sawfly

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Plant genotype often influences plant-herbivore interaction by affecting insect attraction, acceptance and development [1]. Host plant selection by ovipositing females can be crucial for offspring survival [2]. Here, we evaluate the oviposition behavior of the specialist willow sawfly *Nematus oligospilus* (Hymenoptera: Tenthredinidae) on different genotypes of *Salix* spp. (Salicaceae) and discuss how it is affected by leaf micromorphology, nutrient levels and secondary metabolites of the host plant. Through choice and no choice bioassays we analyzed host selection according to willow genotype and leaf surface. We also studied larval performance and adult fecundity. For each genotype, leaf micromorphology was described through light and scanning electron microscopy. Total nitrogen and protein contents were quantified, as well as total phenolics, phenolic glycosides and salicin. Results revealed that *N. oligospilus* clearly prefers to oviposit on *S. nigra* regardless of leaf side. Even though *S. viminalis* was the least preferred genotype, it showed better larval performance and higher adult fecundity. Leaf micromorphology analysis showed a tight association between the egg and the leaf, as it is laid inside the epidermis or between the epidermis and the adjacent chlorenchyma. Regarding leaf toughness, *S. nigra* was lower than *S. viminalis* and *S. babylonica*. Quantification of total nitrogen and protein contents revealed higher concentrations on *S. viminalis* and *S. babylonica*. Total phenolics and phenolic glycosides were higher and more diverse on *S. nigra*. Since salicin content levels correlate with oviposition preference, this suggests a role of salicylates as oviposition stimulants. Altogether, results suggest that oviposition preference may be related to lower leaf toughness and ease of injection of female saw-like ovipositor, motivated by the presence of phenolic glycosides. Nitrogen levels may explain better larval performance and adult fecundity in *S. viminalis*. Thus, a balance among different leaf traits determines the outcomes observed in this study.

[1] Hochwender CG & Fritz RS. 2004. Plant genetic differences influence herbivore community structure: evidence from a hybrid willow system. *Oecologia*, 138 (4): 547-557.

[2] Hilker, M. & Meiners, T. (2006) Early Herbivore Alert: Insect Eggs Induce Plant Defense. *Journal of Chemical Ecology*, 32, 1379-1397.

This research was financially supported by PICT-PRH 247 (ANPCyT) and PNFOR 2212-042121 (INTA).

Tritrophic interaction *Telenomus podisi* – *Euschistus heros* – soybean cultivars

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The cues used by natural enemies to localize their host involve a multimodal communication that include, plant semiochemicals, semiochemicals from host producers and hosts. Studies with *Telenomus podisi* showed that this parasitoid recognize the sex pheromone produced by males of stink bug *Euschistus heros* (1), traces left by walking females of *E. heros* (2), vibratory signals produced during sexual communication by females of *E. heros* (3) and volatiles emitted by plants damaged by *E. heros* feeding female (4, 5). The egg parasitoid *T. podisi* did not recognize volatile emitted by plants damaged by oviposition, but can recognize volatiles emitted from herbivory and oviposition + herbivory damaged plants. In addition, different cultivars present different responses to damages provoked by *E. heros*, or induction from phytohormones, as *cis*-jasmone, what induce different responses from the egg parasitoid. On this symposium we will present the main results obtained with *Telenomus podisi* - *Euschistus heros* and soybean studies, to understand how the egg parasitoid uses plant volatiles to localize its host and how soybean plants respond to different types of induction.

- (1) Borges M. et al, 2003, *Physiology Entomology* 28: 349-355.
- (2) Borges M. et al, 1998, *Physiology Entomology* 23: 202-207.
- (3) Laumann R. A. et al, 2011, *Animal Behaviour* 82: 1175–1183.
- (4) Moraes M. C. B. et al, 2008, *Journal of Plant Interaction* 3: 1742-1756.
- (5) Michereff M. F. F. et al, 2011, *Journal of Chemical Ecology* 37: 273-285.

Acknowledgments: The research was supported by FAP-DF and CNPq

Consumption of *Pteridium* spp and trophic transfer of allelochemicals to specialist herbivorous insects

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Pteridium spp. (Dennstaedtiaceae) popularly known as male fern, bracken and several others, is one of the most prevalent fern species in the world. Its wide distribution and hardiness are due in part to the arsenal of secondary metabolites produced in large quantity and variety, including mono and sesquiterpenes, a cyanogenic glycoside (prunasin), thiaminase, phenolic acids, flavonoids and proanthocyanidins and their glycosides. Many of these compounds are toxic or show deterrent activity, serving a protective function against potential pathogens and predators as well as allelopathic effects¹. Nevertheless, considering all habitats where *Pteridium* spp thrive, there are around 100 species of arthropods that consume different parts of the fern.

Well protected plant species can host some insect herbivores capable of sequestering certain allelochemicals for their own protection at a higher trophic level. In view of the high content of chemical defenses in bracken, we became interested in learning whether their transfer could benefit herbivorous insects as sequestered defenses. In this work we present two tri-trophic systems in this context. First, *Pteridium caudatum*-*Abracris flavolineata* (Orthoptera, consumer)-*Solenopsis geminata* (Hymenoptera, predator) allowed to explore possible associations between fern frond consumption, grasshopper regurgitation and ant deterrence². A second system, which is currently under study, involves the neotropical sawflies *Aneugmenus merida* that we described recently as a possible specialist of brackens *P. caudatum* and *P. arachnoideum* of Venezuela and the behavior of *Odontomachus chelifer* (Hymenoptera, predator) against larvae of the sawfly. The central question is whether this species is chemically protected by constitutive defenses or alternatively by allelochemicals acquired during feeding at larval stages.

[1] Alonso-Amelot M. (2002). *Studies in Natural Products Chemistry*. **26**: 685-739.

[2] Calcagno M. P., Avila J. L., Rudman I., Otero L. D., Alonso-Amelot M. (2004). *Physiological Entomology*, 29, 123-128

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SYMPOSIUM: “Chemical ecology of disease vectors”
Tuesday 4 / 15:20 - 17:30

***Aedes aegypti* responses to co-specific mosquito extracts and a cuticular compound**

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Dengue is considered the most important arboviral disease nowadays and stands out as a major public health problem worldwide, affecting millions of people each year around the world. Since there are no specific drugs for the treatment of the disease, or even a vaccine that prevents virus infection, the main control strategy is to combat the mosquito, *Aedes aegypti*. This control aims to reduce vector populations based on the removal/chemical treatment of breeding sites and the application of adulticides during outbreaks and epidemics. Due the importance of *A. aegypti* in public health, knowledge of their behavior is very important, not only to provide information about the biology of the insect, but also to be able to assist in disease control programs. The recognition of mating partners is described in the literature as being performed by the *A. aegypti* males, which have a sensory organ called Johnston's organ, which identifies the wing beat frequency of conspecific females. The use of chemical attraction in the mating behavior of these insects is not known, however, a recent work has demonstrated the existence of volatiles, which permit the intra-specific recognition in *A. aegypti*. A detailed study of the behavior of males and females to cuticular extracts of conspecific adult mosquitoes, as well as to a synthetic compound identified in these extracts, confirmed the presence of a semiochemical that influences the behavior of *A. aegypti*, which can be used in future vector control strategies.

[¹] Roth L.M. A study of mosquito behavior. An experimental laboratory study of the sexual behavior of *Aedes aegypti* (Linnaeus). *American Midland Naturalist* 40: 265–352. 1948.

[²] Cabrera M. & Jaffé K. An aggregation pheromone modulates lekking behavior in the vector mosquito *Aedes aegypti* (Diptera: Culicidae). *J. Am. Mosq. Cont. Ass.* v. 23 (1), 1 – 10. 2007.

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The complex reproductive behavior of *Lutzomyia longipalpis* complex (Diptera: Psychodidae)

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The sand fly *Lutzomyia longipalpis* (Lutz & Neiva, 1912) is a species complex and is considered the main vector of visceral leishmaniasis in the New World. Male sand flies present an interesting reproductive attraction behavior, with release of sex/aggregation pheromone and song production for copula by wing beating, what makes this insect a fascinating model for studies on reproductive behavior. Usually *L. longipalpis* forms nocturnal leks on or near vertebrate hosts where adult males produce a terpenoid pheromone with the dual function of sex pheromone and male aggregation pheromone. At present two terpenes have been characterized as the main components of sex pheromones blend in some populations of the *L. longipalpis* complex: (S)-9-methylgermacrene-B and 3-methyl- α -himachalene. Other pheromone structures have been proposed to be diterpenes, but the structure has been only partially characterized as cembrene isomers 1 and 2. During copulation, males of *L. longipalpis* vibrate their wings producing two main types of copulatory courtship songs, named Pulse-type and Burst-type. The Burst-type song (B), composed of trains of extremely polycyclic pulses ("bursts") modulated in frequency and amplitude and the Pulse-type song, more variable with three different patterns (P1, P2 and P3) were characterized among Brazilian populations. The sex pheromones and male 'lovesongs' produced during copulation have an important role in the reproductive isolation among closely related species indicating the existence of at least four reproductively isolated populations in Brazil. After copulation, it has been described in many insects that male possess glands associated with their reproductive system which synthesize proteins, including the accessory gland protein (Acp) and the masculine reproductive gland protein (mRGP), which are transferred to the females altering their physiology and behavior. These proteins play a major role in the insect reproduction, acting on induction of oviposition and reduction of sexual receptivity of females after copulation. Although sand flies lack a proper accessory gland, a 'plug-like formation' has been observed in the female spermatheca and probably prevents reinsemination. Transcriptome analysis of *L. longipalpis* male reproductive organs has also identified 14 ESTs encoding putative mRGPs/Acps similar to those produced by *A. aegypti*, *A. gambiae* and *D. melanogaster*. Ultrastructural studies of the male reproductive system suggest that probably the role of the accessory gland in sand flies is played by the seminal vesicle. Mating experiments support this hypothesis, revealing a reduction in sexual receptivity of mated females that indicates the effect of putative Acps/mRGPs acting on reduction of sexual receptivity of females after copulation. It is important to note such proteins that play a role in sexual selection and speciation can also act as good molecular markers for the species complex.

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***Rhodnius prolixus* attraction to volatiles emitted by *in vitro* growing bacteria isolated from human faces**

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Samples from faces of ten human volunteers were obtained to isolate bacteria [1]. Bacteria isolated were identified by amplifying and sequencing 16s rDNA. Bacterial growth curves for each strain were carried out to precisely determine exponential and stationary phases [2]. The attractiveness to adults of *Rhodnius prolixus* of volatiles emitted by single bacterial species were tested during the scotophase using a modified dual choice olfactometer [3].

Seven bacterial species were isolated (*Staphylococcus epidermidis*, *S. caprae*, *S. capitis*, *Citrobacter koseri*, *Micrococcus luteus*, *Propionibacterium acnes* and *Dermacoccus nishinomiyaensis*). The first five species were the most frequently isolated and their times of exponential growth lasted up to 3.5 hours to continue afterwards in stationary phase.

The relative attractiveness showed that volatiles emitted *in vitro* in exponential and stationary phases were not attractive testing *S. epidermidis* but showed to be strongly repulsive using *C. koseri*. Some attractiveness was found in different growing phases testing other bacterial species.

Our preliminary findings with the triatomine *Rhodnius prolixus* confirm similar results obtained from other groups with the mosquito *Anopheles gambiae* [2] and highlights the important role that play volatiles emitted by some bacteria species growing on human skin in attraction and selection of biting sites by bloodsucking insects.

[1] Verhulst *et al.* 2009. Malaria Journal, 8:302 doi:10.1186/1475-2875-8-302

[2] Verhulst *et al.* 2010. PLoS ONE 5(12): e15829.

doi:10.1371/journal.pone.0015829

[3] Ortiz & Molina 2010. Acta Tropica. 113:174-179.

Acknowledgements: Colciencias project # 1204-459-21479 and Faculty of Sciences-Universidad de los Andes for financial support

Development of methods for monitoring and control vectors of Chagas disease using host-odors

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Chagas disease is a serious health problem in Latin America and vector control is the most effective method to prevent it [1]. Vector control 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 the sprayed houses. The use of lured trap devices is a sustainable and an environmentally-friendly method for vector control [2]. We aim at developing effective lures and traps.

Tests carried out in a dual-choice trap olfactometer using a synthetic host odor blend consisting of L(+)-lactic acid, hexanoic acid and ammonia evoked activation, attraction and capture in *T. infestans* and *R. prolixus*. Nevertheless the blend performance could be improved. A pit-fall trap-device inspired on the trap-olfactometer could exploit the host seeking behavior of these insects.

Another method to capture triatomines in the field is the popular Noireau trap, using adhesive tape around a container with the bait (a mouse). When attempting to reach the bait, the bugs get stuck on the tape. However, it is not known if, after being immobilized, the adults stuck become stressed and hence emit their alarm pheromone thus repelling conspecifics. If so, this could result in an overlooked reduction in the trapping performance of this device. We tested if adults stuck in such a way reduce the performance of the trap. Our results suggest that attraction/trapping effectiveness is not compromised by the presence of previously trapped adults bugs.

In order to optimize tests to study attraction and to make results easily comparable between different methods and laboratories, a methodological analysis comparing different variables related to attraction will be presented and discussed.

[1] WHO, 2010. Chagas disease (American trypanosomiasis). Fact sheet N°340. <http://www.who.int/mediacentre/factsheets/fs340/en/index.html>

[2] Guerenstein, P.G., Lazzari, C.R., 2010. The role of olfaction in host seeking of Triatominae bugs, in: Takken, W. and Knols, B. (Eds.), Ecology and Control of Vector- Borne Diseases Volume II: Olfaction in Vector-Host Interactions. Wageningen University Press, pp. 309-325.

We thank Fundación Bunge y Born and Agencia Nacional de Promoción Científica y Tecnológica, Argentina, for funding this project through grants FBB28/10 and PICT-PRH-2009-43, respectively.

Strategies to control triatomines based on sensory ecology principles and community participation

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Triatomines are hematophagous insects and vectors of Chagas disease in the New World. Most behavioral research performed on these arthropods is based on the study of their chemo-sensory ecology. Here a brief review of the advances reported on this subject is presented and new unpublished data is revealed. As several compounds have proven attractive and others toxic to triatomines, a control tool is proposed based on the use of such substances within a trap. A control strategy for the use of this tool is also presented. Given that rural communities could be reluctant to accept the use of new and foreign practices due to cultural prejudices, a community participation strategy associated with a vector control programs is discussed.

Acknowledgements: Thanks to Erick Hernández, Oscar Páez, Antonio Pérez and Ingrid Inciarte for their technical support and to FONACIT for financial support.

**SYMPOSIUM: “Semiachemicals in Pest Management”
Wednesday 5 / 08:55 - 10:45**

**Controlling Insect Pests with Specialized Pheromone & Lure
Application Technology (SPLAT[®])**

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SPLAT[®] is a biologically inert matrix that is used for pest management in agricultural, urban, and forest ecosystems to provide long term release of insect semiochemicals, plant volatiles, insecticides, phagostimulants, biological control agents, and various compounds that are being used for pest control. The amorphous and flowable physical properties of SPLAT can be easily tailored to a wide range of manual and mechanical application methods such as electric and pneumatic grease guns, disposable syringes, caulking guns, tractor and gator application systems and even aerial applications. SPLAT formulations can be designed to provide species-specific control through mating disruption, repellency, attract and kill, and mass trapping techniques. SPLAT is an extremely flexible medium that can increase control efficacy simply by varying a few parameters including size of the chemical emitting point source, placement, and density of point sources applied in the field. The high viscosity allows SPLAT to be applied to almost any application surface and remain in place, releasing the active ingredients over an extended period of time. Just a few hours after application, most formulations will form a protective skin that maintains the shape of the point source as well as protecting the active ingredients from UV rays. All inert ingredients used in SPLAT are suitable for food use based on a US EPA scientific review. Studies also show that SPLAT is non-phytotoxic and safe for the environment. Several SPLAT formulations are labeled for use in organic productions.

Current development and commercial potential of the pheromones in Argentina

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In Argentina, the use of pheromones is restricted to monitoring and control of quarantine pests, in national programs of SENASA: a) *Carpocapsa pomonella* and *Grapholita molesta*, on apple and pear crops. The pheromone is formulated in dispensers for monitoring and mating disruption. b) *Lobesia botrana* S. (grapevine moth), is registered with the same purposes. c) For "fruitfly" using different semiochemicals and traps as fundamental support for sanitary measures diagrammed. d) *Anthonomus grandis* B., the pheromone is a key tool in the monitoring and eradication program of "cotton boll weevil".

The pheromone of non quarantine pest is registered for *Lasioderma serricorne* F., for detection in bales of tobacco, their use is not widespread. *Planococcus ficus* S. pheromone is marketed in vine. Only one company develops technology in horticultural crops and has the pheromone of *Tuta absoluta* M., to apply it in the mass trapping, in final phase of registration.

The commercial potential of this technology is associated with the possibility to identify and synthesize the pheromones of some pests as *Symmetrischema borsaniella* K. in pepper, *Phyrdeneus muriceus* (Germ.) in potato, *Listronotus dauci* Brethes in carrot, *Rhyssomatus subtilis* F., in soybean, among others. In some cases pheromones are available and required technical and commercial development and registration: *Plutella xylostella* L. in broccoli, cabbage and cauliflower; *Spodoptera frugiperda* S. in pepper and sweet corn, *Phthorimaea operculella* (Zeller) in potato and *Helicoverpa zea* B. in corn.

Ethological control, supported by scientific and technological, and a legal framework to facilitate the registration of the pheromones in SENASA, may be more widespread for incorporation into integrated pest management of most important and commercial crops in Argentina, so we can capitalize its effectiveness, specificity, selectivity and its ecotoxicological advantages.

Semiochemicals in the integrated pest management of temperate deciduous fruits in Latin America

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The southern cone of South America is a world leading region in deciduous fruit production (pome, stone fruit, berries and grapes). Integrated pest management of these crops in our region is still largely based on pesticides, but the utilization of semiochemical technologies are fast growing alternatives for a more sustainable fruit production. Consumer demands for fresh fruit without pesticide residues and higher awareness about environmental impacts of agricultural production provide a great opportunity for semiochemical technologies in Latin America. At present, commercial use of pheromones (codlemone, orfamone, grapamone, etc.) for pest monitoring and mating disruption of key pests is used at large spatial scales. New attract and kill, mass trapping, auto-confusion and related technologies based also in pheromones are emerging. Similarly, a new generation of plant derived kairomones (pear ester) and fermentation volatile compounds (acetic acid) are further improving the commercial use of semiochemicals in pest management of fruit crops. The latest research involving microorganism-crop-pest chemical interactions through volatile compounds is a new frontier to explore for the development of commercial technologies for pest management. Finally, the chemical ecology of widespread cosmopolitan pests have been extensively studied in developed countries, but research on native Neotropical pests is scarce with little semiochemical technologies currently available. International trade is increasing the introduction of invasive pests in new geographical regions, and therefore efforts to identify, synthesize and formulate semiochemicals for native pests of local economic relevance will have a growing importance in the future.

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New application tactics to increase the use of semiochemicals in Latin American agriculture

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In Latin-America semiochemicals use in agriculture is still below their potential to pest management. Development of industrial synthesis and formulation, specific tactics of use for different agriculture systems as extensive arable or smallholder crops production and formulations compatible with others pest management tactic, for example biological control, could help to improve and increase the use of semiochemicals in pest management. Two alternative tactics to semiochemical application will presented. Population monitoring of brown stink bugs, *Euschistus heros* (Fabricius) with sex pheromone traps to both record the immigration into soybean areas and early control of population increase are been experimentally tested in different regions of Brazil. Results indicate that early record/control of stink bugs populations has potential to reduce the population levels and number of insecticides application without significant reduction in production. The other tactic aim to integrate semiochemicals with enthomopatogenic microorganism. In this study was used as a model the banana weevil, *Cosmopolites sordidus* (Germar), and the aim was to evaluate a low cost and efficient release device formulated using hydrogenated oils pellets with the specific commercial pheromone (Cosmolure) of the species and the fungus *Beauveria bassiana* (Bals) Vuill. Results showed that this formulation is attractive to the insect and conidia transferred from pellets to banana weevil adults produced satisfactory levels of mortality without negative interactions between pheromone and fungus. This strategy of "attract-kill" in a novel formulation show potential to control a range of cryptic insect pests. The potential of these tactics to be incorporated in pest management as well as other novel alternatives, as nanoformulation of semiochemicals, will be discussed.

Acknowledgments: The research was supported by FAP-DF, CNPq and Embrapa

**MINI-SYMPOSIUM: “Functional Genomics of Chemoreception”
Wednesday 5 / 15:05 - 15:55**

Molecular bases of chemoreception in *Rhodnius prolixus*

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Three protein families are involved in the insect's chemoreception. The odorant receptor (OR) and gustatory receptor (GR) families of seven-transmembrane proteins mediate most of insect olfaction and gustation. In addition, there is a third completely different family, the ionotropic receptors (IRs), which evolved from ionotropic glutamate receptors (iGlurs) and has three transmembrane domains. Insect olfactory neurons, both Ors and IRs, require the co-expression of specific co-receptor proteins in order to be functional: OrCo (for Ors) and IR25a or IR8a (for IRs). Our objective was to characterize ORs, GRs and IRs at the molecular level in *Rhodnius prolixus*, an important vector of Chagas disease. First, receptor sequences of other insects were chosen by searching genomic databases. TBLASTN searches were performed over the predicted proteins from *R. prolixus* genome (unpublished data) using major lineages of insects as queries. Gene prediction programs (Wise2, Augustus and Fgenesh+), Artemis and BioEdit 7.5 were used to create and analyze the gene models. Through applications available on www.cbs.dtu.dk/services/, characteristic structures, including channel and transmembrane domains, were identified. Through the IDT Primer Quest program, specific primers were designed to allow sequencing. New primers were designed to study gene expression in different tissues (antenna, proboscis, front, mid and hind tarsi, genitalia and fat body) by RT-PCR. The OR and GR gene sets consist of 115 (including 5 pseudogenes) and 28 models respectively. Additionally, a total of 18 IRs gene models have been identified. It has been possible to show antenno-specific expression for several ORs and IRs. Finally, the modulation of co-receptor transcription at different physiological conditions has been measured by qPCR. A better understanding of the molecular bases of triatomine olfaction may reveal targets for developing new vector control tools.

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Molecular and sensory correlates of host recognition in mosquitoes

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Olfaction is critical in host location and choice by mosquitoes. Differential attractiveness of hosts to mosquitoes has been attributed to variation in chemical and physical cues. Genome information has enabled molecular characterization of odorant receptors of *An. gambiae* in heterologous expression systems. Through combined bioinformatical, molecular, physiological, chemical and behavioral analyses we aim to identify novel natural ligands from hosts and non-hosts with potential use in the chemical ecological management of this disease vector mosquito. To reach this goal we have characterized the peripheral olfactory system of *An. gambiae* using single sensillum recording (SSR) analysis, and the response of transgenically expressed *An. gambiae* odorant receptors to complex ecologically relevant odors (host, non-host, floral and host environment odors), by SSR and combined gas chromatography-SSR analyses. The tuning of the transgenic ORs reflects relevance to ecologically important signals, but does not seem to fully account for the responses observed ORNs *in vivo*. Thus, the tuning of the olfactory receptor neurons is possibly achieved by additional mechanisms than the receptor expression alone. We are therefore using information from available genome sequencing projects to investigate the structure, evolution and expression characteristics of olfactory genes and their influence on receptor function. These studies will improve our understanding of the role of the peripheral olfactory system in host finding in *An. gambiae*.

ORAL PRESENTATIONS
Abstracts

O1 - Host plant-derived volatile blends: how do changing ratios of constituents influence female insect behavior and neurophysiological responses?

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Specific ratios in plant-released volatile blends are assumed to drive host recognition and location for insect herbivores. Ratios of compounds in host plant odors, however, might fluctuate with different biotic and abiotic conditions (e.g. the phenological stage of the plant). Thus, female insects should have evolved a certain degree of olfactory plasticity to locate their hosts within distinct threshold ratios of volatile blend constituents. We investigated the effect of changing ratios of host plant volatile constituents on herbivore insect attraction and olfactory information processing. We used the oriental fruit moth *Cydia* (□ *Grapholita*) *molesta* as the model organism. Synthetic blends comprising bioactive peach shoot volatiles and differing only in the concentration of one of the blend constituents, benzonitrile, were tested in Y-tube olfactometer bioassays for their attractant effect on female moths. Bioassays indicated that female attraction to the blend was maintained even when the concentration of benzonitrile was increased up to 100 times. Further increases of benzonitrile led to behaviorally ineffective mixtures. We further recorded odor-evoked neural activity patterns in the antennal lobes, the main olfactory center of the insect brain, using calcium imaging. Benzonitrile-containing blends elicited strong activation in two glomeruli, which were found to process mixture-related information in specific ways. Activation in one glomerulus directly paralleled behavioral effects of the different ratios tested. Our results indicate that the ratio of constituents in a volatile blend can be varied to a certain degree without reducing female attraction. Thus, volatile blends in nature might vary quantitatively within a certain range without affecting odor-guided host location. The implications of our findings will be discussed from an ecological and applied perspective.

O2 - Attraction of carrion beetles (*Oxelytrum discicolle*) to carcass volatiles and identification of the male sex pheromone

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Two major groups of insects (Coleoptera and Diptera) are attracted by carcasses and therefore provide the most information in forensic investigations entomological and may be indicators of post-mortem interval (PMI). The beetles of the family Silphidae are known as carrion beetles, in Brazil existing records of the genus *Oxelytrum*, whose larvae and adults feed on and lay eggs on carcasses. Given its importance in forensic entomology, the aim of this study was to determine the structure of the sex pheromone released by males and evaluate the attractiveness of the odor decomposition of the carcass in combination with the pheromone. Ten insects were used for each sex and placed in aeration chambers containing moistened cotton daily. Every other day, they were fed with ground meat, and in the intervals without food, volatiles were captured in columns containing polymer adsorption (HayeSep-Q). The extractions were performed using 400 μL of hexane, concentrated to 160 μL , injected and analyzed by GC-MS and GC-FTIR. The volatile housing were a rat in the swollen phase. In the chromatograms of males was possible to observe a male-specific compound, whose mass spectrum suggested the case of a linear system and molecular weight of 236 g mol^{-1} . In the infrared spectrum, we detected the presence of stretches at 2999 and 3081 cm^{-1} , corresponding to the presence of double bonds. After microderivatization of the catalytic hydrogenation with palladium / carbon was possible to verify the increase of four units in the mass spectrum, confirming the presence of two double bonds. Microderivatization with dimethyl disulfide revealed that the double bonds were at positions 1 and 8. Bioassays revealed that females were preferentially attracted to the male extract aeration combined with the extract decomposition odour of the rat (food source). While males were attracted only to extract the carcass.

[1] ANDERSON, R. S., PECK, S. B. 1985 The carrion beetles of Canada and Alaska: the insects and arachnids of Canada, part 13. Research Branch Agriculture Canada, Ottawa, Canada.

[2] MISE, K. M., ALMEIDA, L. M., MOURA, M. O. 2007 Levantamento da fauna de Coleoptera que habita a carcaça de *Sus scrofa* L., em Curitiba, Paraná. Revista Brasileira de Entomologia, 51: 358-368.

O3 - Activation and orientation of *Rhodnius prolixus* males in response to odors emitted by females

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The existence of a pheromone emitted during copulation was reported for *Rhodnius prolixus* bugs. Adult of this species posses paired metasternal glands (MGs) that emit volatile products recently identified and reported to be released by females at night. In this study, we investigated whether there is a relationship between locomotor activity and volatile signals potentially emitted by adult *R. prolixus*. We first used a shelter based bioassay to characterize whether the activity pattern of groups of males or females is affected by potential chemical signals emitted by opposite gender conspecifics. Furthermore, we used a dual-choice walking compensator bioassay to investigate whether female or male *R. prolixus* show oriented responses to odors from adults. Finally, we tested whether female MG compounds modulate male orientation responses. Males left their shelters more frequently when groups of females were present in the same experimental room. Besides, in this condition their activity was higher and copulation attempts between them increased significantly. Females showed no shelter-related activation in the presence of male odors. Additionally, males confronted with air currents carrying female odors oriented significantly towards them. Females did not show significant orientation to odors emitted by adults. Finally, females with occluded metasternal glands failed to promote significantly oriented trajectories by males. Thus, a volatile chemical signal emitted by females promoted the activation of sheltered *R. prolixus* males and their subsequent orientation when these volatiles were carried by air currents.

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O4 - Bitter taste perception in the blood-sucking insect *Rhodnius prolixus*

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The taste sense plays a crucial role in animals' life informing them about the nutritional quality of a food source. Moreover it provides a means of discrimination between nutrient-rich substrates, from harmful, mostly bitter-tasting. *Rhodnius prolixus* is a hematophagous insect that feeds on blood from small vessels of vertebrate hosts. Once insects pierced the host skin, they move their mouthparts until a venule or an arteriole is reached. Insects pump a small quantity of blood initiating the sampling phase of food. During this period, the taste sense might become crucial in terms of the assessment of food quality. Our goal in this work was to determine whether the bitter taste is perceived by bugs (beyond the well-known phagostimulant ATP). The feeding response of bugs was analyzed by measuring the weight gain of insects to different test diets in an artificial feeder set-up during 10 min. Our results show that *R. prolixus* can perceive different bitter taste sense in dose-dependent manner, although this was only evident in the presence of ATP. Notably, bitter compounds have an inhibitory effect on the feeding behavior of bugs. The importance the taste sense plays in the feeding behavior, for vectors of Chagas disease in Latin America, begins to be understood.

