



Abstracts of the 1st Latin American Meeting of Chemical Ecology

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Organized by:

Latin American Association of Chemical Ecology

Laboratory of Chemical Ecology, Facultad de Química,
Universidad de la República, Uruguay

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Dear Colleagues,

Welcome to Uruguay and to the 1st Meeting of the Latin American Association of Chemical Ecology (ALAEQ). We are proud to host this event, which we hope will stand as a landmark of the field in our region.

Chemical Ecology in Latin America continues to grow. The need for sustainable pest management strategies and the remarkable biological diversity, mostly open for discovery, are key factors that drive our field forward. In the past, the region hosted two ISCE meetings in Chile (1995) and Brazil (2000); and since 1999, the Brazilian Meetings (EBEQs) have become well established. We are now launching the Latin American Association of Chemical Ecology, which will be a platform for intra-regional collaborations and student exchange, as well as a bridge into Latin America for ISCE and APACE members.

We are very excited by the response of our Latin American colleagues; we have about 150 participants from seven countries in the region. We are also proud about the participation of leading scientists from North America and Europe, and we appreciate their support to ALAEQ..

Have a great, productive and fun meeting!!

Jan Bergmann, Carmen Rossini, Andrés González

Welcome from Thomas Eisner, and Jerrold Meinwald

October 4, 2010

Amigos y colegas – bienvenidos a Uruguay!

Chemical Ecology is far from a new subject in Uruguay. Over a half-century ago, Clemente Estable and Maria Isabel Ardao initiated a pioneering study of the chemical defenses of a local opilionid, *Acanthopachylus aculeatus*, in Montevideo. In collaboration with Louis Fieser at the Department of Chemistry at Harvard University, they established the structures of three alkylated p-benzoquinones which serve as the chief bioactive components of the arthropod's defensive secretion. This painstaking chemical characterization required over a hundred milligrams of material. Today this analysis could be carried out on nanograms! This million-fold increase in analytical power has opened the door to studies of biotic chemical interactions that could only have been dreamed of until now.

As a consequence of improved communication, biologists and chemists working anywhere have the opportunity to collaborate as never before. That these cooperative studies will bring forth progress in medicine, agriculture, and environmental preservation is a certainty. At the same time, it will be advances in the basic sciences that will have the largest impact on our field, enabling the pursuit of entirely new areas of both pure and applied research by future generations of scientists. Who knows what the next million-fold improvement in analytical capability will make possible?

With its vast, largely unexplored biological riches, Latin America offers boundless opportunities for chemical ecological research. We applaud your seizing these opportunities, as it is clear from the presentations at this Meeting that you are doing. May this Meeting mark the start of a concerted effort to advance the frontiers of Chemical Ecology in Latin America! There is every reason to hope that the 21st century will be a "golden age," worldwide, for the subject that we all love!

Buena suerte!

Thomas Eisner, Jerrold Meinwald
Cornell University, Ithaca, NY 14853, USA

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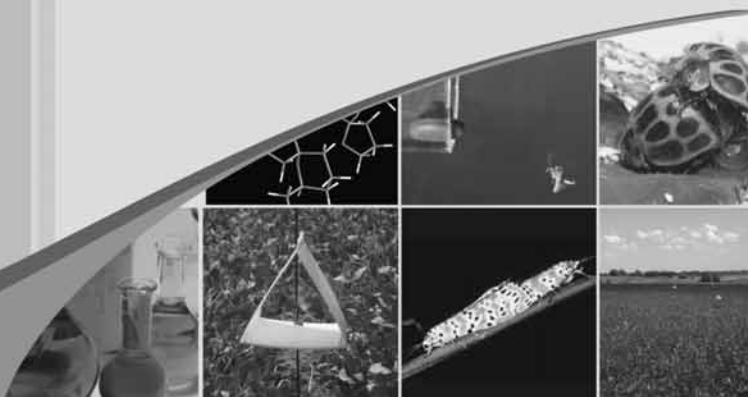
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1st Latin American Meeting of Chemical Ecology

PROGRAM



ALAEQ



Sunday, October 17

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| 13:00 - 17:30 | Registration and Mounting of Posters Foyer of Bastión del Carmen |
| 17:30 - 18:30 | Official Welcome and Opening remarks Jan Bergman President of the ALAEQ Official welcome to Colonia del Sacramento Intendencia Municipal de Colonia Andrés González President of the Organizing Committee of the 1 st Meeting of ALAEQ John T. Romeo Editor-in-Chief of the Journal of Chemical Ecology Meet the Editor: Journal of Chemical Ecology <i>Chair: Andrés González</i> |
| 18:30 - 19:30 | OPENING LECTURE <i>Chair: John T. Romeo</i> Christer Löfstedt Lund University, Sweden. Molecular analysis of the evolution of pheromone communication in moths: new answers to old questions |
| 19:30 - 22:00 | Get together: Welcome reception Foyer of Bastión del Carmen |

Monday, October 18 – Morning

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| 8:30 - 9:15 | PLENARY LECTURE <i>Chair: Jocelyn Millar</i> Thomas C. Baker Penn State University, University Park, Pennsylvania Neuroethology of sex pheromone olfaction |
| Symposium on Neuroethology of Odor Sensing <i>Organizer and Chair: Pablo Guerenstein</i> | |
| 9:15 - 9:25 | Pablo Guerenstein , CICYTTP-CONICET, Argentina Introduction: Why Neuroethology of Olfaction in Latin America? |
| 9:25 - 9:50 | Romina Barrozo , Universidad de Buenos Aires, Argentina Switching Attraction to Inhibition: a Neuroethological Mechanism of Post-Mating Sexual Abstinence |
| 9:50 - 10:15 | Patricia Fernández , Universidad de Buenos Aires-INTA-CONICET, Argentina Learning Related Plasticity in the Antennal Lobe Helps Discrimination of Similar Floral Odors |
| 10:15 - 10:45 | Coffee break |
| 10:45 - 11:10 | Andrés Arenas , Universidad de Buenos Aires-CONICET, Argentina Early Olfactory Experience Modifies Neural Activity and Shapes the Primary Olfactory Center of an Insect Brain |
| 11:10 - 11:35 | Nicolás Pirez , Brandeis University, USA In vivo Control of Olfactory Receptor Neuron Input to the Olfactory Bulb by Presynaptic Inhibition |
| 11:35 - 12:20 | PLENARY LECTURE <i>Chair: Marcelo Lorenzo</i> Rickard Ignell Swedish University of Agricultural Sciences, Sweden Sensory Correlates of Host-seeking Behavior of Mosquitoes |
| 12:20 - 14:00 | Lunch break |

Monday, October 18 – Afternoon

ORAL PRESENTATIONS

Chair: Nancy Barreto

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| 14:00 - 14:15 | Claudia A. Zacharias Role of Cuticular Lipids on the Mating Behaviour in <i>Triatoma infestans</i> (Heteroptera: Reduviidae) |
| 14:15 - 14:30 | Alejandra J. Troncoso Host Mediation in a Mistletoe's Phenotype |
| 14:30 - 14:45 | Rodrigo C. Vergara Into the Wild Sensory World of a Crepuscular Florivore: The Use of Olfactory and Visual Floral Cues in the Flower-Visiting Behavior of <i>B. orientalis</i> (Blattodea: Blattidae) |
| 14:45 - 15:00 | José E. Crespo Ontogeny of a Chemical Cue in a Parasitoid Dipteran |

Symposium: Chemical Ecology of Disease Vectors

Organizers and chairs: Marcelo Lorenzo and Alicia Lorenzo Figueiras

| | |
|---------------|---|
| 15:00 - 15:25 | Lígia M. F. Borges , Universidade Federal de Goiás, Brazil Chemical Ecology of Ticks |
| 15:25 - 15:50 | Alvaro E. Eiras , Federal University of Minas Gerais, Brazil Practical Application of Olfactory Cues for Monitoring and Control of <i>Aedes aegypti</i> in Brazil |
| 15:50 - 16:20 | Coffee break |
| 16:20 - 16:45 | Alicia Lorenzo Figueiras , Universidad de Buenos Aires, Argentina Searching for Aggregation Pheromones in the Haematophagous Bug <i>Triatoma infestans</i> |
| 16:45 - 17:10 | Marcelo Gustavo Lorenzo , CPqRR- FIOCRUZ, Brazil Triatomine Sexual Behaviour is Mediated by Pheromones |
| 17:10 - 17:55 | PLENARY LECTURE Chair: Carmen Rossini Michael Birkett Rothamsted Research, UK Non-host Signalling in Plants, Animals and Humans: A New Paradigm for Pest Control |
| 18:00 - 20:00 | Poster session 1 Odd-numbered abstracts |

Tuesday, October 19 – Morning

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| 8:30 - 9:15 | PLENARY LECTURE <i>Chair: Angel Guerrero</i> Martín Aluja Instituto de Ecología, A.C., México Chemical Ecology of Fruit Flies (Diptera: Tephritidae) and their Natural Enemies |
| Symposium on Pheromone Chemistry <i>Organizer and Chair: Paulo H. Zarbin</i> | |
| 9:15 - 9:40 | Jan Bergmann , Pontificia Universidad Católica de Valparaíso, Chile Larval Secretion of <i>Chilecomadia valdiviana</i> (Lepidoptera: Cossidae): Identification, Synthesis, and Biological Activity |
| 9:40 - 10:05 | Jeffrey R. Aldrich , USDA-ARS, USA Evidence for an Unusual Pheromone from a South American Coreid Bug, <i>Phthia picta</i> (Hemiptera: Heteroptera: Coreidae) |
| 10:05 - 10:30 | Coffee break |
| 10:30 - 10:55 | Jocelyn Millar , University of California at Riverside, USA Pheromones for Cerambycid Beetles in the Subfamily Prioninae |
| 10:55 - 11:20 | Paulo H. Zarbin , Universidade Federal de Paraná, Brasil The pheromone chemistry in the Laboratory of Semiochemicals at the Federal University of Paraná |
| ORAL PRESENTATIONS <i>Chair: Maria Carolina Blassioli Moraes</i> | |
| 11:20 - 11:35 | Valeria Cal Behavioral Studies and Gender-Specific Characterization of Volatiles in the South American Rice Weevil, <i>Oryzophagus oryzae</i> (Coleoptera: Curculionidae) |
| 11:35 - 11:50 | Estefanía Dibello Catalysis in the Synthesis of Insect Pheromones: Preparation of Dominicalure I, Sexual Pheromone of <i>Rhyzopertha dominica</i> |
| 11:50 - 12:05 | Carmen V. Liendo-Barandiaran Identification of Male Sexual Pheromone in <i>Steirastoma breve</i> (Coleoptera: Cerambycidae) |
| 12:05 - 12:50 | PLENARY LECTURE <i>Chair: Pablo Guerenstein</i> Arthur Edison University of Florida, USA New approaches to natural products and metabolomics: Applications to nematode chemical ecology |
| 12:50 - 14:00 | Lunch break |

Tuesday, October 19 – Afternoon

ORAL PRESENTATIONS

Chair: Michael Birkett

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|---------------|---|
| 14:00 - 14:15 | Francisco J. Espinosa-García On the Function of Phytochemical Diversity in Plant Defenses |
| 14:15 - 14:30 | Jorge A. Zavala Plant-Insect Interactions in Soybean Crops: Plant Responses to Stink Bug (<i>Nezara viridula</i>) Attack |
| 14:30 - 14:45 | Diego M. Magalhães Effect of Induced Volatiles in Cotton Plants Damaged by <i>Anthonomus grandis</i> and <i>Spodoptera frugiperda</i> in the Boll Weevil's Behavior |
| 14:45 - 15:00 | Antônio E. G. Sant'Ana Tritrophic Interaction Between <i>Capsicum annum</i> L., <i>Aphis gossypii</i> Glover and <i>Aphidius colemani</i> Viereck |

Symposium on Insect-Plant Interactions

Organizer and chair: Mauricio S. Bento

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| 15:00 - 15:25 | Mauricio Bento , Universidade de São Paulo, Brasil TBA PLANT VOLATILES in PEST MANAGEMENT: to ATTRACT or REPEL? |
| 15:25 - 15:50 | Roxina Soler , Wageningen University, The Netherlands Root Herbivores Interfere with the Development and Behavior of Parasitoids of Foliar Herbivores: Effects, Mechanisms and Future Steps |
| 15:50 - 16:20 | Coffee break |
| 16:20 - 16:45 | Walter Farina , Universidad de Buenos Aires, Argentina Synthetic Mixes of Floral Volatiles Used as Tools to Guide Honey Bees to Specific Crops |
| 16:45 - 17:10 | Eraldo Lima , Universidade Federal de Viçosa, Brasil Oviposition Preference of <i>Ceratitis capitata</i> (Diptera: Tephritidae) to <i>Coffea arabica</i> and <i>Coffea canephora</i> Cultivars |
| 17:10 - 17:55 | PLENARY LECTURE Chair: Jan Bergmann Wittko Francke University of Hamburg, Germany Semiochemicals - Structural Principles and Evolution |
| 18:00 - 20:00 | Poster session 2 Even-numbered abstracts |

Wednesday, 20 October – Morning

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| 8:30 - 9:15 | PLENARY LECTURE <i>Chair: Mauricio Bento</i> Angel Guerrero Institute of Advanced Chemistry of Catalonia (CSIC), Spain Inhibition of the chemical communication in insects: A promising approach to pest control? |
| Symposium: Applications of Semiochemicals in Latin America <i>Organizers and Chairs: Carmen Rossini and Andrés González</i> | |
| 9:15 - 9:40 | Andrés Quiroz , Universidad de la Frontera, Chile Arthropods Chemical Ecology of Economic Importance in Southern Chile |
| 9:40 - 10:05 | Nancy Barreto , Corpoica, Colombia Semiochemicals in Pest Management in Colombia |
| 10:05 - 10:30 | Miguel Borges , EMBRAPA-Brasilia, Brasil Monitoring the Neotropical Brown Stink Bug <i>Euschistus heros</i> (F.) (Hemiptera: Pentatomidae) with Pheromone-baited Traps in Soybean Fields |
| 10:30 - 11:00 | Coffee break |
| 11:00 - 11:25 | Iris B. Scatoni , Universidad de la República, Uruguay Use of Sex Pheromones to Characterize the Spatial Distribution of <i>Argyrotaenia spheropa</i> and <i>Bonagota salubricola</i> (Lepidoptera: Tortricidae) in Southern Uruguay |
| 11:25 - 11:50 | Cam Oehlschlager , ChemTica International, Costa Rica Mass Trapping as a Control Strategy for Coleopteran and Lepidopteran Pests |
| ORAL PRESENTATIONS <i>Chair: Christer Löfstedt</i> | |
| 11:50 - 12:05 | Juan R. Girotti Fusarium Head Blight: Chemical Signals of Early Infection |
| 12:05 - 12:20 | Nathàlia Nocchi Defenses of Tropical Freshwater Macrophytes Against Herbivory |
| 12:20 - 12:35 | Alicia Bautista-Lozada Domestication Status Determines Variation of Induced Volatile Organic Compounds of Tomato Plants |
| 12:35 - 14:00 | Lunch break |