Key words –Taste, feeding behavior, Chagas disease vectors, bitter.

Financial support: Agencia - FONCyT- PICT PRH2009-00081, CONICET.

O5 - Sexual Behaviour in *Rhodnius prolixus* (Heteroptera, Reduviidae): the role of cuticular lipids

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Insect cuticular lipids (CL) are usually involved in intraspecific chemical communication as contact pheromones for mate recognition. Differences in CL composition were observed between sexes for *T. infestans*. However, at present no sexual chemical dimorphism was reported for *Rhodnius prolixus*. We studied here the role of CL in the mating behaviour of *R. prolixus* and we tested the hypothesis that mating is mediated by CL through contact chemoreception.

First, CL dichloromethane (DCM) extracts of the hydrocarbon and non-hydrocarbon profiles of adult bugs were analysed by GC-MS evincing sexual differences only in the non-hydrocarbon profile.

Then, in behavioural assays in which we registered the frequency of copula of a virgin male confronted to a female with different CL compositions (treatments), we found that female CL are responsible for the occurrence of copula in *R. prolixus*. Trying to identify the components responsible for this sexual recognition, we obtained five CL fractions (F1 to F5) of increasing polarity for males and females and tested them individually. Males responded only to one of the fractions acquired from females (F5), which contained cholesterol as major component. The male F5 fraction also contained cholesterol, but in a higher dose. DCM solutions of synthetic cholesterol were then prepared and tested in behavioural assays. Males copulated when one cholesterol female equivalent was added and did not when one male equivalent was added.

Our results suggest that males of *R. prolixus* can recognize the female by a differential proportion of non-hydrocarbon epicuticular lipid compound, cholesterol, present in the cuticle during the copulatory attempt.

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O6 - Effects of biotic and environmental factors on the calling behaviour of virgin *Pseudaletia adultera* (Lepidoptera: Noctuidae) females

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We examined the effects of conspecific female density and environmental factors during the larval stages, on the calling behaviour of virgin *Pseudaletia adultera* (Lepidoptera: Noctuidae) females. To assess the former, females were either placed individually or in groups of 6, and their calling behaviour was observed for 8 consecutive nights upon adult emergence. The same observations were conducted on individual females that had been raised under two conditions of photoperiod and temperature that simulated fall (12:12 L:D; 15 °C) and summer (16:8 L:D; 25 °C). Both the age at which females called for the first time, and the probability of calling, were affected by population density and environmental factors. Grouped females called more, started to call earlier, and spent more time calling, than solitary females. Similarly, females reared under high temperature and long photo-phase initiated calling before and called more than females reared under fall conditions, especially in the first calling nights, while no differences were found in calling duration. Our results suggest that female interactions with conspecific females induce calling. Furthermore, environmental factors such as temperature and photoperiod during the immature stages modulate calling behaviour in young females.

Acknowledgements: Proyecto de Iniciación a la investigación, ANII.

O7 - Pyrrolizidine alkaloids (PAs) sequestered from both native and invasive *Crotalaria* host species provide chemical protection for the arctiid moth *Utetheisa ornatrix*

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Larvae of the specialist arctiid moth *Utetheisa ornatrix* feed on unripe seeds and leaves of both native and invasive *Crotalaria* species (Fabaceae), showing a better performance on the former [1]. Larvae sequester PAs from these hosts, thus getting protection against predation until the adult stage [1,2]. We asked whether adults originated from larvae feeding on native and invasive species would be similarly protected against the golden orb-web spider *Nephila clavipes* (Nephilidae). We fed larvae with seeds and leaves of native (*C. vitelina*, *C. micans*, *C. brevipflora*, *C. incana* and *C. paulina*) and invasive species (*C. pallida*, *C. spectabilis*, *C. juncea* and *C. ochroleuca*), from hatching until emergence, and bioassayed the adults against the spider. Pooling all *Crotalaria* species, *N. clavipes* released unharmed 97.45% adults of *U. ornatrix* from seed diets and 57.80% from leaf diets. These results can be explained by the amount of sequestered PAs, which is high in adults when larvae were feed on seeds (high PA content), and it is low when the larvae were feed on leaves (low PA content). Larvae feeding on leaves of *C. paulina*, *C. vitellina* and *C. spectabilis* were 100% protected against predation. These exceptions may be due to the high PA content in both seeds and leaves in these three species. Our findings showed that *U. ornatrix* is able to use PAs from different *Crotalaria* species for defense, including invasive species, and the most important for improving defense is feeding on plant parts with a high PA content.

[1] Trigo JR, Monteiro WL, Martins CHZ in this volume. [2] see review in Eisner T, Meinwald J (1995) Proc Natl Acad Sci USA 92:50-55

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O8 - Characterization of phenylpropanoid compounds in soybean seeds induced by stinkbugs attack and methyl jasmonate application with HPLC- Mass Spectroscopy

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It is generally accepted that all plant species defend themselves from biotic stresses. Soy bean (*Glycine max* L.) has developed defensive mechanisms such as synthesis of secondary metabolites that confer tolerance or resistance to some herbivore insects. Although, knowledge of regulation of plant defenses in leaves is increasing, isoflavonoid synthesis regulation and induction in developing seeds is not known. The aim of this study was to characterize phenylpropanoid compounds (isoflavonoids and pterocarpan) in soybean developing seeds before and after stinkbugs attack. High performance liquid chromatography-ultraviolet (HPLC-UV) and HPLC-electrospray ionization-mass spectrometry (HPLC-ESI-MS) analysis (positive ion mode) were used to identify these defensive compounds after a stinkbug injured (*Nezara viridula* L., *Piezodorus guildinii*) and the application of exogenous methyl jasmonate (MeJA)(50 μ M). ESI-MS over *cv* Williams seed methanol extract revealed the presence of malonylated-daidzein (m/z 503 $[M+H]^+$) and prenylated-daidzein derivatives (m/z 325 $[M+H]^+$) like glyceollidin I/II, precursor of pterocarpan [1]. Daidzein-7-O- β -D-glucoside, genistein-7-O- β -D-glucoside, daidzein and genistein were identified through HPLC-UV and quantified by correlation with calibrations curves of commercial standards (Sigma-Aldrich). Data were analyzed with an Analysis of Variance (ANOVA), isoflavonoid content were compared by the Tukey test ($P<0,05$) and a Principal Component Analysis (PCA) was applied in order to integrate all information under a much more simplified space (Biplot graphics). MeJA application increased aglycones after 24 h, and stinkbug injured induced aglycones synthesis after 72 h of treatment, showing a strong response of these metabolites as an immediate natural defense.

[1] *Identification of prenylated pterocarpan and other isoflavonoids in Rhizopus spp. elicited soya bean seedlings by electrospray ionisation mass spectrometry.* R. Simons, J.P. Vincken, M. Bohin, T.F.M. Kuijpers, M.A. Verbruggen and H. Gruppen. Rapid Commun. Mass Spectrom. 2011, 25,55–65.

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O9 - Role of volatile compounds in the interspecific interaction of *Naupactus xanthographus* (Coleoptera, Curculionidae) with two host plants

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The grape weevil *Naupactus xanthographus* is a polyphagous insect present in the southern cone of South America, which causes economically important damage to agriculture, mainly in areas of Brazil, Uruguay, Argentina and Chile. Furthermore, it has quarantine status in countries like Japan and The United States.[1,2] The damage is caused by both larvae feeding on the roots of the host plants, and adults feeding on the aerial parts. Its main host species include fruit trees, weeds, ornamentals species and other crops, including economically important species such as grapes (main host), apples and avocados, among others. In the present work, we studied the interaction of adult *N. xanthographus* with some of their host plants, analyzing the behavioural responses of males and females adults to different volatile compounds of the host plants, grape (*Vitis vinifera*) and avocado (*Persea americana*). The olfactory response to the different stimuli was determined in a Y-tube olfactometer and the results were analyzed using the non-parametric χ^2 test. Finally, we identified by GC/MS some volatiles compounds of these host plants.

Our results provide empirical evidence that males adults are attracted to: i) host plants volatiles v/s control (hexane), ii) herbivore-induced host plants volatiles v/s control (hexane) and iii) herbivore-induced host plants volatiles v/s host plants volatiles. Also, males showed no significant preference when presented to volatiles of both host plants. Females, showed no significant preference to host plants volatiles v/s control (hexane). The analysis GC/MS allowed us to identify some host plants volatiles and herbivore-induced host plants volatiles, such as: α -pinene, β -pinene, limonene, mircene, (Z)- β -ocimene, (Z)-3-hexenyl acetate.

Finally, we conclude that *N. xanthographus* is using plants volatiles as olfactory cues to locate their host plants. The preference to particular stimuli may be associated with qualitative and quantitative changes of the volatile compounds emitted. Studies aimed at determining the role of individual compounds are underway.

[1] Ripa, R. Boletín N°192, INIA (1992) 74.

[2] Sazo, L. Publicación Facultad de Ciencias Agrarias y Forestales. U. de Chile (1996) 63-65.

Acknowledgements. WV is grateful for doctoral fellowships provided by the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT).

O10 - Differential expression of gut trypsin of *Anticarsia gemmatalis* larvae in response to soybean (*Glycine max*) protease inhibitors

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Anticarsia gemmatalis is one of the major pests of soybean (*Glycine max*). However, soybean has digestive trypsin protease inhibitors (TPIs) as a defense against insects attack. The most important TPIs in soybean are Kunits and Bowman Birk. Although these TPIs are an effective defense to many herbivores, *A. gemmatalis* larvae tolerate these defenses and have low mortality level when feed on diet with high TPI levels. Overexpression of trypsins in the gut of insects is one of the defense mechanism to tolerate TPIs. The aim of this study was to clone trypsins from *A. gemmatalis* and analyze enzyme expression and activity from guts of larvae that fed on soybean cultivars with different TPIs levels. While the survival of larvae that fed on a cultivar without Kunitz TPI (PI542044) was 60%, those larvae that fed on plants that normally express Kunitz (Williams) was 40%. The digestive enzyme activity assays of larvae showed no differences between treatments. However, larvae that fed on different cultivars showed differential expression of three different isoforms of gut trypsin. Isoforms Try 1 and Try 2 invert their expression patterns while Try 3 was overexpressed in Williams. These results suggest that *A. gemmatalis* is able to compensate trypsin inhibition from soybean TPI by overproduction insect trypsin in gut that are not inhibit by TPI.

O11 - The sex pheromone of the Poplar moth *Condyloorrhiza vestigialis* (Lepidoptera: Crambidae)

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The Poplar crop has gaining attention in the southern region of Brazil, principally to supply the match industry and manufacturing matchsticks boxes. The poplar moth *Condyloorrhiza vestigialis*, is considered the major pest of this culture. The defoliation caused by this Lepidoptera occurs on the higher phase of plant grown, affecting seriously the production. The natural pheromone was extracted from female pheromonal glands by the use of solvent extraction, between the 7th and 10th hours of scotophase. Y-olfactometer bioassays showed great attractiveness of males to extracts with notable behavioral changing with exposure to natural compounds. The electroantennographic activity of these compounds in male antennae was tested in GC-EAD assays, and it was observed the presence of six possible pheromonal components. The chemical structure of the compounds was studied by analyzing their chromatographic profiles, mass (GC-MS) and infrared spectra (GC-FTIR), as well as by analysis of microderivatizations products with 4-methyl-1,2,4-triazol-3,5-dione (MTAD), with DMDS and isomerization of double bonds employing thiophenol. The spectroscopic and chromatographic information obtained indicated that the compounds refer to aldehydes belonging to the type I Lepidoptera pheromonal class and their structures were identified as: *E*-hexadec-12-enal (**1**), (11*E*,14*Z*)-hexadeca-11,14-dienal (**2**), *Z*-hexadec-14-enal (**3**), (10*Z*,12*E*)-hexadeca-10,12-dienal (**4**), (10*E*,12*Z*)-hexadeca-10,12-dienal (**5**) and (10*E*,12*E*)-hexadeca-10,12-dienal (**6**). The chemical structure of the compound **2** has to be confirmed by its respective synthesis which is underway.

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O12 - Alarm pheromones of *Pachycoris torridus*

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The aim of this research was to evaluate qualitatively the defensive compounds (alarm pheromones) emitted by 5th instar nymphs and adults of *Pachycoris torridus*. The dorsal abdominal glands (DAGs) of the 5th instar nymphs were extracted in redistilled hexane. Female and male stink bugs were kept in a freezer at -20°C for 10min before preparing the extracts [1]. Adult hexanic extracts were prepared from the whole abdomen, last abdominal segments, metathoracic glands (MTG), salivary glands, adipose tissue, oocytes (females only). Aliquots of 1 µL samples were injected into the Gas Chromatograph (GC), Shimadzu 2010, equipped with Flame Ionization Detector containing capillary column RTX-1 (0.25mmx30m). The Kovats retention index was calculated using standard hydrocarbon (C7-C30). The chromatogram profiles showed that the macerated extracts had a lesser amount and relative concentration (area) of the compounds than those that were not macerated. Then 1µL of extracts not macerated were injected into Mass Spectrometer (GC/MS-QP2010 Ultra). The compounds identified in extracts of adult insects were: tridecane, 2-hexenal, E-2-hexenal, dodecane, hexacosane, tetradecanal, and 2-methyleicosane. While the compounds identified in DAGs were: heptenal, dodecane, undecane, hexanal, 2-heptenoic acid, Z-3-hexadecene, tridecane, tetradecane, octanal, pentadecane, tetradecanal, hexacosane, hexadecane. These volatiles are described in the literature as alarm pheromones of many species of Heteroptera, including some of those described in *Pachycoris stalii* [2]. The results showed that the putative defensive compounds of males, females and nymphs are produced in several anatomical structures.

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Acknowledgements

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O13 - Cuticular compounds recognition and mating behavior of the rice water weevil *Oryzophagus oryzae* (Coleoptera, Curculionidae)

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O. oryzae is the main pest of irrigated rice crops in Brazil because larvae destroy roots of rice plants. Insecticide spray on leaves and granulated insecticides formulations applied in the irrigation water are used as control methods. However, the development of new control and monitoring system based on semiochemicals could be a viable alternative to chemical insecticides. Providing basic information for Chemical Ecology studies, three initial experiments were performed to determine the period of sexual activity, if weevils were able to find each other and mate in water free environment; and to learn if one sex is preferentially attracted to the other. Recognizing when and how matings occurred, we investigated the presence of semiochemicals emitted by the weevils performing four types of extractions: aeration, water, abdomen and cuticle. With these four types of extractions we examined if weevils emitted semiochemicals directly into the air and/or directly into the water; we tested the direct extraction of the insect and the recognition of the compounds present on the cuticle of females by males. Bioassays revealed that couples had intense sexual activity in the first 3 hours of photophase and scotophase; adults mated and re-mated at any time of day in the presence of water, although in the absence of water, only 10% of adults mated; females were always attracted to males, seeking them, before males tried to mate; and males showed guarding activity which prevented female sexual activity with rival males and allowed remating. Four steps were observed before mating occurred (a-d): female approached male (a); male mounted female (b); male walked over female's back and tapped her with his forelegs (tapping or stroking mechanism) (c); female accepted male and they mated (d). Extractions revealed that males and females had similar cuticular chemical compounds, though in different concentrations. Bioassays suggested that males recognized females by their cuticle composition.

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O14 - Identification and formulation of pheromones of the ambrosia beetle *Megaplatypus mutatus* and field management using reservoir and monolithic delivery systems

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Megaplatypus mutatus (Chapuis) (Coleoptera: Curculionidae: Platypodinae) is an ambrosia beetle native to South America, but it has recently been introduced to Italy and represents a serious problem in commercial poplar and fruit tree plantations. Male *M. mutatus* emits a sex pheromone composed of (+)-6-methyl-5-hepten-2-ol [(+)-sulcatol], 6-methyl-5-hepten-2-one (sulcatone), and 3-pentanol. We made reservoir and monolithic type dispensers for pheromones to be deployed in the field during the flying period. The release rates of the dispensers were measured in a wind tunnel at controlled temperature and wind speed.

The polymeric reservoir-type dispensers had constant release rate (zero order kinetics) in the range of milligrams per day for periods of until 15-20 days. The monolithic dispensers were made with different mixtures of waxes and polymers with inert components. They followed first-order kinetics.

We performed field trials of mating disruption of *M. mutatus* and trapping in baited traps in highly infested hazelnut and poplar plantations of Italy and Argentina. Different shapes and colour of traps were tested. Taking into account that the beetle is relatively immobile, that males are monogamous, and that the pheromones are of very low commercial cost, stable in field conditions and can be formulated in controlled released systems with relatively high release rates, we evaluated the potential management by disruption of communication. After the treatment, the number of galleries where mating took place was significantly higher in control than in treated areas, indicating that pheromone application had interfered with female behavior and male localization. As damage reduction was greater than 56% in both countries, these results show the potential for the strategy of pheromone-mediated mating disruption of *M. mutatus* in commercial poplar and hazelnut plantations. Also, our study provides the first evidence for successful pheromone-mediated mating disruption in a forest beetle.

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O15 - Olfactory Memories Promote Toxic Bait Ingestion

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The use of olfactory cues has been extensively studied for controlling harmful insects. The case of ants is particular because, being social insects, only a low proportion of workers leave the nest for resources. They forage and transport the food into the nest where it is distributed to their nest-mates. Given that the workers are infertile, *alimentary baits* are recommended for ant chemical control. Sugar solutions are effective baits for a large number of ant species. Some commercial baits for residential control often contain sugar as an attractant and boric acid as toxicant. However, *Camponotus mus* ants, unlike other species, frequently reject this toxicant.

Although some pheromone compounds have been tested to supplement chemical control for invasive ants with current global distribution, the use of these compounds is limited as they tend to be species-specific, and there are many species to control. Therefore, we proposed to use olfactory cues not belonging to the ants -but that could be learned by them- to examine whether this memory can promote higher acceptance of a toxic bait that presents the same odor.

We studied the behavioral responses of *C. mus* ants under controlled conditions. First, we proved that the presence of linalool in a boric acid bait did not generate an increase in its acceptance. Then, we evaluated if the memory established in the trophallaxis context during recruitment could promote a higher ingestion of scented toxic bait while foraging. Results showed that ants that learned the odor of the sugar solution received from a nest-mate ingested more toxic bait containing the same odor than control ants confronted with non scented sugar solutions.

These results, applied to a protocol for commercial baits, could increase the range of species in which boric acid is effective.

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O16 - Reverse Chemical Ecology of *Hylamorpha elegans* Burmeister (Coleoptera: Scarabaeidae)

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Three-dimensional structure of proteins can give a major understanding about functions and their regulation when ligands are involved. An inexpensive choice to obtain protein structures are computing modeling approaches, such as *ab initio*, threading and homology or comparative modeling. On the other hand, molecular docking can be used to predict how and where small molecules are bound to proteins. Odorant-binding proteins (OBPs) are biomolecules involved in the olfactory perception of insects, acting as a first filter of olfactory information in insects during their search of host, potential mate and prey. From the function and characteristics of OBPs, reverse chemical ecology has emerged as a novel concept to determine semiochemicals [1]. To date, Lepidoptera, Diptera, Hemiptera and Coleoptera are the orders with a broad research developed on OBPs. Within the Coleopterans, in Chile the endemic beetle *Hylamorpha elegans* (Coleoptera: Scarabaeidae, Rutelinae) belongs to the white grub complex, which causes large economic damages mainly in wheat and grass crops. This phytophagous insect feed on roots during its larval stage, resulting in a decomposition of plants and a subsequently occurrence of yellow spots. Likewise, these beetles during the adult stage are defoliators of *Nothofagus obliqua*, where the distribution of the *H. elegans* correlates with the distribution of the tree between regions VII and X. Conventional Chemical Ecology studies allowed to determine that a blend of 1,4-benzoquinone, released only by unmated females, mixed with *N. obliqua* essential oil was attractive to conspecific males [23]. On the other hand, recent results indicate that *H. elegans* have three antennae-specific proteins, where currently one of them is related with a PBP. The results are discussed in terms of selecting high affinity semiochemicals to *H. elegans* OBPs by homology modeling and molecular docking.

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O17 - Influence of UV-B radiation in chemical and photosynthetic response of the freshwater macrophyte *Nymphoides indica* (Menyanthaceae)

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Climate changes over the past decade reduced the ozone layer, increasing the intensity of Ultra-Violet B (UV-B) radiation. Increased UV-B radiation can directly affect the photosynthetic rate and secondary metabolite production. The most common mechanism of protection against possible damage is their biosynthesis and / or bioaccumulation of secondary metabolites, including phenolic compounds. *Nymphoides indica* has phenolic compounds besides presenting a chemical defensive against herbivores. The aim of this study was to evaluate the photosynthetic efficiency and chemical response of the *N. indica* against oxidative stress caused by UV-B radiation. Twenty specimens of the *N. indica* collected in Lagoa Comprida, Parque Nacional da Restinga de Jurubatiba (Rio de Janeiro, Brazil) underwent cultivation in two conditions: 1) with photosynthetic active radiation PAR plus UV-B radiation (311nm) and 2) only PAR. To evaluate the photosynthetic efficiency, measurements of chlorophyll *a* fluorescence was conducted with a portable Pulse Amplitude Modulation Fluorometer- Mini-PAM. The secondary metabolites profile of organisms was analyzed by High-Performance Liquid Chromatography (HPLC-UV-DAD) and their correlations were summarized using Principal Component Analysis (PCA). Our results showed that the application of a UV-B dose twice as stronger than the observed in middle summer did not induce significant differences of photosynthetic efficiency in *N. indica*. It suggested a strong acclimation capacity of this specie to conditions of high UV-B radiation. The chemical profiles from the leaf extracts of *N. indica* showed to be highly complex, but the PCA was able to discriminate the individual samples from plants treated with and without UV-B radiation, according to the first principal component. The factor loadings help to identify the substances with retention time of 28.3 minutes (higher concentration in individuals cultivated without UV-B) and 29 minutes (higher concentration in individuals cultivated without UV-B) as the mainly responsible for the separation of groups. The UV spectrum (HPLC-UV-DAD) suggested that these metabolites belong to the group of phenolic compounds. The results suggeste the influence of UV-B radiation on the biosynthesis and/or bioaccumulation of secondary metabolites that are possibly acting as a chemical defense in *N. indica*.

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O18 - Interaction of Chemical and Structural Components Providing Defenses to Sea Pansies *Renilla reniformis* and *Renilla muelleri*

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Chemical and structural defense mechanisms are reported to co-occur in both terrestrial and marine systems¹. Among benthic marine organisms, the common co-occurrence of secondary metabolites and calcium carbonate (CaCO₃) in soft corals provides an opportunity for testing synergistic interactions between these traits. Defensive properties of crude extracts, chemical fractions and CaCO₃ sclerites from two sympatric species of soft corals, *Renilla reniformis* and *R. muelleri*, from Guanabara Bay, southeastern Brazil, were examined against fishes. To evaluate a potential interaction (secondary metabolites versus sclerites), both crude extracts and sclerites were evaluated as isolated defenses and in combination in field assays against generalist fishes during the austral summer of 2007. While neither sclerites nor crude extracts from *R. reniformis* deterred feeding when offered individually in artificial food, both traits from *R. muelleri* offered individually provided effective defense. For both species, however, the combination of secondary metabolites and sclerites significantly deterred feeding, indicating that these traits are more effective in combination than in isolation.

Key words: Chemical defense, Physical defense, Synergism, Sea Pansies.

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O19 - Intraspecific variation on herbivory defenses in freshwater plant *Typha domingensis* Pers.

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Secondary metabolites play multiples roles in the nature and vary remarkably as a function of space and time. Freshwater plants are chemically defended against herbivores. *Typha domingensis* Pers. has a cosmopolitan distribution and shows relevant structural and metabolic importance in aquatic ecosystems. Many herbivores species are observed living associated with this specie but there is no evidence that these organisms feed on plant. The aim of this study was 1- evaluate if the secondary metabolites of *T. domingensis* are activity against herbivory, 2- investigated the geographical variations on the chemical defense of crude extract and 3- evaluate there is variation at chemical profile of secondary metabolites in the extracts. For this study four different populations were collected at Cabiunas lagoon, Parque Nacional da Restinga de Jurubatiba, Rio de Janeiro, Brazil in May 2011. Methanol extracts were analyzed by High-Performance Liquid Chromatography (HPLC-UV-DAD) and comparison with standard compounds. The herbivory bioassays were performed with *Biomphalaria sp* The artificial foods (control – without crude extracts – and treatment – with crude extracts in natural concentrations) were offered simultaneously to one herbivory per replicated. The HPLC analyses showed complex chemical profiles and a high concentration of phenolic compounds in all the extracts. Qualitative and quantitative differences were observed to compounds in each population. All the extracts of *T. domingensis* presented activity against *Biomphalaria sp*. ($p < 0.05$, $N = 14$ to 19 , Wilcoxon test). However, intraspecific variations on the chemical defense were observed between the populations. Abiotic factors varied among locations in Cabiunaslagoon and may be affect the secondary metabolites composition in *T. domingensis*. Changes on the secondary metabolites in freshwater plants can influence the structural of a community and driven evolutionary aspects in plant-herbivores interactions.

POSTERS
Abstracts

P1 - A first approach on the evolution of chemical defense of benthic invasive marine invertebrates in Brazil

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Marine Chemical Ecology aims to investigate the ecological interactions mediated by secondary metabolites present in marine organisms. It is known that chemical defense strategies provide protection against consumers and competitors, enhancing the successful on colonization of exotic species and their dispersion into new environments [1]. The scleractinian corals *Tubastraea coccinea* Lesson, 1829 and *Tubastraea tagusensis* Wells, 1882 are worldwide distributed. Both species were originated from the Indo Pacific region and they were first reported in the Brazilian coast in the 1980's encrusting oil and gas platforms in the Campos Basin, state of Rio de Janeiro. More recently, *Tubastraea* spp. has expanded to the rocky shores of Ilha Grande Bay and Arraial do Cabo, both located in Rio de Janeiro. This genus is considered harmful to the native species and its invasion can represent a threat to the marine biodiversity [2]. The present study has compared the chemical defense potential of *T. coccinea* and *T. tagusensis* face to generalist consumers from two different sites. The colonies were collected in the rocky shores of Arraial do Cabo and Ilha Grande Bay. Their secondary metabolites were extracted using solvents (methanol and dichloromethane) and then artificial food was prepared to the development of the field trials to analyze consumption by generalist fishes. Among all the treatments conducted with extracts of *T. tagusensis*, none of them were able to significantly minimize artificial food consumption, so there was not defensive potential. However, the only trial that exhibited significant reduction of consumption was the treatment carried out with dichloromethane extract of *T. coccinea* from Ilha Grande Bay. Thus, considering invasive species as substantial opportunities to explore ecological aspects, the comparison of the defensive response of species from different sites may contribute to the understanding of factors related to the evolution of chemical defense.

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P2 - Resistance to Herbivory: Comparison between a Cultivated and a Wild Potato

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Plants have developed many strategies to counteract herbivory during evolution. However, in cultivated species the domestication process may result in the loss of such defensive capacity. This can be assessed by comparing these traits between wild and cultivated congeners. The objective of this study deals with the anti-herbivore resistance of two solanaceous plants, the cultivated potato *Solanum tuberosum* and its wild congener *S. commersonii*, which grows as a weed and is native to Uruguay. A field study (6 weeks) was done to compare the types and amounts of herbivores and natural enemies in alternated plots with cloned plants for both species. Insects were sampled weekly from the aerial part of the plants and transferred to the laboratory for identification and recording of parasitoids. Herbivory levels and the abundance of leaf trichomes were estimated at the end of the field experiment. In addition, the preference of *Myzus persicae*, one of the most abundant insect herbivores found in the field experiment, was studied in a two-choice laboratory bioassay. The results showed that more herbivores (specifically aphids) and natural enemies were obtained in the cultivated species in the field, which parallels the preference of *M. persicae* for the cultivated species in the laboratory bioassay. The wild species, however, was more consumed (2.4 times) due to a chewing herbivore which was exclusively found on *S. commersonii*. Both plant species showed equal types of simple and glandular trichomes, although their density was higher in *S. commersonii*. Finally, chemical analysis (GC-MS) of volatiles from intact specimens showed that both species emit different odor blends, both quantitatively and qualitatively. While in *S. tuberosum* the main compound was β -cariophyllene, *S. commersonii* emitted D-germacrene as its main volatile. Further studies are needed to evaluate both direct and indirect (volatiles) defense mechanisms employed by these *Solanum* species against herbivorous insects.