Wednesday, 20 October – Afternoon

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|--|---|
| 14:00 - 14:45 | PLENARY LECTURE <i>Chair: Paulo Zarbin</i> Jocelyn Millar University of California, USA Chemistry and Applications of Mealybug Pheromones |
| ORAL PRESENTATIONS <i>Chair: Andrés Quiroz</i> | |
| 14:45 - 15:00 | Warley S. Borges Metabolites Produced by <i>Escovopsis</i> sp., a Parasitic Fungus Found in Ants Nests |
| 15:00 - 15:15 | Carla F. Fávoro Chemical Ecology of the Stink Bug <i>Pallantia macunaima</i> Grazia (Hemiptera: Pentatomidae) |
| 15:15 - 15:30 | Michely F. S. de Aquino Kairomones from <i>Euschistus heros</i> Eggs Induced Host Selection Behavior on Parasitoid <i>Telenomus podisi</i> |
| 15:30 - 15:45 | Leonardo J. Parra Electrophysiological and Behavioral Response of <i>Aegorhinus superciliosus</i> (Coleoptera: Curculionidae) to Volatiles Released from Conspecific and its Host Plant |
| 15:45 - 16:15 | Coffee break |
| 16:15 - 17:00 | CLOSING LECTURE <i>Chair: Thomas C. Baker</i> Jeremy McNeil The University of Western Ontario, Canada Using Chemical Ecology to Study Lepidopteran Migration |
| 17:00 - 17:30 | Closing remarks Andrés González President of the Organizing Committee of the 1 st Meeting of ALAEQ |
| 17:30 - 18:00 | ALAEQ business meeting |
| 20:00 | Final dinner Country House: Los 3 Botones. Transportation provided. Pick up points: Bastión del Carmen (2 buses) Posada Don Antonio (1 bus) |

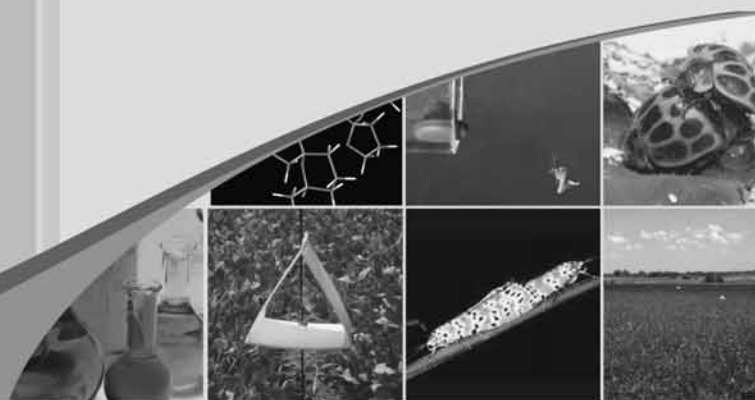
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PLENARY LECTURES



ALAEQ



Molecular Analysis of the Evolution of Pheromone Communication in Moths: New Answers to Old Questions

Christer Löfstedt

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Sex pheromones are species-specific mate recognition signals that play a role in reproductive isolation. The nature of variation in pheromone communication systems is critical for the evolution of these systems and for our understanding of the evolutionary processes [1]. In the 1980's several cases of significant within- and between-population variation in moth pheromones had been demonstrated, which opened up for a detailed analysis of the genetic control of pheromone systems and how and why pheromone differences evolve. The European corn borer *Ostrinia nubilalis* (Crambidae) entered the scene as a model species for research on the evolution of pheromone communication [2].

I will review the development of research on variation in moth pheromone systems with an emphasis on how molecular tools have provided new answers to old questions during the last 10 years. Recent molecular, phylogenetic and genomic analyses have revealed the control of moth pheromone production by two multigene families, encoding fatty acyl desaturases and fatty acyl reductases respectively [3-7]. In *O. nubilalis* and its congeners the ratio of pheromone components is modulated by substrate preference of the fatty acyl reductase (FAR) involved in pheromone production. The pgFAR gene is under positive Darwinian selection and mutational changes at sites under selection cause both gradual and saltational shifts in enzyme activity associated with divergence in pheromone composition. Exploration of olfactory receptor genes, another multigene family, may bring about a similar understanding of variation in pheromone detection. The genetic control of variation in behavioural response, however, largely remains a black box.

- [1] Löfstedt, C., Ent. Exp. Appl. 54, 199-218 (1990).
- [2] Roelofs, W.L., Glover, T., Tang, X-H., Sreng, I., Robbins, P., Eckenrode, C. Löfstedt, C., Hansson, B., and Bengtsson, B. O. Proc. Natl. Acad. Sci. USA 84, 7585-7589 (1987).
- [3] Roelofs, W.L, Rooney A.P. Proc. Natl. Acad. Sci. USA 100, 9179-9184 (2003).
- [4] Liénard, M., Strandh, M., Hedenström, E., Johansson, T., and Löfstedt, C. BMC Evol. Biol. 8, 270:1-15 (2008).
- [5] Lassance, J.-M. and Löfstedt C. BMC Biol. 7, 10:1-12 (2009).
- [6] Lassance, J.-M., Groot, A.T., Liénard, M.A., Antony, B., Borgwardt, C., Andersson, F., Hedenström, E., Heckel, D.G., and Löfstedt, C. Nature 466, 486-489 (2010).
- [7] Liénard, M.A., Hagström, Å.K, Lassance, J.M., and Löfstedt, C. Proc. Natl. Acad. Sci. USA 107, 10955-10960 (2010).

Neuroethology of Sex Pheromone Olfaction

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There have been several puzzling questions in the field of sex pheromone olfaction over the past several decades. One of these is why, in nearly every species, are the majority of olfactory receptor neurons (ORNs) tuned to the most abundant pheromone component in a species' sex pheromone blend? If detection sensitivity is what has determined ORN abundance, then should not the most abundant ORNs be tuned to the *least* abundant pheromone components, since these will be the components that are most difficult to detect in any blend? A related question concerns ORNs that are co-compartmentalized in the same sensillum: why does the ORN apparently tuned to the most abundant pheromone component in the blend have a larger diameter dendrite and produce a larger amplitude action potential than the ORN tuned to the minor component? Our group has come up with a hypothesis for why these relationships between ORN number and dendrite diameter have been sculpted over evolutionary time in this fashion. The hypothesis applies nicely not only to the evolution of sex pheromone olfactory systems, but also to olfactory systems for general odorants involved in host-finding. We also have examined another issue, that of pheromone components as 'agonists' or 'antagonists', and have concluded that understanding the evolution of sex pheromone blends is facilitated by taking the view that a positive olfactory signal to promote upwind flight behavior depends on balanced olfactory antagonism.

Sensory Correlates of Host-seeking Behavior of Mosquitoes

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Mosquitoes that act as disease vectors rely upon olfactory cues to direct several important behaviors that are fundamentally involved in establishing their overall vectorial capacity. Using the single sensillum recording (SSR) technique we have functionally characterized the peripheral olfactory system of some of the major disease vectors in the world, including *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*. By building on this research we are currently performing gas chromatography coupled SSRs in order to identify novel olfactory cues used by the mosquitoes to drive their host-seeking behavior. This analysis has so far allowed us to identify a number of novel human-derived volatile cues, which elicit significant behavioral responses in *Aedes aegypti* and *Culex quinquefasciatus*. As an extension of our research, we believe that we will be able to identify behaviorally active attractants, repellents and attractant ‘masking’ agents that may be used in the development of a mosquito novel management system based on push-pull technology.

Non-host Signalling in Plants, Animals and Humans: A New Paradigm for Pest Control

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In pioneering chemical ecology studies in our laboratory, we have demonstrated that arthropod pests negatively affecting (i) crop and (ii) animal/human health actively avoid unsuitable hosts through olfactory perception of non-host derived volatile semiochemicals.

In seminal studies on the interaction of aphids, and their natural enemies, with host plants, we have shown that non-host avoidance can be switched on by activation of defence signaling using *cis*-jasmone, a volatile plant natural product. The mechanisms underlying activation of defence by *cis*-jasmone have been elucidated in *Arabidopsis thaliana* [1] and in wheat. Furthermore, the potential for using *cis*-jasmone as a practical tool for activation of defence in other major world crops has been explored, e.g. in cotton, soybean and maize, and results to date demonstrate real promise for the use of small lipophilic molecules as natural plant activators in the sustainable control of aphid, stinkbug and nematode pests.

For animals and humans, we have demonstrated that semiochemicals released specifically by non-host species interfere with host location, e.g. for the sea louse, *Lepeophtheirus salmonis*. Furthermore, individuals within a bovine or human population can be extremely unattractive, even to arthropods (horn flies, mosquitoes and biting midges) highly adapted to these species. We have proved this to be caused by the presence of additional compounds which can themselves be used as repellents on “attractive” animals and human beings. The identification of semiochemicals involved in non-host avoidance can be exploited for the development of new repellents [2], with the prospect of their deployment alongside host-derived attractants and attractant pheromones in a push-pull system for pathogen vector control.

[1] Bruce, T.J. et al. (2008) *P. Natl. Acad. Sci. USA*, 105, 4553-4558.

[2] Pickett, J.A. et al. (2010) *J. Chem. Ecol.*, 36, 113-121.

Chemical Ecology of Fruit Flies (*Diptera: Tephritidae*) and their Natural Enemies

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Insect behavior is closely tied to their chemical ecology. Aspects such as foraging (e.g., food or host-seeking), courtship and mating, as well as oviposition are modulated by chemical stimuli. This conference provides a broad overview of fruit fly (*Diptera: Tephritidae*) chemical ecology using along the way examples of how females find hosts or mates using chemical cues or how chemical signals (e.g., sexual pheromones, host volatiles, host marking pheromones) can be used to manipulate the behavior of these insects in an attempt to reduce the significant damage they inflict on many commercially valuable commodities (particularly fruit and vegetables). Examples are also provided on some of the natural enemies of fruit flies, particularly parasitoids (*Hymenoptera*) that also rely on chemical cues during host-seeking and utilization.

Acknowledgements: Campaña Nacional Contra Moscas de la Fruta (SAGARPA – IICA), Consejo Nacional de Ciencia y Tecnología (CONACyT), Consejo Nacional de Investigaciones Científicas, y Técnicas (CONICET, Argentina), Fondo de Investigaciones Ricardo J. Zevada, United States Department of Agriculture (USDA-ARS), International Foundation for Science (IFS), International Atomic Energy Agency (IAEA)

New Approaches to Natural Products and Metabolomics: Applications to Nematode Chemical Ecology

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The nematode *Caenorhabditis elegans* is one of the best-studied animals in the world. It was the first metazoan to have its genome sequenced. Its entire cell lineage from a single fertilized egg to an adult is known and has been related to the animal's anatomy, and its anatomical ultrastructure has been comprehensively described by thin-section electron microscopy. *C. elegans* is particularly tractable for genetic studies, and as a result many signal transduction pathways have been identified. In the past 3-4 years, *C. elegans* has emerged as an outstanding organism for chemical biology studies. Over a dozen related small molecules called ascarosides regulate multiple behaviors, including dauer formation, mating behavior, and aggregation (reviewed in [1]).

We are extending work in *C. elegans* to other species, and I will describe our recent efforts in this area. Moreover, I will summarize our work on NMR technology and data analysis to facilitate studies in nematode natural products and metabolomics.

[1] Edison, A. S. (2009) *Caenorhabditis elegans* pheromones regulate multiple complex behaviors, *Curr Opin Neurobiol* 19, 378-388.

Semiochemicals - Structural Principles and Evolution

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Molecular recognition is prerequisite to the beginning of life, and therefore, “chemical signalling” is the oldest means for the transmission of information.

Principles of chemical signalling may have evolved several times and for different reasons. Typical elements are represented by a broad spectrum of secondary metabolites originating from the ubiquitous streams of classic catabolism or metabolism [1].

As a result, striking similarities are found among molecular structures of semiochemicals which point to general concepts in the establishment of “chemical languages” - and the development of appropriate receptor systems.

In a conservative scenario, most of the relevant compounds are represented by acetogenins, polyketides, and terpenoids, the biosynthesis of which is not restricted to animals, but is also valid in plants and microorganisms - in terrestrial as well as in aquatic ecosystems. The role of (endo)symbionts in the production and transformation of animal associated chemical signals as well as in (co)-evolutionary processes is yet unknown.

- Acetogenins show unbranched carbon skeletons made up of acetate units. They frequently occur as rows of bis-homologues.
- Polyketides show methyl-branched carbon skeletons. Biosyntheses involving propanoate units will result in typical 1,3-dimethyl-branching.
- Terpenes involve isoprene units as substructures which may be produced along three different ways.

Due to processes mentioned above, the structures of volatile signals from plants, insects, and microorganisms can be identical. The same compound may be used as a chemical messenger by quite different organisms and in entirely different ecological contexts. The information linked to a specific chemical is not necessarily constant and may change during evolution.

[1] W. Francke, S. Schulz. Pheromones of terrestrial invertebrates. In: L. Mander, H.-W. Liu (Eds) *Comprehensive Natural Products Chemistry II*, Vol. 4 (2010) pp 153-224, Oxford, Elsevier

Inhibition of the Chemical Communication in Insects: A Promising Approach to Pest Control?

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Enzymatic inhibition is a well-known tool to block many key biochemical and physiological processes. For this reason, the development of potent enzyme inhibitors is an important area of research in the pharmaceutical and agrochemical fields. Incorporation of polyfluoroketone moieties into compounds structurally close to the parent substrates has resulted in a useful strategy for developing strong inhibitors of enzymes involved in pheromone degradation [1].

Fluorinated ketones function as transition-state analogues of the enzyme, with the inhibition arising by formation of one adduct of tetrahedral geometry between a serine residue of the active site of the enzyme with the highly electrophilic carbonyl. Among the fluorinated ketones, the trifluoromethyl derivatives (TFMKs) are known to inhibit reversibly the antennal esterases responsible for the pheromone catabolism in male olfactory tissues, and therefore proposed as a potential approach for insect control [2]. A variety of TFMKs have been prepared and their inhibitory activity has been established in different moth species, such as *Spodoptera littoralis*, *Plutella xylostella*, *Thaumetopoea pityocampa*, *Sesamia nonagrioides*, *Ostrinia nubilalis*, *Mamestra brassicae*, *Antheraea polyphemus*, *Bombyx mori*, and *Cydia pomonella*, in the laboratory and in the field. Following our current research on this topic, I will present an overview of our latest results to answer the question of whether these pheromone antagonists are real candidates to consider in future pest control strategies.

[1] A. Guerrero and G. Rosell, *Curr. Med. Chem.* **12**, 461 (2005).

[2] E. Plettner, *Curr. Med. Chem.* **9**, 1075 (2002); A. Guerrero, M.P. Bosch, G. Rosell, M. Riba and A. Sans, Sp. Patent 200301667 (2003).

Chemistry and Applications of Mealybug Pheromones

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Vineyards in many parts of the world have chronic problems with mealybugs (Homoptera: Pseudococcidae). These small, soft-bodied insects damage plants by feeding, by the accumulation of insect detritus, and by the growth of sooty molds on the honeydew excreted by the insects. They also vector diseases such as the leafroll viruses that are becoming increasingly important in vineyards in the United States and New Zealand. This has led to renewed interest in the identification and development of applications for pheromones for monitoring and possibly controlling mealybugs. Over the past several years, we have identified the pheromones of some of the most economically important species, including the obscure (*Pseudococcus viburni*), longtailed (*P. longispinus*), and grape (*P. maritimus*) mealybugs, all of which are worldwide pests in vineyards and other crops. The pheromone of each species consists of a single compound unique to that species, and all three of the pheromones have highly unusual monoterpene skeletons. The identification and synthesis of each pheromone, including determination of relative and absolute configurations, will be briefly described. Two of the three pheromones are now commercially available, and are being widely used for detection and monitoring of mealybug population cycles. Economic and biological factors affecting their commercial development will be discussed, including the development of more efficient strategies for synthesizing each pheromone once the basic structure of each had been determined. Pilot projects to examine the use of mealybug pheromones in nurseries for ornamental plants will also be discussed.