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P3 - Infection by *Soybean Mosaic Virus* alters soybean-aphid interaction

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Virus infection induces changes in plant phenotype that can affect the interaction with the vector. Virus-induced alterations in plant morphology and physiology lead to changes in vector behavior (attraction, arrestment and performance) that helps virus dispersal. The aim of our work was to investigate if infection by *Soybean Mosaic Virus* (SMV, an aphid-borne virus) alone and in co-infection with *Bean Pod Mottle Virus* (BPMV, a beetle-borne virus) alters the behavior of the aphid *Aphis glycines* (Hemiptera:Aphididae) toward soybean plants. In arena dual choice tests (visual+olfactory cues), wingless aphids clearly showed a preference for orienting towards SMV-infected plants over healthy plants although they did not discriminate between BPMV-infected or SMV+BPMV-infected soybean and healthy plants. Volatile collections showed that SMV and BPMV both reduced the total volatile emissions of soybeans, although our choice test indicates that for SMV, this does not reduce attractiveness to *A. glycines*. We also found that aphid growth was significantly reduced on SMV-infected plants compared to healthy or co-infection (SMV+BPMV). This observation may be due to lower amounts of both amino acids and sugars found in the phloem sap of SMV-infected soybean, but reductions in these nutrients does not explain aphid performance on SMV+BPMV-infected plants, which is nutritionally poor as well. In terms of defense, SMV-infected and SMV+BPMV-infected plants have reduced levels of JA and increased levels of SA compared to the healthy plant. Thus, infection by SMV causes changes in soybean volatile profile, nutritional quality and defense system, which may influence aphid performance (e.g., for SMV-infected plants). Our data also suggest other factors, such as the ease of feeding on the plant, may also be important to consider. Future experiments should include electrical penetration graphing to measure feeding by aphids, as well as choice tests that separate visual and olfactory plant cues in aphid preference assays.

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P4 - Comparison between floral and extrafloral nectar composition of *Passiflora* species (Passifloraceae) and their licking insects

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Studies on nectar chemistry concerning floral nectar are abundant, fewer ones deal with extrafloral nectar and only very few compare floral vs. extrafloral nectar in the same taxa. Relationships between floral visitors and floral nectar concentration are well known, while relationships between floral visitors and floral nectar constituents are rarer. In contrast, relationships between extrafloral visitors (mostly ants, but also wasps, flies and other insects) and extrafloral nectar features have not been thoroughly studied. To ascertain if the chemical characteristics respond to the different visitors preferences or to phylogenetic constraints, the composition of floral nectar was compared to that of extrafloral nectar within each of three *Passiflora* species with both kind of these glands. Screening with HPLC for the three main sugars in nectars and 17 proteinogenic amino acids was performed in 53 samples (28 floral and 25 extrafloral) of *P. caerulea*, *P. suberosa* and *P. misera*. Fructose, glucose and sucrose occurred in both nectar types but their proportions varied within two species. Total amount of sugars in floral nectar was similar to that of the corresponding extrafloral nectar in all species; it was slightly lower in *P. caerulea* and *P. suberosa*. A larger range of variation of sugar total amount occurred in the extrafloral nectar of each species, while that of floral nectar was more consistent. The amino acids had a higher concentration in the floral nectars (the double or the quadruple), the number showed no clear tendency, their quality and frequency were alike in each counterpart. The chemical properties of each nectar type are discussed in relation to the plant traits associated with the interactions involved (pollination vs. antiherbivore defence) and the nutritional requirements, morphological features and behaviour of the insect partners. Evolutionary aspects of these secretory structures and of the plant taxa studied are also considered.

P5 - Is *Haplopappus platylepis* (Asteraceae) killer resin an effective barrier against herbivores?

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Plants experience herbivory by insects in all its life cycle. This can directly affect its reproductive success; so it is proposed that the existence of traits that confer resistance against insects arise as a result of herbivores evolutionary pressure¹. We discovered a mixture of resinous compounds in the outer surface of the inflorescence buds of the plant *Haplopappus platylepis* that maybe considered as a chemical-mechanical resistance against herbivory. Many different insects die by getting stuck in to this layer. However there are others that visit the inflorescence as it opens and are able to feed on its floral resources. Our analysis detected that the resin is dominated by a complex mixture of labdane diterpenes, with small amounts of lipophilic flavonoids (flavonols) and a monoterpene (limonene). In addition, we found that most of the insect killed by the bud resin corresponded to larcenists such as *Arthrobracus* sp. beetles and argentine ants, those opportunistically feed on *Haplopappus* pollen. In a minor extent these corpses corresponded to the fly *Dioxya chilensis* and the microlepidopterans *Lioptilodes friasi* and an unidentified Tortricidae species. Moreover, we scored opened-inflorescence visitors that do not touched the resin barrier such as pollinator candidates and some of the above-mentioned parasites. We concluded that *H. platylepis* resin is an effective barrier against some larcenist but not towards pollinator or specialized endophagous floral parasites.

[1] Coley, P.D., Bryant, J.P., Chapin, F. S. I. (1985). Resource Availability Plant Antiherbivore Defense.

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P6 - *Apis mellifera* cuticular hydrocarbon analysis according to food source profitability: linking released compounds with communication

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When a honeybee (*Apis mellifera*) collects nectar with high sucrose concentration from a flower, she returns to the hive and dances vigorously, raising her corporal temperature. This display promotes the release of certain cuticular hydrocarbons which have been postulated as a relevant stimulus for the inactive foragers inside the hive to start foraging. Our study consisted of identification, comparison and quantification of the cuticular hydrocarbons released by honeybees while collecting at a low or high profit food source (0.5M or 2M sucrose concentration). We used artificial feeders where honeybees were trained and captured. The honeybees were killed using CO₂. Cuticular hydrocarbons were extracted by immersing the honeybees in dichloromethane. Samples were analyzed using a CG-MS.

We found differences in cuticular hydrocarbons between honeybees captured directly from the hive and those that had collected sucrose solution. Moreover, honeybees that collected high profit food sources showed different profiles from those in contact with 0.5M sucrose solution.

We propose that the presence of these cuticular hydrocarbons could be relevant in the recruitment context. The differences found according to food source profitability suggest that there could be a modulation in the release of chemical cues associated to a differential recruitment.

This study was partly supported by grants from ANPCYT, Universidad de Buenos Aires and CONICET.

P7 - Volatile organic compounds produced by native fluorescent *Pseudomonas* improves essential oils in *Mentha piperita* L.

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Some bacteria, including fluorescent *Pseudomonas*, live in the rhizosphere and promote plant growth. In the present work, fluorescent *Pseudomonas* were isolated from rhizospheric soil of a commercial crope of *Mentha piperita* L located in Villa Dolores, Córdoba. Explants of *Mentha piperita* L were exposed to volatile organic compound (VOCs) produced by these native bacteria. The aim of this work was to determinate if VOCs emitted by bacteria affects the production or composition of essential oil (EO) in *Mentha piperita* L.

A total of 40 bacterial strains were tested where only 11 strains produced an increase in plant growth parameters. In this sense, shoot fresh weight of plants exposed to bacterial VOCs was increased 3 to 4.5 folds relative to control, plants exposed to distilled water. These plant growth promoting strains were further studied and selected for the analysis of EO. Gas chromatography was used to determinate qualitatively and quantitatively the composition of monoterpenes. Plants exposed to VOCs of SJ25 and SJ27 strains displayed a significant increase ($p < 0,05$) in total content of EO, relative to control. The amount of limonene was increased in plant subjected to VOCs of SJ04, SJ28, SJ7b and SJ48 strains, whereas that the amount of menthol and menthyl acetate was increased in plants exposed only to VOCs of SJ04 and SJ7b strains, respectively. All strains displayed a decrease in content of menthofurane. No significant variations in the content of de menthone, terpineol, pulegone and menthofurane were observed. The sequences of nucleotide of 16S RNAr gene were analyzed on these strains in order to establish their identity and taxonomic position in a phylogenetic tree constructed with fluorescent *Pseudomonas* used as reference. We conclude that some native fluorescent *Pseudomonads* are able to improve the production of essential oils in micropropagated *Mentha*, even when knowledge of plant promoting-growth activity of VOCs and its mechanism of action are very poor.

P8 - Jasmonic acid regulates defenses in soybean (*Glycine max L.*) developing seeds against stinkbug (*Nezara viridula*) attack

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Insect attack increases the production of defenses, which are regulated by the phytohormone jasmonic acid (JA). Southern green stinkbug (*Nezara viridula*) is one of the most important pests in soybean crops and affects both yield and seed quality. However, little is known about soybean response to stinkbug attack on developing seeds. Lipoxygenases 1, 2, and 3, which regulate JA accumulation, are only present in soybean seeds. Our aim was to determine the role of JA in the regulation of induced defenses, such as proteinase inhibitors (PI), when soybean seeds are attacked by stinkbugs. We used the soybean mutant BRM 926600 genotype, without *lox1*, 2 and 3; and Williams that normally express gene *lox*, as a control. Four treatments were applied on seed pods: i) no damage; ii) mechanical damage; iii) stinkbugs damage and; iv) methyl jasmonate (MeJA) applied on pods. The transcriptional expression of *pi* genes was estimated by the RT-PCR reaction. Stinkbug attack and MeJA induced gene expression and activity of trypsin and cysteine proteinase inhibitors (TPI and CysPI respectively). On the other hand, no induction of either TPI or CysPI expression and activity was observed in BRM 926600 mutant, except under MeJA treatment where the application recuperated in part the PI expression, suggesting that JA regulates PIs in soybean developing seeds.

P9 - Nitric oxide modulates the ingestion of ATP in *Rhodnius prolixus*

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Animals in the presence of a food source can test its quality by means of taste, and this allows them to make the final decision of feeding on that source or searching for another one. Nitric oxide (NO) is a neuromodulator which participates in a great number of physiological and biochemical processes in almost all living organisms. In insects, NO modifies sensory inputs and behavioural responses associated to chemical stimuli, both olfactory and gustatory ^[1]. In this work we studied the role of NO in the recognition and acceptance of a food source by the bug *Rhodnius prolixus*. A solution of 10⁻³ M of ATP was offered as a food source. Insects were previously fed for 1 minute on solutions of the NO donor S-nitroso-acetylcysteine (SNAC) of different concentrations. Pre-treated insects were then allowed to feed on the solution of ATP for 5 minutes. Ingestion was determined by weighing insects before and after feeding on ATP. Insects with different starvation periods were used (15-20 days and 30-45 days). We observed a dose-dependent decrease in the ingestion of ATP in insects starved for 15-20 days. The ingestion of ATP was almost suppressed in insects pre-treated with the highest dose of SNAC tested (200 mM). However, insects starved for 30-45 days and pre-treated with this same concentration of SNAC did not show any decrease in the ingestion of ATP. A role of NO in the evaluation of the quality of a food source in *R. prolixus* is suggested. Moreover, this effect varies depending on insect's motivation for feeding given by its starvation period.

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P10 - The herbivory activities of sterols isolated from *Potamogeton illinoensis* (Potamogetoneceae)

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Aquatic plants are an important spatial refuge for many aquatic invertebrates. However the secondary metabolites in plants show deterrent activity against these herbivores. The co-evolution between plants and herbivorous arthropods resulted in a diversity of secondary metabolites. *Potamogeton* is a widespread genus of 100 predominantly perennial aquatic plant species. Most of them are valuable fodder plants for waterfowl and fish. The phytochemical study has already led to the isolation of several diterpene lactones, phenolic compounds and sterols, some of them showing a high antialgal, antiviral and allelopathic activities. Previous studies by our research group showed that the crude extract of *P. illinoensis* has defenses against herbivory. The aim of this work was evaluate which compounds are involved in the chemical defense observed to plant extract. The macrophytes were collected in the Parque Nacional da Restinga de Jurubatiba (PNRJ), Macaé, in Rio de Janeiro, Southeastern Brazil. The hexanic extract of *P. illinoensis* was analyzed by Nuclear Magnetic Resonance (NMR) and Gas Chromatography-Mass Spectroscopy (GC-MS). Its phytochemical study was conducted and the secondary compounds were isolated. The hexanic extract was also evaluated in herbivory bioassays with *Biomphalaria* sp. (Planorbidae), a generalist herbivorous known as the vector of *Schistosoma mansoni* (Schistosomatidae). The results were analyzed using the Wilcoxon statistical test for paired samples. The ¹H and ¹³C NMR and GC-MS analyses revealed the presence of labdane diterpenes type furan, sterols and fatty acids as major compounds. The fractionation of the crude extract of *P. illinoensis* yielded a mixture of sterols stigmasterol and ergosterol, identified by comparison of NMR data and mass spectrum with the literature. Both the hexanic crude extract and the mixture of sterols showed chemical defense against herbivory (p=0.0002, N=18 and p=0.0009, N=32, respectively). The results of this study indicate that the production of these compounds may be related to plant defense system, contributing to the ecological success of this species.

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P11 - Composition and fumigant activity of essential oils of *Tagetes terniflora* against the stored-grain pest *Tribolium castaneum* and *Sitophilus oryzae* (Coleoptera)

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As is well known, *T. castaneum* and *S. oryzae* cause serious damage to stored grain products throughout the World, and the two species are the major pests found in Ingeniero White Port, Bahía Blanca, Argentina, one of the most important overseas ports of the country [1]. *T. terniflora* is a native specie of northwestern Argentina. As part of a screening programme to evaluate the bioactivity of native plants of Argentina, the objective of this work was to determine the composition of the essential oil from *T. terniflora* and to analyse the fumigant activity against *T. castaneum* and *S. oryzae* adults. To evaluate the fumigant toxicity on adults, Whatman N° 1 filter papers (5 cm²) were impregnated with hexane solution (50 µl) at different concentrations. Hexane was used as a control. The filter paper were palced on small vials (0.8 cm diameter x 2.8 cm height) standing inside a 40 ml vial. The insects were released in the bottom of the outer vial to avoid direct contact with the products tested, and assess the toxicity of vapours. Three replications were set up. Mortality was determined after 72 h of treatment, and LC₅₀ values were calculated by probit analysis [2]. Analysis by GC-MS of the steam distilled oils showed that the major components of *T. terniflora* were *cis*-Ocimene (27.3%) and *cis* + *trans*-tagetone (26%). The essential oil demonstrated fumigant toxicity for *T. castaneum* and *S. oryzae* (CL₅₀=362.8 µg cm² and 322.61 µg cm² respectively). Statistical analysis of the data did not show significant differences between LC₅₀ values obtained for any of the species tested. It can be concluded that the essential oil of *T. terniflora* have potential application in the control of different stored-product pests.

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[2] Pascual Villalobos, M.; Ballesta-Acosta, M y Soler, A. (2004). Toxicidad y repelencia de aceites esenciales en plagas de almacén del arroz. Boletín de la Sanidad Vegetal Plagas 30: 279-286

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P12 - Ovicidal activity of *Thymus vulgaris* (Labiatae) against *Pediculus humanus capitis* (Anoplura, Pediculidae)

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In Argentina, pediculosis is a serious health problem that affects more than 30% of school-age children. The prevalence of head lice (*Pediculus humanus capitis* De Geer) in kindergarten from Bahía Blanca (Province of Buenos Aires, Argentina) was 42.7% [1]. Today, essential oils (EOs) are an interesting alternative in the control of this insect. The aim of this study was to evaluate the ovicidal activity of EO from *T. vulgaris*. Lice were collected using metal comb from infested children 3-5 years old. The oil was obtained by hydrodistillation in a modified Clevenger type apparatus and analyzed by gas chromatography coupled to mass spectrometry (GC-MS HP5972A). The analysis revealed the presence of major components such as p-cymene (26%), γ -terpinene (21.8%) and 1-terpinen-4-ol (16.3%) and minor components such as carvacrol (3.1%) and δ -terpinene (3.3%). Eggs were held at 31 ± 1 °C and $65 \pm 5\%$ RH and darkness. Filter papers (5.5 cm diameter Whatman No. 1) were treated with 100 μ l of hexane solution. Four concentrations were used (0.21 mg/cm², 0.08 mg/cm², 0.05 mg/cm², 0.02 mg/cm²). Control filter papers received hexane alone. After solvent evaporation, filter papers were placed in the bottom of a Petri dish. Groups of 10 eggs (3-4 days old) attached to hair were placed in each Petri dish and covered with a lid. All treatments were replicated three times. After 8 days, the percentage of inhibition of hatch (PIH) was calculated from the formula $PIH (\%) = [(C - T)/C] \times 100$, where C is the control percentage hatch and T is the treated percentage hatch [2]. The PIH were 100%, 87%, 57% and 33%, respectively. These results indicate that the essential oil of *T. vulgaris* could be considered as an alternative to control this parasite.

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[2] Werdin González, J.O., Gutiérrez, M.M., Murray, A.P., Ferrero, A.A., 2011. Composition and biological activity of essential oil from Labiatae against *Nezara viridula* (Hemiptera: Pentatomidae), soybean pest. Pest Manag Sci. 67: 948-955.

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P13 - Synthetic and biostabilized sulfakinin peptides affect food intake in *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

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Insect sulfakinins (SKs) are short neuropeptides with a conserved C-terminal hexapeptide (Y[SO₃H]GHMRamide). These peptides show structural similarity with the cholecystokinin hormone of vertebrates that regulates food uptake and digestive processes. In several insects, SKs have been shown to reduce food intake [1] and they are associated with several ganglia and the CNS of insects, affecting food intake and digestion.

In our project, we injected synthetic and biostabilized SK peptides in the lesser grain borer, *Rhyzopertha dominica* and analyzed accumulative food intake and mortality after 3 days. We evaluated the effects of single and combined peptides to understand the function of these novel molecules.

We found that peptides as FDDYGHMRFa and FDD[A]GHMRFa with the conserved sequence GHMRFa, homologous to that of the SKII GEEPFDDYGHMRFa, had an SK agonistic effect reducing food intake. On the other hand, peptides as DGY[SO₃H]RPLQFa had a SK antagonistic effect, increasing food intake. Previously, similar results were found on another important pest, *Tribolium castaneum* [2].

More studies should be done to elucidate the mode of action, of these peptides with SK agonistic and antagonistic activity.

The data obtained, provide tools to better understand the process of feeding and growth of insects.

[1] Downer K.E., Haselton Q.T., Nachman R.J. & Stoffolano Jr. (2007) Insect satiety: Sulfakinin localization and the effect of drosulfakinin on protein and carbohydrate ingestion in the blow fly, *Phormia regina* (Diptera:Calliphoridae). *Journal of Insect Physiology*, 53: 106-112.

[2] Yu N., Benzi V., Nachman R.J. & Smagghe G. (2012) Expression profile and role of sulfakinin and receptor in model and pest insect *Tribolium castaneum*. 26th Conference of European Comparative Endocrinologists. Zürich, 21-25 August 2012.

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P14 - Chemical profile of cotton plants involved in the foraging behaviour of *Anthonomus grandis*

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Anthonomus grandis is the main pest on cotton crops around the world, in Brazil; millions of tons of pesticides are used to control this pest every season. To try to minimize the abusive use of insecticide in cotton crops the MIP is encouraging the use of the aggregation pheromone to monitoring the population (Tumlinson et al., 1969). However, when the plants start to emit the reproductive structures, males and females of *A. grandis* are not caught any more in the aggregation pheromone trap and go directly to the plants. Several studies have been proposed that volatile compounds emitted by reproductive stage of the cotton plants are the responsible for this attraction. However, we still not have a blend of cotton volatiles that could be the responsible for this attraction. Therefore, the aim of this work was to evaluate the chemical profile from different cultivars of cotton plants that could be involved in the attraction of *A. grandis*. Two different cultivars Delta-OPal and BRS293 had the chemical profile of volatiles studied considering the treatments: damaged and undamaged by herbivory by *A. grandis* in both physiological stage (reproductive and vegetative). The chemical analysis of air-entrainment extracts from cotton plants (undamaged and damaged by *A. grandis*) showed that the cotton plants released quantitative different chemical profile when compared between damaged and undamaged plants, and the main compound involved in this difference are monoterpenes, such as (E)-ocimene, when the both physiological stages were compared, the monoterpenes did not varied, but homoisoprenoids, such as DMNT and TMTT, were released in different amounts. These compounds might be involved in the attraction of *A. grandis*.

P15 - Effects of extract of *Fuorensia oolepis* (chilca) on oviposition behavior of *Helicoverpa gelotopoeon* (Lepidoptera Noctuidae)

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Synthetic insecticides are the most used tool for pest control. However, it has been found that indiscriminate use of these chemicals has resulted in irreversible damage to the environment, non-target species, etc. Currently, botanical insecticides are one of the environmentally acceptable alternatives. Extract of *Fuorensia oolepis* (Asteraceae) has shown to be effective against several species of insect pests. The purpose of this work was to assess the extract crude effect of *F. oolepis* on oviposition behavior of *Helicoverpa gelotopoeon*. This butterfly is polyphagous pest of several economically important crops. Choice tests were performed using the extract of "chilca" [1] on the surface half of the breeding recipients at different doses (1% to 10%), and the other half were sprayed with acetone. Two pairs of butterflies per cone were placed and performed 10 repetitions per dose. Daily observations were made; eggs were counted and established the period of pre-and post-oviposition. The data were analyzed through the ANOVA or Kruskal Wallis, t test or Wilcoxon test, and the inhibition rate was calculated. The results showed significant differences in the number of eggs laid on the control surface relative to the extract treated for any of the doses used. Females chose particularly the wall and ceiling of the recipient to attach their eggs, places that can interact with the host plant preferred [2]. Furthermore, the inhibition index showed that the dose of 2.5% had a marked effect on the oviposition deterrent, while 7.5% behaved opposite. Comparable responses were observed with other flavonoids.

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[2] IGARZÁBAL, D., P. FICHETTI, F. NAVARRO, G. MAS & J. MORRE. 2011. Manejo de orugas defoliadoras. DuPont, Buenos Aires, pp. 43-46.

P16 - Toward the Chemical Characterization of the Sex Pheromone of *Pseudaletia adultera* (Lepidoptera: Noctuidae)

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Pseudaletia adultera (Lepidoptera: Noctuidae) feeds on cultivated grasses such as wheat, barley, oat and rice, all of which are important commodities in the agricultural sector in Uruguay [1]. The frequent attacks of *P. adultera* to these crops often call for preventive chemical control, due in part to the lack of an appropriate monitoring system based on sex pheromones. As in most Lepidoptera, in which sex pheromones are emitted by the females from abdominal glands [2], *P. adultera* females adopt a characteristic calling position during the scotophase, and males are known to respond by flying upwind with a typical zigzag pattern.

The extraction of the sex pheromone was performed by removing the glands from calling females with forceps, and extracting them with hexane for 30 min. The extract was then concentrated under N₂, and analyzed by GC-MS and GC-EAD. The analyses showed a major EAD-active compound which was identified as (Z)-11-hexadecenol, and two minor derivatives, (Z)-11-hexadecenyl acetate and (Z)-11-hexadecenal, which may also be involved in the sex pheromone blend (96:6:1 relative ratio). The three synthetic compounds were individually tested with male antennae using GC-EAD, and all elicited a clear response.

Wind tunnel bioassays were carried out with different mixtures of the synthetic compounds. The behavioral response of individual males was observed for ten minutes, recording the activation time (flying onset), oriented flight, and arrival to the emission source. The mixture that elicited the highest male response was the one mimicking the natural proportion found in the female gland extracts.

Field tests may be required to prove the efficiency of *P. adultera* sex pheromone as a tool for the development of a management strategy for this species in Uruguay.

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P17 - Increase of secondary metabolite content in marigold (*Tagetes minuta*) by inoculation with plant growth-promoting rhizobacteria

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The effects of single inoculation and co-inoculation of two plant growth-promoting rhizobacteria (PGPR) (*Pseudomonas fluorescens*, *Azospirillum brasilense*) on growth and essential oil (EO) composition and phenolic content were evaluated in marigold (*Tagetes minuta*). Plant growth parameters (shoot fresh weight, root dry weight, leaf number, node number) were measured. EO yield increased 70% in *P. fluorescens*-inoculated and co-inoculated plants in comparison with control (non-inoculated) plants, without altering EO composition. The biosynthesis of the major EO components was increased in the inoculated plants. Shoot fresh weight and EO yield were significantly higher in *P. fluorescens*-inoculated and in co-inoculated plants than in control plants. The total phenolic content was 2-fold higher in singly-inoculated or co-inoculated treatments than in controls. In view of the economic importance of monoterpenes and phenols compounds for a variety of applications in the food and cosmetic industries, *P. fluorescens* and other PGPR have clear potential for improving the productivity of cultivated aromatic plants. Better understanding of the processes that affect secondary metabolites accumulation will lead to increased yields of these commercially valuable natural products.

Keywords: rhizobacteria, PGPR, aromatic plant, *Tagetes minuta*, essential oil, total phenolic content, *Pseudomonas fluorescens*, *Azospirillum brasilense*.

P18 - Volatile compounds of Brazilian Holstein cattle herds infested by local *Hematobia irritans* (L., 1758)

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In Brazil, the horn fly, *Hematobia irritans* (L., 1758), is a major pest of grazing cattle and affects livestock production. To control this pest producer, most of the time, makes use of conventional chemicals. This strategy, however, is not always effective, causing many producers do and indiscriminate use of pesticides in a wrong way, having thus reducing their effectiveness. Aggregate is also the potential public health problems, considering that the producers do not notice the grace periods for products used. Thus, the search for alternatives to conventional pesticides is necessary, aiming to reduce or eliminate those problems. Previous studies in Europe and Chile have shown that cattle flies, including horn fly, are differentially attracted to individual cattle within herds and that volatile semiochemicals are responsible for this phenomenon[1]. This study provides evidence that similar differential attractiveness occurs for the interaction between Brazilian Holstein cattle herds and local *H. irritans* populations. Thus, Holstein dairy cattle, *Bos taurus*, which were of similar age and physiological condition, were shown to possess an uneven distribution of horn fly. Volatile samples were collected from sixteen heifers in Maceio, Brazil. Gas chromatography coupled mass spectrometric analysis of samples collected from heifers revealed the presence of 50 different compounds and detected difference between the compositions of these compounds in the animals evaluated. This study provides evidence for the hypothesis that the natural differential attractiveness within herds of Holstein heifers, i.e. a single host species, for cattle flies is partly due to differences in volatile semiochemicals emitted from the host. This study represents a first step for research into the application of semiochemicals in monitoring and control of cattle flies in Brazil. The research activities will assess the future activities of the compounds identified in relation to the behavior of the fly both in olfactometer and in EAG.

[1] Oyarzún MP, Palma R, alberti E, Hormazabal E, Pardo F, Birkett, MA, Quiroz A. (2009) Olfactory response of *Haematobia irritans* (Diptera: Muscidae) to cattle-derived volatile compounds. Journal of Medical Entomology 46(6): 1320-1326.

P19 - Importance of Organic Chemistry in biological interactions

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In Organic Chemistry subject from the Faculty of Natural Sciences (UNSa), the important element is not only science that is taught, but also that teachers develop their research through interdisciplinary work. Since 1996, we have been developing research related to biological interactions: 1) Mediated by secondary metabolites exuded to the atmosphere, called allelopathy. We determined whether allelochemicals may influence the distribution and abundance of *Trichocereus atacamensis*. Secondary metabolites were identified by spectroscopic, mainly from plant extracts of *Baccharis boliviensis*, *Plectrocarpa rougesii*, *Senna crassiramea*, *Aphyllocladus glutinosa*, *Gochnatia spartioides* which could be responsible for the negative interaction with *Trichocereus atacamensis*. The importance of this work is the management and conservation studies of the *Trichocereus atacamensis* and the determination of secondary metabolites that could eventually be used as bioherbicides. 2) Standardization of the methodology for the determination of fecal bile acid patterns in carnivores by Thin Layer Chromatography (TLC). 3) Relationship with other research groups to study snow leopard feces in Armenia, discrimination of puma and jaguar feces from Amboró National Park in Bolivia, and determination of the presence of Pampa's cat in samples from Las Cuevas (Mendoza). By TLC we established the presence and distribution of species of *Xenarthra*, *Carnivora*, *Artiodactyla* and *Perissodactyla*, in the region of Bahía Blanca and National Parks. These investigations have demonstrated that TLC can be applied to the study of the distribution and abundance of mammal species, being possible to perform interdisciplinary work with researchers from Argentina and abroad. Research on allelopathy has allowed the application of basic laboratory techniques to separate, purify and identify the metabolites responsible for the interaction.

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Araujo, S., Cazón, A. y Casanave E. 2010. Differentiation of *Xenarthra* (Mammalia) species through the identification of their fecal bile acid patterns: An ecological tool. Rev. Chilena de Historia Natural 83: 335-347.

P20 - Role of plant volatiles in host plant selection by the pea weevil *Bruchus pisorum* (Coleoptera: Bruchidae)

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Peas (*Pisum sativum* L.) are cultivated worldwide as an important source of protein for human consumption, and as feed for animals and are an important component of arable crop rotations. The pea weevil *Bruchus pisorum* (L.) (Coleoptera: Bruchidae), is considered the most damaging insect pest of the pea cultivars, this insect adversely affects its yield and quality by consuming a large part of the seed (Clement et al., 1996). *B. pisorum* adults emerge from hibernation in spring and visit exclusively pea flowers for pollen feeding prior to oviposition on immature pea pods (Michael et al., 1993). This strong association implies that this relationship could be either behavioral or biochemical. Olfactometric experiments were conducted to determine the role of volatiles released by *P. sativum* on host-plant selection by the pea weevil. Volatiles from the aerial part of different phenological stages of the host (vegetative, flower and pod) were collected on Porapak Q and analyzed by coupled gas chromatography-mass spectrometry. Several chemical groups were identified including green leaf volatiles, aromatic compounds, and terpenes. The olfactometric responses of *B. pisorum* toward different odor sources were studied in a four-arm olfactometer. Volatiles from all phenological stage elicited an attractant behavioral response on both male and female weevils. The strongest attraction was observed on female with volatiles released at pod stage. The results suggest the host location behavior of *B. pisorum* could be mediated by volatiles derived from *P. sativum*, and the specific chemicals emitted by pods are used as oviposition-cue. Some of the volatiles from *P. sativum* pods could be used as an attractive source for *B. pisorum* in integrated pest management programs (IPMs). Future work should aim to determine the electrophysiological response of *B. pisorum* to individual odors and combinations of multiple odors released by *P. sativum*. This information will provide an understanding of the role of volatiles in mediating the host selection and oviposition process of the weevil *Bruchus pisorum*.

[1] Clement, S., Evans, M. and Lester, D. (1996) Settling and Feeding Responses of Pea Weevil (Coleoptera: Bruchidae) to Flowers of Selected Pea Lines. *J. Econ. Entomol.*, **89**, 775-779. [2] Michael, P., Hardie, D. and Mangano, P. (1993) Growing field peas. *Insect and mite control*. (ed. J. Carpenters), pp. 65-77. Western Australian Department of Agriculture.

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P21 - Intraspecific chemical diversity associated to latitude in two *Smallanthus* (Heliantheae, Asteraceae) species

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Smallanthus macroscyphus and *S. connatus* are wild species closely related to an Andean crop, yacon, *Smallanthus sonchifolius*. These species are rich in sesquiterpene lactones (STL), compounds that serve as deterrents to herbivores and have many biologic activities. *S. macroscyphus* grows in the yungas, deciduous forests in Southern Bolivia and NW Argentina. The STL variability in *S. macroscyphus* was explored in 20 populations distributed along a latitudinal gradient from southern Bolivia up to the limit between the provinces of Catamarca and Tucumán in northwestern Argentina. Populations towards the north of 24.3°S where found to be chemically diverse with enhydrin, uvedalin and fluctuanin as major STL, while populations southward of that latitude were chemically uniform with polymatin A as the main and largely dominant sesquiterpene lactone. A similar behavior was found for *S. connatus*, a related specie from forests and disturbed habitats in Southern Brasil, Paraguay, Uruguay and NE Argentina. Populations from Misiones province were found to be chemically more diverse than populations from Buenos Aires province in Argentina. The distribution of the chemotypes of these species is consistent with the hypothesis that leaves of tropical forests have both higher overall levels of defense and a greater diversity of metabolites when compared to their temperate counterparts. Lower commitment to chemical defense could be an evolutionary response to less predation and lower predator diversity in Southern -less tropical- populations.

P22 - Phosphate solubilization by rhizobacteria

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Phosphorus (P) is the nutrient that most limits the growth of plants and microorganisms, second only to nitrogen. In soil, phosphorus is subject to numerous biogeochemical processes that alter their availability. Among these processes, stands dissolution of phosphates by microorganisms, making it available to plants [1]. Some microorganisms present in the soil, such as bacteria and fungi have a role important in the natural cycle of P, due to hydrolysis of phosphate compounds to inorganic form, becoming unavailable to plants, being the phosphatases enzymes mediating these processes. Mineral phosphate solubilization by rhizobacteria acting on the insoluble phosphate, by phosphatase enzymes, making the phosphate available to plants [2]. To test the ability of phosphate solubilization of the isolated, the method used was adapted from Katznelson & Bose (1959), where the medium used was 1/10 NYDA plus CaHPO₄. The fine precipitate of CaHPO₄ resulted from the reaction of 50 mL of 0.57 M K₂HPO₄ solution and 100 mL of 0.90 M CaCl₂ solution added to 850 ml of 1/10 NYDA. The solutions and the medium were autoclaved separately. The pH was adjusted to 7.0 with 1 N NaOH, sterile. When soluble they were transferred to the petri dish using a sterile straw leaving a 100µL circle in the middle of the petri dish with a diameter of 0.6mm containing the desirable isolates. Then the isolate was transferred to the plate and incubated at 28 ° -30 ° C for seven days. The rhizobacteria *Providencia rettgeri* (identified through biochemical characterization in VITEK 2 with 99% reliability) produced clear halo with a diameter of: 8.3, 8.2, 8.5 e 9.4. proving its phosphate solubilizing activity. Later studies will be undertaken *in nature* (inoculation of rhizobacteria isolated) in seedlings of vegetables.

[1] WHITELOW, M. A. Growth promotion of plant inoculated with phosphate-solubilizing fungi. **Advances in Agronomy**, 69: 99-151, 2000.

[2] NAUTIYAL, C. S. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. **FEMS Microbiology Letters**, 170: 265-270, 1999.

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P23 - Automated Identification (System Vitek2) rhizobacteria isolated in Alagoas: biochemical characterization

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The VITEK 2 Compact uses an “Advanced colorimetry” technology, it compares the new substrates to a range of previously identified substrates that are stored in a wide phenotype banc. The lineages were analyzed with a GN card using NaCl 0,85% in order to prepare the bacterial suspension as recommended by the producers. The final analysis of the microorganism was classified as excellent (99% reliability), which identified a single species for each analysis. Two rhizobacteria were identified: *Pseudomonas putida* group and *Proteus vulgaris*. The details biochemical obtained from the Analysis of the two samples were VITEK 2: *Pseudomonas putida*: 2/APPA/-; 10/H2S/-; 17/BGLU/-; 23/ProA/+; 33/SAC/-; 40/ILATk/+; 46/GlyA/-; 58/O129R/+; 3/ADO/-; 11/BNAG/-; 18/dMAL/-; 26/LIP/-; 34/dTAG/-; 41/AGLU/-; 47/ODC/-; 59/GGAA/-; 4/PyrA/-; 12/AGLTp/-; 19/dMAN/-; 27/PLE/-; 35dTRE/-; 42/SUCT/+; 48/LDC/-; 61/IMLTa/-; 5/IARL/-; 13/dGLU/+; 20/dMNE/+; 29/TyrA/+; 36/CIT/+; 43/NAGA/-; 53/IHISa/-; 62/ELLM/-; 7/dCEL/-; 14/GGT/+; 21/BXYL/-; 31/URE/-; 37/MNT/-; 44/AGAL/-; 56/CMT/+; 64/ILATa/+; 9/BGAL/-; 15/OFF/-; 22/BALap/-; 32/dSOR/-; 39/5KG/-; 45/PHOS/-; 57/BGUR/-. *Proteus vulgaris* group: 2/APPA/-; 10/H2S/+; 17/BGLU/-; 23/ProA/-; 33/SAC/+; 40/ILATk/+; 46/GlyA/-; 58/O129R/+; 3/ADO/-; 11/BNAG/-; 18/dMAL/+; 26/LIP/-; 34/dTAG/-; 41/AGLU/+; 47/ODC/-; 59/GGAA/-; 4/PyrA/-; 12/AGLTp/-; 19/dMAN/-; 27/PLE/-; 35dTRE/-; 42/SUCT/+; 48/LDC/-; 61/IMLTa/-; 5/IARL/-; 13/dGLU/+; 20/dMNE/-; 29/TyrA/-; 36/CIT/-; 43/NAGA/-; 53/IHISa/-; 62/ELLM/+; 7/dCEL/-; 14/GGT/+; 21/BXYL/-; 31/URE/+; 37/MNT/-; 44/AGAL/-; 56/CMT/+; 64/ILATa/-; 9/BGAL/-; 15/OFF/-; 22/BALap/-; 32/dSOR/-; 39/5KG/-; 45/PHOS/+; 57/BGUR/-. This identification using biochemical characterization allowed the initiation of studies involving isolated rhizobacteria and trying to analyze the production of auxin and phosphate solubilization in vegetables.

We are grateful for the help that was received from the Alagoas of Laboratory Central (LACEN), in particular for the analysis that were conducted at VITEK 2.

P24- Colorimetric assay for determination of auxin by *Serratia marcescens*

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The rhizobacteria inoculated plants can promote plant growth and biocontrol of diseases, reducing production costs and reducing the impact of pesticides on the environment [1]. A research study was conducted in the Laboratory of Reseach in Natiral Resources (LPqRN), Maceio-AL, in 2012, where a colorimetric assays for determined of auxin (AIA) was conducted. The main objective of this study was to determine the production of auxin (AIA) by rhizobacteria *Serratia marcescens* (using biochemical characterisation in VITEK 2 with 99% reliability). The levels of auxin (IAA) secreted by the bacteria in the culture medium were analyzed by cultivating them in 50 ml medium YEPD supplemented with tryptophan (1mM). Cultures were maintained at 30 ° C in the dark under constant agitation of 140 rpm for 24 hours and 10% of the bacterial inoculum was transferred to 20 mL vials containing 5 ml of the same medium supplemented with 200 µg mL⁻¹ of tryptophan (1mM). After culturing for 48 hours, the liquid cultures were centrifuged at 6.000xg for 10 minutes at 15 ° C and the supernatant was collected. The evaluation of AIA by mL of culture was estimated according to the protocol Gordon and Weber (1951) with some modifications mixture consisting of 2 mL of reagent Salkowski (FeCl₃.6H₂O 2% + 37% H₂SO₄) with 1 mL of supernatant culture, which was reserved for 30 minutes in a dark environment for reaction and manifestation of red color. The presence of the red color enforced the conclusion that it the rizobacteria that produces the AIA. Subsequent to this study the *Serratia marcescens* was inoculated seeds (Early Ball) of *Capsicum annum* L, *Lycopersicon esculentum*, *Lactuca sativa* Linne to evaluate the effects this rhizobacterium has on the development of these plants and production of their bulbs.

[1] Harthmann, Oscar Emilio Ludtke *et al.* Tratamento de sementes com rizobactérias na produção de cebola. *Ciência Rural*, Santa Maria, v.39, n.9, p.2533-2538, 2009.

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P25 - Effect of UV-B radiation on chemical defense of freshwater *Salvinia auriculata* (Salviniaceae) against herbivory

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Secondary metabolites play an important role as defense against herbivory in freshwater plants. Their biosynthesis and/or bioaccumulation are strongly influenced by environmental factors, such as UV-B radiation. Since the genus *Salvinia* has a cosmopolitan distribution and shows relevant structural and metabolic importance in aquatic ecosystems, this work focused on evaluating the effect of UV-B radiation on chemical defenses against herbivory in *S. auriculata*. For that, twenty plants collected in the Lagoa Comprida (Parque Nacional da Restinga de Jurubatiba, Rio de Janeiro state, Brazil), were cultivated in aquarium at laboratory. Two light conditions were applied during 14 days of cultivation where (i) ten plants were placed under an illumination for ca. $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, and (ii) other ten plants had the same illumination but received an additional daily dose of 1,93 times of UV-B radiation ($\lambda=311\text{nm}$). Crude extracts in methanol from all cultivated plants and also from ten individuals of natural environmental were prepared. To evaluate the activity against herbivory, the extracts were tested for food preference using the gastropod *Biomphalaria sp.* as a model organism. The foods were offered simultaneously to herbivore control (control - without crude extract and treatment - with crude extract in natural concentration). The results showed that the UV-B radiation increase the consumption of exposed plants by *Biomphalaria sp.* (in cultivation experiments ($p<0.05$, Wilcoxon test), probably changing negatively their chemical defenses against herbivory. Additionally, the natural UV-B dose in field is not enough to knock out the chemical defenses, since there was no significant differences between the consumption of artificial food made of field plants and from cultivated plants without UV-B radiation. Our results suggest strongly that the UV-B radiation modified the secondary metabolites composition in *S. auriculata* reducing the defense capacity of this species against herbivory in cultivated plants. Such kind of response could have effects on plant- herbivore interactions in freshwater ecosystems. For a better understanding of this phenomena, analysis of the chemical profiles from plant extracts of natural and cultivated plants are going on at our laboratory.

P26 - Chemical ecology studies in *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae)

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Diabrotica speciosa is a polyphagous species whose adults damage the aerial parts of various cultures as bean, soybean and sunflower. The larvae consume the roots, being important pest of potato and corn. Studies were conducted aiming to develop chemical ecology strategies for management of this pest. One strategy is oriented to the identification and evaluation of volatile compounds from a cucurbitaceous plant *Lagenaria vulgaris* L. for attracting *D. speciosa* adults, volatile of opened (hand cuted) and intact fruits were collected by air entrainment. The other strategy was oriented to the identification of sex pheromone of *D. speciosa*. Air entrainment of adults of both sexes was conducted. The extracts from air-entrainment were analyzed by GC-FID and GC-MS to quantify and identify the compounds present in the samples. Bioassays were conducted in "Y" olfactometer with extracts of *L. vulgaris*, where males and females, previously fed and not fed, were released and observed during 10 minutes. Initial choice, the number of times that the insect came in each area, and residence time were recorded. Composition of *L. vulgaris* extracts showed a high diversity of compounds: hydrocarbons, alcohols, aldehydes, ketones, monoterpenoids, sesquiterpenoids and homoisoprenoids. Bioassays showed that *L. vulgaris* volatile extracts significantly attract males of *D. speciosa*. Results from the chemical analysis of the volatiles present on *D. speciosa* adult air entrainment extracts showed female specific compounds that are currently under investigation to chemical structure elucidation.

P27 - Volatile organic compounds emitted by *Cucurbita maxima* as potential clues for its specialist herbivore, *Epilachna paenulata* for host finding

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The ecological relationships among phytophagous and their host plants can - among other stimuli- be mediated by volatile and non-volatile semiochemicals. As a part of an ongoing research program aiming to typify the ecological relationships between the specialist *Epilachna paenulata* (Coleoptera: Coccinelidae) and their Cucurbitaceae host plants, we have characterized the volatile organic compounds (VOCs) emitted by leaves and flowers of *Cucurbita maxima* (Cucurbitaceae). Sampling was performed by enclosing *in vivo* leaves (3-5 two-week old plants) or flowers (1) into a polyester oven-bag (Wyda Practic), and the VOCs were adsorbed on a HayeSep Q (100 mg) trap connected to a battery-operated vacuum pump (Casella) operated at 1.6 mL/min (23 h). HayeSep Q elution was done with hexane, and the hexane solution (previously concentrated under N₂) was analyzed by GC/MS. The identification of VOCs was based on their retention indexes and mass spectra compared to the NIST library (2008) and those reported in literature [1]. Chemical profiles differed between both plant organs. Whereas main flower VOCs were aliphatic alcohols, leaves emitted mainly terpenes and ketones, and smaller amounts of organosulfur compounds. Preliminary results of behavioral bioassays using a Y-tube olfactometer showed that those chemicals do not elicit *E. paenulata* adults' choice of their host plant.

[1] Adams R (2007) Identification of Essential Oils Components by Gas Chromatography/Mass 340 Spectrometry. Allured Publishing Corporation Illinois

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P28 - Repellent activity of native plants of central Argentina against leaf cutting ant, *Acromyrmex lundii*

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Leaf-cutting ants (Hymenoptera, Formicidae) are considered the most important herbivores in the Neotropics, due to the high level of defoliation that they cause. Beside leaves, they cut and carry enormous amounts of other plant materials to their nests, including stems, fruits, seeds and flowers. Because of its spectacular defoliator activity, leaf-cutting ants are considered pests in agriculture, forestry, and urban household. So far, the most used management strategies for the control of leaf-cutting ants include cultural and mechanical control and especially synthetic insecticides. The prolonged presence of these insecticides in the environment, has led to problems of contamination and bioaccumulation in the food chain. The growing awareness of these and other undesirable effects associated to the use of synthetic insecticides has stimulated the search for new tools, more benign to the environment, human health and the for the ecological balance. In this context, plants could be a source of eco-friendly pesticides.

In order to find new substances for the control of *A. lundii*, a screening of ethanolic extracts derived from 110 native plants, was performed. This study showed that 8% of the extracts repelled the foraging, with a repellency index (RI) greater than 80%. Likewise, 10 extracts showed repellency with IR between 70 and 80%, while 79 extracts were inactive. The 13% of the extracts showed a strong attractant activity to *A. lundii*.

One of the most active extracts, was that derived from the Anacardiaceae, *Lithrea molleoides* (IR = 100%), while one of its major components, (Z,Z)-5-trideca-4, 7-dienyl resorcinol (1), showed IR = 100% to 50 $\mu\text{g}/\text{cm}^2$ of the substrate offered to ants.

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P29 - Evaluation of Preference and Consumption by *Anticarsia gemmatalis* larvae in two soybean (*Glycine max*) commercial genotypes with contrasting rutin foliar content

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Insect feeding on foliage can be importantly affected by the presence of constitutive or inducible defense molecules. Rutin is a phenolic compound present in soybean leaves that has been associated, in assays with artificial diets, with a reduction in both consumption and digestibility and with higher mortality in *Anticarsia gemmatalis* larvae [1]. The objective of the present work was to evaluate *A. gemmatalis* preference and consumption in two field-grown soybean commercial genotypes. Cultivars DM 4210 y DM 5.8i were planted (60pl/ m²) in the experimental field of Facultad de Agronomía, UBA. At beginning bloom (R1) three 5th instar *A. gemmatalis* larvae were placed in tulle transparent bags containing fully expanded leaves from both cultivars. 48hs after the start of the treatment, larvae were removed and leaves were photographed, for later calculation of the consumed area, and immediately frozen in liquid N₂. Control leaves from both cultivars were also sampled. Leaf tissue was extracted with methanol and analyzed by reverse-phase HPLC to evaluate the phenolic compound profile. *A. gemmatalis* damage was lower in cv. DM 5.8i with respect to cv. DM 4210 (7% and 30% consumed area, respectively). Rutin concentration in cv. DM 4.2 of control leaves was 0,118 mg rutin/ g leaf while in cv. DM 5.8i was significantly lower (0,009 mg rutin/ g leaf). Although a similar induction of genistein-malonylglucoside and of an unidentified compound was found in both cultivars, rutin concentration remained constant after 48hs of larvae damage. We conclude that the difference found in foliar rutin concentrations between both cultivars did not have the expected effect. Further studies are needed to be made to elucidate if rutin can act as a phagostimulant at certain concentrations or if other type of defenses are important to determine the consumption of *A. gemmatalis* at field.

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P30 - Activity of the *Duguetia lanceolata* against *Atta sexdens rubropilosa*

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Leaf-cutting ants, *Atta sexdens rubropilosa*, are known as a serious pest for agriculture due to the high amounts of vegetal matter used as substrate for the symbiotic fungus development [1]. Different methods and new strategies to control these pests have been requested for controlling them such as natural products.

The genus *Duguetia* consists of approximately 70 species, of which 50 are found in Brazil. *Duguetia lanceolata* is used in medicine as anti-inflammatory and antimicrobial [2]. Interest in the study of plants of 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 *Atta sexdens rubropilosa*. The ethanolic extracts of leaves (DLEF), branches (DLER), fruit (DLE Caf), and seeds (DLES) were tested in incorporation into artificial diet (2 mg mL⁻¹). According to median survival (Md) of *A. sexdens rubropilosa* submitted to bioassay, significant differences according to the statistical test "log rank" ($p < 0.05$, Md-control = 18 days) were observed for Md-DLEF = 9 days, Md-DLER = 11 days, Md-DLE Caf = 12 days, Md-DLES = 8.5 days.

Each extract was subjected to liquid-liquid partition and those in hexane, dichloromethane, ethyl acetate and hydroalcoholic were tested (1 mg mL⁻¹). The active fractions were hexane and dichloromethane with Md in relation to Md-control (25 days) were Md-DLEF-hex = 15 days, Md-DLEF-dichloro = 7 days, Md-DLER-hex = 20 days, Md-DLER-dichloro = 12.5 days, Md-DLE Caf-hex = 18 days, Md-DLE Caf-dichloro = 17 days and Md-DLES-dichloro = 10 days. The extracts and fractions with higher activity are being subjected to chromatographic processes accompanied by new tests in the search for potential substances with insecticidal properties.

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FAPESP, CNPq, CAPES, INCT-CONTROLE BIORRACIONAL DE INSETOS PRAGAS

P31 - Role of guava volatiles on *Anastrepha fraterculus* male sexual display and pheromone release

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The South American fruit fly *Anastrepha fraterculus* is a fruit crop pest in Argentina and neighboring countries with a complex courtship in which males cluster in leks and release a sex pheromone while performing sexual displays (Lima *et al.* 2001). Although preexposure of males to certain fruit volatiles has been shown to increase their mating success (Vera *et al.* 2010), the mechanism associated to this phenomenon is unknown. Here, we evaluated the hypothesis that this increase in mating success is mediated by an increase in pheromone emission and/or a change in the associated sexual displays. *A. fraterculus* males preexposed to guava volatiles were individually offered to virgin females together with not exposed males. Under such conditions, females chose one partner and males were assigned into one of four possible categories: according to treatment (guava exposed or not exposed) and mating success (successful or unsuccessful). On the following day, we collected volatiles emitted by each male type in groups of ten individuals and registered behavioral parameters of the sexual display. Male volatiles were adsorbed in SuperQ filters and desorbed with dichloromethane to be analyzed by gas chromatography. Pheromonal compounds were identified by standards. The results show that the intensity of the sexual display positively correlates to the amount of released pheromone. Successful males performed a more intense sexual display (and released more pheromone) than unsuccessful ones. Preexposure to guava volatiles increased the probability of male mating success. This phenomenon appears to be mediated by an increase in the proportion of males able to release pheromone to levels that conferred a greater acceptance by females. Further studies should look into differences in short distance cues as the cuticular composition of males after exposure.

Keywords: male sexual competitiveness, semiochemicals, sexual selection, fruit flies, Tephritidae **Acknowledgements:** We are grateful to C Cabrera, J Cuezco, C Jalil and F Stegmayer from Facultad de Agronomía y Zootecnia, Univ. Nac de Tucumán, for their assistance during the experiments. **References:** Vera, M. T.; Ruiz M. J.; Oviedo A.; Abraham S.; Mendoza M.; Segura D. F.; Kouloussis N. A. and Willink E. (2010). Fruit compounds affect male sexual success in the South American fruit fly, *A. fraterculus* (Diptera: Tephritidae). J Appl Entomol. doi: 10.1111/j.1439-0418.2010.01516.x.

P32 - Volatile Organic Compounds of *Corythucha gossypii* (Heteroptera, Tingidae)

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The goal of this work is to identify volatile compounds (pheromones) released by *Corythucha gossypii* using two methodologies (Met¹ and Met²) extraction (Aeration type dynamic headspace - Met¹ and wash of cuticle - Met²). For the extraction of volatile compounds through Met¹ were used 299 adults being 61 females (F) and 238 males (M). The desorption of volatiles retained in the adsorbent was performed with assistance redistilled hexane solvent and volatiles eluted in 2 µL per insect. For Met² [1] was used 60 (30M and 30F) adults who were anesthetized and subsequently dipped in redistilled hexane by 30min. Aliquots of 1 µL samples were injected into Gas Chromatograph (GC), mode split, containing capillary column RTX-1 (0.25 mm x 30m) with initial temperature of 50 °C maintained for 5min with a progressive increase of 5 °C / min up a final temperature of 250 °C held for 10min. The Kovats retention index was calculated using standard hydrocarbon (C7 - C30). Next, 1µL of each extract was injected into Mass Spectrometer with a ramp of 50 °C for 5 m in, 5 °C per minute to 250 °C and maintaining this temperature for 10 minutes to identify the volatile compounds which showed higher peaks in the 7 extracts. Hexadecene and 2,5-Dimethylheptane were present only in extracts of males, while compounds: Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester, (Z,Z)-9,12-Octadecadienoic acid methyl ester, 2-dodecenoic acid, undecan-5-ol, hexadecanoic acid, octadecanoic acid, docosane and 1-tetracosane were found only in females. The hexacosane was present in both sexes, being described in pherobase as alarm pheromones. There is evidence that some compounds identified are alarm pheromone [2] by frequency contained in extracts of males and females and /or sexual by the presence of volatiles at high concentrations only in one sex.

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Acknowledgements

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P33 - Comparison of volatile organic compounds from *Cyclocephala melanocephala* identified by calculating the KI and Library Mass Spectrometer

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This study aimed to identify the volatile compounds released by this species of beetle through the calculation of the KI (identified by the database Pherobase) and Library Massa Spectrometer. The collection of volatiles was performed by aeration type dynamic *headspace*. The airings occurred during 24h. The desorption of volatiles retained in the adsorbent was performed with assistance redistilled hexane solvent and volatiles eluted in 10 µL per insect, five females (50 µL) and two male (20 µL). The samples were injected into Gas Chromatograph (GC), containing capillary column RTX-1. The Kovats retention index was calculated using a standard hydrocarbon (C7 - C30). Were detected 36 different volatile compounds being 20 males in the extract and 22 females in the extract, 6 compounds were present in both extracts. The volatile with more area (relative concentration) detected and identified according with database "pherobase" through the KI only in extracts of males, were 3,3-Dimethyl-cyclohexanone (KI = 1036, Area = 23142.7) and Butyl 3-methylbutanoate (KI = 1042, Area = 16376.0). The extract of the males showed the compound 4,4-Dimethylheptane (KI = 826, Area = 10529.2) in an area greater than the female (Area = 7717.5). While the extract of females showed the compound 2,2,6-Trimethylcyclohexanone (KI = 1011, Area = 10566.9) in an area greater than the male (Area = 3762.2). The compounds identified in the pherobase by KI diverged of volatiles identified by library do GC/MS. The organic compounds found in both sexes, according to GC/MS were: carvacrol, 3-ethylacetophenone, 1-4-ethylphenylethanone. While, 1,2-dimethylpropyl acetate, 2-methylbutyl acetate, Isoamyl acetate, ethylidene diacetate, propyl butyrate, isoamylbutyrate, propyl butyrate were observed in females and 1,2-diethylbenzene, 4-1-Hydroxyethylbenzaldehyd found only in males. These results highlight the divergence in the identification of volatile compounds, being necessary to check the identifications with the literature and with the standards of synthetic compounds.

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P34 - Male sex pheromone structural identification of the carrion beetle *Oxelytrum erythrurum* (Coleoptera: Silphidae)

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The beetles of the family Silphidae are known as carrion beetles due to its abundance of vertebrate carcasses in the tropicals and sub-arctic zones, may be indicators of post-mortem interval (PMI) when are part of entomological trace. In Brazil, the family is represented by the genus *Oxelytrum* spp., where larvae and adults feed on and lay eggs on carcasses exposed. *Oxelytrum erythrurum* is a species recorded at criminal scenes in Argentina, also occurring in south of Brazil. Given its importance in forensic entomology, the aim of this study was to determine the structure of possible pheromonal compound. Were separated 10 specimens of each sex, and placed in aeration chambers containing moistened cotton daily, and airflow of 1 Lmin⁻¹. The volatiles were captured in the adsorption columns containing polymer (HayeSep-Q). Every two days the insects were fed ground meat without the adsorption column, which was placed 24 hours later, when the meat had already been consumed. The extractions were performed every 24 hours at 400 µL of hexane. The extracts were then concentrated to 160 µL with a slight airflow, injected and analyzed by GC-MS and GC-FTIR. In chromatograms of males was possible to observe a male-specific compound with a molecular mass of 264 g mol⁻¹. In the infrared spectrum, there was the presence of stretches at 3039 and 3060 cm⁻¹, corresponding to the presence of double bonds. A microderivatization catalytic hydrogenation with palladium / carbon confirmed the presence of two double bonds and one microderivatization of partial reduction with hydrazine, followed by reaction with dimethyl disulfide revealed the positions 1 and 9 or 10 the possible locations of the double bonds. The proof will be done by the synthesis of the two proposed structures and bioassays activity.

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P35 - Activity of limonoid cedrelone against the fall armyworm, *Spodoptera frugiperda* (J.E. Smith)

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The fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) occurs annually in tropical and subtropical areas. In Brazil, *S. frugiperda* is a pest of maize in favorable conditions and increase its population, occurring from seedling emergence, destroying leaves and cartridge plants, causing damage to 34% of production [1]. The use of synthetic insecticides has been the primary method of pest control, but their indiscriminate use and misuse has increased the number of applications and reduced efficiency. Thus, its importance is due not only to the damage, but especially the difficulty of control. Because of the good results seen with neem (*Azadirachta indica*), other Meliaceae also started to attract the interest of researchers in order to find new species and new molecules with insecticide activity [2]. Thus, control measures that cause less environmental impact are paramount importance, which has spurred a resurgence in the use of plants as a promising tool insecticides for insect control. The aim of this study was to evaluate the activity of limonoid cedrelone isolated from *Toona ciliata* (Meliaceae) of stem in different concentrations on the larvae of *S. frugiperda* when incorporated into artificial diets. The mortality and average weights of larvae fed from the first instar larvae on artificial diet treated with cedrelone after seven days of testing were 40% / 1.6 mg at 156 ppm, 54.6% / 1.2 mg at 312 ppm and 100% mortality at 625 and 1250 ppm, compared with the control. The comparison between treatment were performed using the statistical analysis using the Tukey test at the 5% level of probability.