This work was supported by grants from the Viticulture Consortium West, the Oregon Wine Board, the American Vineyard Foundation, and the UC Integrated Pest Management Project.

Using Chemical Ecology to Study Lepidopteran Migration

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There are a number of insects species that migrate in response to habitat deterioration, but given the long distances they cover it is not always easy to observe them directly. However, there are behavioural and physiological processes that may be modified by environmental cues that indicate habitat quality and these systems may be used to gain insight into different facets of insect migration. In my talk I will discuss how different environmental cues, such as temperature, daylength, smoke and host plant volatiles, modulate the pheromone-mediated mating systems of several migratory moth species. I will also present data comparing migrant and non migrant populations of the same species, as this system should allow us to gain a better understanding of adaptations that may reduce the cost of migration on future reproductive output.

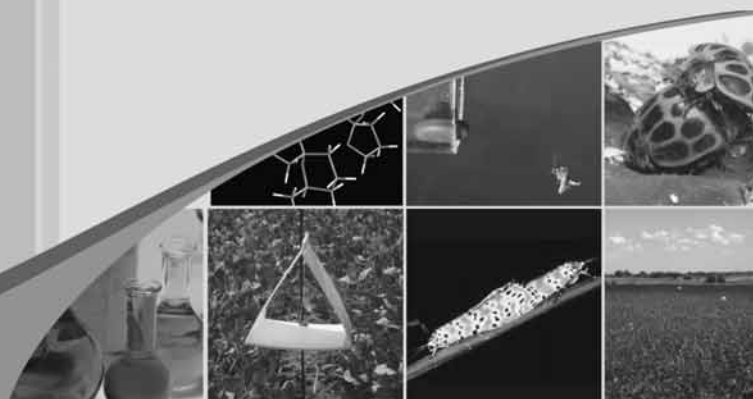
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st Latin American Meeting of Chemical Ecology

SYMPOSIA



ALAEQ



Symposium: Neuroethology of Odor Sensing

Coordinator: Pablo G. Guerenstein

Introduction: Why Neuroethology of Olfaction in Latin America?

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Briefly, neuroethology studies the neural bases of natural behavior, while also dealing with development and evolution of neural circuits and behaviors. Emphasis is given to consideration of the natural environment of the animal in all experimental work. The top-down approach starts from behavioral/ecological questions and attempts to answer them even at the neural level, while the bottom-up approach starts from neurophysiological questions and attempts to understand the behavioral and ecological role of the neural circuits under study. Applications range from pest control to robotics, disciplines that need to be further developed in Latin America. In particular, studies on the neuroethology of olfaction and multimodal sensory integration are gaining ground all around the world, and Latin America has the potential to be part of this endeavor.

Switching Attraction to Inhibition: a Neuroethological Mechanism of Post-Mating Sexual Abstinence

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In animals, mating involves detection and central processing of relevant chemical cues that lead to appropriate reproductive behaviours. In many species, the most prominent use of olfactory signals is the sex pheromone communication, with males generally attracted by a female-produced pheromone. Male reproductive success depends not only on the ability to locate and copulate with a female, but also on their ability to effectively transfer an ejaculate. However, males are limited with respect to the number of ejaculates they can deliver and the time required to restore depleted reserves. Although males of many species are known to enter in a refractory post-ejaculatory interval (PEI), the mechanisms that lead to this sexual abstinence are far from being understood. In the male moth *Agrotis ipsilon*, mating induces a transient inhibition of behavioural and central nervous responses to sex pheromone. Using non-pheromonal (plant) odours, pheromones, and their mixture, we showed that the observed lack of pheromone response originates from differential post-mating odour processing in the brain. Although mated males still respond to plant odours alone, their response to mixtures depends on the added pheromone concentration. Below a specific threshold, sex pheromone is not detected at the brain level; above this threshold, it becomes inhibitory. This PEI can thus be interpreted as a “refusal to respond”, but not as a sleep-like/exhaustion behaviour. A sex pheromone, which is generally considered as an attractant, can become inhibitory depending on the physiological state of the receiver. We provide evidence for a neuroethological mechanism based on transient odour-selective central nervous processing leading to post-mating sexual abstinence in males.

Learning Related Plasticity in the Antennal Lobe Helps Discrimination of Similar Floral Odors

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Flower odors are highly variable combinations of several volatile components. No two flowers smell exactly alike, even examples from the same species and cultivar. Pollinators must therefore establish if a newly encountered flower is similar enough to a previous rewarded one, turning foraging decisions into a fine tuned generalization-discrimination task. Sensory and neural systems must provide mechanisms for very precise odor recognition, allowing perceptual stability (i.e. generalization to prevent all experiences from being independent and novel) [1]. We hypothesize that experience with odors tunes sensory processing and thereby improves odor recognition and classification of newly encountered flowers [2]. In the present work we designed artificial floral blends that mimic the components, proportions and variability of 2 natural varieties of snapdragon flowers. All designed blends share the same components. But they can be differentiated based on the relative concentration of the components, which were more similar within than between cultivars. We trained restrained honey bees using the proboscis extension response paradigm (PER). Bees were differentially conditioned using examples of both cultivars. After training we tested the ability to recognize a new example from each cultivar. The duration of PER was lower and the latency longer when an example of the non-rewarded cultivar was offered to the trained bee. Odor induced activity patterns were measured in Projection Neurons of the Antennal Lobe by calcium imaging. Consistent with behavior, results suggest that the neural network in the AL is tuned via differential conditioning to decorrelate mixtures representing different floral varieties. Experience-dependent plasticity at the level of the Antennal Lobe may help animals categorize a newly flower as belonging to a class related to reinforcement. This kind of mechanism may allow bees a quick adaptation to a different and constantly changing environment.

[1] Barnes et al 2008. *Nat Neurosci* 11(12):1378-1380

[2] Fernandez et al 2009. *J Neurosci* 29(33):10191-10202

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Early Olfactory Experience Modifies Neural Activity and Shapes the Primary Olfactory Center of an Insect Brain

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The antennal lobe (AL), the first olfactory centre of an insect brain, is organized in glomeruli. These neuropiles are well-defined structures that encoded odor information in spatiotemporal patterns of activity. Whether and how such patterns are modified in the long term after an olfactory experience during early adulthood (i.e. in the first days of adulthood) remains unknown. We used *in vivo* calcium imaging technique to measure the odor-evoked responses in the AL of 17-day-old honeybees which either experienced a scented food at the age of 5-8 days after emergence or were left untreated. Additionally, we evaluated whether early olfactory experiences could modify the shape of the primary processing center by measuring the glomerular volume in a confocal microscope. Physiological experiments showed that precocious olfactory experiences associated to reward enhanced the activity and the number of activated glomeruli in an adult bee while modify the spatial response of the patterns. Morphological experiments also revealed changes in the volume of specific glomeruli of early-experienced bees. Interestingly, those glomeruli that most increased their size after the odor experience appeared as newly recruited in odor-evoked patterns of activity suggesting a positive relationship between the functional and the structural properties of the AL. Long-lasting changes after the appetitive experience with odors may induce the reorganization of the neural networks in the AL of an adult insect, a fact that may result from the interaction between timing and nature of early sensory inputs [1, 2].

[1] Arenas A & Farina WF (2008) Age and rearing environment interact in the retention of early olfactory memories in honeybees. *J. Comp. Physiol. A.*, 194, 629–640.

[2] Arenas A, Fernández VM, Farina WM (2009) Associative Learning during Early Adulthood Enhances Later Memory Retention in Honeybees. PLoS ONE 4(12): e8046. doi:10.1371/journal.pone.0008046.

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In vivo Control of Olfactory Receptor Neuron Input to the Olfactory Bulb by Presynaptic Inhibition

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The first major reorganization of odor representations in the nervous system occurs at the synapse between olfactory receptor neurons and second-order neurons in the olfactory bulb. Signal transmission at this synapse is modulated by signaling pathways that regulate the amount of transmitter release from the receptor neuron, controlling the strength of sensory input to the brain. Using *in vivo* calcium imaging, we imaged from the receptor neuron terminals of anesthetized mice before and after blocking GABA-ergic presynaptic inhibition and investigated what features of neural activity control the strength of this inhibition. As expected from earlier studies, blocking presynaptic GABA_B receptors *in vivo* increased odorant-evoked presynaptic calcium signals, confirming that GABA_B-mediated inhibition modulates the strength of receptor inputs. We found that the strength of this inhibition was affected little by the nature of the input, being independent of the spatial distribution of activated glomeruli, independent of the sniff frequency used to sample the odorant, and similar for weak and strong odorant-evoked inputs. Instead, we found that tonic inhibition was a major determinant of receptor input strength. This finding establishes a neural substrate by which the strength of sensory input to the brain may be modulated as a function of behavioral state.

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Symposium: Chemical Ecology of Disease Vectors

Coordinators: Alicia N. Lorenzo Figueiras and Marcelo Lorenzo

Chemical Ecology of Ticks

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The medical and economic importance of ticks has long been recognized due to their ability to transmit diseases to humans and animals. When ticks are seeking for the host they recognize a variety of stimuli from prospective hosts which in turn excite their host-finding behavior. Aggregation pheromones can also participate in the process of locating the host. Nidicolous ticks are those which live in caves, burrows and nests of their hosts. To contact the host and initiate successful parasitism they use an active strategy which involves running towards the host, so they are referred to as hunter ticks. Exophilic ticks, which occupy open habits, generally use the ambush strategy to find the host. Some tick species feed only on specific hosts, or a narrow range of closely related species, others are relatively indiscriminate in their choice. Some hosts can develop resistance to ticks and be less parasitized than others of the same species. We will examine the chemical ecology of two tick species, the nidicolous and host-specific *Rhipicephalus sanguineus* and the non-nidicolous and non-host specific *Amblyomma cajennense*. The appetence behavior in the cosmopolitan tick *R. sanguineus* is mediated by CO₂. The host urine, tick faeces, or their constituent compounds such as guanine, xantine and hipoxantine are not arrestant for this species. To ascertain whether *R. sanguineus* shows different behaviors against resistant (beagle) and susceptible (English cocker spaniel) dogs, five animals of each breed were maintained in a kennel whose walls were infested with ticks. Five times more ticks were found on cocker spaniels than on beagles. Substances were collected by rubbing pieces of clean flannel on the dogs and these were tested for arrestment and attractiveness to the ticks. Three choices were offered: cocker extract vs. control; beagle extract vs. control, and cocker extract vs. beagle extract. When allowed to choose between substances rubbed from dogs and a control, more ticks were arrested by extracts from the cockers than from beagles. In the arrestment tests with only a choice between substances from dogs of each breed, more ticks were arrested by cocker substances. To test for attraction/repellence, capsules containing adsorbent were used and the tests were carried out in a Y-olfactometer. The ticks were not attracted to the odor of either breed; however the odor of the Beagle was repellent. These results indicate that *R. sanguineus* can use substances from the dogs to differentiate susceptible English Cocker Spaniels from resistant Beagles. *A. cajennense* also demonstrates appetence to CO₂ and equine odors. Contrary to other *Amblyomma* species, the pheromone (AAAP) produced by fed males of *A. cajennense* neither acts as an attractant nor causes aggregation of males and females. The only role

shown to be played by the pheromone was to increase the attachment of adults. All compounds already identified in AAAP of *Amblyomma* were also observed in *A. cajennense* extract, such as benzoic, nonanoic and salicylic acids and methyl salicylate. However, o-nitrophenol, which is responsible for attraction and aggregation behavior in the genus *Amblyomma*, was not found in *A. cajennense*.

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Practical Application of Olfactory Cues for Monitoring and Control of *Aedes aegypti* in Brazil

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Existing control programmes of the mosquito *Aedes aegypti* rely on larval surveys for population information, but this is often unreliable and becomes available too late for the application of control measures to have a significant impact on disease transmission. Olfactory cues from oviposition sites are now being used to lure gravid *A. aegypti* females into sticky traps for population assessments and indices of risk of dengue. The development of the so-called Mosquitrap which incorporates an oviposition attractant is described and its efficiency relative to more traditional monitoring methods is discussed. Semiochemical attractants have also been isolated and identified for blood-seeking female *A. aegypti*. These have been formulated for controlled release (“BG-Lure”) and incorporated into novel trap designs (“BG-Sentinel”) with much improved capture of *A. aegypti* compared to the standard traps that have been used previously. Both trapping systems are now being used for monitoring and surveillance of these important disease vectors. The Mosquitrap is also an important corner-stone of an online GIS-based surveillance system called MI Dengue (Dengue Intelligent Monitoring System) which is beginning to be implemented in Brazil. Results to date have demonstrated that the accuracy of its predictions relating to dengue risk is much greater than that obtained using more traditional methods. Real-time decision making related to vector management is now possible using the new surveillance systems which should greatly impact the management of the disease. At the moment, 45 Brazilian cities are using such system for monitoring female *A. aegypti* base on olfactory cues.

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[2] Kröckel U, Rose A, Eiras AE & Geier M (2006) New tools for surveillance of adult yellow fever mosquitoes: Comparison of trap catches with human landing rates in an urban environment. *J Am Mosq Control Assoc* 22:229-238.

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Searching for Aggregation Pheromones in the Haematophagous Bug *Triatoma infestans*

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Triatoma infestans is the main vector of Chagas disease in South America. Insects of this species show an aggregation behaviour mediated by olfactory cues present in their dry faeces and by contact chemoreception cues present on their cuticle [1, 2, 3]. In this work, we present the analysis of the chemical composition of these two signals, as well as their effect on the assembling behaviour of *T. infestans*. We found that recently deposited faeces do not induce aggregation on the insects. Instead, they induced rejection by the insects. This response changed to attraction after 3 h of faeces deposition. Interestingly, only insects starved for more than eight hours assembled around dry faeces [3]. This attractiveness persisted for 10 days and could be recovered by the rehydration of faeces. Using GC-MS and SPME technique, the volatiles emitted by faeces were sampled for six days and identified. Afterward, they were tested with bugs through behavioral bioassays. All substances tested, i.e., 2, 3-butanediol, acetamide, acetic, isovaleric and hexanoic acids, showed behaviour modifying effects at particular doses. In addition, a blend made of these substances showed to be attractive in tests performed to individual insects. We also investigated the aggregation response of *T. infestans* larvae after exposure to different amounts of either total epicuticular lipid extracts or their fractions. Insects significantly aggregated around the total extract of epicuticular lipids. Nevertheless, only the free fatty acid fraction promoted a significant aggregation on the bugs. The behavioural tests with different doses of single fatty acid showed that hexadecanoic acid did not induce a significant aggregation, but a significant assembling effect was observed with octadecanoic acid. hexacosanoic acid was significantly attractive at low doses, although a repellent effect was observed at higher ones [4]. Our results suggest that blends of these substances may be useful for the development of baits for triatomine bugs. These lures may show useful for allowing an early detection re-infestation process, a highly relevant aspect of Chagas disease control programs.

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Triatomine Sexual Behaviour is Mediated by Pheromones

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Triatomine bugs transmit *Trypanosoma cruzi*, the etiological agent of Chagas disease, to humans. This health burden affects more than 15 million people in Latin America. We show here that the sexual behaviour of several triatomine species is mediated by pheromones. We characterized a sequence of behaviours that mediate male/female encounters and mating. Furthermore, we identified an assembly of compounds secreted by the metasternal glands (MG) of *Triatoma infestans*, *Triatoma brasiliensis* and *Rhodnius prolixus*, suggesting that they act as sexual pheromones. SPME assays showed that *R. prolixus* females emit MG odours during the night. In response to these, sheltered *R. prolixus* males were activated, i.e. left their refuges and showed increased activity. On the other hand, females did not respond to male emitted odours. *R. prolixus* males presented oriented take-off towards airstreams laden with female odour and this behaviour was triggered by MG signals. Olfactometer experiments revealed that walking *T. brasiliensis* males choose female odour laden airstreams and female MG secretions were necessary for the expression of this behaviour. Similar results were obtained with *R. prolixus* males tested on a locomotion compensator. Mating pairs of *T. infestans* and *R. prolixus*, known to promote the aggregation of other males, also emitted MG odours. Experiments showed that mating success is drastically affected by the occlusion of MGs of *R. prolixus* males or females. Moreover, the occlusion of female MGs abolished male aggregation. GC-EAD studies determined that several MG compounds promote antennal responses in *T. brasiliensis* males. In conclusion, we suggest that the volatile blends produced by triatomine MGs act as sexual pheromones. We suggest that MG odours may be used for developing Chagas disease vector detection or capture tools.

Acknowledgements: CAPES-MYNCyT (Argentina-Brazil), FAPEMIG, FIOCRUZ, INCT Entomologia Molecular-CNPq (Brazil), SIDA (Sweden), CONICET and UBA (Argentina).

Symposium: Pheromone Chemistry

Coordinator: Paulo H. Zarkin

Larval Secretion of *Chilecomadia valdiviana* (Lepidoptera: Cossidae): Identification, Synthesis, and Biological Activity

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The carpenterworm *Chilecomadia valdiviana* (also known as butterworm) is a polyphagous insect native to Chile. It is associated with bushes and trees, among them economically important species like eucalyptus, apple, or avocado trees. The larval stage feeds on the wood of the host species, weakening the bole and making it more susceptible to wind breakage. In the course of a study aimed at the identification of the sex pheromone of adult *Ch. valdiviana*, the composition of volatile compounds produced by the larvae was analyzed and a possible function of the secretion was investigated.

As shown by gas chromatography–mass spectrometry, the larval secretion contains saturated and unsaturated straight-chain acetates, with (*Z*)-5,13-tetradecadienyl acetate and dodecyl acetate as the main components, while the corresponding alcohols are also present in a minor proportion. The identification and the synthesis of the compounds will be presented. Laboratory bioassays showed that the extract had no adverse effect on the growth of different species of phytopathogenic and entomopathogenic fungi. Similarly, no effects on the growth of gram-positive and gram-negative bacteria (one species each was tested) were observed. However, when tested in a Y-tube olfactometer, larvae were attracted to their own secretion, suggesting a possible role of the secretion in intraspecific communication. Possible implications for the biology of the species will be discussed.

Acknowledgements: The authors thank Dr. Ricardo Ceballos (INIA Chillán, Chile) for carrying out the assays with entomopathogenic fungi, and Dr. Cristian Mandiola for carrying out the assays with bacteria and phytopathogenic fungi. Financial support was provided by the Dirección de Investigación of Pontificia Universidad Católica de Valparaíso. MFF is grateful for a doctoral fellowship provided by CONICYT and LJR for a doctoral fellowship provided by MECESUP (Project UCH0601).

Evidence for an Unusual Pheromone from a South American Coreid Bug, *Phthia picta* (Hemiptera: Heteroptera: Coreidae)

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When disturbed, true bugs (Heteroptera) characteristically produce defensive secretion from metathoracic scent glands (MSG). The MSG consists of a pair of lateral accessory glands (LAGs), each of which is connected to a median reservoir by a duct. Typically the LAGs produce acetate or butyrate esters that are enzymatically cleaved in the reservoir to derive the final more irritating aldehydes and acids. However, in some heteropterans (e.g. Alydidae and Lygaeinae) males or females produce unusual esters, in addition to esters commonly produced by both sexes, and release these ester mixtures via the MTG openings directly to the outside bypassing the median reservoir. In these broad-headed and seed bugs, respectively, the ester blends are aggregation pheromones (in male-produced species) or sex pheromones (in female-produced species). Here we report evidence for a new chemical type of pheromone produced from the LAGs of male *Phthia picta* coreids. Esters were not detected in extracts of the LAGs in males of this species, even though hexanal and hexanoic acid were present in the median reservoir as in females, and the LAGs of females produced relatively large amounts of hexyl acetate. Instead of producing hexyl acetate in their LAGs, males produce the unusual hydrocarbon, 5,9,12,16-tetramethyleicosane. This tetramethyleicosane was also abundantly present in aeration extracts of live *P. picta* males, among other compounds, but was absent from aeration extracts of live females. Gas chromatography-electroantennogram detector (GC-EAD) experiments showed that the antennae of females responded solely to 5,9,12,16-tetramethyleicosane. Final confirmation of pheromonal activity awaits field-testing of synthetic 5,9,12,16-tetramethyleicosane.

Pheromones for Cerambycid Beetles in the Subfamily Prioninae

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There are ~35,000 described species of cerambycid beetles, many of which are important pests of timber and orchard trees. They are particularly important as invasive species because the long-lived larvae are readily transported between continents in wooden packing materials and wood products. Evidence from a handful of species within the subfamily Prioninae suggested that female beetles in this subfamily produce powerful sex attractant pheromones. We identified nanogram quantities of the sex pheromone of the species *Prionus californicus* as 3,5-dimethyldodecanoic acid,¹ which is present along with a number of analogs in extracts from female beetles. Field tests have shown that the pheromone is highly attractive to males, that the mixture of all four stereoisomers is as attractive as the pure stereoisomer produced by the beetles, and that the analogs do not appear to increase or decrease attraction. Nonstereoselective and stereoselective syntheses of the pheromone and the analogs will be described. Because this beetle is a major pest of hops and a sporadic pest of orchard crops in the western US, the pheromone is being commercially developed by several companies for monitoring and possible control of the beetles. Furthermore, this pheromone structure appears to be highly conserved within the genus, because at least 8 congeneric species from locations across North America are attracted to lures baited with 3,5-dimethyldodecanoic acid.

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This work was supported by grants from the Hop Research Council, USDA/Western Region IPM grant number 207-03623, the USDA National Research Initiative Arthropod and Nematode Biology and Management Program grant 2006-35302-17457, and the Alphawood Foundation.

The Pheromone Chemistry in the Laboratory of Semiochemicals at the Federal University of Paraná

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The Laboratory of Semiochemicals at the Federal University of Parana (UFPR) in Brazil was established in 2002, and the aim of the studies conducted in this laboratory are: study the biological behavior of the insects when different odors sources are used; isolate and identify the semiochemicals involved in the communication system; accomplish bioassay to evaluate the biological activity of the semiochemicals; study the mechanism of plant-insect interaction as well as synthesize the identified pheromones and other semiochemicals. During eight years several species were studied including lepidopteran like *Lonomia obliqua* (Saturniidae), *Bonagota salubricola*, *Crociosema aporema*, and *Grapholita molesta* (Tortricidae), the hymenopteran *Paravespula vulgaris* (Eumenidae) and the coleopteran *Sphenophorus levis*, *Sternechus subsignatus* and *Pseudopiazuros obesus* (Curculionidae). There are ongoing studies with *Anastrepha fraterculus* (Diptera: Tephritidae), *Prionus californicus* and *Hedypathes betulinus* (Coleoptera: Cerambycidae), *Cratosomus subfasciatus* and *Oryzophagus oryzae* (Coleoptera: Curculionidae), *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), *Diatraea saccharalis* and *Condylorrhiza vestigialis* (Lepidoptera: Crambidae), and *Loxa deducta*, *Pellaea stictica* and *Pallantia macunaima* (Hemiptera: Pentatomidae). Several advances are still expected in studies aiming at the application of the semiochemicals as a control of pest-insects, as well as the use of these compounds to increase the activity of the natural enemies. This presentation explains some of the main results achieved with the insects studied in our laboratory.

Acknowledgments: INCT de Semioquímicos na Agricultura, CNPq e CAPES.

Symposium: Plant-insect interactions

Coordinator: Mauriscio S. Bento

Plant Volatiles in Pest Management: to Attract or Repel?

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Plants are able to produce an enormous diversity of chemical compounds that are released into the atmosphere. Sufficient evidence has been accumulated in recent years showing that plant volatiles can repel or attract herbivores, and are also responsible for attracting natural enemies, parasitoids and predators of herbivores, both in aerial as in roots parts. In addition, changes in the emission of volatiles in plants after infection by pathogens have been demonstrated, affecting the insect vectors and possibly their natural enemies, although few studies have been reported in this direction. A view of these interactions and possibilities for use these volatile in the insect pest management will be discussed.

Support: *INCT de Semioquímicos na Agricultura (CNPq and Fapesp)*

Root Herbivores Interfere with the Development and Behavior of Parasitoids of Foliar Herbivores: Effects, Mechanisms and Future Steps

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For many years, ecology has been dominated by studies of communities focusing on bi/tri-trophic interactions on aboveground systems, involving plant-herbivore and plant-herbivore-parasitoid associations, whereas the soil has been considered as a “black box”. More recently there has been increasing interest to cross the barrier between the soil and aboveground domains. However, most studies focus on interactions between soil organisms directly related with the plant-root and leaf herbivorous insects, overlooking potential consequences for parasitoids. Using as a model system the wild cruciferous plant *Brassica nigra* L. (Brassicaceae), we studied the effects, and mechanisms, of root herbivory by *Delia radicum* L. (Diptera:Anthomyiidae) on the performance and behaviour of the parasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae), the parasitoid of *Pieris brassicae* (Lepidoptera: Pieridae). I will present cases studies where root herbivory interfere (1) with the growth and development of the parasitoid; the parasitoid developed slower and attained a smaller size when parasitizing hosts feeding from root-infested plants. The foliage of *B. nigra* plants attacked by *D. radicum* belowground possessed two-fold higher concentrations of 2-propenyl glucosinolate (sinigrin) than the foliage of plants that were not exposed to the root herbivore, while shoot biomass and primary plant compounds remained similar in plants with and without root herbivores. Root herbivory also influenced the plant preference and foraging efficiency of the parasitoid via the shared host-plant (2a), but more interestingly, via the surrounding environment (2b). Our results revealed that *C. glomerata* is able to distinguish between plants on the basis of the presence or absence of root herbivores, and preferred root-undamaged plants. Interestingly, the volatile blend of plants exposed to root herbivores was characterized by low levels of known-attractant compounds and high levels of specific sulphur volatiles. Remarkably, the foraging behavior of the parasitoid was also strongly influenced by the presence of root-herbivores in the surrounding environment (2c). The parasitoid females located the

host-infested plants significantly faster in the environments where the root-herbivore was present compared with the environment composed entirely by root-undamaged plants. This shows that cues triggered by antagonistic root-associated organisms might play a key role influencing the behaviour of aboveground parasitoids, even when the root herbivore and the parasitoid host feed from different plants.

Finally, I will discuss how soil organisms, whose presence passes habitually unnoticed, can participate with the interactions of plants, aboveground herbivores and their parasitoids, generating complex processes that still are far to be unraveled.

Synthetic Mixes of Floral Volatiles Used as Tools to Guide Honey Bees to Specific Crops

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Honeybees *Apis mellifera* are important pollen vectors for many crop plants. In some crops, productivity is ensured only through the pollination service of this insect species. Beekeepers use to condition honeybee colonies by feeding them with syrups containing crushed flowers of the species that pretend to be pollinated. Despite the use of this procedure little is known about the process that guide bees to the flowers of crop plants or about the dynamic and extent to which floral scent contributes to pollinator attraction in agricultural settings. Recent studies show that insect pollinators need only a few volatile compounds to recognize a specific floral scent. Based on this idea, we developed synthetic mixes of volatile compounds that bees confound with the natural floral fragrances of some specific crop plants. Our hypothesis is that memories established by the offering of synthetic-mix odor within the colonies (i) are used to orientate pioneer foraging bees while search for food nearby the crop field; and (ii) facilitate the transference of crop-related information through recruiting mechanisms such as dances. As an example, we will show evidences at the levels of cognition, orientation and navigation that suggest a prompt foraging task in sunflower crops, a fact that opens the possibility for an improved efficiency in pollination and crop yield.

This study was supported by funds from CONICET, UBACYT and ANPCYT grants to WMF. Also a Guggenheim Fellowship supports WMF

Oviposition Preference of *Ceratitis capitata* (Diptera: Tephritidae) to *Coffea arabica* and *Coffea canephora* Cultivars

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Finding a suitable place for oviposition is a challenging task for a female insect herbivore. The decision of the site has positive or negative consequences on their offspring. These consequences may be related to the immature development, survival and fecundity of adults. The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is a high polyphagous tephritid that oviposits and develops in more than 300 species of fruits and vegetables. We have the evidence that medfly oviposits and develops in coffee fruits of *Coffea arabica* but was never found in fruits of *Coffea canephora*. The aim of this study was to evaluate the chemical cues for oviposition of *C. capitata* with combinations of *C. arabica* and *C. canephora* cultivars. The headspace volatiles were collected in all combinations of cultivars and analyzed by GC-MS. To assess the oviposition preference we used three cultivars of *C. arabica* (Mundo Novo, Bourbon Vermelho and Catuaí Amarelo in combination with two cultivars of *C. canephora*, (Conilon and Robusta). At least 10 couples of *C. capitata* were used in cages for different combinations of coffee fruits. When females reached a peak of oviposition (the third day after emergence), rosettes containing five mature fruits of each cultivar were introduced in cages. After 44 hours, the rosettes were removed from the cages and the eggs counted. The headspace volatiles analysed by GC-MS shows a strong divergence in the chemical profile of coffee species. *C. arabica* cultivars were strongly preferred to oviposition when compared to the *C. canephora* cultivars.

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Symposium: Applications of Semiochemicals in Latin America

Coordinators: Andrés González and Carmen Rossini

Semiochemicals in Pest Management in Colombia

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The research and use of semiochemicals for agricultural pests management in Colombia begun in the late 70s with the use of pheromone traps for the detection and monitoring of pests in cotton and fruit crops. Traps with the synthetic sex pheromone “gossypure” were employed to catch the pink bollworm, *Pectinophora gossypiella*, and the strategy of mating disruption was evaluated. Management traps with the aggregation pheromone “grandlure” were also used for the boll weevil, *Anthonomus grandis*. Detection of the Mediterranean fruit fly *Ceratitidis capitata* was carried out as a national program developed around the border areas, using Steiner traps with the attraction pheromone Trimedlure. In the 80s and 90s Integrated Pest Management research programs were developed to be implemented on different crops through the use of pheromone traps for the surveillance and monitoring of pests such as the potato tuber moth, *Phthorimaea operculella*, the armyworm, *Spodoptera frugiperda*, the tomato fruit borer, *Neoleucinodes elegantalis*, the palm weevil *Rhyncophorus palmarum*, the tomato leaf miner, *Tuta absoluta*, and the Guatemalan potato moth, *Tecia solanivora*. In all these cases imported synthetic pheromones were used and only during the last decade, Colombian research groups have been created in institutions such as Corpoica, Universidad Nacional and Universidad de los Andes, which in alliance with foreign research groups such as the Agricultural University of Sweden, the University of Goettingen in Germany, and the National Semiochemicals Institute of Brazil, are focusing their research on topics such as identification of volatiles from potato plants (*Solanum tuberosum*) as attractants of females of *T. solanivora*, attraction and repellency technics (push-pull) for *T. solanivora* in potato and for *S. frugiperda* and *Copitarsia decolora* in cape gooseberry (*Physalis peruviana*), and in medical entomology research on sensorial ecology of blood sucking insects as *Rhodnius prolixus* and *Lutzomyia longipalpi*.