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FAPESP, CNPq, CAPES, INCT

P36 - A methodological study to assess attraction and trapping in vectors of Chagas disease

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**Equal contribution*

The most effective strategy to prevent Chagas disease is by vector control [1], and one sustainable, environmentally-friendly, method for this is the use of trap devices lured with attractants. To find out effective attractants, different olfactometers were previously used, in which attraction is quantified as walking displacement towards a test zone. In our laboratory we are using a dual-choice trap olfactometer [2], consisting of an arena with 3 holes. A tube is connected from below to each hole and the arena can virtually be divided in 3 zones: neutral (where a “refuge” –starting point- tube is connected; this is the only tube containing a piece of cardboard to allow insects reach the arena), test and control, each with a hole to which a trap-tube is connected. The test and control zone, opposite to the neutral zone, are separated by a partition. Below the “test trap-tube” a test odor source is placed, whereas below the “control trap-tube” the odor control is placed. A single insect is tested overnight. Attraction is quantified in terms of number of insects trapped in the control and test tubes. By video-recording the experiments, we could for the first time: 1) compare our trapping results with results obtained measuring attraction in other ways; 2) study the behavior dynamics preceding trapping. Two stimuli (mouse and a synthetic odor blend) were tested in *Triatoma infestans* and *Rhodnius prolixus*. An analysis of the insects’ first choice (i.e., test or control side) after leaving the refuge suggests that in our device this way of measuring attraction may overestimate results in *R. prolixus* when comparing to capture data. Capture occurred mainly during the first half hour of experiment. The two stimuli caused the insects to spend less time in the neutral zone than in tests in which there was no odor stimulus below either trap-tube.

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P37 - Study of Neem oil and their nanocapsules on *Lycopersicon esculentum* mill. on attack of *Bemisia tabaci* biotype b.

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The *Bemisia tabaci* biotype B is one of the factors that contribute to the decline in yield of tomato¹. For the chemical control of *B. tabaci* has been used a variety of synthetic insecticides and a few pesticides derived from plants. It is well known the effect of aqueous extracts of seeds of Neem (*Azadirachta indica*) on all life stages of *B. tabaci*² beyond the Neem oil and derivatives. However, there is any chemical study of its mode of action on *B. tabaci*. Despite the efficiency, the constituents of Neem have a low residence time in the field, which prevents its application in crops that demand for an extended protection. In view of this instability, a methodology was developed for the nanoencapsulation of Neem oil. The nanoformulations were analyzed systemically on *Lycopersicon esculentum* Mill. with *B. tabaci* in the leaves. A LC-MS/MS method was developed to analyze the nanoformulations and oil content in all organs of tomatoes plants on attack of *B. tabaci*. Nanoformulations were prepared by emulsion diffusion. The nanocapsules and Neem oil were applied in tomato cv. Santa Clara infested with *B. tabaci*. After evaluation of the rate of mortality, tomato samples were stored at -80 °C and analyzed by API 2000 triple quadrupole LC-ESI-MS/MS to quantify Azadirachtin A. The nanoformulations were more active than the Neem oil against *B. tabaci*. For analysis by LC-MS/MS was used experiment SRM optimizing azadirachtin A and B. Azadirachtins were quantified in various parts of the plant except the stem where these compounds were below the limit of quantification. The results show the possibility of applying nanotechnology to combat *B. tabaci*. A method for LC-MS/MS was developed to quantify Azadirachtin A and B with LD and LQ at 2.7 and 9.0 ng / mL, respectively.

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P38 - *Aedes aegypti* larvae respond to Natural and Synthetic Odorants

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The ability to detect a wide range of sensory cues is essential for the survival and vectorial capacity of mosquitoes. Of these sensory stimuli, chemosensory inputs, especially olfactory cues, are crucial in food detection, mating, predator avoidance and other behaviors. This investigation focuses on the analysis of olfactory-driven behavior in dengue vector mosquito *Aedes aegypti* (L.) larvae to respond to synthetic or natural odorant stimuli. Mosquitoes have four distinct developmental stages: egg, larva, pupa and adult. Immature stages of mosquitoes require water to complete their life cycle. The larvae will go through four developmental stages called instars. We established a sensitive olfaction-based behavior assay, following the technique of Xia *et al.* [1] with minor modifications, that tracks the ability of *Ae. aegypti* larvae to respond to a range of synthetic and natural odorant stimuli. In this assay, the distribution of 100 2nd or 3rd instar larvae was monitored to a range of odorants stimuli as well as appropriate negative controls throughout a 60 minute time course. The number of larva in both test and control zone was counted throughout all time-point and performance index (PI) at 40min was calculated to represent the response characteristics of each odorant with +1 indicative of full attraction while -1 represents complete repulsion. We tested various odorants, studying behavioral olfactory response of the larvae exposed. The widely used insect repellent N, N-diethyl-m-toluamide (DEET), consistently evoked highly significant negative PI and when using yeast extract, a know food source, the larvae responded with a significantly positive PI. Little is known about olfactory processes and the relevant behaviors of pre-adult stage dengue vector mosquitoes. It is important to study the behavior of attractant and repellency, as it could contribute to the understanding of the mechanism of olfaction in *Ae aegypti* larvae.

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P39 - Contextual and scale-dependent use of relative humidity in two hawkmoths from the desert

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In the Sonoran Desert, *Manduca sexta* (Lepidoptera, Sphingidae) larvae feed on *Datura* sp. plants, whose large, fragrant flowers are an important nectar source for the adult moths. As flowers open during sunset, they release elevated levels of CO₂ [1], which have behavioral effects on foraging adults [2]. We have recently shown [3] that these responses to CO₂ can be: 1) Scale-dependent, as CO₂ plumes in a wind tunnel elicit upwind tracking responses at a distance, but has no effect as a local feeding stimulus, and 2) Context-dependent, as in dual-choice assays female adults prefer to first visit flowers emitting high-CO₂ only when host-plant odors are present (oviposition context). We are now investigating the scale- and context-dependent functions of a ubiquitous environmental cue – water vapor – in the *Manduca-Datura* system and in a similar association between *Hyles lineata* and *Oenothera* sp. Besides of CO₂, these flowers also deliver at anthesis a “cloud” of water vapor resulting in a transient increase of relative humidity (RH) in their headspace. As with CO₂, the transient increase of RH has the potential of being an “honest” cue of nectar presence. Additionally, hawkmoths in the Sonoran Desert are known to travel back and forth between the wet, high woodlands and the low, dry grasslands, which together with the highly localized monsoon showers could make RH a significant sensory cue at the landscape scale during habitat selection. Here we present results from experiments on these two hawkmoth species, in which we are investigating the scale-dependency and contextual value of RH as a sensory cue during nectar foraging and as an environmental cue during habitat selection.

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P40 - Odor emission from a trap for the vectors of Chagas disease depending on its physical attributes

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Chagas disease vector's management is currently mainly accomplished by insecticide spraying of infested houses. However, this is not ideal considering that the insects could develop resistance to the insecticides, and that these chemicals could affect people living in sprayed houses. The use of trap devices to capture the insects could help monitor and control the bugs without generating any resistance or toxic effect for humans. One method to capture walking bugs is based on the use of adhesive tape. This method has been used to intercept bugs moving between different habitats [1]. Adhesive tape is also used as the trapping agent of the popular Noireau trap, baited with a mouse [2]. However, it is not known if, after being immobilized, the adults stuck become stressed and hence emit their alarm pheromone thus repelling conspecifics. If so, this could result in an overlooked reduction in the trapping performance of the device being used. To study this, we evaluated the effect of the presence of bugs stuck in adhesive tape on the capture performance of a trap lured with a mouse. Fifth-instar *Triatoma infestans* bugs starved for 1 month were tested, singly, in an experimental arena, overnight. Three experimental series were carried out: in series 1 the trap just contained a mouse, in series 2 the trap contained a mouse plus 10 *T. infestans* adults free to move inside it (but not allowed to contact the mouse), and in series 3 the trap contained a mouse plus 10 *T. infestans* adults stuck to adhesive tape (importantly, those bugs were stuck to the tape by their own). Results show that the presence of insects (stuck or not) inside the lured traps do not affect capture. The percentage of capture in all series was 100%. Our results suggest that the use of adhesive tape as immobilization method in traps could be used without compromising attraction/trapping effectiveness.

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P41 - The role of the presence of a host in the oviposition behavior of a vector of Chagas disease

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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 near a host. In fact, the triatomine *Rhodnius prolixus* often lays (sticks) their eggs on the feathers of avian hosts. The physical characteristics of the substrate certainly play a role in the oviposition behavior [1]. We tested if the presence of a host has an effect on the spatial distribution of the stuck eggs in an experimental arena. The arena consisted of a PVC tube (0.2 m diameter, 1.70 m length). Across the tube, little holes (behind which, a fine mesh was placed to avoid the escape of bugs or eggs) were made to allow air circulation and entrance of sensory cues from a host. 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. A host was placed below one end of the test tube while the other end remained empty; no host was placed below the control tube. Surprisingly, results show that the presence of a live host (mouse) significantly stimulate oviposition in *R. prolixus* (total eggs test, 254; total eggs control =172, N=11, $\alpha < 0.05$, Wilcoxon one tailed test). There are significant differences in the distribution of eggs between zones in both test and control ($P < 0.05$, χ^2 Goodness of fit). However, host sensory cues are not responsible for the spatial distribution of eggs. Preliminary tests using fresh hen feathers showed a similar oviposition stimulation, suggesting that host odor could be involved in the responses obtained.

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P42 - A CO₂-free synthetic host-odor blend evokes trapping-related behavior in triatomines, vectors of Chagas disease

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Chagas disease is a serious health problem in Latin America. The vectors of the 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 treated houses. The use of lured trap devices is a sustainable and an environmentally-friendly method for vector control [2]. Thus, it is possible to monitor and control the bugs without generating any resistance or toxic effect for humans.

Using two of the most important Chagas vectors, *Rhodnius prolixus* and *Triatoma infestans*, we test potential odor attractants in a dual-choice trap olfactometer. In this olfactometer, the insects have to be activated, attracted and captured in order to obtain a response to an odor source. We tested a blend of synthetic odors that did not include CO₂, a compound that is either expensive or unpractical to use in the field. The synthetic blend, consisting of L(+)-lactic acid, hexanoic acid and ammonia, was assayed using live mouse odor as positive control. The positive control, as well as the synthetic blend, evoked significant activation and attraction (capture) in *R. prolixus* and *T. infestans*. The fact that the blend was able to significantly trap the insects would imply that it is a strong attractant. This is the first time a CO₂-free synthetic host odor blend is able to capture a significant number of triatomines in a trap-olfactometer.

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P43 - Evidence that honeybee elemental and non-elemental associative learning is impaired by glyphosate traces

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Glyphosate (GLY) is a broad spectrum herbicide used for weed control. During the evaluation stages for product approval, only lethal effects studies on invertebrates were reported. However, the possibility that GLY causes sub-lethal damage to non target organisms such as insects that forage in agricultural ecosystems cannot be discounted. Honeybees are the main agricultural pollinators and a well known model for learning and memory research. We studied the putative effects of GLY on the behavior of honeybees, specifically on their associative learning abilities through different integrative experiments: 1) Laboratory reared bees of 15 days of age and exposed to GLY at least during their first week as adults, were trained to establish an elemental association through an olfactory conditioning of the proboscis extension response (PER), which consisted in three odor-reward association trials. 2) Bees captured at the hive entrance were trained to a longer elemental learning protocol (PER conditioning of eight odor-reward association trials and five extinction tests) in which the sucrose reward contained sub-lethal doses of GLY. 3) Bees were trained to a non-elemental learning paradigm (negative patterning) also with contaminated sucrose reward offered during PER conditioning. Mortality, solution uptake and locomotor activity (LA) were recorded for laboratory reared bees and acquisition curves and memory retention were recorded for all the tested subjects. Mortality, food uptake and LA did not differ between treated groups. Elemental and non-elemental associative learning was however, impaired by GLY traces. Memory retention at short term differed between treatments for experiment 2), but no differences were found at a longer time scale for the rest of the experiments. These results imply that GLY at concentrations found in nature as a result of standard spraying could impair associative learning between a floral odor and its nectar, which in turn would have negative consequences on honeybee foraging.

P44 - Chemical composition and bioactivity of *Achillea millefolium* and *Eucalyptus* spp. essential oils on *Aegorhinus nodipennis* (Coleoptera: Curculionidae)

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Aegorhinus nodipennis (Coleoptera: Curculionidae) is a poliphagous insect, native of southern Chile. This insect causes a significant damage to the foliage of the host plants during its adult stage. However, the most significant damage is produced by the larval stage, feeding on roots and rootlets, drilling holes and forming galleries¹. This insect has been associated with introduced productive species, such as *Corylus avellana* in the south of Chile. Traditional control methods have not been fully satisfactory at present. Therefore, the searching of new control alternatives is imperative.

The use of plant extract is a valuable tool for the pest control, due to the large amount of bioactive compounds present in them. Essential oil is a kind of plant extract obtained by hydrodistillation. In the literature there are a number of reports showing the biological properties of the essential oils against insects. Thus, these botanical extracts, could be used for the monitoring and the control of *A. nodipennis*².

In this research, we propose the study of two non-host plants of *A. nodipennis* introduced into Chile: *Achillea millefolium*, native from Eurasia and *Eucalyptus* spp. native from Australia widely naturalized in template regions. Both species have a wide range of properties for their use in pest management and might be used as a low risk alternative for pest control in sustainable agriculture.

Based on this background, this research aims: 1) to evaluate the biological activity of both essential oils on *A. nodipennis*, and 2) to identify the chemical compounds present in these essential oils. The attractant or repellent effect showed by the essential oil was evaluated using an olfactometer of four arms, and the compounds were identifying by GC-MS. The results allow elucidating the role of the volatiles identified in these oils on the behavioural response of *A. nodipennis*.

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P45 - Interaction between multimodal sensory signals – study of the invasive fruit fly, *Bactrocera invadens*

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Multimodal sensory attraction is the combinational effect of several sensory cues that guide an insect toward the object, known to be important in insects in general. Most sensory ecology studies with pest of true fruit flies (Tephritidae), conducted to date, have nevertheless focused separately on odours, colours or shape with few examples of combinations of sensory information. Attraction of fruit flies to traps is common practice of pest reduction, primarily for male annihilation. Our study aims at obtaining knowledge about the importance and the interaction of visual and olfactory cues for attraction of the African invasive fruit fly *Bactrocera invadens*. We study if a colour increases in attractiveness with a specific odour or if an odour is more attractive with a specific colour. The goal is to develop measures to enhance the attractiveness to an object and to increase the number of insect that get trapped. In our field study we combine different colours and odours (protein baits and para-pheromones). Odourless glue and buckets with entrance holes are means by which the number, and the sex, of attracted insects are trapped and counted. Measurements of the visual aspects of the objects/traps are compared with the vision capacity of the related species *B. dorsalis*. Preliminary results from laboratory assays show a preference for blue and black colours during oviposition, while attraction to a feeding source is governed by other colours, also showing a sex discrepancy in preference. Our knowledge of behaviourally modifying cues involved in attraction can be used to manipulate the fruit fly behaviour and to be a base for development of traps that are design to effectively reduce the population of the devastating pest.

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P46 - Candidate Kairomones for *Bactrocera invadens* (Diptera: Tephritidae) From *Mangifera indica* and *Sclerocarya birrea* (Anarcadiaceae)

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B. invadens is regarded as one of the most destructive insects of fruits and vegetables (Mwatawala et al 2004). These investigations were aimed at identifying semiochemicals from its hosts so as to establish their potentials as one tool in its control strategies. GC-EAD and GC-MS were carried out to identify the active constituents from ripe fruits of two hosts viz. *Mangifera indica* and *Sclerocarya birrea*. Responses of the antennal olfactory receptors of male and female *B. invadens* were carried out using coupled gas chromatography-electroantennographic detector (GC-EAD). A number of peaks were identified from the two hosts and the identified EAG-active compounds included terpenes, alcohols, esters and aldehydes. It was concluded that the two hosts demonstrated to be potential source of attractants for *B. invadens* and has laid down ground work for further investigations in order to identify the most active blend.

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P47 - Interspecific interactions mediated by semiochemicals between *Dichelops melacanthus* and *Euschistus heros* (Hemiptera: Pentatomidae)

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Additionally to intraespecific role mediating communication and interactions, semiochemicals could be used in interspecific interactions. For example, cross-attraction to sex pheromone of heteroespecifics was reported several times in stink bugs. Two hypotheses are been proposed to explain this behaviour, one postulate the use of sex pheromone of heteroespecific as a kairomone to search for food plants and the other suggest that it could be related to a mechanism of passive defence in stink bug aggregations. Field experiments with a formulation of *Euschistus heros* sex pheromone showed high attractiveness to *Dichelops melacanthus* females. To better understand this phenomenon in this work we evaluate the response of *D. melacanthus* to semiochemicals from *E. heros*. Volatiles of 40 *E. heros* males were collected by air entrainments and identified using a GC-MS and a GC-FID. Aeration extracts containing defensive compounds and the three male specific compounds (methyl 2S,6R,10S-trimethyldecatrienoate, methyl 2,6,10-trimethyldodecanoate, and methyl 2E,4Zdecadienoate) were selected for an initial set of bioassays . The behavior of *D. melacanthus* females and males was observed in a Y shape olfactometer when exposed to the collected volatiles vs. n-hexane, and the first choice was recorded. Results showed that *D. melacanthus* adults were not attracted to volatiles from *E. heros* males, however males avoid the arm of the olfactometer treated with the *E. heros* male volatiles that could suggest that some compounds from defensive secretions or sex pheromone of *E. heros* could be recognized by males of *D. melacanthus* and used to discriminate places where potential competitors are. This hypotheses need to be tested by behavioral studies oriented to identify the specific compounds related to this behavior. Further studies are being conducted using blend and individual synthetic compounds in olfactometer bioassays .

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P48 - Chemical Ecology of *Anastrepha obliqua* (Diptera: Tephritidae) in mango cultivars

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The selection process by herbivorous insects comprises several steps as host location, recognition and acceptance. The location of suitable host for a female is of crucial importance for the development and survival of their offspring. The knowledge of the interaction of *Anastrepha obliqua* with their hosts is scarce, but is known that females prefer to lay their eggs in Anacardiaceae fruits, especially mango, *Mangifera indica*. The aim of this study was to understand the chemical ecology of this fruit fly. To accomplish this goal we collect volatiles of three mangoes cultivars "Carlota" (susceptible), "Keitt" (intermediate) and "Espada" (resistant). The response of females of *A. obliqua* to these volatiles was evaluated in a "Y" olfactometer, oviposition and GC-EAD. Because these mango cultivars show different amounts of resin, we also test the survival of the embryos and larvae in fruits of *M. indica*. We collect the headspace fruit volatiles from third stage fruit ripening in filters of Super-Q®, which was subsequently washed with hexane. To assess the viability of the embryo eggs were placed "Espada" and "Carlota" resin for periods of 1, 3, 9, 17 and 24 hours. Then, the eggs were fixed and dehydrated in aqueous ethanol for histological evaluation of the embryo. The first instar larvae were conditioned to different diets, containing skin, pulp, resin and "Espada" and "Carlota" for a period of 96 hours. The olfactometer shows that the females prefer volatiles of the cultivars when contrasted with control (hexane). When the volatiles of cultivars were contrasted two-by-two there is a clear preference to "Carlota" and no preference to "Keitt" or "Espada". In the oviposition test, females showed the same preference as in the olfactometer. The chromatographic profiles are distinct among cultivars, as well as the antenna response. Females respond to ten compounds from "Carlota" and five of "Espada" and "Keitt". In the survival test embryos die when they were conditioned in resin of "Espada" showing total deterioration in 24 hours. However when the eggs were exposed to resin of "Carlota" and the control (water) the embryos developed normally. Likewise when larvae fed diets containing "Espada" resin the mortality is 100% contrasting with the normal development in "Carlota" and controls. The "Carlota" proved to be a good host for *A. obliqua* both in location, acceptance and development of offspring. The "Espada" exhibits resistance to attack of this fruit fly, demonstrating a natural protection against the eggs and larvae.

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P49 - Effects of natural products from northwestern Argentinean plants on the behavior of *Myzus persicae*

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The green peach aphid, *Myzus persicae* (Sulzer) (Homoptera, Aphididae), is a major pest for potato crops (*Solanum tuberosum*); it has a worldwide distribution and causes damage mainly indirectly by its ability to transmit plant viruses. Repellence surface factors will avoid probing by the aphid and therefore will avoid viruses' transmission. Currently, to control this pest, synthetic insecticides are used that cause adverse behavioral effects and also insect resistance. There are native northwestern Argentinean plants known for its insecticidal or bactericidal activity that has not been studied against aphids. Our goal is to find natural products with repellency against *M. persicae* from new plant sources that will be more effective against viruses' transmission. Here we studied the effect that aqueous extracts of *Synandropadix vermitoxicus* and *Flourensia tortuosa* have on the behavior of *M. persicae*. Different plant extracts were probe for repellency by choice-test and to study aphid probing behavior Electrical Penetration Graph (EPG) monitoring¹ was used. Choice-test data were analyzed by binomial test and repellent indexes (RI) were calculated². EPG monitoring was analyzed by Mann–Whitney U-test. Potato plants were treated with aqueous extracts from *S. vermitoxicus* (0.5%) and *F. tortuosa* (0.5%); Imidacloprid (0,015%) and Tween 20 (0.05%) were used as controls. We found that *S. vermitoxicus* extracts were the most repellent (RI >86%). *F. tortuosa* extract was repellent after 45 min (RI <60%). The EPG results combined with the repellency effect results will be discussed during the meeting.

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P50 - Repellence and toxicity properties of Eugenol, Isoeugenol, Carvacrol and Thymol against *Triatoma infestans* Klug and *Ceratitis capitata* Wiedemann

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Essential oils, which are volatile oils from plants, have been shown to be a potent source of botanical pesticides. Among its constituents, some derived from benzene (phenylpropanoids) such as thymol, eugenol and carvacrol are recognized by their properties against insects. In this work, we evaluated the repellent and toxicity properties of carvacrol, thymol, eugenol and isoeugenol against *Triatoma infestans* and *Ceratitis capitata*, which are insects of health and agronomical importance. The lethal dose LD50 was determined on *C. capitata* using Probit analysis based on mortality data at 24 h and lethal time of toxicity LT50 was done on assessments at 10 µg/fly by topical application against females and males. The LD50 were <15 µg/fly both for males and females with the exception of isoeugenol in females (25.5 µg/fly). The most active compound was carvacrol on both sexes, followed by thymol in males. The LT50 in males were 2.8 h and 3.3 h for carvacrol and thymol respectively, while in females 12.9 h and 17.4 h for carvacrol and eugenol respectively. Repellency on *T. infestans* nymphs was determined according to standard method. Isoeugenol and eugenol were the most active compounds with repellency values of the 96 and 70% respectively. Carvacrol and thymol showed a faint repellent activity.

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P51 - Accumulation of Apigenin-7-O-rutinoside in leaves of Murcott Tangor inoculated with *Alternaria alternata*

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The fungus *Alternaria alternata* “tangerine pathotype” causes the disease known as alternaria brown spot (ABS), which affects tangerines and their hybrids. The fungus produces the host-selective toxin (HST) ACT, which is responsible for the pathogenesis [1]. The symptoms include brown to black necrotic spots surrounded by a yellow halo. Severe cases of ABS can lead to fruits and leaves abscission or death of the entire shoot [2]. In plants, antibiotic compounds can be both preformed (phytoanticipins) and inducible (phytoalexins). The purpose of this study was to investigate the differences in chemical accumulation between leaf extract of Murcott tangor with symptoms and control. Leaves of Murcott tangor were inoculated with cells of *A. alternata*, and resulted in 90% of symptomatic plants. NMR-600 MHz studies showed that the concentration of the apigenin-7-O-rutinoside in leaves with symptoms of ABS was in markedly higher concentration than in those from control plants. This result was confirmed by analyzing the distribution of Apigenin-7-O-rutinoside in intact leaf tissue by MALDI-IMS. These data suggest that apigenin plays a role in plant-pathogen interaction, probably as a phytoanticipin.

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CAPES, CNPq, FAPESP, INCT

P52 - Semiochemicals involved in the interactions between cowpea (*Vigna unguiculata* Walp) and black cowpea aphid (*Aphis craccivora* Koch)

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Cowpeas are widely grown in the tropical and subtropical regions of the world for human and animal food [1]. 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 because transmit virus, and this can compromise growth after attack. Volatile compounds originating from the plant may play a role in the orientation of its insect pests towards the plant and in ultimate recognition of the plant for feeding and oviposition [2]. The aim in this job was to analyze qualitatively the volatiles compounds released by cowpea (VITA 7 susceptible cultivar). Volatiles compounds of cowpea plants were collected by drawing air over the plants in a polyester chamber during 24 hours, trapping the volatiles compounds on Porapak Q (80/100 mesh, 0,05 g; Supelco) adsorbent, and the desorption was realized using 700µl of hexane as solvent. GC-MS was used to analyze the volatiles. Some volatiles were identified such as: Dodecane, Tridecane, pentadecanoic acid, octadecanol, farnesol, hexadecenoic acid, oleic acid, heptadecene, undecane, 2-ethyl hexanol, β-myrcene, 3-hexanone, nonanal, decanal, naphthalene, 2-ethylnonane, limonene. This compounds can reveal important information's because this Cowpea cultivar is susceptible and we can know what the specifics compound (s) are responsible to increase the attractiveness of the black aphid, *Aphis craccivora*. In the next studies we will study the role of cis-jasmone in the resistance inducing of cowpea, *Vigna unguiculata* Walp.

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[2] Costa, João Gomes da. Caracterização química e genética da interação *Capsicum* spp. (Solanaceae), pulgão *Aphis gossypii* Glover (Hemiptera: Aphididae) e o Parasitóide *Aphidius colemani* Viereck (Hymenoptera, Braconidae, Aphidiinae)/ João Gomes da Costa.- Maceió, 2010.

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P53 - Antifungal study of the resinous exudate and of meroterpenoids isolated from *Psoraleaglandulosa* (Fabaceae)

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The antifungal properties of the resinous exudate from aerial parts of *Psoraleaglandulosa* were tested against eight clinical yeast isolates. Comparison of the antifungal activities of bakuchiol and 3-hydroxy-bakuchiol, both compounds isolated from the plant, with that of the crude extract suggested that the latter 3-hydroxy-bakuchiol was the major active component in the exudate. These results validate the vernacular medicinal use of *Psoraleaglandulosa* as an antiseptic in treatment of infections and skin diseases.[1]

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P54 - Antimicrobial activity of metabolites produced by the gland metapleural of leaf-cutting ant *Atta sexdens rubropilosa* against pathogenic microorganisms isolates of the fungus garden

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Leaf cutting ants are dominant herbivorous in neotropical regions and constitute one of the main forest plagues in the Americas¹. These eusocial insects are vulnerable to pathogenic attack as the colonies are constituted by million of individuals genetically similar living in humid environment under the soil, where numerous microbial pathogenic communities are present². One of their defense mechanisms is the liberation of organic secretions produced by exocrine gland metapleural (MG)³. In the present work, it has been demonstrated that the main constituents of the metapleural gland secretions of leaf cutting ants possess strong inhibitory activities. The compounds detected was carboxylic acids from acetic acid to the long-chain fatty acids but comprise also some alcohols, lactones, and keto acids. These compounds contain oxygen atoms in their molecules, which present the possibility of binding to the micro-organisms membrane via hydrogen bonds, and thus promoting the breakdown of the membrane structure. The compounds from secretion of metapleural gland were tested against of entomopathogenic fungi *Escovopsis weberi* and *Fusarium sp.*, *Cunninguanella sp.*, showed a general broad-spectrum antibiotic function, with most of its over 12 compounds being effective antimicrobial chemicals, were both conidia and hyphae of these pathogens are inhibited similarly by different classes of chemical compounds from the MG secretions. As the secretions flow out over the ant cuticle, it is likely that they primarily protect the ants themselves, although an additional function in protecting the fungus garden cannot be ruled out.

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P55 - Diversity of endosymbiont microorganisms of larvae and adults of *Diabrotica speciosa* (Coleoptera: Chrysomelidae): Hierarchical clustering analysis by MALDI-TOF

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Diabrotica speciosa, is a species which occurs in most Brazilian states, standing out as one of the most important polyphagous pest of beans and corns [1]. To understand the functions of the insect digestive tract microbiota through the identification of the communities members, enable to understand possible interactions among plants and insects, microorganisms and plants and also insects and microorganisms, which may lead to the development of a new mode of action to control this insect pest. Therefore, this study aim to evaluate the microbial diversity of endosymbionts present in the digestive tract of larvae and adults *Diabrotica speciosa*. As a result 20 bacterial strains and 7 fungal strains were isolated, although some of these microorganisms are still unpublished in the literature. The isolated bacteria were characterized and taxonomically grouped by their morphological characteristics such as colony color, Gram staining and cell form. Furthermore, isolated microorganisms were grouped taxonomically through a new technique for microbial identification and classification, assessing the protein profile by mass spectrometry desorption ionization and matrix assisted (MALDI-TOF), enabling classify the strains in a hierarchical way (Figure 1) through analysis of the main components related to the spectras obtained. It resulted in the division of the microorganisms into 9 groups, which confirmed their morphological informations. These microorganisms are in phase of identification by DNA sequencing.