Arthropods Chemical Ecology of Economic Importance in Southern Chile

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From 2007 salmon culture, fruit growing and food industry are considered by the Chilean government as economic development areas (cluster). The natural Chile isolation: Atacama Desert, the Andes Range, Glaciers, and the Pacific Ocean along with a rich geography and diverse climate, have given a wide range of industries such as fishing, copper, wines and farming products. Everything is in line with high levels of efficiency and competitiveness. Nowadays, Chile is the main berry exporting country in the southern hemisphere and the second biggest one in both raspberry and salmonid species production worldwide, and natural beef production is one of the emerging markets. However, berries, salmonid species, cattle and forage plants are heavily attacked by insects and crustacean. Because of the problems associated with the use of synthetic pesticides, including environmental degradation, development of resistance, and chemical residues, there is a growing use of semiochemicals (ethologic control) in integrated pest management (IPM). Therefore, the Chemical Ecology Group of the Universidad de La Frontera has focused their studies on insects and crustacean pest of economic importance.

I will show the chemical ecology advances of the: a) raspberry weevil, *Aegorhinus superciliosus* (Guerin) (Coleoptera: Curculionidae), a native species from southern Chile and Neuquen district of Argentina. In Chile, the species is a pest of blueberries, raspberries, and similar berry crops [1], b) root borer *Hylastinus obscurus* (Coleoptera: Scolytidae), the most important pests of red clover (*Trifolium pratense*) throughout the world, c) horn fly, *Haematobia irritans* (Diptera: Muscidae), the major global hematophagous pest of cattle, and d) copepod *Caligus rogercresseyi* (Copepoda Caligidae), the dominant species affecting aquaculture, which is present in 99% of the established cultured cages, and is distributed on both salmonid and non-salmonid hosts [2].

Chemical substances involved in both intra and inter specific relationships will be reviewed, and behavioural and molecular methodologies will be discussed.

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Monitoring the Neotropical Brown Stink Bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with Pheromone-baited Traps in Soybean Fields

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The effectiveness of the synthetic sex pheromone of the Neotropical brown stink bug, *Euschistus heros*, was evaluated both in laboratory and field assays. Lures loaded with 1 mg of methyl 2,6,10-trimethyltridecanoate continuously attracted female bugs for more than thirty days to pheromone-baited traps in field trials. The pheromone-baited traps were effective in field tests even at low bug population densities, as compared with the usual monitoring technique, shake cloth sampling. Traps around borders or in the centre of soybean fields caught similar numbers of bugs. Trap captures showed a positive relation with field populations, as monitored with the shake cloth technique, during the reproductive phase of the soybean crop, i.e., from the R1 to R5 developmental stage (pod formation to pod fill). The physiological state of the trapped migrating insects was determined. The first insects arriving in the field had fewer eggs in the reproductive tract compared to later arrivals. Some cross-attraction was also observed, with *Piezodorus guildinii* and *Edessa meditabunda* also being caught in pheromone-baited traps, suggesting that these insects respond to the sex pheromone or to the defensive compounds released by *E. heros* captured in traps. The results showed that traps baited with 1 mg of the sex pheromone efficiently caught bugs, that the lures lasted for more than one month under field conditions and that placement of traps around the borders of the crop area was as effective as placement inside the crop area. Border-placed traps were effective at a density of one trap every 200 meters.

Use of Sex Pheromones to Characterize the Spatial Distribution of *Argyrotaenia sphaleropa* and *Bonagota salubricola* (Lepidoptera: Tortricidae) in Southern Uruguay

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Argyrotaenia sphaleropa and *Bonagota salubricola* are native polyphagous^{1,2} pests found with yearly variations on fruit trees and in vineyards³. A sex pheromone⁴ synthesized locally is used to bait traps for monitoring them. Knowledge of their spatial-temporal distribution optimizes the adoption of control measures in micro areas, while minimizing the environmental impact of the measures. Contemporary geostatistical tools allow analysis of large databases of insect captures to describe the spatial distribution of these tortricids. The objective of this research was to characterize the spatial distribution of these tortricids by geostatistical methods, in the south fruit tree area using pheromone traps. In apple and pear orchards, 130 pheromone traps per species were separated by 500 and 1000m in 50.000has, forming a grid. The traps were georeferenced and checked weekly during consecutive field seasons (september-april, 2007-2010). The accumulated captures were used to develop spatial analysis for the three seasons, using the geostatistic software (GS+). The model was validated by the method of maximum verisimilitude (SAS software). A variogram and a spatial distribution map were obtained for each species and for each season. Spatial autocorrelations were significant in most cases. The populations of both species were very low in the first two years, varying according to the area. The larger populations of *A. sphaleropa* were found in Canelon Chico, Juanico, and Progreso, probably related to the proximity of vineyards. *B. salubricola* was focused in Melilla, although in the last two years the populations grew toward the north. These data show the annual variations in infestations by these pests. Permanent monitoring is recommended to determine the risk population level.

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Mass Trapping as a Control Strategy for Coleopteran and Lepidopteran Pests

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Pheromone-based mass trapping is established as an economical method of management for Coleoptera such as palm weevils, banana weevils, coconut rhinoceros beetles and bark beetles. This strategy is presumed to be successful because these insects produce and respond to aggregation pheromones making it possible to remove both sexes from a crop. Pheromone-based mating disruption is usually the preferred method of used in management of Lepidopteran pests. This presentation will give examples of operational pheromone-based mass trapping of Coleoptera, a proposal that mass trapping of Lepidopteran pests should be more economical than mating disruption, and an example of mass trapping of a Lepidopteran pest of tomatos, *Tuta absoluta* M.

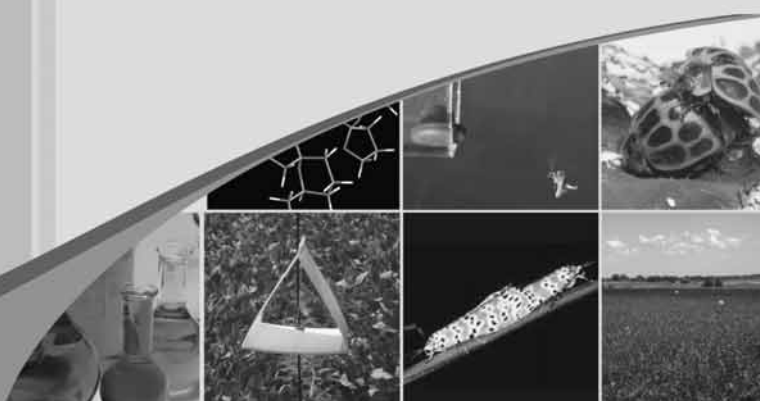
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ORAL PRESENTATIONS



ALAEQ



Role of Cuticular Lipids on the Mating Behaviour in *Triatoma infestans* (Heteroptera: Reduviidae)

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Cuticular lipids in insects have an important role in chemical communication. In fact, they are involved in aggregation behaviour [1], sexual attraction and mate recognition as contact pheromones. We studied bugs' behaviour to determine whether a cuticular chemical cue, acting as contact sex pheromone, is present in the haematophagous bug *Triatoma infestans*. The aim of this work was to analyse the role of cuticular lipids during the mating behaviour of this species. Under controlled experimental conditions, a female submitted to any of the following treatments was presented to a male: alive; dead (experimentally killed); washed (dead and their cuticular lipids removed with hexane) and painted (dead, their cuticular lipids removed and afterwards painted with an equivalent of one female hexane extract). Mating attempts frequencies were quantified for each treatment. As expected, the highest frequency (94%) of mating attempts was observed in presence of an alive female. Mating attempts were observed in half of the dead females. In the washed treatment, the frequency of mating attempts decreased and males only tried to mate in 24% of the assays. When males were in presence of a painted female, no significant differences were found between this result and the mating frequency observed with dead females. Meanwhile, males in assays with dead or painted females showed significantly higher frequencies of mating attempts than in presence of washed ones. As shown for *Rhodnius prolixus* [2], behavioural evidence revealed that cuticular lipids of the female are involved in sex recognition by the male during the mating behaviour of *T. infestans*.

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- [2] Lorenzo-Figueiras AN & Manrique G (2009). Are Cuticular Lipids Involved in Mate Recognition in *Rhodnius prolixus* (Heteroptera, Reduviidae)? *Acta del VI Encontro Brasileiro de Ecologia Química*. p. 73.

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Host Mediation in a Mistletoe's Phenotype

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Parasitic plants are physically linked to their hosts through the haustorium, through which they derive water, nutrients and a variety of primary and secondary metabolites from their hosts. Through this intimate connection, hosts have the capacity to modify genetically unrelated individuals, a phenomenon which has been described as phenotype canalization. In a previous study, we showed that the hemiparasitic mistletoe *Tristerix verticillatus* (Loranthaceae) sympatrically growing within a small area (and hence exposed to similar abiotic and biotic conditions) on three different hosts (*Schinus montanus*, *Fabiana imbricata* and *Berberis montana*) could be discriminated as distinct chemical populations on the basis of their volatile chemical profiles. We continue to address the phenotypic canalization idea by examining the influence of these three host species on flower and fruit phenotype traits (colorimetry, morphometry, and nectar and fruit pulp sugar composition) of this single mistletoe species. Flowers and fruits were detached from the plant, kept in moisturized cotton in separate vials and photographed against a millimeter-grid paper. The flower measurements taken were: flower length, flower tip diameter, mean open-colla diameter, pistil length, stamen length and anthers length and width. Fruit measurements were: weight, length and diameter. Nectar and fruit pulp samples were collected in the field, diluted and filtered under laboratory conditions, and analyzed by HPLC coupled with an RI (refractive index) detector. Finally, reflectance measurements were taken on flowers and fruits with the use of a optic fiber spectrophotometer (colorimetric variables: brightness, hue and chroma). Our results reveal that the three host species have a significant influence on some flower and fruit phenotypic traits. The relevance of our results for the interactions maintained between the mistletoe and their pollinators and dispersors are discussed.

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Into the Wild Sensory World of a Crepuscular Florivore; the Use of Olfactory and Visual Floral Cues in the Flower-Visiting Behavior of *B. orientalis* (Blattodea: Blattidae)

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In plant-insect interactions such as pollination, signals of different sensory modalities (olfactory, visual, tactile, etc), mediate the establishment of reciprocal influences between flowering plants and their insect visitors [1]. In this work we studied the relevance of visual and olfactory stimuli from flowers of the evening primrose *Oenothera acaulis* (Onagraceae) to the flower-visiting behavior of a non-native florivorous insect, the cockroach *Blatta orientalis* (Blattoidea: Dictyoptera), in coastal central Chile. We performed manipulative experiments that decoupled visual (corolla) and olfactory (fragrance) stimuli utilizing paper corollas and green mesh bags, with or without a freshly-picked natural flower inside. We contrasted the behavioral responses of roaches to these treatments with those to the natural combination of traits and their respective controls, measuring the frequency of visits, mean and total residence time spent on the treatments by the cockroaches. We found that *B. orientalis* were mostly attracted towards treatments that included the floral fragrance, thus these animals utilized mainly olfactory cues when approaching *O. acaulis* flowers. Nevertheless the presence of a paper corolla increased the time spend on artificial flowers, and then visual cues would augment the conspicuousness of these flowers. In addition, we discovered that the presence of conspecifics had a strong influence on cockroach flower-visiting behavior. Our results suggest the existence of a hierarchy in the use of flower-derived stimuli in *B. orientalis*. Olfactory cues were the primary attractant for these insects; meanwhile visual cues corresponded to a secondary source of flower-derived signals, which must be coupled with olfactory cues to have any relevance as multimodal stimuli for cockroach. In our study system, olfactory and visual stimuli may act in synergy [2] in the attraction of introduced roaches to a novel food source.

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Acknowledgements: We thank F. Salinas and A. Villagra for their help finding *Oenothera acaulis* populations in Coastal Central Chile. Also we thank S. Herrera and R. Yury for their help in the field experiments. We are in debt with J. C. Ortúzar for his helpful statistical advisory, J. Alcayaga, M. Cannals and D. Dominguez, A. Jimenez and R. Suarez for their advices and comments on this work. This research was funded by postdoctoral FONDECYT grant N° 3095002 to C. A. Villagra, NSF grant DEB-0317217 to R. A. Raguso. and ICM P02-005 fund to Instituto de Ecología y Biodiversidad.

Ontogeny of a Chemical Cue in a Parasitoid Dipteran

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The robber fly *Mallophora ruficauda* is an ectoparasitoid of scarab beetle larvae. As adults they prey on honey bees, while as larvae they parasitize mainly third-instar larvae of *Cyclocephala signaticollis*. Females of *M. ruficauda* lay eggs away from the host on tall grasses. After being dispersed by the wind, larvae drop to the ground and search for their hosts. The hosts are readily available during summer time, but they are not fully grown when the parasitoid larva appears. It is known that second-instar larvae of *M. ruficauda* exhibit active host searching behaviour towards their host, but it is unknown if they can orientate to the first two host instar odours. The aims of this work were to characterize the chemical profiles of the three larval instars of *M. ruficauda*, and to determine if the parasitoid larva orientates to odours from these instars with different levels of intraspecific competition. For this work, we performed GC-mass analyses and behavioural experiments in the laboratory using host body extracts. We found that the chemical profiles of the three host instars have different kinds and amounts of large saturated hydrocarbons. We also found that the parasitoid larva orientates generally to odours from third instar hosts. However, under a high competition situation, larvae also orientate to odours from second instar hosts. Finally, when given a choice between second and third instar hosts, parasitoid larvae orientate to third instar odours when competition was low, but similarly to both odours under high competition. Our results show that although *M. ruficauda* orientates preferentially to third instar host odours due to its chemical properties, and its behavioural decisions are also influenced by external factors such as the level of competition.

Behavioral Studies and Gender-Specific Characterization of Volatiles in the South American Rice Weevil, *Oryzophagus oryzae* (Coleoptera: Curculionidae)

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Rice production has become an important economic activity in the Uruguayan economy. The rice water weevil, *Oryzophagus oryzae*, is an important pest that affects production yields and rice quality, due to the attack of both mature and immature stages.

Previous studies about the chemical communication in this family have shown a general pattern of emission of aggregation pheromones by the males, attractive to both males and females, and modulated by the presence of the host plant.

In this study we present the chemical analysis of volatiles emitted by virgin *O. oryzae* males and females, as well as behavioral assays of cross-gender attraction. Rice plants were collected in the field and *O. oryzae* pupae were separated from the roots and maintained in water at room temperature until emergence of the adults. The adults were immediately sexed and separated, and their volatiles were adsorbed in Haysep-Q in the presence of rice plants. The volatiles were analyzed by gas chromatography coupled with mass spectroscopy (GC-MS). Behavioral assays were performed in a Y-tube olfactometer, using live males in rice plants as the stimulus and plants as control.

Two male-specific compounds were tentatively identified by their mass spectra and retention index. In the behavioral studies, males showed a tendency toward co-specific males (not significant), which was not the case in the females. Pheromone studies in *O. oryzae* may provide tools for the development of a management strategy for this species in rice crops in Uruguay.

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Catalysis in the Synthesis of Insect Pheromones: Preparation of Dominicalure I, Sexual Pheromone of *Rhyzopertha dominica*

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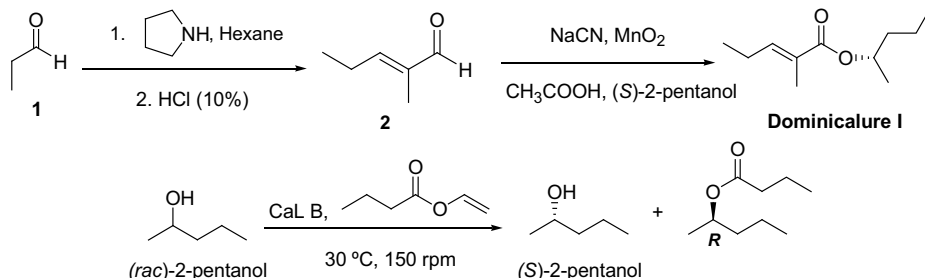
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Insect pheromones play important roles in intraspecific chemical communication, being valuable tools for pest management programs. Many of these compounds have high stereochemical purity, which is essential for activity. Although pheromone chemistry has been widely developed during the last decades, stereoselective syntheses are still challenging tasks for classical organic chemistry [1].

Here we report a strategy that combines both bio- and organocatalytic approaches for the stereoselective synthesis of Dominicalure I, sexual pheromone of *Rhyzopertha dominica* [2].



The key step in the synthetic route to Dominicalure I was the pyrrolidine-catalyzed self-condensation of propanal, giving the corresponding α,β -unsaturated aldehyde 2. Further oxidation under Corey conditions using (S)-pentanol as solvent furnished stereochemically pure Dominicalure I [3]. Enantiomerically pure (S)-pentanol (> 99%) was obtained through lipase-catalyzed kinetic resolution of the corresponding racemic mixture, under solvent-free conditions.

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Support for this work from PEDECIBA (Programa de Desarrollo de Ciencias Básicas) and ANII (Agencia Nacional de Investigación e Innovación) is gratefully acknowledged.

Identification of Male Sexual Pheromone in *Steirastoma breve* (Coleoptera: Cerambycidae)

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The cocoa beetle, *Steirastoma breve* Sulzer (Coleoptera: Cerambycidae), is one of the main pests of cocoa in Venezuela. Previous work showed that female beetles were attracted to male adults in olfactometric experiments. Analyses of male prothorax extracts by gas chromatography (GC) linked to electroantennographic (EAG) recording from a female beetle antenna showed two male-specific components. Only one of them was identified as 6,10-dimethyl-5,9-undecadien-2-ol using GC-mass spectrometry (MS) and RI in chromatographic columns of different polarities. Electrophysiological activity of this component and their isomers was confirmed by EAG bioassays. Both isomers of this alcohol showed significant electroantennographic activity compared with the respective controls. Additionally, 6,10-dimethyl-5,9-undecadien-2-ol was isolated from individually male prothorax using solid phase microextraction (SPME). We propose this technique like an alternative to quantify the components of sexual pheromone in this species per individual.

This research was financially supported by the “Proyecto de Investigación en Red en el marco de la Ruta del Chocolate N° 200500898; Subproyecto 7: Estudio y validación de alternativas de control etológico de insectos plagas del cacao: *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae) y *Carmenta foraseminis* (Busck) (Lepidoptera: Sesiidae) en el estado Miranda”, and GID-012 from the Universidad Simón Bolívar.

On the Function of Phytochemical Diversity in Plant Defense

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Several models have been proposed to explain the usually rich phytochemical diversity in plant tissues and populations. Two of them are at extremes: at one end one model postulates that herbivore and pathogen (henceforth Plant Consumers) pressure selects for high phytochemical diversity and high secondary metabolite (SM) concentration (the adaptive diversity hypothesis)¹, thus, high phytochemical diversity and high SM concentration protect plants. On the other end, other model postulates that one or two “effective” SM’s fend off all plant enemies and all the other SM’s within the plant are useless (the screening hypothesis)². We present experimental or correlative evidence showing that phytochemical diversity functions differently for fungi and insect herbivores independently of their specialization on the host. Experimental maize SM mixtures of increasing richness do not necessarily reduce growth or oviposition of maize plant consumers; specific compounds inhibit some plant consumers whereas specific mixtures are effective against some others. Correlative patterns among *Persea americana* phytochemical diversity and some of its major pests and pathogens suggest that some plant consumers are insensitive to phytochemical diversity of Hass avocado plants or orchards, whereas other plant consumers are affected by either phytochemical diversity of the plant or the orchard. The incidence of the specialist leaf gall insect *Trioza anceps* on *P. americana* var. *drymifolia* (criollo avocado) planted in germplasm banks suggests that foliar phytochemical diversity functions based on specific compound combinations that affect various life stages of the insect. None of these results support the screening hypothesis, whereas some support partially the adaptive hypothesis. Apparently, the antagonistic relationships among plant tissues and populations and their consumers are mediated more by specific SM or mixtures of them than by increasing or decreasing phytochemical diversity.

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Plant-Insect Interactions in Soybean Crops: Plant Responses to Stink Bugs (*Nezara viridula*) Attack

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Soybean (*Glycine max*), the world's most widely grown seed legume, can be attacked by *Nezara viridula* that decreases crops yields and quality of seeds. Soybeans have constitutive and inducible defenses against insect attack, such as proteinase inhibitors (PI) and isoflavonoids, which decreases amino acids assimilation in the gut and, in turn, insect performance. These chemical defenses are regulated by the hormones jasmonic acid (JA) and ethylene (ET). While ET in soybean seeds is regulated by the expression of 1-aminocyclopropane-1-carboxylate synthase (*acc-s*), JA is regulated by lipoxygenase 1, 2 and 3 (*lox1*, *lox2* and *lox3*). However, insects can suppresses plant defenses through manipulating the defense hormones ET and JA. To test this hypothesis developing soybean seeds were either i) attacked by adult stink bugs collected from the field, or ii) treated with MeJA, or iii) mechanically damaged, or iv) no damaged (control). Plant material was collected 24h and 72h after treatments, and flash frozen in liquid nitrogen. While only insect attack and mechanical damage increased *acc-s* expression, the highest induced expression of *lox1*, 2 and 3 was found after insect attack. Furthermore, insect attack induced the highest levels of isoflavonoids (daidzin y genistin) in developing seeds. However, although mechanical damage and JA increased *pi* expression, insect attack did not increase *pi* transcript and activity levels. Our results suggest, although *N. viridula* attack suppresses PI elicitation, plants still respond to insect damage increasing isoflavonoid production, which is a good defense against these insects.

Effect of Induced Volatiles in Cotton Plants Damaged by *Anthonomus grandis* and *Spodoptera frugiperda* in the Boll Weevil's Behavior

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The boll weevil, *A. grandis*, is an important pest of cotton in Brazil and its colonization in cotton occurs after the formation of the first flower buds [1, 2]. The aim of this study was to evaluate whether the cotton in the reproductive stage produces volatiles that are involved on the attraction of the boll weevil and if the herbivory damage cotton could produce different blend of volatiles. Volatile collections of plants damaged by boll weevil and *Spodoptera frugiperda* were conducted every 24 hours for four consecutive days. Undamaged plants were used as control. The extracts were analyzed by GC and GC-MS. The chemical profile was evaluated by principal response curves followed by ANOVA. The attractiveness of these extracts to adults of boll weevil was tested in "Y" tube olfactometer bioassays and analyzed by chi-square and paired *t* test. The analysis of volatiles showed that the treatments emitted a volatile blend different over the time from 48 hours onward when compared to the control ($F_{16, 20} = 1.05$, $p = 0.005$). The bioassays analysis of the first choice showed that males responded only to extract from plants damaged by *A. grandis* after 96 hours ($p = 0.016$), while females were not attracted. These extracts, in addition to the plants compounds, showed the presence of the aggregation pheromone. Whereas the analyses of the residence time showed that males and females spent more time in the arm containing extracts from plant damaged by boll weevil herbivore after 24 and 96 h. The extracts of plants damaged by *S. frugiperda* did not show significant difference in the attraction of *A. grandis*. The results suggest that the boll weevil recognize the volatiles from plants damaged by different insects and that could be a synergism with the aggregation pheromone.

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Tritrophic Interaction Between *Capsicum annuum* L., *Aphis gossypii* Glover and *Aphidius colemani* Viereck

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Pest control of cultivated plant species has been usually performed by insecticides, which is undesirable because of economical and environmental concerns, since successive applications affect natural enemies and increase the possibility of development of resistant population toward insecticides. These problems can be minimized with alternative control methods as the use of resistant varieties, use of substances that induce resistance and biological control. Those studies involving the interaction of plant, pest and natural enemies are of fundamental importance. Thus, this study aimed to study the effect of volatile organic compounds in tritrophic interactions between pepper *Capsicum* spp., the aphid *Aphis gossypii* and its parasitoid *Aphidius colemani*. Pepper varieties were evaluated for resistance to the aphid *A. gossypii* and their volatiles were collected before and after infestation. Volatile compounds were tentatively identified by gas chromatography/mass spectrometry. Olfactometry bioassays were performed with volatile regarding the behavior of *A. gossypii* and *A. colemani*. The main conclusions obtained in this work were: a) there is genetic variability among genotypes of *Capsicum* in relation to the release of volatile compounds and in the susceptibility toward *A. gossypii*; b) genotype Cambuci can be used in breeding programs aiming *Capsicum* cultivars more resistant to *A. gossypii*; c) there were significant differences between the effects of volatiles from the two cultivars on behavior of *A. gossypii* and *A. colemani*; d) the volatiles emitted by Cambuci cultivar after infestation produced repellent effect on *A. gossypii* and were attractive to *A. colemani*; e) the genetic variability

between genotypes, after infestation indicates that volatile organic compounds present as variables can be used for selection and development of bell pepper cultivars resistant to the aphid *A. gossypii*.

Fusarium Head Blight: Chemical Signals of Early Infection

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In Argentina, *Fusarium* head blight (FHB) is a major destructive disease of wheat and other cereal cultivars. *Fusarium* species infect wheat during the flowering period; in addition to losses of yield, mycotoxins produced by these fungi in suitable environmental conditions, can threaten animal and human health. Fungi produce volatile organic compounds (VOC), during both primary and secondary metabolism, VOC appear as intermediate and end products of various metabolic pathways, among them mono- and sesquiterpenes, alcohols, ketones, lactones, esters or C8 compounds, can be used for their detection and identification. Solid phase microextraction (SPME) coupled to capillary gas chromatography (CGC) and mass spectrometry (MS) was used to study its potential to detect volatile precursors of mycotoxins released by *Fusarium graminearum* either grown in culture medium or in wheat plants [1]. The aim was to develop a new tool to predict *Fusarium* head blight in wheat cultivars grown in greenhouse or in the field. We also investigated the utility of fungal volatiles to discriminate *Fusarium* species.

The chromatographic profile of *F. graminearum* VOC showed a variety of sesquiterpenes, including trichodiene, major precursor in the synthesis of trichothecene mycotoxins; together with minor amounts of short chain alcohols, esters, ketones, and hydrocarbons.

When wheat cultivars in greenhouse or experimental field assays were artificially infected, trichodiene was detected within 24h after fungal infection, before disease signals are evident. The VOC sesquiterpene pattern of *F. graminearum*, *F. poae*, *F. equiseti*, *F. verticillioides* and *F. oxysporum* was useful in their differentiation. Trichothecene-producer and non-trichothecene producer *Fusarium* spp. were separated by the presence of trichodiene in their VOC fingerprints.

We conclude that this technique is helpful to detect *F. graminearum*, the major head blight disease-producing fungi in the region.

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Defenses of Tropical Freshwater Macrophytes Against Herbivory

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Herbivory show strong impacts on ecosystem and can be important in controlling the structural properties and dynamics of freshwater macrophytes communities. Defense mechanisms against predation in plants are know for marine and terrestrial ecosystem, but few studies are conducted with freshwater macrophyte. The aim of this study was investigate the role of secondary metabolites of 12 freshwater macrophytes of Parque Nacional da Restinga de Jurubatiba (Rio de Janeiro, Brazil) against *Biomphalaria sp.* The assays were conducted with crude extract and reconstituted plants (specimens whose overall plant architecture was scrapped). The secondary metabolites profile of organisms was analyzed by Thin Layer Chromatography (TLC). Our results showed that 9 of the 12 species studied were deterrents against herbivory ($p < 0,05$, statistic test accordin according each experimen). Although most of the extracts were deterrent, the crude extract of *Ceratopteris thalictroides* was the only one to be able to inducing the consumer ($p = 0,01$, Wilcoxon, $N = 21$). Among all species chemically defended, *Potamogeton montevidensis*, *Xyris brevifolia*, *Eleocharis interstincta* and *Paepalanthus sp* showed greatest defense potential against herbivory. The TLC analysis indicated presence of phenolics and terpenoids - known to act as defenses against herbivores - correlated positively with those more deterrent. Tests using reconstituted plants showed that despite the mischaracterization of their architectures, the chemically defended species *E. azurea*, *P. montevidensis* e *Utricularia gibba* maintained its defensive potential, with reduced rates of consumption ($p = 0,001$ ANOVA, $N = 30$). However, the species *Typha domingensis* and *X. brerifolia* that showed defensive properties, were the most consumed ($p = 0,001$ ANOVA, $N = 30$), pointed that despite the production of chemical defenses, other factors such as nutritional content may be involved in preference and consumption by the herbivore. Our results suggest that freshwater macrophytes can show deterrent properties and significantly influence on the food herbivores preference interfering in ecological relationships on ecosystems in which they are inserted.

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Domestication Status Determines Variation of Induced Volatile Organic Compounds of Tomato Plants

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Induced resistance is an example of adaptive phenotypic plasticity which allows plants to respond dynamically to attackers. Induced resistance allows plants to respond dynamically to changing attacker identities and it is more effective than possessing no resistance, and more flexible and less costly than possessing high constitutive resistance [1]. The emission of volatile organic compounds (VOCs) induced by herbivores is one resistance mechanism by which plants becomes less susceptible to herbivores by the recruitment of natural enemies of phytophagous insects; such a mechanism is predicted by the indirect defense hypothesis which states that plants should give natural enemies a reliable signal to facilitate prey/host finding to reduce herbivory. Domestication syndrome can be used as a model to test for differences in plant traits related to plant defence against herbivores. We performed a series of experiments damaging cultivated tomato varieties (*Solanum lycopersicum* L.) and populations of its wild relatives (*S. lycopersicum* var. *cerasiforme*) with nymphs of a phloem-sucking insect (*Bactericera cockerelli* (Sulc.), Homoptera: Psyllidae) to compare the ability of plants to change after herbivory according to their domestication status. Although feeding by *B. cockerelli* nymphs did not increased VOCs, plant domestication status significantly influenced the inducibility of VOCs and the trade-off between constitutive and induced VOC emission. Cultivars had a homogeneous induced VOCs emission while wild-types showed variability in this response. We found that variation at the individual and population level in VOCs were related to plant domestication status. Reduced plasticity and trade-offs between constitutive and induced VOCs in cultivars can be the result of selection pressures for limited allocation of resources to induced responses that constrain the quantity of VOC release after herbivory producing homogeneous responses.