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Acknowledgements: FAPESP, CNPQ, CAPES, INCT.

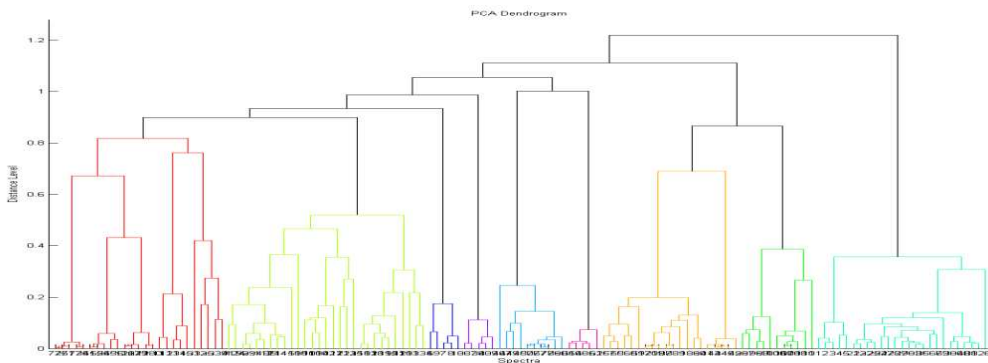


Figure 1. Dendrogram obtained from the mass spectras of strains and cluster analysis.

P56 - VOCs emitted by *Eucalyptus benthamii* are influenced quantitatively and qualitatively by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae) feeding activity

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The *Eucalyptus* plantations pest *Thaumastocoris peregrinus* has no effective control strategy available in the regions where it was accidentally introduced (South Africa and South America). Thus, understanding how plants and insects interact may be useful, in the future, as basic information for new pest management strategies. To determine how *T. peregrinus* influences the emission of volatiles of its host plant *Eucalyptus benthamii* we investigated the effect of *T. peregrinus* feeding activity (herbivory) and compared it with control plants. Hence were investigated the effect of mechanical damage and if there were qualitative and quantitative differences of VOCs emitted during photophase and scotophase after herbivory. The extractions were done every 24h for five days for the herbivory and mechanical damage treatment and every 12h for 4 days for the photophase/scotophase treatment. Chromatographic profiles of all treatments were compared and the volatile organic compounds were identified and quantified. Bioassays testing the response of virgin and mated females to extracts of herbivory and control plants were performed on Y olfactometer. Three major compounds, α -pinene, aromadendrene and globulol were released after herbivory and detected in lower quantities after mechanical damage and control. In total, 15 compounds were quantified for all treatments. In general, after herbivory plants released a greater quantity of compounds when compared with other treatments. There were no differences between VOCs released after photophase and scotophase. Bioassays revealed a preference of mated females to control plants extracts when compared with herbivory extracts. Virgin females did not show any preference. Bioassays using the plant and not the extract as source of odor are in course.

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P57 - I know what I like, and I like what I known: A specialist herbivore performs better in native host plants in comparison with invasive ones

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Larvae of the neotropical specialist moth *Utetheisa ornatix* (Arctiidae) feed on unripe seeds and leaves of both native and invasive *Crotalaria* species (Fabaceae). They sequester pyrrolizidine alkaloids (PAs) from them, and use these alkaloids for defense until the adult stage [1]. We hypothesized that native herbivores would have a better performance on native plants when compared with invasive ones, since they are co-adapted with the former. In order to test this hypothesis, we fed larvae of *U. ornatix* with leaves of native (*C. micans*, *C. paulina*, and *C. vitellina*) and invasive species (*C. juncea*, *C. ochroleuca*, *C. pallida*, and *C. spectabilis*), from hatching until emergence, and recorded the corresponding larval development time and pupal weight. Couples from each host-plant were mated and the number of eggs was recorded. Our results showed that the performance of *U. ornatix* was better on native hosts when compared with invasive ones, which agrees with the former hypothesis. These findings cannot be explained by different PAs in native and invasive hosts. For example, the native host *C. paulina* - in which *U. ornatix* showed the best performance, has a similar PA amount and structures as those found in the invasive *C. spectabilis*, where *U. ornatix* showed one of the worst performances. We suggest that other compounds, such as protease inhibitors and lectins, may be involved in the chemical defense of invasive *Crotalaria* species against *U. ornatix*. Such hypothesis is currently under investigation in our lab.

[1] see review in Trigo JR (2011) *Phytochemistry Reviews* 10: 83-98.

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P58 - Behavioral Response of the infesting stage of *Caligus rogercresseyi* (Copepoda: Caligidae) towards semiochemicals

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Sea lice (Copepoda: Caligidae) are crustacean ectoparasites associated to wild and cultured salmonid species that feed on the mucus, skin and blood of the host [1, 2]. In Chile, the dominant species affecting aquaculture is *Caligus rogercresseyi* which is present in 99% of the established cultured cages, and is distributed on both salmonid and non-salmonid hosts [3]. Current control of sea lice is through the application of chemicals, such as antibiotics and pesticides incorporated in the food. However, this approach has generated resistance to chemical treatments. Therefore, is urgent the searching of new alternative control methods. Chemical ecology studies of the interspecific interaction between *C. rogercresseyi* and salmonid species, such as Atlantic salmon, *Salmo salar*, Coho salmon, *Oncorhynchus kisutch* and Rainbow trout, *O. mykiss*, determined that semiochemicals mediate the parasite-host interaction between *C. rogercresseyi* and salmonid species [4]. The aim of this work was to study the behavior of the infesting stage (copepodid) of *C. rogercresseyi* towards both a repellent semiochemical and a chemical analogue. Ovigerous females of *C. rogercresseyi* were collected in a Culture Center of Atlantic salmon in Calbuco, Región de Los Lagos (Chile). This adults females were transferred to the laboratory in containers with sea water (12°C), and they were remained until hatching copepodids. The specimens were subjected to behavioral testing [5] (n = 30). For this, an acrylic Y-tube was used in a vertical position. Y-tube was divided into four zones: two arms, one decision zone and the leg. Treatments consisted of sea water (33 ppt) conditioned with the semiochemical (stimulus) versus seawater (control). The results indicated that copepodids showed a repellent response to the compound used ($P < 0.05$). These results are the first report on the effectiveness of semiochemicals for the control of sea lice.

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P59 - Olfactory response and morphological differences of *Dioxya chilensis* (Diptera: Tephritidae) as result of the use of different host plants from the genus *Haplopappus*

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Dioxya chilensis is a generalist endophagous inflorescences parasite of several plant species among Asteraceae, where the larval stages feed and develop. Species from the genus *Haplopappus*, like *H. foliosus* and *H. decurrens* are some of its frequent host in Central Chile. These plant species differ in their inflorescence morphology, scent and phenology¹. Thus, these constitute a propitious model for the study of the behavioral and morphological consequences of the use of different host plant. Traits that have been suggested as keys for the origin and conservation of host-races insects varieties. Using a Y olfactometer we evaluated the behavioral responses of *D. chilensis* adults derived from *H. foliosus* and *H. decurrens* towards the inflorescence scent from these two alternative larval hosts. We used purified air as control and scored the proportion of time spends in each arm as response variable. In addition, we evaluated the differences in the geometric morphometrics of the dorsal view of the wings. Flies did not show a statistically significant preference towards its developmental host plants. Imagines developed from *H. foliosus* displayed a positive chemotaxis towards the alternative host *H. decurrens* in detriment of his larval host. In addition, adults derived from *H. decurrens* did not show preference for any host presented. Responses were not affected by insect sex. Contrastingly, we did find differences in wing geometrics morphometrics among insects from different developmental hosts. Our result does not show a clear tendency of host fidelity towards the developmental host, however they suggest the possible generation of sensorial bias that could induce different behavioral responses in the adult stage. On the other hand, larval development in two different hosts influenced differentially *D. chilensis* wing morphogeometrics. This may correspond to a plastic response as results of the use of different hosts. Previous studies in the genera *Dioxya*² discard the presence of sexual pheromone, thus, these morphological differences may be of key importance in visual recognition and mate selection.

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P60 - Effect of imidacloprid on associative learning during the early adulthood of honeybees (*Apis mellifera*)

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Imidacloprid (IMI) is a neonicotinoid used for its high selective toxicity in insects. However, its effect on non-target and beneficial insects, such as the honeybee *Apis mellifera*, is still controversial. IMI acts as a nicotinic agonist. Moreover, these cholinergic pathways take part in the acquisition and retention stages of associative learning. Therefore, we used IMI to assess this system's role and the changes it suffers during the first days of the honeybee's adult life. Harnessed bees 2-10 days old were exposed to sub-lethal doses of IMI (0, 0.25 or 0.50ng per bee) administered topically on their thoraxes 15 minutes prior to an olfactory conditioning procedure based on the proboscis extension response (PER). Conditioning consisted of 5 consecutive presentations of a pure odour paired with sucrose solution as a reward. Retention tests were performed immediately after conditioning or 24 hours later. Acquisition and retention were impaired as a result of acute exposition to both treatment doses of IMI. These outcomes differed amongst age groups. We propose that IMI interferes with learning and memory processes in recently emerged bees and that the nicotinic cholinergic pathways seem to suffer modifications during the first days of adulthood, along with other changes in the nervous system.

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P61 - Study of the immunostimulant activity of the aromatic geranyl derivative Filifolinone

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Fish farming crops are constantly exposed to infectious diseases due to heavy conditions of production, such as enclosed spaces in which microorganisms develop allowing easy dissemination of lakes to seas and representing a problem often difficult to control, producing high mortality and the decrease in productive parameters. These diseases can be caused by viruses, bacteria, parasites and fungi, and causing each year major production losses.

The salmon industry is one of the main exports in Chile. However, there have been heavy losses due to the emergence of viral such as IPNV and ISA. Infectious diseases can be controlled and even eradicated by uses of immunomodulators able to of regular, promote and enhance the immune response. The search for compounds with activity immunostimulant, which are biodegradable and do not have adverse effects on fish and the environment, has been a challenge.

In this work we show the results obtained of the evaluation of immunostimulant potentiality of 3 H-spiro [1-benzofuran-2,1'-ciclohexane] derivative called Filifolinone, obtained from Filifolinol, the main component of the resinous exudates produced by plant species *Heliotropium filifolium*. We evaluated the effect of the filifolinone in mouse dendritic cells and tested the level of expression of MHC molecules class II by flow cytometry. These results indicate that filifolinone increased the expression of class II MHC in dendritic cells, promoting the maturation of dendritic cells.

In relation to the fish immunomodulatory activity, we evaluated the effect of filifolinone on expression of cytokines, observing significant results in the activation of them.

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P62 - Evaluation of antifungal activity of volatile compounds produced by saprophytic fungus

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Saprophytic fungus play an important role in ecosystems because of their enzymes and secondary metabolites production involved in the decomposition of dead organic matter and their role in competition behaviors with other microorganisms to colonize a niche. Because of these characteristics, saprophytic fungi have the potential to provide novel candidates compounds with antifungal activities.

Botrytis cinerea and *Fusarium oxysporum*, are phytopathogenic fungus causing a severe damage in both agricultural crops and postharvest management of fruits. The control by synthetic fungicides could be cause of environmental damage and favoring the developing of resistance in phytopathogenic fungus. Therefore, is necessary to search new environment friendly control methodologies.

The aim of this investigation was to evaluate the antifungal activity of volatile metabolites isolated from saprophytic fungus against phytopathogenic fungus, by using two-compartmented plate bioassays [1]. Volatile compounds collection was carried out by solid phase microextraction (SPME) and the mixture was analyzed by gas chromatography coupled to mass spectrometer (GC-MS).

Our results showed inhibition on plant pathogens that infects post-harvest berries and others crops in Chile.

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P63- Anti-Saprolegnia activity of essential oil of *Laurelia sempervirens* and *Laureliopsis philippiana*

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Saprolegnia parasitica is an opportunistic pathogenic Oomycota that attaches to the dead eggs and damaged tissues, grows and leads to Saprolegniosis in fish. Since chemical compounds that are used in prevention and treatment of Saprolegniosis infection causes cancer and is harmful to human health, in this research, for alternative materials that affects *S. parasitica*, the essential oil of *Laurelia sempervirens* and *Laureliopsis philippiana* were tested on this fungus. *S. parasitica* has inhibited completely in agar saboraud dextrose. The essential oil was analyzed by GC- mass. [1-3]

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P64 - Inhibitory response of saprophytic fungi against phytopathogenic fungus *Botrytis cinerea* and *Fusarium oxysporum*

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Botrytis cinerea and *Fusarium oxysporum*, are considered within of “top ten” of more harmful fungus in agriculture for their wide host ranges and environmental persistence. They cause vast economic damage during crop cultivation as well as in harvested produced, killing host plant cells and then colonizing the dead tissue. *B. cinerea* attacks over 200 different plant species and their action is manifested by necrotic areas with extensive fungal growth, giving the characteristic appearance of grey mould. Meanwhile, *F. oxysporum* infects a wide variety of plant species by directly penetrating roots, invading the cortex and colonizing the vascular tissue.

As a result of problems associated with the continuous applications of synthetic fungicides, including environmental and health risks and residues on harvested fruit, alternative strategies of management for these fungus are needed. The main objective of this work was to find in Chilean saprophytic fungi, biological control agents through generation non-volatile compounds that act by inhibiting the growth and development of phytopathogenic agent. The antifungal activity of saprophytic fungi collected in Parque Nacional Conguillio (Región de La Araucanía, Chile), was assessed by antagonism bioassay in Petri dishes with PDA culture medium and subsequent incubation at 25°C for one week [1].

The results showed an inhibition of growth of phytopathogenic fungus, *B. cinerea* and *F. oxysporum* for secondary metabolites produced by saprophytic fungi.

[1] Schalchli Heidi, Horamazabal Emilio, Becerra Jose, Birkett Michael, Alvear Marysol, Vidal Jorge and Quiroz Andrés (2011): Antifungal activity of volatile metabolites emitted by mycelia cultures of saprophytic fungi, *Chemistry and Ecology*. 27:6,503-513.

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P65 - Biological activity of volatile compounds of *Homalinotus depressus* (Coleoptera: Curculionidae)

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Homalinotus depressus larvae, due to their feeding behavior, promote damage in coconut leaves and inflorescence peduncles. The aim of this study was to evaluate *H. depressus* behavior to elucidate the chemical communication for this species. *H. depressus* adults were collected in a commercial area at Sococo Company, and kept under laboratory conditions. Volatile collections from males and females adults were done using aeration chambers and the extracts were used to test the biological activity in a Y-tube olfactometer. The male volatiles with or without natural food and natural food showed different results regarding co-specific attraction. It was possible to confirm the presence of male-specific compounds in *H. depressus* and that these compounds act as an aggregation pheromone, because attracted both sexes.

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P66 - Behavioral and Toxicological Effects of Monoterpenes on *Triatoma infestans*

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Some essential oils have been used against plagues since millennia. They are complex mixtures of monoterpenes, phenols and sesquiterpenes, both safe to non-invertebrate organisms and environment.¹ Monoterpenes have a broad spectrum of insecticidal activity, in which triatomines are already included.² This work belongs to a project focused in the development of new diagnostic and control tools for Chagas disease. First instar nymphs of *Triatoma infestans*, main vector of Chagas in Argentina, were exposed to: carvacrol, (-)-carveol, citronellol, eugenol, geraniol, linalool, menthol, α -terpineol or thymol. To evaluate the effect on locomotor activity, groups of four nymphs were allowed to walk on filter papers impregnated with acetone solutions of each substance and recorded with a video camera connected to an image analyzer. Deltamethrin was used as a positive control (it produced hyperactivation from 0.39 $\mu\text{g}/\text{cm}^2$). All monoterpenes produced hyperactivity from 390 or 3,900 $\mu\text{g}/\text{cm}^2$. The knock-down effect was evaluated in plastic containers with lids. Groups of ten nymphs were exposed to filter papers impregnated with acetone solutions of the substances at 3,900 $\mu\text{g}/\text{cm}^2$. The number of knocked-down insects was recorded every ten minutes. Replicates were interrupted when 90% of the insects were knocked-down or 7 hours after the start of the exposure. Values of Knock-down Times for 50% of the exposed insects (KT50) were calculated. Dichlorvos, a volatile organophosphate, was used as a positive control. Dichlorvos was significantly more toxic than any other substance and monoterpenes presented this order of toxicity: α -terpineol < eugenol < carvacrol < linalool < thymol. (-)-Carveol, citronellol, geraniol and menthol exceeded the 7 h of exposure with less of 50% of the insects knocked-down. In the immediate future we planned to assess the ability of these monoterpenes to produce flushing-out effect on semi-field and field conditions. Thymol and linalool are worthy of further study as potential active ingredients of triatomaticides formulations.

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[2] Sfara, V., E. N. Zerba and R. A. Alzogaray. 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus*. *Journal of Medical Entomology* 46(3): 511-515.

P67 - Chemical Composition of the Defensive Secretions of *Metagyndes longispinia* and *Parabalta cristobalia* (Laniatores, Gonyleptidae) two Chilean Harvestmen

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The arachnid order Opiliones is represented in Chile by around 93 species with 56 representatives of the family Gonyleptidae, they produce a very unpleasant smelling secretion which is considered defensive. The chemical composition of the Gonyleptidae secretion is characterized by the characteristic presence of 1,4-benzoquinones derivatives¹, not associated with the unpleasant smell.

In this work the chemical composition of the defensive secretion of two species of Chilean Gonyleptidae is presented. The analysis was performed by adsorbing the components of the secretion from a closed system containing the harvestmen using SPME and GC-MS. From the secretion of *Parabalta cristobalia*, several derivatives of 1,4-benzoquinones (the principal components), phenolic compounds, aldehydes and ketones were identified. A difference between the composition of the secretion between males and females was observed. From the secretion of *Metagyndes longispinia* only 1,4-benzoquinones were identified and the identification of minor components is in progress. No difference between the composition of the secretion between males and females was observed.

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P68 - Female attraction to host plant odors in an invasive moth has a genetic basis: ecological and practical implications

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Most attractant-based approaches for insect pest management rely on the assumption that the responses of adult insect herbivores to volatile blends are a species-specific trait, neglecting the option for the existence of potential significant intra-variations. Knowledge of such potential variation in insect olfactory attraction could then determine the promise and the long-term success of tactics based on behavioral manipulation with plant volatiles. In this study we used the oriental fruit moth *Grapholita* (= *Cydia*) *molesta*, an oligophagous lepidopteran invasive insect that attacks stone and pome fruit trees, as a model organism to investigate whether and to what degree female attraction to host plant odors has a genetic component. We used a full-sib/half-sib approach to estimate the heritability of female attraction to plant odors and of a key fitness-related life-history trait, the females' fecundity. Furthermore, we calculated the genetic correlation between these two traits. Results showed a considerable genetic basis for female olfactory attraction as well as a genetic trade-off with fecundity. These estimations were empirically corroborated when comparing two strains maintained in the laboratory for different numbers of generations. A long-term reared strain lost its olfactory discrimination ability but achieved significantly higher fecundity compared to a short-term reared strain. Our results highlight that genetic studies are relevant for understanding the evolution of odor-guided behavior in herbivore insects and for judging the promise of pest management strategies involving behavioral manipulation with plant volatiles.

P69- Terpenes from *Eucalyptus* essential oils: Electrophysiological and enantioselective response on *Aedes albopictus* (Skuse) antennae

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Dengue and yellow fever are viral diseases transmitted by mosquitoes; one of them is *Aedes albopictus* (Skuse). Despite this mosquito is originally from Asia and India, it has distributed to other continents, included Latin America. The World Health Organization recommends the application of repellents in order to prevent the bites of mosquitoes that may, probably, be infected. Repellents based on plant-derived products is one alternative to reduce mosquito bites. Gas-chromatography coupled to electroantennographic detection (GC-EAD) is a useful tool to find components in essential oils that elicit a signal on mosquito antennae, and may be good candidates for repellent formulations.

Female mosquitoes (4-7 days old, not blood-fed) were placed on a stage with plasticine and immobilized with U-shaped wires. The tip of one antenna is removed and inserted into a glass capillary tube filled with a conductive solution and connected to a silver-recording electrode. The indifferent electrode is inserted through the cervix. The signal was amplified and processed with Syntech® software (Ver. 4.6). GC-EAD was performed on a chiral stationary phase, Cyclosil- β , with FID detector. The GC-MS profile of the essential oil was also obtained, using the same column.

Previous results obtained by our research group have demonstrated that (R)-(+)- α -pinene elicited an electrophysiological response on *Aedes albopictus* antennae. The essential oil of *Eucalyptus tereticornis* and *E. grandis* are tested for the electrophysiological activity of its components. In addition to this, we have previously isolated terpenes from *E. tereticornis*: spathulenol, β -phellandrene and cryptone. The main components found in *E.tereticornis* essential oil are *p*-cymene, 1,8-cineole, β -phellandrene, cryptone and spathulenol. Meanwhile, in *E. grandis* essential oils we found α -pinene and 1,8-cineole as mayor components. In both essential oils (R)-(+)- α -pinene and 1,8-cineole elicited an electrophysiological response on *A. albopictus* antennae. The components isolated and their electrophysiological activities are also presented here.

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P70 - Sexual dimorphism in chemical defenses of a Neotropical harvestman (Arachnida: Opiliones)

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Sexual dimorphism in immunocompetence, with males showing lower immune function than females, may be understood as a result of resource-based trade-offs between male mating effort and immune defense, a trade-off that females do not take [1]. Given that chemical defenses are costly, a similar trade-off should also be found between male mating and chemical defenses. We tested this hypothesis using the harvestman *Acanthopachylus aculeatus* (Gonyleptidae) as model organism. Individuals of this species release a blend of three benzoquinones, which are produced in a pair of glands located at the anterior margin of the dorsal scute [2]. We predict that: (1) females would produce more secretion than males, and (2) females would replenish their glands more readily than males. We collect 31 females and 45 males in a salt marsh area close to Punta Gorda, Uruguay, and took them to laboratory where they were maintained in individual recipients. We measured the width of the dorsal scute and collected all the exudate released by each individual using capillary tubes. Then we allowed the individuals to replenish the exudates for 18 days and repeated the process of extraction. The exudates were subject to gas chromatographic mass spectrometric (GC-MS) analysis to quantify the amount of benzoquinones in the samples. The amount of benzoquinones released by each individual was log-transformed and compared between males and females using an ANCOVA. According to our predictions, females produce more exudates than males both in the first (intercept: $F = 15.476$; $p < 0.001$) and in the second extraction (intercept: $F = 17.4916$; $p < 0.001$). Males of *A. aculeatus* heavily invest in weaponry, i.e., large spines and tubercles on the fourth pair of legs that are used in male-male fights, and probably gain more from sacrificing chemical defenses than do females.

[1] Stoehr & Kokko 2006

[2] Eisner *et al.* 2005

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P71 - Sequestration of aristolactams from *Aristolochia chilensis* by *Battus polydamas archidamas* (Papilionidae, Troidini)

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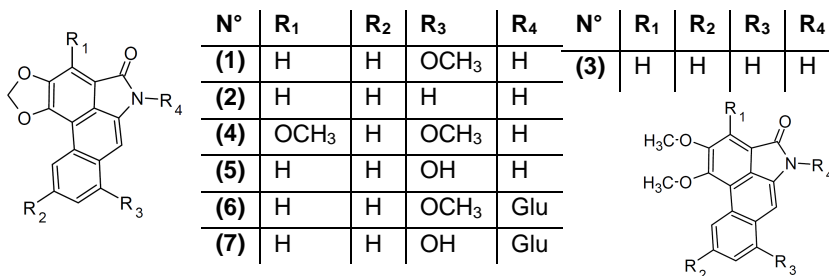
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Insect specialization regarding its host plants is of common occurrence in nature. Among insects, the majority of Lepidopterae lay eggs and feed in the larval state in a single plant, or only in plants of a few genera or families.¹⁻⁴ *Aristolochia chilensis* is the host of *Battus polydamas archidamas* in central Chile and the aristolochic acids (AAs) found in the plant are sequestered by larvae and have been found in wild adults and pupae of *B. polydamas archidamas* reared in *A. chilensis*^{5,6}. Surprisingly, although the sequestration of AAs by larvae of the genus *Battus* of the Troidini tribe (Papilionidae) has been studied, nothing is known about the possible sequestration of aristolactams (ALs). These compounds biogenetically related with the AAs also are ingested by the larvae from the host plants, but nothing is known about their sequestration and if they play any role as semiochemicals.

In the light of these observations, the following questions may be formulated:

- 1- Do larvae of *B. polydamas archidamas* sequester aristolactams from their host plant *A. chilensis*?
- 2- Does the distribution of the aristolactams in the insect vary with its development, from larvae to pupae?

Extracts of basic and neutral compounds of *A. chilensis* and *B. polydamas archidamas* were fractionated by PTLC and the fractions analyzed by HPLC-DAD and LC-MSⁿ, standards of ALs were used. AL-I (1), AL-II (2) AL-BII (3), 2-OMe-AL-I (4), AL-Ia (5), AL-I (6) and AL-Ia (7) N-glucosides were found in the five instar larvae, no ALs were found in the pupae.



Sequestration is selective and some metabolites of ALs were found.

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P72 - Differences in Profile of Volatile Organic Compounds in two Genus from Cactus

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The cacti forage is an important culture in the North-East Brazil. It's also a crucial food source for cattle as it provides nutrition particularly throughout the dry season when it is the only food source available for the animals. In Brazil, there are two main genus produced from the Cacti: *Opuntia* and *Nopalea*. Most species within the *Opuntia* genus are susceptible to the cochineal insect, *Dactylopius opuntiae*, whilst most species within the *Nopalea* genus are susceptible to the cochineal insect, *Diaspis echinocacti*¹. The cochineal insects are the main cacti pest causing irreversible damage on the plant making it nearly impossible to grow healthy cacti. The Brazilian Agriculture Research Center has estimated that within three years 50% of the cacti plantations will be damaged due to the pest caused by the different cochineal insects hence making it a major agricultural concern. The main objective of this research study was to evaluate and produce a profile of the different organic volatiles produced by four varieties of cacti; two from the *Opuntia* genus and two from the *Nopalea* genus. This was conducted through a 24h air entrainment/plant using: a constant air flow of 800mL/min (entry) and 400mL/min (exit). The volatiles collected on the Porapak Q (60/80 mesh, 0.06g, Supelco) were eluted using 750µL of hexane and then analyzed using GC/FID and GC/MS. This analysis made it possible to understand that there is a difference between the compared genus and also within each genus. About 25 VOCs were extracted only in Orelha de Elefante (genus *Opuntia*), while 9 are specific compounds of Negro Michoacan (genus *Nopalea*). The presence or absence of volatiles could be related to infestation caused by this pest. This could also explain the resistance and the susceptibility of the different cacti species and the different cochineal species.

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P73 - The sesquiterpene (*E*)- β -caryophyllene is emitted by sugarcane in response to attack by *Diatraea saccharalis*

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The sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera:Crambidae), is a key pest of sugarcane (*Saccharum* sp.) in the Americas, contributing for losses of around 10% of this culture. Plants often release a blend of volatile organic compounds (VOCs) in response to damage by herbivorous insects that may serve as location cues to their natural enemies, both predators and parasitoids. The elucidation of such defense mechanisms in sugarcane might result in useful tools for biological control of pests. In trying to unravel the defense mechanisms of sugarcane in response to attack by *Diatraea saccharalis*, we have performed an herbivore damage assay by infestation 40-45-days sugarcane plants with second instar sugarcane borer larvae, and collected the VOCs emitted during the first 48 hours by infested and non-infested control plants. The emitted VOCs were adsorbed in Porapak Q traps, desorbed with hexane, and analyzed by GC/MS. The chromatogram comparisons revealed a compound differentially emitted by infested-plants, which was identified as β -caryophyllene by comparison with a mass spectrum standard library. The identity of this herbivore-induced compound was confirmed both by comparison of retention times and by co-injection with an authentic β -caryophyllene standard on two GC-columns of different polarity. Interestingly, (*E*)- β -caryophyllene has been previously demonstrated to be emitted by maize leaves and roots in response to attack by herbivores [1], and to act as a signal to attract the natural enemies of maize herbivores from both above and below ground. Accordingly, laboratory bioassays are currently underway to assess the ecological role played by (*E*)- β -caryophyllene during attack of sugarcane by *Diatraea saccharalis*. Our aim is elucidate the defense mechanisms of sugarcane in order to pave the way for developing, through genetic engineering, of sugarcane varieties with enhanced resistance to insect pests.

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Acknowledgements: Instituto Nacional de Ciência e Tecnologia (INCT) Semioquímicos na Agricultura.