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Metabolites Produced by *Escovopsis* sp., a Parasitic Fungus Found in Ants Nests

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In nests of Attini ants, actinobacteria, the fungus *Leucoagaricus gongylophorus* and ants live in mutualistic symbiosis. In this association, they face the parasitic fungus of the genus *Escovopsis* which can limit the fitness of a colony and devastate a garden in a few days. *Escovopsis* are specialized and potentially virulent parasites of the ancient mutualism between attini ants and their fungal cultivar¹ acts as a necrotrophic parasite that destroys the cultivar's hyphae and exhibits a complex pattern of co-evolution with the cultivar². *Escovopsis*, unlike invasive necrotrophs which always penetrate host hyphae, can secrete compounds that break down host mycelium before contact occurs¹. This work investigates the production of metabolites by *Escovopsis* sp. that can elucidate the interaction between the parasitic fungus and *Leucoagaricus gongylophorus*. *Escovopsis* sp. (ES001) was isolated from the nest of *Trachymyrmex* ant. It was cultivated in rice medium for 30 days at 25 °C. After this period the culture was macerated with EtOH for 24 hours. The EtOH was evaporated and the extract, after several chromatographic procedures, resulted in some metabolites that were elucidated by NMR techniques and GC-MS. The structural elucidation of these metabolites and their role in the interaction between microorganisms are discussed.

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Acknowledgements: Fapesp

Chemical Ecology of the Stink Bug *Pallantia macunaima* Grazia (Hemiptera: Pentatomidae)

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Brazil is one of the major soybean producers in the world. Among the main soybean pests are stink bugs that feed directly from grains, affecting seed quality. In the piercing-sucking complex that occurs in soybean crops, *Pallantia macunaima* is considered a secondary pest. The aim of this work was to study the chemical structure of the defensive compounds and sex pheromone from *P. macunaima*. Therefore, defensive compounds from metathoracic scent glands (MTG) of adults of both sexes with different ages (10, 20 and 30 days old), and dorsal abdominal glands (DAGs) of the five nymphal instars were extracted, identified, quantified and compared. Among the identified compounds there were saturated and unsaturated aliphatic hydrocarbons, unsaturated aldehydes, oxo-alkenals and unsaturated esters. From the sex pheromone studies, the volatiles produced by males and females adults were collected by aeration techniques, and it was found a sex-specific compound only in males extracts. Bioassays performed with Y-Tube olfactometer and electroantennograms showed the biological activity of this male-specific compound only for females. Through mass spectrum analysis of the pheromone compound and micro-derivatizations it was possible to conclude that this compound is a ketone with aliphatic, saturated and branched carbon chain. The synthesis of this molecule has been initiated to allow us to confirm its chemical structure.

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Kairomones from *Euschistus heros* Eggs Induced Host Selection Behavior on Parasitoid *Telenomus podisi*

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Kairomones play an important role in host selection behavior that could be used by the egg parasitoids [1]. The aim of this study was to identify and assess the influence of contact kairomones present in eggs of *Euschistus heros* (Hemiptera: Pentatomidae) in host selection behavior of the egg parasitoid *Telenomus podisi* (Hymenoptera: Scelionidae). Extracts of the egg mass of the host were obtained submerging in hexane, methanol, dichloromethane and acetone for 24 hours. The extracts obtained were used in behavioral tests with female *T. podisi*. Glass beads (n=5) of similar size to eggs of bugs were treated with 5 uL (1 uL/bead) of different extracts and positioned in the center of an arena and the behavior of the insects was evaluated: encounter (movements of the parasitoid from their liberation area until physical contact with the artificial egg mass), drumming (examination of pearls by drumming using the antennae) and attempt of oviposition (ovipositor extrusion and attempt to pierce the glass beads). Chemical analyses were conducted using GC and GC-MS to quantification and identification of the different extracts. All extracts induced behavioral responses in egg parasitoids. However, samples taken in methanol and acetone produced longest drumming in glass beads and increase frequency in the oviposition attempts. Kairomonal activity observed in different extracts indicates the presence of important compounds for recognition and acceptance of hosts, most notably for the solvents acetone and methanol.

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Electrophysiological and Behavioral Response of *Aegorhinus superciliosus* (Coleoptera: Curculionidae) to Volatiles Released from Conspecific and its Host Plant

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The raspberry weevil, *Aegorhinus superciliosus* (Guérin) (Coleoptera: Curculionidae), is the most important pest in blueberry fields in the south of Chile. The objective of this study was to evaluate the electroantennographic and behavioral responses of *A. superciliosus* to semiochemicals released from 1) conspecific individual adult and, 2) host volatiles. Volatiles from the aerial part of different phenological stages of blueberry and individual adults were collected on Porapak Q and analyzed by coupled gas chromatographic-mass spectrometry (GC-MS). Electroantennogram (EAG) recording showed that males of *A. superciliosus* possess olfactory sensitivity for the *R* isomer of limonene and α -pinene, whereas females only perceived *R*-limonene. Other volatiles that elicited responses from weevils' antenna were 2-nonanone, eucalyptol, and 4-ethyl-benzaldehyde. However, there were no sexual differences of the antennal response between male and female to any of these compounds. Field experiments confirmed the results obtained in the laboratory, showing that *R*-limonene was attractive to the weevil. These host plant and conspecific volatiles may be potentially useful as attractant in a control and management programme of *A. superciliosus*.

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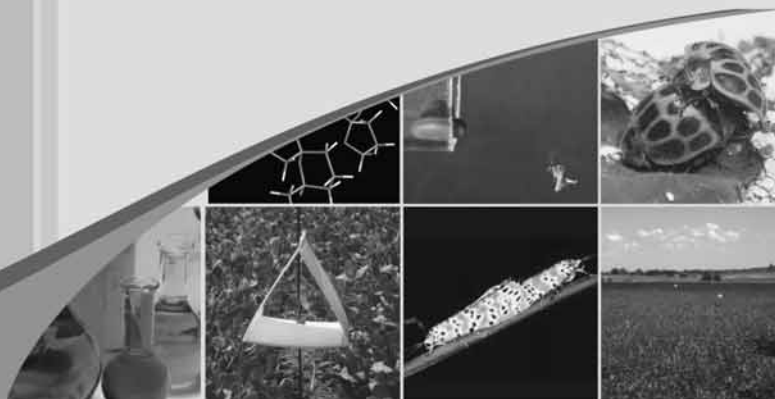
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Chemoenzymatic Approach to the Oviposition Pheromone of *Culex* Mosquitoes

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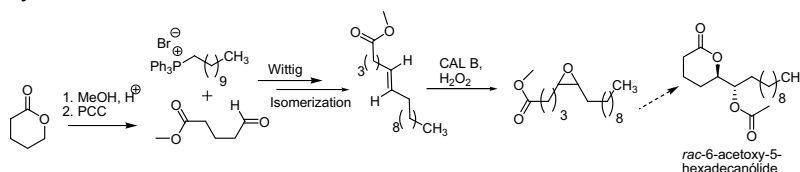
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The use of pheromones is an important tool for controlling insect proliferation, particularly those that are vectors of crop pests or serious diseases like filiasis, malaria or dengue fever [1]. The compound (5*R*,6*S*)-6-acetoxy-5-hexadecanolide is a pheromone used by *Culex* mosquitoes (Diptera: Culicidae) to locate an appropriate oviposition sites. The pheromone is released by the mature eggs to attract females to lay their eggs in a suitable location.

Studies have demonstrated that the only biologically active stereoisomer is (5*R*,6*S*), and that its activity is not affected by the presence of other isomers [2]. The molecule exhibits an interesting synthetic challenge: the introduction of two contiguous chiral centers at the C5 and C6 position of a 16 carbon fatty acid that is a probable biosynthetic precursor of the pheromone.

In our laboratory we have initiated a project to develop a brief chemoenzymatic synthesis of the pheromone. The methodology begins with the preparation of the fatty acid precursor by an olefination reaction. This is a key step because the required 5-hexadecene acid is not a natural or commercially available compound. The methodology then follows with the chemoenzymatic epoxidation of the double bond and concludes with the formation of the lactone ring and the acetylation of the hydroxyl at position C6.

Synthesis



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Trophic Interaction Between *Meloidogyne javanica* and *Tuta absoluta* in Tomato

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Plants respond to herbivory when subjected to the attack in an attempt to defend. Among the responses, the induced defense is a mechanism widely used by plants in these conditions. This defense may be localized or systemic. When there is systemic defense she can go from root to leaves and fruit or vice versa, so the parts damaged for parts not attacked. Therefore, the objective was to determine whether the defenses of the tomato, *Solanum lycopersicon* when attacked by *Meloidogyne javanica* root induces a response in leaves and whether this response changes the behavior of pregnant females of a specialist herbivore, *Tuta absoluta*. Knowing that the plant response may vary depending on the intensity and duration of exposure to attack, so the plants were evaluated at different levels of infestation with nematode eggs (0, 1000, 3000 and 5000) and days after infestation (10, 20 and 30). As an indication of induced defense quantify protease inhibitor, nitrogen and photosynthesis. There was the oviposition behavior of the herbivore to shoot through a preference test. Tomato plants infested in the root system responded to attack by inducing systemic defense by increasing the amounts of protease inhibitors, nitrogen and the decrease of photosynthetic rate. Given these responses the oviposition behavior of the specialist herbivore in the shoot was affected. The results show that interactions between spatially separated herbivores may be mediated by the response of induced plant defense.

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Concise Strategy for the Synthesis of (7Z,9Z)-Dodecadienol and (7Z,9Z)-Dodecadienyl Acetate, Components of the Sex Pheromone of *Epinotia aporema*

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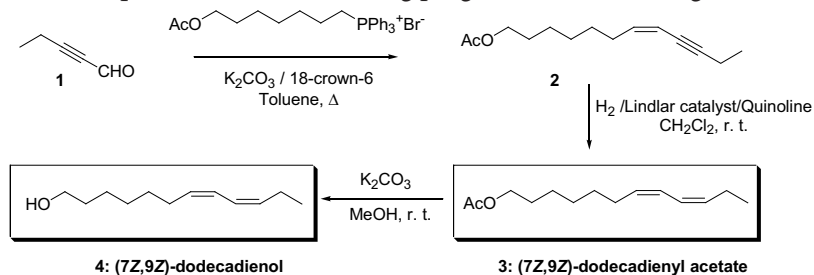
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Soybean production has rapidly increased in the last decade in southern South America, thus causing a dramatic expansion in the use of insecticides such as endosulfan and chlorpyrifos.

Epinotia aporema (Walsingham, 1914) (Lepidoptera: Tortricidae) originates from Costa Rica, but currently is widely distributed in Argentina, Chile, southern Brazil and Uruguay, being the major pest of soybean and other legumes.

The sex pheromone of *E. aporema* was identified as 15:1 mixture of (7Z,9Z)-dodecadienol and its corresponding *O*-acetyl-derivative [1], and a concise synthetic route to prepare the pheromone in adequate scale for monitoring programs was then designed.



The key step in the synthetic route to (7Z,9Z)-dodecadienyl acetate was the Wittig reaction of 2-pentynal (**1**) with the adequately functionalized phosphonium salt under Bodem conditions. Further stereoselective reduction of **2** furnished the corresponding (7Z,9Z)-*O*-acetyl ester **3**, which was subsequently hydrolyzed through chemical transesterification to give **4**, the main component of the pheromone.

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Identification of Signaling Molecules in *Xylella fastidiosa* by GC/MS

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Macroscopic organisms such as insects and mammals use chemicals in your communication. However, microorganisms also depend on chemical communication to survive. The literature shows several examples of bacteria that do not exist as independent cells, but as colonial microorganisms that exploit elaborate systems of intercellular communication to facilitate adaptation to environmental conditions [1]. The most significant diseases caused by *Xylella fastidiosa* are the citrus variegated chlorosis (CVC) and grapevines (Pierce's disease). The mechanism of pathogenesis is through formation of biofilms. However, the processes that mediate the formation and maintenance of these biofilms are still unknown. The present study refers to the identification of molecules produced by the bacterium *X. fastidiosa* by gas chromatography-mass spectrometry (GC/MS). *X. fastidiosa* (strain 9a5c) was grown in PW (Periwinkle wilt), centrifuged at 10.000 rpm for 15 min and subsequent extracted with organic solvents and analyzed by GC/MS. Fatty acid derivatives were identified in unpolar fraction. The literature suggests that fatty acids can make an important role as chemical mediators in bacterial signaling system [2]. Thus, the results become very relevant, which together with data already reported, can be a major advance in understanding the mechanism of action and biofilm formation in *X. fastidiosa*.

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Evaluation the Addition of CO₂ for Capturing *Culex quinquefasciatus* Say with BG-Sentinel® Trap in the Urban Area of Manaus (AM, Brazil)

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The mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) is the main vector of lymphatic filariasis and of various arboviroses. The immature stages (larvae and pupae) develop mainly in permanent eutrophic breeding sites. The urban area of Manaus (AM, Brazil) shows characteristics that are favorable for the development and proliferation of this species. The BG-Sentinel® trap has been used as an important tool for capturing adult mosquitoes, especially *Aedes aegypti*, *A. albopictus* and *Culex* spp. The trap contains the synthetic attractant (BG-Lure™) that are found on the human skin like ammonia, lactic acid and caproic acid and CO₂ are used only for some mosquito species. The objective of the study was to evaluate the effectiveness of BG-Sentinel traps with and without carbon dioxide (CO₂) for capturing *Culex* mosquitoes. The field studies were carried out in 15 areas within the city of Manaus (Amazonas State, Brazil) in January 2009. In each area, four houses were randomly chosen to receive one trap for 24h. Two traps per area baited with BG-Lure and approximately 3kg dry ice (flow rate: 2.08g/min), and two traps baited only with BG-Lure (control). The results showed that the total of 2,699 individuals of *C. quinquefasciatus* were captured, 1,487 (55%) were females and 1,212 (45%) males. The mean number of captured females per trap lured with CO₂ (42.73 ± 12.23) was significantly higher than control traps (7.32 ± 2.26) (Mann-Whitney; $p=0.001$). Traps with CO₂ also captured higher numbers of male *C. quinquefasciatus* (32.03 ± 12.06), than the traps without CO₂ (8.96 ± 2.38), but the difference was not significant ($p=0.095$). We concluded that the addition of CO₂ to BG-Sentinel baited with BG-Lure increased the collection of female and male *C. quinquefasciatus*.

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Determination of Chemical Defense of the Freshwater Macrophyte *Utricularia gibba* (Lentibulariaceae)

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Freshwater vascular plants contain a diversity of secondary metabolites that could function in defense and several authors have suggested that chemical defenses against herbivory may be important in structuring freshwater plant communities [1]. *Utricularia gibba* (Lentibulariaceae) is known as a carnivorous plant due to their trapping mechanism. In the present study we determinate the chemical profile of the methylene chloride - methanol (1:1) extract of *U. gibba* by HPLC and investigated its activity against herbivores. The macrophytes were collected in the Parque Nacional da Restinga de Jurubatiba (PNRJ), Macaé, in Rio de Janeiro State, Southeastern Brazil. The bioassay experiments were performed with *Biomphalaria* sp. (Planorbidae), a generalist herbivorous known as the vector of *Schistosoma mansoni* (Strigeiformes). In the HPLC analyses we observed the presence of rutin, kaempferol-7-O-glicosídeo, apigenin, luteolin and gallic acid, on 53%, 2,5%, 16%, 8,0% and 9,9%, concentrations respectively. The extract of *U. gibba* presented activity against *Biomphalaria* sp. ($p = 0,016$, $N = 23$, Wilcoxon test) probably due the high levels of flavonoids. In order to determinate in wich fraction the chemical activity is concentrated, fractionation of the crude extract was guided by feeding assays using *Biomphalaria* sp. Our results show that the crude extract of *U. gibba* act as defensive agent, protecting the plant from herbivory, supporting the postulated idea about importance of chemical defenses to the establishment of biotic communities.