P74 - Olfactometric response of Aphids toward volatiles of three ecotypes (Quiñequeli-INIA, Superqueli-INIA and Syn II Pre III) of red clover (*Trifolium pratense* L.)

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Aphids (Hemiptera: Aphididae) are insects of agricultural importance because of the direct and indirect damage on their hosts, such as incorporation of toxic saliva, extraction of sap from the plant and viruses transmission. The most affected vegetal species by aphids belong to the families Poaceae, Solanaceae and Leguminaceae. *Trifolium pratense* (Leguminaceae) is an important forage species, widely cultivated in southern Chile for its quality forage and for the exportation of their seeds. For this reason, the agricultural research institute (INIA Carillanca) has developed a breeding plan, providing new lines and cultivars of *T. pratense* species. Field observations have shown a differential population pattern of different aphid populations colonizing both lines and cultivars of red clover. During host selection by aphids, visual and olfactory stimuli play an important role in host recognition. In addition, several studies have demonstrated the response of aphids to plants volatiles, and secondary metabolites could explain the distribution of aphids on different environments and host plants.

Based on this background, the objective of this work was to analyze the response of aphids to volatile released from the aerial part of red clover plants. For this, three red clover ecotypes were transferred from the field to the laboratory. After one month of acclimation, the plants were introduced into a glass chamber connected to an olfactometer [1] for determining the behavioral response of apterous adults aphids towards volatiles released from the aerial parts of the plants. Preliminary results indicate that aphids were attracted to volatiles released from the most susceptible to the insect attack, Quiñequeli-INIA [2]

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P75 - Biochemistry effects on *Spodoptera frugiperda* larvae by three germacranolides isolated from *Angelphytum aspilioides* (Griseb.) H. Rob.

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From a study of the insecticidal potential of 71 native plants to province of Córdoba, Argentina, against *Spodoptera frugiperda* (Lepidoptera, Noctuidae), *Angelphytum aspilioides* (Asteraceae) was one of the most active species with an antifeedant effective concentration 50 (CA₅₀) of 24.4 µg/cm². Bioguided separation of the ethanol extract of *A. aspilioides*, three germacranolides, 8-(19-hydroxymetacryloxy)-10-acetoxymethyl-4-methylgermacran-4,10,11,17-tetraen-6,12-olide (**2**) and 8-(19-hydroxymetacryloxy)-10-hydroxymethyl-4-methylgermacran-4,10,11,17-tetraen-6,12-olide (**3**), were isolated as antifeedants. The CA₅₀ of these compounds against *S. frugiperda* were 20.5, 21.5 and 20.0 µg/cm², respectively.

We evaluated the potential of the three compounds on a biochemical levels, from neonate larvae of *S. frugiperda* fed with artificial diet and treated with sub-inhibitory dose (95 ppm) of each compound through the non-choice tests for 17 days. Compound **1** showed the greatest effect on the larvae biochemical levels, causing a severe reduction in the lipid content, protein and total carbohydrate. This result demonstrate that **1** negatively affects the feeding behavior and the nutrition of *S. frugiperda* larvae, disturbing their development, affecting the three major metabolisms involved in the insect growth.

P76 - *Triatoma infestans* (Hemiptera: Reduviidae) Larvae Responses to Volatiles Released by Disturbed Adults: Searching for Active Compounds of the Alarm Pheromone

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Triatoma infestans adults possess two pairs of exocrine glands: metasternals and Brindley's glands. According to previous studies, both glands may release their volatiles when adults are disturbed, suggesting an alarm and/or defense function. Behavioural experiments from those studies support this hypothesis. Although a vast number of volatiles emitted by each gland during disturbance have been already identified [1], only the behavioural response to isobutyric acid (the major compound of Brindley's glands) has been analyzed [2]. In this study, using a double choice olfactometer, the orientation response of larvae submitted to individual or mixtures of volatiles released by both glands, was analyzed. Firstly, we studied the response of larvae when odours coming from disturbed adults were presented in one side of the experimental arena. In a second step, we analyzed the behavioural response against different doses (1000, 10 y 0.1 µg) of the main compounds of the secretion released during disturbance: isobutyric acid, butyric acid, propionic acid, 2-methyl-1-butanol, 3-pentanone and 2-butanone. Finally, we examined the behavioural response of larvae when binary combinations of compounds were presented (only doses in which an evident escape response was observed were used). As expected, results showed that odours coming from disturbed adults evoked an escape response in *T. infestans* larvae. Similar results were observed in bioassays with isobutyric acid (10 µg), butyric acid (10 µg) and 2-methyl-1-butanol (10 µg). Mixtures evoked diverse effects in the behaviour of *T. infestans* larvae. Results presented here suggest that Brindley's gland volatiles are mostly involved in an alarm context, although 2-methyl-1-butanol is present in both glands. The identification of active compounds of the alarm pheromone of this species could be useful for the formulation of a repellent blend and/or to develop new tools for vector detection to be used in programs of Chagas disease control.

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P77 - Antifouling activity of *Plocamium brasiliense* (Rhodophyta) monoterpenes and its possible presentation mechanism at the thallus surface

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In the present work, we performed a cellular characterization of *Plocamium brasiliense* (Rhodophyta) cortical cells and related it to ecological processes. Biofouling assays were performed using different polarity extracts from *P. brasiliense*. We observed for the first time the sphere structure (SS) in cortical cells of this seaweed by optical and electron microscope. These organelles are circular with a tendency to a round shape (Diameter = $10 \pm 2 \mu\text{m}$, Circularity = 0.92 ± 0.027 and Area = $24.12 \pm 1.30 \mu\text{m}^2$). Nuclear Magnetic Resonance and Mass Spectrometry analyses have shown that the major compounds in extracts are: monoterpenes in hexane (HE) extract; sterols, aldehydes and monoterpenes in dichloromethane (DI) extract; alcohols, long chain hydrocarbons and glycerols in ethyl acetate (EA) extracts and esters of saturated and unsaturated fatty acids in methanol (ME) extract. The attachment of the mussel *Perna perna* bioassay was inhibited mainly by HE and DI extracts. The EA extracts concentrations of $1 \mu\text{g.mL}^{-1}$ inhibited biofilm-forming bacteria (*Halomonas marina*, *Polaribacter irgensii*, *Pseudoalteromonas elyakovii*) and microalgae (*Chlorarachnion globosum* and *Exanthemachrysis gayralia*). The ME extract concentrations of $0.1 \mu\text{g.mL}^{-1}$ inhibited bacteria (*Vibrio aestuarianus* and *P. elyakovii*). We are proposing that the SS found in cortical cells of *P. brasiliense* are involved in storage and release of chemical compounds to the thallus surface, which implies in an efficient chemical defense system improving the biofouling protection in seaweeds.

Key words: Chemical defense, storage structures, *P. brasiliense*.

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P78- Effect of feed on endophyte-infected (E+) and endophyte-free (E-) pastures on the population of *Haematobia irritans* (Linnaeus, 1758) (Diptera: Muscidae)

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The horn fly, *Haematobia irritans*, is an obligate bloodsucking ectoparasite of pastured cattle. The biting and nuisance behavior of this insect reduce both cattle weight gain and milk production. Several studies have reported a variation in the fly-load/animal which appears to be heritable. Therefore, the selection of cattle with low fly-load could offer an alternative to chemical control of this ectoparasite pest. Due to this background, the aim of this study was to determinate variation in the fly-load of *H. irritans* in cattle feed on different pastures with presence (E+) or absence (E-) of endophyte. For this, fly-load evaluations were performed during two seasons. In 2011 season the fly-load was compared in animals grazing bromine (E-), mixed pasture (ryegrass/red clover) (E-) and (E+) tall fescue pastures. The results showed that *H. irritans* was not distributed homogeneously over the animals in any of the pastures evaluated. Because this, two groups of animal were phenotyped: low and high carriers of *H. irritans*. However, when the fly-load on cattle grazing in E+ tall fescue was compared with that on bromine pasture ($P < 0.05$), a decreasing in the fly-load was observed. In 2012 season was evaluated the fly-loads in animals grazing (E+) tall fescue versus (E-) tall fescue pastures. Field experiments confirmed the results obtained in the previous season, showing that animals feed on (E+) tall fescue pasture were less attractive to horn flies compared with animals feed on (E-) tall fescue ($P < 0.05$). Our results, based in two season of evaluation, suggest that the infestation of *H. irritans* in bovine could be manipulate through the use of pastures with endophyte presence, which would produce compounds that exert a negative effect on the flies-load on animals.

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P79 - Evaluation of artificial diets for *Hylastinus obscurus* (Marshall) (Coleoptera: Curculionidae)

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Red clover (*Trifolium pratense* L.) is a valuable forage legume in temperate regions of the world. In Chile, is used primarily in short-rotation pastures and as hay, silage and soiling. The low persistence of *T. pratense* is attributed to the infestation of the red clover root borer, *Hylastinus obscurus*. This curculionid bore and feed into the roots. Unfortunately, a pesticide application is not a control option because this insect is an underground pest. There are several works reporting a number of semiochemicals associated to this insect-plant interaction [1-2]. This work explored the phagostimulant or antifeedant characteristics of long-chain free fatty acids identified in red clover roots incorporated in an artificial diet. The first step was to test two artificial diets: 1) pure agar, and 2) agar, glucose and lignin (composed diet). To perform the bioassays, the insects were removed from the root pieces 12 hours before their use and placed in humid chambers consisting of glass Petri dishes with pieces of filter paper humidified with distilled water and held at 7°C. Then, the insect were weight and placed in transparent glass tubes which were maintained in a vertical position. Evaluations were performed for each treatment for nine days. The results indicated that there was a significant feed response by *H. obscurus* towards composed diet compared with agar diet ($P < 0.05$), showing that composed diet would be an appropriate matrix to perform feeding tests for *H. osbcurus*. This diet was used for testing different fatty acids and azadiractin as antifeedant control.

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[2] Palma, R., Mutis, A., Manosalva, L., Ceballos, R. & Quiroz, A. 2012. Behavioral and electrophysiological responses of *Hylastinus obscurus* to volatiles released from the roots of *Trifolium pratense* L. *Journal of Soil Science and Plant Nutrition* 12: 183-193.

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P80 - Chemical content identification of dorsal abdominal glands and metathoracic glands of *Leptoglossus zonatus* (Dallas, 1852) (Hemiptera: Coreidae)

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The identification and manipulation of chemical compounds which mediate insects' vital activities offer many opportunities for developing control strategies less harmful to the environment. The coreid *Leptoglossus zonatus* is distributed throughout the American continent and in Brazil it is an important maize pest. The contents of the dorsal abdominal glands in nymphs and the metathoracic glands in adult males and females were identified and quantified. The immature leaffooted bugs produce their defensive compounds in the dorsal abdominal glands (DAGs) and their content is transferred to the exuviae after ecdysis. Thus, the extraction of exuviae is an efficient method to obtain DAG secretions to analysis. They were collected ≤ 24 h after ecdysis and extracted in hexane for 24h. The number of collected exuviae varied according to the instar, decreasing with increasing instar. The adult leaffooted bugs produce their sexual pheromone compounds in the metathoracic gland (MTGs). The extraction of the MTG compounds was performed through a dissection process, where the whole gland was removed and extracted in hexane for 24h. The identified compounds of both DAGs and MTGs are oxo-alkenals, unsaturated aldehydes and linear chain acids. The results obtained for the DAGs content showed almost no qualitative differences between instars, however the first instar showed different pheromonal blend composition from the following instars.

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P81 - Behavioural and Chemical Studies of Volatile Compounds produced by *Annona* sp.: its role as oviposition attractants of *Cerconota anonella* (Lepidoptera:Oecophoridae) mated females

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Cerconota anonella limits the production of *Annona* sp. due to the damages they cause to fruits which thus reduces the production of *Annona* cultivars and therefore is considered the main insect pest of Anonaceae cultivated on the Neotropical region [1]. The goals of the presented study were: to determine which organic volatile compounds (COVs) are produced by custard apples (*A. squamosa*) and soursop (*A. muricata*) and to verify the oviposition preference of *C. anonella* females towards these volatile compounds, under laboratory conditions. Larvae of *C. anonella* were obtained from damaged custard apples and soursops, collected in orchards located in the estate of Maragogi, Alagoas and maintained on an artificial diet until adult emergence. The COVs were collected from non-infested custard apples and soursops using the dynamic headspace collection (aeration), using activated charcoal as adsorbent. Desorption of volatiles was carried out using hexane and thus two extracts were obtained: one from custard apples (**P**) and another from soursop (**G**). The behavioural bioassay were conducted using a double choice Y shaped olfactometer and the number mated *C. anonella* females (1-5 days-old) attracted to treatments **P** and **G** were recorded and submitted to statistical analyses (Mann-Whitney, $P < 0.05$). The extracts were analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The results showed that there is no significant differences on the oviposition response of *C. anonella* mated females to **P** and **G**. Fifty-six compounds were found to be present on these extracts. These results suggest that the volatile constituents responsible for the attraction of *C. anonella* mated females are among the compounds which were identified on both extracts.

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P82 - What do taste receptors tell blood-sucking bugs about food quality?

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Chemosensory systems like smell and taste are essential senses for most animals to find food, locate a sexual partner and refuge. Though, taste sense enables them to detect safe-nutritious food sources and to avoid toxic substances present in the environment. Taste receptors in insects are widely distributed over the animal's body (antenna, proboscis, legs, ovipositor, wings). In triatomine insects, previous reports based on morphological evidences, have suggested the presence of taste receptors on the antenna, although also on the proboscis. However, the functional nature of these receptors was still unknown. In the present work, we characterized through single sensillum recordings (SSR), the response of taste antennal sensilla in *Rhodnius prolixus* to different tastants, like salts (NaCl, KCl), sweet (sucrose) and bitter compounds (quinine and caffeine). Subsequently, we analyzed, by means of behavioral assays, whether the taste antennal/proboscis receptors might have a role in food acceptance. The feeding response of insects (measured as weight gain) to a well-established artificial diet (i.e. saline solution added with ATP, adenosine triphosphate) was evaluated in insects that their antenna or proboscis had been previously and gently touched with a toothpick soaked with water, NaCl, quinine or caffeine: Our results reveal the existence of taste receptors sensitive to salts and bitter compounds, (caffeine and quinine) that respond in a dose-dependent manner. Furthermore, we begin to uncover the role of taste antennal/proboscis receptors in the decision of insects to accept or to reject a potential meal.

Key words: triatomines, blood sucking, feeding, electrophysiology.

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P83 - Use of VOCs identified from Chilean native saprophytic fungi for controlling post-harvest diseases

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The production and bioactivity of fungal volatile organic compounds (VOCs) depend strongly on different factors, such as the species, substrate, age of the fungal culture, fruit body development stage and the different parts of the mushrooms. The antifungal activity of a native strain of *Schizophyllum commune* (*Sch-1*) on the plant pathogenic fungus *Botrytis cinerea* grown in different substrates was investigated using a bi-compartmented Petri dish bioassay. The chemical composition of VOCs released by *S. commune* was investigated by headspace solid-phase microextraction (SPME) and gas chromatography/mass spectrometry (GC-MS) analysis. Then, an artificial mixture and individual compounds of *Sch-1* VOCs were tested. Volatiles released from *Sch-1* growing in potato-dextrose-agar (PDA) media significantly inhibited the *B. cinerea* growing. The artificial mixture calculated from the natural releasing ratio from *Sch-1* grown in the best substrate, had a minimum inhibitory concentration (MIC) and median lethal dose (LD₅₀) values of 0.70 and 0.29 µl per ml of aerial space, respectively. The compound classified as C-4 was the most active against *B. cinerea* growth. These results suggest that there is an additive and/or synergistic effect among the *Sch-1* volatiles resulting in an antifungal activity on *B. cinerea*. The results of inhibition of *B. cinerea* produced by the artificial mixture confirmed that volatile compounds released from *Sch-1* elicited an antifungal activity that could be used for controlling the growth of this plant pathogen.

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P84- Study of chemical communication of *Proeulia auraria* (Lepidoptera: Tortricidae)

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Proeulia auraria is a Lepidopteran species native to Chile, whose immature stages feed on the foliage, flowers and new fruits of economically important fruit tree species such as apricot, peach, kiwi, apple, pear and others [1]. Control of this pest species is achieved mainly by insecticides targeted at other important tortricid pests, such as *Cydia* or *Grapholita* spp. present in central Chile. Additionally, pheromone traps for the tufted apple bud moth (*Platynota idaeusalis*), consisting of a 1:1 mixture of E11-14Ac and E11-14OH, are commercialized in Chile as monitoring tool for *P. auraria*. The aim of this study is to characterize the composition of the pheromone of *P. auraria*, in order to improve existing traps and to set the basis for development of mating disruption. We studied the calling behaviour of 1 to 3-d-old virgin female moths and subsequently analyzed the content of the pheromone glands excised from calling females by gas chromatography–mass spectrometry (GC-MS). Field test confirmed the attractivity of the main compound produced by calling females, E11-14Ac, while addition of the second compound present in commercial traps, E11-14OH, lowered trap catches. Additional studies are needed to detect other minor compounds that may play a role in the communication of the species.

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P85 - Behavioral, electrophysiological and chromatographic studies evidences mediation by semiochemicals in chemical communication of bean weevil, *Zabrotes subfasciatus* (Boheman, 1838) (Coleoptera: Bruchidae)

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Brazil is the largest world producer of beans with an average annual production of 3.5 million tons¹. It is estimated that in Brazil 20% of annual grain production is lost between harvesting and storage, and that half of those losses are due to attacks by pests during storage². Among them, the weevil *Zabrotes subfasciatus* (Boh., 1833) larvae stands out causing damage by penetrating and feeding within beans grains. Here, we have highlighted the semiochemicals that mediate the communication between conspecifics of *Z. subfasciatus*, and we are looking forward to using weevil pheromones in integrated management programs for beans. We have used a total of 500 males and 500 virgin females in our experiments, which were aerated separately in two aeration glass chambers under an air flow rate of 1.0L/min and a photoperiod of 12/12h. Volatile compounds were collected in separate from males and females by using an adsorbent polymer. Our results pointed out the presence of a male-specific compound, and also that insect communication in *Z. subfasciatus* is possibly mediated by the presence of semiochemicals, more specifically, by sex pheromone, since there was significant attraction of female insects to male emitted volatiles in the bioassay. Furthermore, EAD analysis of the volatile compounds emitted by virgin males of *Z. subfasciatus* suggested the presence of an active component.

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P86 - The interaction between *Diatraea saccharalis* (Lepidoptera, Crambidae) and Sugarcane: Changes in Plant's Proteome

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The crescent global demand for renewable energy sources to replace fossil fuels has given a great interest to sugarcane (*Saccharum* sp.). Brazil is the main world producer, where sugarcane has been cultivated in 8.5 million hectares producing up to 600 million metric tons in 2012/2013. Biotic stress is responsible for significant sugarcane losses and it has been estimated that around 10% of this crop losses are caused by insect pests, from which the sugarcane stem borer (*Diatraea saccharalis*) is the most important. In order to reduce insect damage, plants have evolved complex and varied defense mechanisms, including, physical barriers, toxic and volatile metabolites, and defense proteins. Here, by using a two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/TOF), we identified the proteins in phenolic extracts of leaves that were both wounded and treated with oral secretion (OS) of *Diatraea saccharalis*. The phenolic extracts yielded approximately 650 protein spots, and 169 of them were altered by elicitation. In general, proteins that had an increased expression were found to be involved in primary metabolism, defense, and transcriptional and translational regulation; while those that had a decreased expression were found involved in photosynthesis. Systemic suppression of photosynthesis in herbivory by caterpillars has often been described for other plants. We concluded that the response of the plant's proteome to herbivory is complex, however the integration of proteomics and the chemical ecology may facilitate the understanding of this ubiquitous ecological interaction and so enable the pest management.

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P87- Biosynthetic pathway of *Magnispina neptunus* benzoquinones (Arachnida: Opiliones)

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Opiliones or harvestmen are found in all continents and are the third major group of arachnids, with 6500 known species. They secrete a mixture of volatile compounds, that in the suborder Laniatores are benzoquinones, alkyl phenols, and vinyl ketones, while in the suborder Eupnoi are alcohols, naphtoquinones, and other acyclic short chain substances.¹ The biosynthetic pathways of these metabolites have never been studied before.² The scent gland exudate of the harvestman *Magnispina neptunus* contains 2-ethyl-1,4-benzoquinone as the major compound. We studied the biosynthetic origin of this benzoquinone by feeding individuals with [1-¹³C]acetate and [4-¹³C]methylmalonate. Our results based on ¹³C NMR spectroscopy showed the polyketide pathway pattern, bearing labels at alternating positions of the benzoquinone ring (Scheme 1). The C-5 labeling scrambling can be explained by a compartmentalized PKS (polyketide synthase) that incorporates the second extender unit from a different pool of malonate. There are few reports of biosynthetic studies with non-insect arthropods, such as arachnids, and this is the first one involving harvestmen.

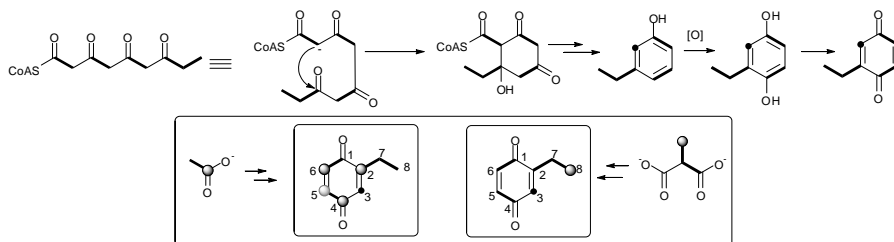


Figure 1. Proposed biosynthetic pathway for 2-ethyl-1,4-benzoquinone from *Magnispina neptunus* and labeling pattern with [1-¹³C]acetate and [4-¹³C]methylmalonate feeding.

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P88 - Absorption and metabolism of *Eucalyptus cinerea* essential oil by *Musca domestica* L.

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The house fly, *Musca domestica* (L.), is one of the most common insects, associated with vectoring of etiological agents of bacterial, protozoan and viral infections converting this insect in a threat to public health. Intense applications of a variety of synthetic insecticides to control *M. domestica* have led to the development of resistance to most of them around the world. Natural insecticides are a great alternative to conventional ones because are safe to the environment and to human being. Essential oils (EOs) are part of this new proposed as fumigants because of their high volatility, low toxicity to warm-blooded mammals and toxicity to *M. domestica*¹.

After a fumigation bioassay was performed (n=100), dead flies were collected in a vial (10 ml volume) with a septum, and then washed with hexane. The vial was placed in a bath at 60 °C for 10 min. With the aim of quantify (GC-FID) the compounds absorbed by the fly, 20 µL of a solution containing 5 mg/mL of camphor (internal standard) in acetone was added to the vial. Terpenes desorbed from *M. domestica* were captured using a SPME microfiber in the headspace of the vial. The assay detected two new compounds 2,3-dehydro-1,8-cineole and terpineol (Fig. 1), this result demonstrated that, in *M. domestica*, 1,8-cineole was transformed to these metabolites probably by the oxidative detoxification pathway P450 (Table 1).

SPME Analysis of	Relative amount (%)			
	1,8-cineole	α-pinene	terpineol	2,3-dehydro-1,8-cineole
<i>E. cinerea</i> EO ^a	90.4 ± 1.5	1.9 ± 0.2	9 ± 0.1	nd
Flies died by action of <i>E. cinerea</i> EO	74 ± 1	0.1 ± 0.3	24.7 ± 0.2	1.2 ± 0.1
Flies died by action of <i>E. cinerea</i> EO + PBO	90.8 ± 1	0.1 ± 0.01	9.1 ± 0.1	nd
1,8-Cineole	99 ± 0.5			
Flies died by action of 1,8-cineol	79.5 ± 1.4		11.4 ± 0.2	9.1 ± 0.1
Flies died by action of 1,8-cineol + PBO	100 ± 1			

Table 1: Amount relative of 1,8-cineole, α-pinene, terpineol y 2,3-dehydro-1,8-cineole recovery from died flies by treatment with *E. cinerea* EO, 1,8-cineole with or without piperonylbutoxide

^a: More terpenes were detected but only 1,8-cineole, α -pinene, terpineol and 2,3-dehydro-1,8-cineole were considered because these were the only four terpenes detected in flies. nd: not detected.

To assay *E. cinerea* EO, 1,8-cineole in combination with an inhibitor of P450 (PBO), insects were anesthetized with a CO₂ current, and then a solution of PBO in acetone (20 mg/ml) was applied topically to the thoracic notum at a dose of 10 μ g (0.5 μ l) per fly, 1 h before the insecticide treatment. *E. cinerea* EO (LC₅₀ de 5.5 a 2.2 mg/dm³) and 1,8-cineole (LC₅₀ de 3.3 a 1.6 mg/dm³) showed more toxicity by action of this inhibitor of P450. In other words, the insect detoxification system contributed to decrease the toxicity of the *E. cinerea* EO, the metabolites presented less toxicity than 1,8-cineole (LC₅₀ de terpineol: 36.8 mg/dm³)².

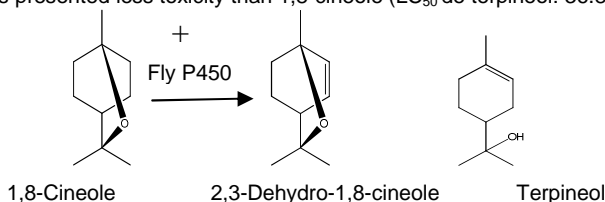


Figure 1. Conversion of 1,8-Cineole to 2,3-Dehydro-1,8-cineole and terpineol mediated by fly cytochrome P450.

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P89 - Effect of Volatiles from Lemon Peel in the Viability of the South American Fruit Fly, *Anastrepha fraterculus* eggs

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The chemical properties of fruits, especially the compounds present in the essential oils glands from the peel, are considered the most important resistance mechanism of citrus against fruit flies infestation and impacts on the condition of a given fruit as a larval host. In several fruit fly species the viability of eggs and larvae are affected by these compounds. In *Anastrepha fraterculus*, it has been shown that lemon is not a host and chemical resistance has been proposed as the involved mechanism. We evaluated the effect of volatile compounds of essential oil of lemon, ether extracts from the lemon peel and pure compounds such as limonene and citral in the viability of *A. fraterculus* eggs. We also evaluated ether extracts from grapefruit. The eggs were exposed to different concentrations of the extracts or pure compounds in Petri dishes for 24 hours. Then, they were transferred into a clean capsule and evaluated on the 5th day to score the number of hatched and unhatched eggs. Values of Lethal Dose 50% (LD50) were calculated for each extract or pure compound tested. In addition, we chemically characterized the extracts using a Hewlett Packard II 5890 mass gas chromatography (GC-CM). The LD50 for the lemon oil and ether extracts (either lemon or grapefruit) were similar ($p > 0.05$) and both pure compounds showed to be toxic. The gas chromatography showed that the relative amounts of limonene, the citrus oils major compound, were similar for the lemon oil and the ether extracts. In spite of this, the fruit extracts presented differences in their minor compounds and the extract from grapefruit presented a higher diversity of compounds. We show that the compounds present in the flavedo of the citrus species evaluated are toxic to the eggs of *A. fraterculus*, confirming the presence of chemical resistance mechanisms.

P90 - Structural identification of the compounds male-specific of *Leptoglossus zonatus*

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In seeking to develop alternative methods for replacing and / or reducing the use of insecticides on maize crops in the state of Paraná, the study of chemical ecology of insect pest of this crop since the extraction of the pheromone, structural identification, characterization and synthesis of compounds of the pheromone is crucial to combat this plague. The bug *Leptoglossus zonatus* is regarded as a major pest of maize cultivars. The natural pheromone of this insect pest was extracted through airings of males and females separately for subsequent comparison. After several extractions, were found by gas chromatography, nine compounds male specific. The chemical structures of these compounds were identified by spectroscopic information obtained by gas chromatography coupled with mass spectrometry (GC-MS) and infrared (GC-FTIR). Testing performed photophase and scotophase showed that the production of these compounds released by males occurs during the photophase. The activity of these compounds in electroantennographic antennas females was tested in GC-EAD. It was possible to observe a low response to the male specific compounds *L. zonatus*.

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Acknowledgements: CAPES, INCT, Embrapa.

P91 - Matricaria and lachnophyllum esters in the essential oil of *Baccharis concava*

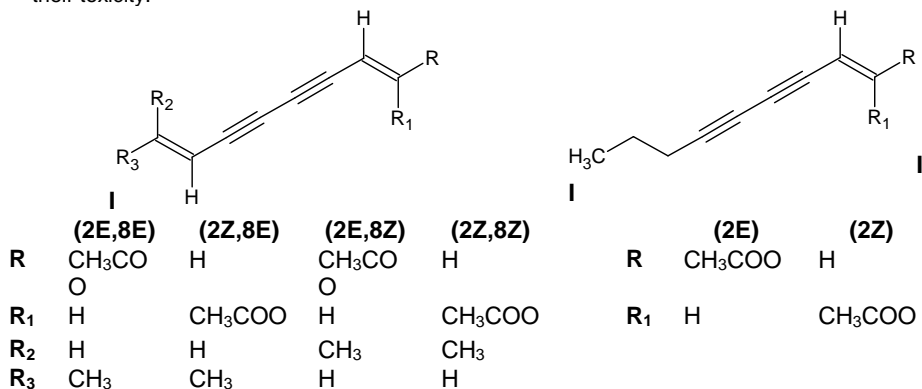
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Baccharis concava grows in Central Chile ranging from the Pacific sea shore to the Andes Mountains. Is a dioecious plant that dominates the ecosystem where grows^[1]. The common name is "rabbit killer" due to the known property of being toxic to rabbits and field observations have shown that it suffers no herbivory. This species may have allelopathic properties, preventing the growth of other plant species and could be toxic or repellent to herbivores insects. In order to detect potentially toxic secondary metabolites responsible for its properties the phytochemical study was initiated, starting with the composition of the essential oils of male and female individuals. From the essential oils of both individuals around 15% was an amorphous solid mass which after crystallization from MeOH yield a pure crystalline compound : MW= 174.0679 (C₁₁H₁₀O₂) identified by 1D and 2D NMR spectroscopy as (E, Z)-matricaria ester. Since in the literature the structural determination of matricaria esters is unclear and inconsistent, the stereochemistry was obtained by X-ray spectroscopy. In addition, GC-MS of the essential oil shows a mixture with the other isomers of matricaria ester I and the two isomers of lachnophyllum esters II (MW= 176). The other components of the essential oil were identified as common mono and sesquiterpenoids. (E, Z)-Matricaria ester is a very toxic compound to insects, mollusks and other organisms [2] and their presence in *Baccharis concava* may explain, at least in part, their toxicity.



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P92 - Feeding behavior of *Nezara viridula*: a resistance mechanism against soybean protease inhibitors?

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Nezara viridula is a widespread pest of several crops, mainly legumes, among which is soybean (*Glycine max*). Nymphs and adults feed via stylet insertion primarily in seeds, reducing seed yield and quality, as well as a phenomenon known as leaf retention. Soybean plants, in turn, have a wide array of anti-herbivore defenses, such as protease inhibitors (PIs). These bind to the target enzymes, slowing protein hydrolysis, and negatively affecting insects' growth and development rates. However, insects have developed their own strategies to minimize the effects of plant defenses, and *N. viridula* has its own set of behavioral tactics to increase its fitness. The aim of this work was to assess the effects of developing seeds of soybeans on stinkbug behavior and digestive proteases. Briefly, adults of *N. viridula* were starved for 24h and either allowed to eat freely from whole soybean plants, or forced to feed on pods of different ages for 24 and 72h. Protease extracts were obtained from the gut of the insects, and cysteine-protease activity was measured with a chromogenic substrate. After 24h feeding, a slightly significant difference in protease activity was found; those insects that had been feeding on the youngest pods showed lower activity ($p=0,073$). But after 72h, insects that had been allowed to eat freely had the highest protease activity, while those that had been forced to feed on certain pods showed a variable degree of inhibition ($p=0,0004$). Our results suggest that PI levels of pods effect feeding behavior of the stinkbug *N. viridula*, which allows them to tolerate and feed on soybean developing seeds.

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P93 - Extract effect of *Flourensia oolepis* (Asteraceae) on the nutritional parameters of the bollworm *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae)

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Among the chemicals with low environmental impact, the botanical insecticides are a good alternative for the control of insect pests. The extract from of "chilca" (*Flourensia oolepis*), endemic in the Cordoba province, has shown insecticide activity. The aim of this work was to analyze the effect of this extract on nutritional parameters of the caterpillar bollworm of *Helicoverpa gelotopoeon*. These polyphagous larvae are considered important pests for agriculture. It have has been reported recently generating damaged significant on chickpea. Forced feeding tests were performed. Larvae III were placed into Petri dishes, for 10 days, and fed with chickpea leaves treated with different doses of the extract (1% to 5%), and with water/acetone (control). Eight repetitions were performed using three larvae per treatment. The data were analyzed using ANOVA or Kruskal Wallis. Consumption, growth and mortality were determined. The variables fresh and dry weight of each larva, their feces and leaves supplied every 48 hours were considered. Also, the consumption index, approximate digestibility, efficiency in conversion of ingested and digested food, the relative growth rate and mortality were calculated. The extract affected the larval weight, causing a significant decrease in the growth of the larvae with increasing dose and it influenced negatively on the efficiency of conversion of ingested food and deferred, although digestibility was not affected. Furthermore, the mortality increased significantly from the 4th day of experimentation. With such promising results we plan field trails to assess the response of the extract under natural conditions.

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P94 - Effect of nitric oxide on the activity of the antennae of *Blattella germanica* in response to DEET

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N, N-diethyl-3-methyl benzamide (DEET) is an insect repellent used worldwide. Its effectiveness has been proved in a number of insect species, including haematophagous and non-haematophagous^[1]. There are two contrasting hypotheses regarding the mode of action of DEET: the more classical hypothesis proposes that DEET interferes with the detection of odors, in particular host odors in haematophagous, instead of having a repellent effect. On the other hand, recent works demonstrated that DEET acts as an odorant molecule and elicits a behavioral response in the absence of other stimuli. In this work we show with electrophysiological recordings that the antennae of *Blattella germanica* respond to DEET and become adapted when stimulated with long pulses of the same substance. We also found that treatment of the antenna with the NO donor S-nitroso- acetyl cysteine (SNAC) causes a temporary decrease of the electrical response to DEET.

To determine whether continuous stimulation with DEET decrease the response of the antennae to the same compound, we delivered a long pulse of DEET and recorded the response of the antennae before and after the stimulation. We found that a stimulation of 6 or 60 seconds with DEET produced a decrease of 58% in the amplitude of the response to the same compound registered after stimulation. The application of 20 μ l of 40 mM SNAC decreases electrical response in a 68 % and the effect is reversible after approximately 15 minutes post-treatment.

These results are in agreement with the role of DEET as an odor molecule, since it produces electrical response of the antennae of *B. germanica* and it is possible to adapt this response with continuous stimulation of the antennae.

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P95 - Monolithic matrix dispensers for *Megaplatypus mutatus* pheromones based on natural and biodegradable waxes

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We have developed monolithic matrix devices in a half sphere shape for controlled release of the *Megaplatypus mutatus* known pheromones; these are 6-Methyl-5-hepten-2-one (sulcatone), 6-Methyl-5-hepten-2-ol (sulcatol) and 3-pentanol. The devices were made with different mixtures of waxes and inert compounds, mixing the inert substance at the melting temperature of the waxes and finally adding the pheromone. The waxes used in these experiments were: paraffins of different melting points, paraffin oil, polyethylene glycols, pentaerythritol ester, lanolin and carnauba wax. The inert substances employed were: kaolin, glass spheres, molecular sieve and activated carbon.

The release rates of these monolithic systems were measured in the lab in a wind tunnel using a wind speed of (0,5-0,6) m/sec at temperatures of 29-30°C. The monolithic devices released significant amounts of pheromone during a period of 33-60 days. The release rates found are in the 4-400 mg/day range. Graphs of pheromone release were plotted and we found that it followed a first-order kinetics: the best equation that fits the release curves (with an R^2 between the values 0,8-0,9) in these experiments is the exponential equation $y = ae^{-bt}$, where a is a constant that determines the amplitude of the release curve, b is a decay constant and t is the time in days.

We found correlations between the parameters of the release rates with certain waxes and different amounts of inert substances.

P96 - Sex Pheromone of the True Bug *Phthia picta* is an Unusual Hydrocarbon

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Phthia picta (Heteroptera: Coreidae) is part of a complex of true bugs that attack tomato crops, being considered one of the main pest of the culture, since nymphs and adults feed from both leafs and fruit. Aeration of males and females adults showed the presence of a male-specific compound. GC-EAD indicated this male-specific compound as a bioactive molecule on female antennae. GC-MS and GC-FTIR analysis suggested the compound as being a methyl branched hydrocarbon structure. After the synthesis of three differed proposed structures, the natural produced was indentified as 5,9,17-trimethylhenicosane, that showed to be strongly attractive to females in Y-tube olfactometer bioassays. In addition, we determined the site of pheromone accumulation. Analysis of dissected parts of insect body revealed that the pheromone is produced in the lateral gland of the metathoracic scent gland of males of this species. Identification of this pheromone may eventually be useful for integrated pest management in tomatoes, one of the crops most heavily treated with pesticides in the country, and the discovery reveals a new and novel type of pheromone molecule for the Heteroptera.

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P97 - Electrophysiological and behavioral response of *Grapholita molesta* (Lepidoptera: Tortricidae) to the apple varieties 'Gala' and 'Eva' volatiles in different stages of development

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The oriental fruit moth, *Grapholita molesta*, occurs throughout the year in Brazil, attacking peach trees and migrating to apple trees. There is evidence that moths are guided by volatiles in host-location and the emission of these compounds during fruit maturation influences these insects' orientation. The aim of this work was to identify volatiles emitted by fruits of varieties 'Eva' and 'Gala' at different stages of development and determine *G. molesta* behavioral and electrophysiological response to these compounds. Volatiles from immature, maturing and physiologically mature fruits of both varieties were extracted via headspace and analyzed by gas chromatography. To test insects' response to different volatiles, virgin males and virgin and mated females antennae were exposed to volatiles released by the three stages of fruit development and the response was registered by gas chromatography coupled to an electroantennogram. "Y" tube olfactometer bioassays evaluated the behavioral response of adults stimulated by volatiles. The oriental fruit moth did not respond electrophysiologically to volatiles released by immature fruits of both varieties. Adults responded electrophysiologically to two compounds of 'Eva' maturing fruits: isoamyl hexanoate and α -farnesene. Amongst the volatiles released by physiologically mature fruits, twelve compounds elicited a response in adults. Virgin females did not respond to volatiles in olfactometer bioassays and mated females were attracted to volatiles released by physiologically mature fruits. This may be due to mated females' sensitivity, stimulated by the need to locate oviposition sites. Males were also attracted to volatiles of maturing and physiologically mature fruits, possibly acting as partner-location cues.

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P98 - Direct effect of ozone on cereal aphids

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Our understanding of ozone pollution effects on plant-insect-interaction is limited to the separate response of each organism to the pollutant. On one hand, ozone effect on plants is highly dependant on plant developmental age and exposure conditions. On the other hand, both positive and negative effects of ozone have been reported on aphids, which were in all cases mediated by the plants. In this study we evaluated the effect of direct exposure to ozone on *Metopolophium dirhodum* (Hemiptera: Aphididae). Insects fed on artificial diets were exposed to ozone in open top chambers (OTCs) at three doses: $166,6 \pm 1,3$ ppb, $45,3 \pm 1,9$ ppb and $0,1 \pm 0,1$ ppb during 6 hours. After exposure, insects were recounted and the proportions of dead and escaped insects were calculated. Lipid peroxidation (TBARS) and total reactive antioxidant potential (TRAP) were assessed as oxidative stress markers on the surviving insects.

Our results show that ozone directly affected both aphid behavior and population size: the proportion of insects escaping from diet cages was highest in the control treatment ($F_{2, 25} = 4,15$, $p = 0,0278$; DMS Tukey $_{25 \text{ gl. } \alpha:0,05} = 0,069$). Insect mortality was higher in both ozone doses than in the control treatment ($F_{2, 25} = 10,01$, $p = 0,0006$; DMS Tukey $_{25 \text{ gl. } \alpha:0,05} = 0,111$, DMS Tukey $_{25 \text{ gl. } \alpha:0,1} = 0,097$). Additionally, TBARS were higher at 166 ppb than in the 45 ppb treatment, although not different from the control ($F_{2,8} = 8,55$, $p = 0,0103$, DMS Tukey $_{8 \text{ gl. } \alpha:0,05} = 0,402$). Besides, no significant differences were detected on aphid TRAP among ozone treatments ($F_{2,8} = 0,68$, $p = 0,5310$).

Ozone exposure negatively affected aphids. These results on the direct effect of ozone on insects add relevant insights for the understanding on how this pollutant affects plant-aphid interaction.

P99 - Analysis of larvicidal and adulticide potential of crude venom from *Phyllomedusa vaillanti* (Anura: Hylidae) Against *Aedes aegypti* and *Anopheles darlingi* (Diptera: Culicidae)

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The mosquitoes are important vectors of several diseases that affect humans, such as dengue and malaria. *Aedes aegypti*, the vector of dengue, is highly endophilic, adapted to several artificial breeding sites and this is the key factor in their evolutionary success. The mosquito *Anopheles darlingi*, captured in high density in the Amazon is the main malaria vector there,. Substances secreted by animals as *Phyllomedusa vaillanti* (HYLIDAE), whose venom is described as an analgesic, antibiotic and healing have been prospected as potential larvicide and adulticide. The aim of this study was to evaluate the larvicidal and adulticide activity of the crude venom of *Phyllomedusa vaillanti* against *Aedes aegypti* and *Anopheles darlingi*. Toxicity tests on *Aedes aegypti* and *Anopheles darlingi* were conducted with three groups of 25 individuals per replicate, using five different concentrations (ppm): 100, 50, 10, 5 and 1 control and four replicates. Lethal concentrations (LC₅₀ and LC₉₀) after 24-96 h were calculated. Overall there was a higher mortality of larvae and adults of *Anopheles darlingi* for crude venom of *Phyllomedusa vaillanti*. At the highest concentration tested (100 ppm) larvicide and adulticide mortality was observed at 86% and 74% for *Anopheles darlingi*, respectively, and for *Aedes aegypti* 74% and 69%, respectively. The LC₅₀ and LC₉₀ (ppm) were 0.4 and 325; 2.10 and 364 , both larvicide and adulticide for *Anopheles darlingi*, 1.79 and 378; 2.11 and 468, for *Aedes aegypti*. The results of the toxicity tests on larvae and adults are satisfactory with crude venom of *Phyllomedusa vaillanti* against *Anopheles darlingi* and *Aedes aegypti*, i.e. high mortality rate at low concentrations. However, further studies to evaluate its toxicity in detail are needed.

P100 - Evaluation of the potential larvicidal of fractions obtained from leaves *Humirianthera ampla* (icacinaceae) against *Aedes aegypti* (Diptera: Culicidae)

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Dengue is the most important viral diseases and currently is the largest public health problem in the country. The high incidence of dengue in the Americas is due to the mosquito *Aedes aegypti*, found that in the modern world environmental conditions very favorable to its rapid expansion. The strategy used to control epidemics of dengue vectors for reducing combat the mosquito *Aedes aegypti* are alternative methods that are being researched using plants that have bioactive compounds with insecticidal effects. Some plants that are being researched and promising results for vector control, including, in the present work *Humirianthera ampla* (Icacinaceae), which is used in folk medicine as anti-snake, antinoceptivos, anti-inflammatory, anticancer compounds, being common in South America and predominant in the Amazon, is also popularly known for surucuína or surucucuína. In the search for new alternatives to integrate control the *Aedes aegypti* larvicidal tests were performed with fractions of ethanol extract of leaves of *Humirianthera ampla* (Hexane, Ethyl Acetate, Acetone and Chlorine) solubilized in ETOH on larvae of 3rd – 4rd instar concentrations (ppm): 100, 50, 10, 5, 1 and control. Observations of mortality were 24-96 hours, in order to determine the lethal concentration for the test larvicidal, which were calculated by MINITAB using the PROC PROBIT procedure for obtaining LC₅₀ and LC₉₀ were (ppm): 128 and 193; 17 and 66; 84 and 127; 104 and 133, respectively. Fractional *Humirianthera ampla* have good larvicidal activity (high and low concentrations and mortality) with potential use, and the ethyl acetate fraction of the more efficient. However need is further toxicity tests for use in field situations.

P101 - *Eupatorium buniifolium* essential oil to control tomato pests and diseases

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Given their effectiveness and low persistence in the environment, essential oils (EOs), as botanical pesticides, seem to be a good source to develop alternative strategies for pest and disease control. Tomato crop is affected by many insects and fungal diseases, among which, the insects *Trialeurodes vaporariorum* and *Tuta absoluta*, and the fungi *Alternaria* spp. and *Botrytis cinerea* are of great incidence. In this work, products (EO and hydrolate) extracted from the local species *Eupatorium buniifolium* (Asteraceae) were characterized in their insecticidal and antifungal activities. The EO was also characterized in its chemical composition, being its major components α -pinene ($22 \pm 2\%$) and (E)- β -guaiene ($10 \pm 1\%$), similarly to the report from Lorenzo et al. [1]. The biological activities were studied by different bioassays in which insects and fungi were in contact either to the EO or to its vapors. For the hydrolate, only its potential as antifungal agent was studied by media poisoning, but no activity was found at the concentrations tested. For the EO, lethal doses 50 and 99 (LD₅₀ and LD₉₉ respectively) for insects, and inhibitory concentrations 50 and 99 (IC₅₀ and IC₉₉) for fungi, showed that activity improves by direct contact (Table 1).

	Contact (mean \pm standard error)		Volatility (mean \pm standard error)	
	LD ₅₀ (mg/cm ²)	LD ₉₉ (mg/cm ²)	LD ₅₀ (mg/cm ³)	LD ₉₉ (mg/cm ³)
<i>T. vaporariorum</i>	0.02 \pm 0.01	0.08 \pm 0.02	0.06 \pm 0.02	0.29 \pm 0.06
<i>T. absoluta</i>	0.65 \pm 0.06	1.5 \pm 0.2	NT	NT
	IC ₅₀ (mg/mL)	IC ₉₉ (mg/mL)	IC ₅₀ (mg/cm ³)	IC ₉₉ (mg/cm ³)
<i>Alternaria</i> sp.	0.67 \pm 0.04	1.9 \pm 0.1	Inactive	
<i>Botrytis cinerea</i>	1.46 \pm 0.09	4.9 \pm 0.5	0.08 \pm 0.01	1.8 \pm 0.5

Table 1: *E. buniifolium* EO activity by direct contact or volatility. LD_xs (at 24h) and IC_xs (at 48h) were calculated following a linear model regression ($p < 0.001$). NT: no tested.

[1] Lorenzo D, Paz D, Davies P, Villamil J, Vila R, Cañigueral S and Dellacassa E (2005) Phytochem Anal 16: 39-44.

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P102 - Studies of Synthesis, Characterization and Pheromone Release from Alginate-Chitosan Polymer Spheres. Its Application in *Megaplatypus mutatus* control

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Megaplatypus mutatus is a beetle of the Platypodidae family causing major economic losses in productive forest poles of Argentina due to tunneling inside the trunks of trees. This species uses a blend of pheromones as a sexual caller identified as 6-methyl-5-hepten-2-ol (sulcatol), 6-methyl-5-hepten-2-one (sulcatone) and 3-pentanol. The objective of the present work was to synthesize and characterize polymeric devices for controlled release of one of the compounds of the pheromonal blend (sulcatol) and in this way know their potential application as devices in the control of this pest. Polymeric spheres were synthesized using a combination morphology of a core of alginate and a shell of chitosan. These polymers were chosen due to its natural origin, its biodegradability, and their recognized ability in the controlled release of compounds from different devices. In the first instance, synthesis conditions were optimized to obtain the spheres, then they were characterized and finally, we studied their ability to control sulcatol release. The main results of the first two stages (synthesis and characterization) led to the conclusion that those synthesized at a concentration of 4% P/V of alginate and a pH of 9, showed very good stability and low size dispersion. For that reason these spheres were chosen for the following studies of sulcatol release. Using FT-IR and "Watershed-transformation" we verified that both polymers were in the devices and that the core consisted of amorphous characteristics polymer (alginate), whereas the shell of a sphere was of crystalline characteristics (chitosan). Finally, sulcatol release studies conducted by SPME indicated that sustained release is obtained for seven days. As above, and upon reaching the appropriate release profile of the other two pheromonal compounds of the blend, is promising to consider the use of these spheres as controlled release devices in *Megaplatypus mutatus* control.

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P103 - Identification of a Pheromone-binding Protein of *Brachysternus prasinus* (Coleoptera: Scarabaeidae)

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White grubs from Scarabaeidae family are serious pests of cereals and grasses in Chile, which feed on roots of these crops. The main scarab beetles related to the white grub group are *Phytoloema herrmanni*, *Hylamorpha elegans* and *Brachysternus prasinus*. To design novel strategies for insect control, molecular approaches have arisen to use odorant-binding proteins (OBPs) and pheromone-binding proteins (PBP). These proteins, involved in perireceptor events, are located in the antennae of insects, transporting hydrophobic molecules (odorants) across an aqueous environment called lymph [1]. Despite of the broad identification of these proteins in several insect orders, including Coleoptera, there are no OBPs and/or PBPs reported for these endemic beetles. Therefore, the objective was to determine PBP presents in antennae of *B. prasinus*, the three-dimensional structure and its evolutionary relationship with other proteins reported to date. Native polyacrylamide gel electrophoresis analysis of protein extracts from antennae and legs of *B. prasinus* showed the occurrence of an antennae-specific band which had a mobility similar to PBPs previously identified in other studies. Total RNA was extracted from antennae by RNeasy Kit and first-strand cDNA was synthesized by Smart Race cDNA Kit. cDNA was amplified by PCR using degenerated primers previously reported [2]. PCR products were sequenced and comparison was performed by BLAST. The three-dimensional structure of the protein was carried out by homology modeling, using Modeller and ProCheck to assess the model. Multiple sequence alignment was generated with amino acid sequences of scarab beetles from Rutelinae subfamily. Thus, a neighbor-joining tree was constructed using MEGA5. Results indicate that *B. prasinus* has an antennae-specific protein belonging to PBPs family. This protein shares similar features with other PBPs, such as six α -helices and three disulfide bridges from six cysteine residues (theoretical pI: 4.47; MW: 12,909). Further perspectives regarding to these proteins are discussed.

[1] Pelosi, P., Maida, R. 1995. Odorant-binding proteins in insects. *Comp. Biochem. Physiol.* 111, 503 – 514.

[2] Wojtasek, H., B. Hansson, and W.S. Leal. 1998. Attracted or Repelled?—A matter of two neurons, one pheromone binding protein, and a chiral center. *Biochemical and Biophysical Research communications* 250: 217–222.

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P104 - Male response of two *Spodoptera* species to conspecifics and heterospecific pheromonal blends

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Spodoptera species (Lepidoptera: Noctuidae) provoke serious damage to several crops such as cotton, maize, and grains in general. The sex pheromone for monitoring some of these species is commercially available, but its use in Brazil is still limited, mainly because of high cost, and specificity. This study has as aim to evaluate if *S. frugiperda* and *S. cosmioides*, which has a different pheromonal blend, should respond to a blend, containing pheromone components produced by both species. The chemical analysis of the volatiles present on glands extracts obtained from *S. frugiperda* indicated the presence of only three components of the pheromonal blend of this species reported in the literature (1), the compounds are (Z)-7-C12OAc, (Z)-C14OAc and (Z)-11-C16OAc at the ratio of 0.5:80:20, and the analysis of volatiles present on glands extracts of *S. cosmioides* showed the presence of the following components (Z)-9-C14OAc, (E)-9-C14OAc, (Z,E)-9,12-C14OAc, (Z)-11-C16OAc and C16OAc, at the ratio 72:2:12.9:12.6:0.5. Electroantennography assays showed that the male antenna of *S. frugiperda* (N=3) responded to all pheromone components present on the female gland extract of conspecific and heterospecific, and a similar result was obtained when male antenna of *S. cosmioides* was tested (N=5). Bioassays in wind tunnel showed that 60% of *S. frugiperda* males responded to heterospecific females (N=40) and to volatiles present on the gland extract of *S. cosmioides* (N=40); whereas males of *S. cosmioides* neither responded to heterospecific females nor to volatiles present on the gland extract of *S. frugiperda* (N=40). To evaluate blends with synthetic compounds (Z)-7-C12OAc, (Z)-9-C14OAc, (Z,E)-9,12-C14OAc, (Z)-11-C16OAc e C16OAc were used in different combinations and ratios. The presence or absence of the compounds C16OAc e Z11C16OAc did not appear to affect the response of males of both species in the wind tunnel. The presence of (Z)-7-C12OAc increase the response of *S. frugiperda*, but inhibit the response of *S. cosmioides*. Therefore, a final mix was tested containing 10:1 of (Z)-9-C14OAc and (Z,E)-9,12-C14OAc, this mixture induced the behavioural response in more than 60% of males of both species (N=40 for each species).

(1) Batista-Pereira et al., *Journal of Chemical Ecology*. 2006

P105 - Identification and Synthesis of the sex pheromone of *Chauliognathus* sp. (COLEOPTERA: Cantharidae)

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Beetles of the genus *Chauliognathus* were found near Eucalyptus trees in Curitiba. The behavioral responses of males and females beetles to aeration extracts from conspecifics suggested the presence of a male sex pheromone. GC analyses of aeration extracts of both sexes revealed the presence of a male-specific compound. GC-EAD experiments reinforced the behavioral data, showing that this compound is bioactive on female antennae. Crude extracts were fractionated on a silica gel column and the male-specific compound eluted on the hexane fraction, suggesting that the compound was highly apolar. Analysis of the GC-MS spectrum confirmed the hydrocarbon function suggested a linear carbonic chain of 23 atoms, containing a carbon-carbon double bond (molecular ion at m/z 322). The presence of the double bond was confirmed by submitting the extract to a Pd/C hydrogenation, with the increasing of two units on M^+ (m/z 324). GC-MS analysis of the DMDS product showed two intense fragments (m/z 201 and 215), indicating the position of the double bond between the carbons C11-C12. The presence of a broad band at 3005cm^{-1} is typical on monounsaturated compounds containing of a *cis* double bond¹. Analysis of the data set allowed us to develop a possible structure: (*Z*)-11-tricosene (1). A synthetic route was proposed to produce a synthetic standard of the compound (1), starting by coupling 1-bromodecane to the propargyl alcohol in 5 steps. This synthetic route is underway.

[1] Attygalle, A. B.; *Pure Appl. Chem.*, Vol. 66, N10/11, pp. 2323-2326, **1994**.

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P106 - Preliminary evidence of jasmonate-induced changes in inflorescence fragrance of *Haplopappus foliosus* (Asteraceae)

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Plants are able to dynamically respond to different biotic stressors. Such responses include the release and/or the *de novo* production of volatile organic compounds (VOCs). Plant release of VOCs has been shown to modify the behavior of higher trophic level organisms, such as herbivores and predators. Among others, the production of VOCs in plant-herbivore systems has been shown to be under the orchestrations of the phytohormone jasmonic acid (JA). Despite the highly studied effect of JA in mediating the release of VOCs in vegetative organs, relatively little is known on the role of JA in mediating flower VOCs release, and how this might affect subsequent flower visitation. In this work we explored JA effects in *H. foliosus* inflorescence fragrance in the field. Undamaged and protected flower buds were exposed to the following treatments: i) 10 µl JA 95% injections in buds (Injection); ii) air-borne application of 10 µl JA from cotton tip during 24 hrs (Cotton); and iii) unaltered bud treatment (Control). Two days after treatments, we performed 4 hrs headspace collection following Raguso & Pellmyr (1998). Eluted VOCs were then analysed by GC-MS. To detect fragrance differences between treatments, we next performed principal component analysis (PCA) on proportions of each compound and kept the nine first PCs generated for canonical discriminant function analysis (DFA) using treatment as classifier (Vereecken, 2010). With DFA we were able to separate Control, Cotton and Injection treatments in different spaces formed from the function based on the combination of compounds released by these groups. This suggests that is experimentally possible to change inflorescence fragrance in the field through the use of phytohormones. Future experiments will aim at assessing the ecological costs/benefits of fragrance changes in flowers.

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P107 - Identification and evaluation of plant volatile compounds as feeding attractants of *Aedes aegypti*

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Aedes aegypti is considered one of the main vectors of dengue fever, yellow fever and other diseases. Transmission occurs when an infected female mosquito bites a person to blood-feed in order to complete its reproductive cycle. Many of the mosquito species within the genus *Aedes* are capable infection vectors, but the most important one is *A. aegypti*, due to its domestic habitat preference.

Nectar derived sugar feeding, as an energy source, has been documented for male and female mosquitoes. Due to the sharing of living space with humans, it is probable that they will encounter ornamental plants as potential nectar sources. The goals of this work is to identify which plants act as feeding attractants, obtain their volatile composition and asses individual compounds for feeding attraction.

The behavioural response to plant volatiles was tested in a two-choice feeding assay. A bait of volatile compounds from a whole plants or plant flowers with a 10% sucrose and 10 ppm Imidacloprid solution versus the plain 10 % sucrose solution were offered for feeding. The plant and volatiles which were preferred by *A. aegypti* over the plain sucrose solution were *Lobularia maritima*.

Plectranthus neochilus and *Tagetes patula* were tested without obtaining any measurable feeding preference.

Closed loop Head space and SPME GC-MS spectra were used for the determination of volatile compounds of *L. maritima*. Of those identified, two were tested (Acetophenone and 2-Phenylethanol) in the two-choice feeding assay. The 0.1 mg of Acetophenone proved to be attractive, while the same amount of 2-Phenylethanol had no effect.

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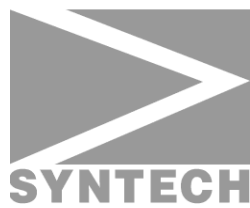
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