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Insecticidal Activity of *Limonium brasiliense* (Plumbaginaceae) against *Rhizopertha dominica* (Coleoptera)

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The lesser grain borer, *Rhizopertha dominica* F. is arguably the most important insect pest of stored grains worldwide. The conventional way to control this pest has been the use of synthetic insecticides. However, the persistence of them in grains and the resistance of insects have derivate in the research of natural products as an important source of botanical insecticides. *Limonium brasiliense* Kuntze (Plumbaginaceae) is a medicinal plant, known as “Guaycurú”. It is a shrub that grows in Argentina, Uruguay and south of Brazil. Infusions from the roots are used in the treatment of hemorrhage, menstrual disorders, rheumatism and it is believed to have cardioprotective properties [1]. In this work we report the insecticidal activity of the methanolic extract, aqueous subextract, and a fraction derivates from this subextract. To determine the fumigant toxicity the protocol was follow as previously described by [2] with some modifications. Mortality was evaluated daily during 5 days. Probit analysis was used to estimate LC₅₀ (lethal concentration) by Micro Probit 3.0. Dried roots from *L. brasiliense* were milled and extracted with refluxing methanol. This extract was partitioned with water and hexane to obtain sub-extracts and then these were fractionated by silica gel column chromatography, to obtain fractions and to isolate and identified active compounds [3]. The elucidation of the isolated compounds was determined by ¹H and ¹³C NMR spectra and confirmed by comparison with literature data [4-5]. LC₅₀ values are shown in table. The hexanic subextract was not toxic against *R. dominica*. However the fraction, the aqueous subextract and the methanolic extract showed an increment in the toxicity, respectively. These could be a synergism between components that make the extract more toxic than the subextracts and fractions. Because of these results *Limonium brasiliense* should be considered as a resource of biological importance.

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Defensive Compounds from Nymphs and Adults of *Pachycoris torridus*

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Pachycoris torridus is an important pest of *Jatropha curcas* in Latin America. The seeds of *J. curcas* contain around 50% oil which is being used as biodiesel. Nymphs and adults of *P. torridus* suck the seeds provoking a reduction in 70% in the oil. The semiochemicals could be used to try to manage and control the population of *P. torridus*. Thus, the volatiles semiochemicals obtained from air-entrainment from nymphs and adults and fresh exuvias extracts were quantified by gas chromatography (GC) and identified using GC-mass spectrometry. Males and females adults emitted a series of compounds such as (*E*)-2-hexenal, (*E*)-4-oxo-2-hexenal, (*E*)-3-octenyl acetate, n-undecane, n-dodecane and n-tridecane, the last one being the most abundant. (*E*)-2-hexenyl acetate a less common compound in Hemiptera also was identified in males and females of *P. torridus*. In addition, three compounds were identified only in males and one compound was identified only in the females extracts. The gender-specific compounds are alcohol with a terpenoid structure. The air entrainment and the exuvias extractions of nymphs showed that the main compounds present in nymphs are (*E*)-2-hexenal, (*E*)-4-oxo-2-hexenal, n-tridecane and tetradecanal. (*E*)-4-oxo-2-hexenal is the predominant compound in the first and second instar, decreasing in the last instars, and tetradecanal and tridecane are the main compounds in the fourth and fifth instar. The pattern of the components in *P. torridus* is similar to that observed for other stink-bugs species (Pentatomidae) [1, 2]. The specific compounds in males and females could have a sexual or aggregation function because this species have a strong aggregation behavior.

[1] Borges, M.; Aldrich, J. R. *Experientia* 48, 893, 1992.

[2] Moraes MCB, Pareja M, Laumann RA, Borges, M. *Neotropical Entomology*. 37, 5 2008.

Acknowledgements: FAP-DF, CNPq, IFS, Embrapa.

Production of a Biopesticide from Botanical Origin in Small-Scale for Horticultural Crop

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*Ecological interactions in nature suggest a long history of plant synthesized chemical use by insects which have been molded during the course of evolution. These phytochemicals, are often distasteful and toxic to many insects [1]. Environmental current problems related to agriculture make it necessary to search for alternatives to the use of synthetic pesticides. The insecticides of botanical origin appear like an option of considerable interest, due to their effectiveness, low persistence and being environmentally safe. The fruits of “Paraiso” *M. azedarach* like the Neem Tree (*Azadirachta indica*) has proven to have insecticide effect and repellence to insects, due to limonoids, their active principles [2]. In this research, was developed a pilot process for extraction of active principles from extracts of *Melia azedarach* L. (Meliaceae). The stages of the research included: selection of the raw material, optimization of the extraction conditions in a pilot plant, adaptation of an analytic methodology for its standardization. Effectiveness of the biopesticide was evaluated at field experiments against *Thrips tabaci* in onion crop for two seasons and showed a control similar to the pyrethroid lambda-cyhalothrin. According to results obtained the bioinsecticide can be used for moderate control of insects in organic horticulture and become part of IPM systems.*

[1] Koul, O and Dhaliwal, G.S. 2001. *Phytochemical Biopesticides*. Harwood Academic Press. Amsterdam.

I Carpinella, M.C., et al. 2006. *Role of Melia azedarach L. (Meliaceae) for the control of insects and acari*. In *Naturally Occurring Bioactive Compounds*. Pp 81-123- Edited by M. Rai and M.C Carpinella. Elsevier, Amsterdam.

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Involvement of Coumarin as a Defense Strategy in *Pteridium caudatum*. Evaluation of Allelopathic Potential

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Pteridium called bracken fern, is considered the fifth most widely distributed weed in the world and it is proposed that this is due to different adaptive strategies, both physical and chemical [1]. Our objective in this study is to determine whether the content of coumarin present in leachate from *Pteridium caudatum* fronds can participate as a strategy to colonize land, excluding other species by phytotoxic action of coumarin as a chemical defense. Thus, we developed a method of quantitative analysis of coumarin by HPLC of aqueous macerated *P. caudatum* in different phenological stages. The quantitative determination was performed with coumarin pattern in the range of 0.5 - 100 μM and the effect of the parent plant was dismissed. The results showed that the content of coumarin in aqueous macerated ranged from 0.7 to 75 μM (0.08 - 5.40 mol/g de biomass). To evaluate the phytotoxic activity of macerated aqueous bioassays were performed on germination and growth of lettuce (*Lactuca sativa*) and tomato (*Solanum esculentum*) in a concentration range of 7-125 mM. The results indicated that the macerated obtained a greater impact on lengthening the stem and root of lettuce compared with coumarin standard. There is a correlation between coumarin levels present in the extracts with the observed phytotoxic activity. In conclusion, coumarin released into the aqueous environment through leaching (eg, by action of the rain) participates as a metabolite of defense, serving as a possibly allelopathic effects against other species in the environment.

[1] Alonso-Amelot, M. E., Oliveros, A., Calcagno M. P., and Arellano, E. (2001) *Pure and Applied Chemistry*.

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Reproductive Success in Wild *Mus musculus* Exposed Conspecific Odors in Overcrowding Conditions

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The regulation of natural populations is frequently mediated through density dependent responses in reproduction or mortality. One of the mechanisms proposed is a physiological effect of conspecific odors on reproductive parameters at different stages: pregnancy rate, offspring development and survival, birth success and post- birth survival. We studied the pregnancy and birth success under laboratory conditions in two groups of wild *Mus musculus* individuals that were captured in poultry farms in the Province of Buenos Aires, Argentina. One group was maintained under ventilated conditions with few animals in the same room, which were fed ad libitum. The other group was subject to crowding conditions and not ad libitum food. After an acclimation period we paired females and males belonging to the same group, each mating was of one female- one male which were maintained in separated cages from other animals. For each mate we registered pregnancy success and offspring birth, weight and survival. Both groups did not differ in pregnancy success (control:3/15; crowding: 6/16) ($X^2_{(1)}=1,1507$, $p>0,05$). The proportion of birth with respect to the number of pregnancies ((control:0.66 (2/3); crowding:0.66 (4/6) were similar.

Mean litter size was 5 (5-5) and 4,5 (3-7) for the control and crowding group, respectively. We observed cannibalism in one of the control females and in 4 of the crowded females. In the crowding group one male killed the female during the mating period. The low pregnancy success may have been related to captivity conditions that affected both groups in a similar way. There were not differences between groups in pregnancy and birth success, but crowding may be related to aggressive behavior of both males to females and females to offspring, but more data are needed to confirm this trend.

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Biocatalytic Oxygenation of Cineols Modulates Anti-Coleopteran Activity

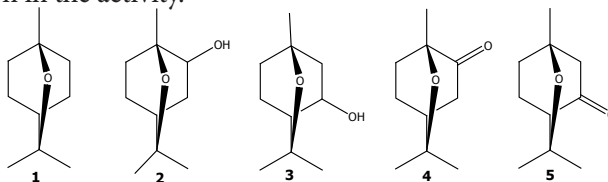
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Eucalyptus globulus is one of the main species planted as paper pulp source in Uruguay where the paper industry has greatly expanded in recent years. When trees are cut, the leaves are left behind as debris creating environmental problems. As a part of a program aimed to look for alternative uses of remains, leaf essential oils (EOs) and their major compounds biotransformed by fungi were tested in their potential capacity as fumigant, deterrent and toxic agents against three coleopterans. Indeed, EOs and its components have been proved to be active against many insect species and are a promising alternative to synthetic insecticides. We obtained derivatives of the major component of *E. globulus* essential oil, 1,8-cineol (**1**): 2-hidroxy-1,8-cineol (**2**); 3-hidroxy-1,8-cineol (**3**); 2-oxo-cineol (**4**) 3-oxo-cineol (**5**) and using *Aspergillus terreus* and *Gymnosporium spectabilis* 7423 as biocatalists. The fumigant and contact activity of these products was evaluated against *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus sp.* (Coleoptera: Curculionidae), two important pests of stored grains. Deterrent activity was tested against *Epilachna paenulata* (Coleoptera: Coccinellidae), which feeds on cucurbitaceae plants and represents a problem for organic farmers. Our results show that 2-oxo-cineol and 3-oxo-cineol (**5**) are active against *T. castaneum* and *Sitophilus sp.* Both products were also deterrent for *E. paenulata*. From the hidroxyl derivatives tested, 2-hidroxy-1,8-cineol (**2**) was active only against *E. paenulata* while 3-hidroxy-1,8-cineol (**3**) showed no activity against any of the insect species. As a general conclusion, fungal biotransformations of the basic structure of 1,8- cineol resulted in a significant increase in deterrence against *E. paenulata*. Among derivatives, the presence of a ketone function (**4**, **5**) appears to either mantain or increase this activity, while an hydroxyl moiety in the structure (**2**, **3**) would lead to a reduction in the activity.



Bioguided Fractionation of *Clytostoma callistegioides* (Bignoniaceae) Led to Six Compounds with Differential Anti-Insect Activity

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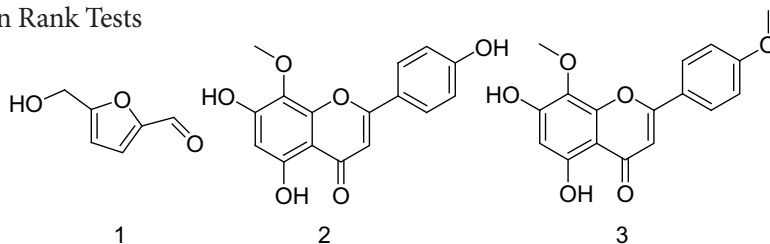
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During the course of a bioprospecting search for biopesticides from the local flora, members of the family Bignoniaceae native from Uruguay and Southern Brazil were reported to have anti-insect activity [1]. Among them, leaf extract from *Clytostoma callistegioides* (Cham.) showed anti-settling activity against two species of aphids, *Myzus persicae* and *Rhopalosiphum padi* (Hemiptera: Aphididae), both of them important pests in agriculture. In order to test its potential anti-insect properties, choice tests bioassays were done. Leaf pieces were offered either coated with the product test (T) or with solvent (C). The settling inhibition indexes (SI), calculated as the percentage of aphids settled on T and C surfaces as $SI = (C-T)/(C+T)$, was 0.29 ± 0.09 ($p = 0.01^a$) and 0.21 ± 0.08 ($p = 0.02^a$) for *M. persicae* and *R. padi* respectively.

As a part of a subsequent bioguided fractionation, an acid hydrolysis (HCl 1 N) of the leaf extract from *C. callistegioides* led to an aglycone extract active against the two aphid species, with a $SI = 0.45 \pm 0.07$ ($p = 0.0003^a$) for *M. persicae* and a $SI = 0.44 \pm 0.06$ ($p = 0.0002^a$) for *R. padi*. Further purification resulted in the isolation of six different compounds. We here present the results of the anti-settling activity of the six isolated compounds: 5-(hydroxymethyl)-2-furaldehyde (1) was identified as well as the flavonoids: 4'-hydroxywogonin (2) and galangustin (3). We are currently working on the structure elucidation of the other three structures.

^a Wilcoxon Rank Tests



[1] Castillo, L., et al., *Screening of Uruguayan plants for deterrent activity against insects*. Industrial Crops and Products, 2009. **29**: p. 235-240.

Volatiles from Flowers of *Manilkara zapota* and Electroantennographic Response of *Zamagiria dixolophella*

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This study investigated differences in volatile organic compounds (VOC) from chicozapote flowers (*Manilkara zapota*) along its phenological development in an orchard at Chiapas, México and the response of *Zamagiria dixolophella* (Pyrilidae) female antenna to these volatiles. This insect drills buds, buttons and small fruits causing economic damage [1]. In a chicozapote tree, five groups of flowers in bud phase were enclosed in a plastic jar. The air with volatiles inside plastic jar was extracted through a filter with SuperQ®. The volatiles were collected *in situ* every two days at same hour until flowers dried out. Chemical determinations were made by GC-MS analyses. The electrophysiological response of the antenna of female to volatiles was registered through coupled GC-EAG. Terpenes and benzenoids were the most abundant VOCs and constitute about the 75% of all VOCs. Few VOCs and small quantities were dispensed at the beginning of floral development and they reach the maximum when booming and both, VOCs and quantities declined when the flowers dried out. The antenna of females of *Z. dixolophella* responded to four compounds: benzaldehyde, eucalyptol, camphene, and linalool.

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Volatile Attractants to Neonate Larvae of *Copitarsia decolora*

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The objective of this work was to know the behavioral response of neonate larvae of *C. decolora*, to volatiles obtained from five plants; cabbage (*Brassica oleracea* L.), maize (*Zea mays* L.), “huauzontle” (*Chenopodium nuttalliae* Saff.) and “epazote” (*Teloxys ambrosioides* (L) and a non-host, tomato (*Solanum lycopersicum*). In one-choice bioassays in a “Y” tube, we tested if volatiles obtained from the plants and impregnate on a filter paper lured the larvae. The volatiles were obtained through dynamic ventilation and captured in a filter filled with Super Q®, and eluted with dichloromethane. Neonate larvae were significantly more attracted by the volatiles from cabbage, corn, “huauzontle” and “epazote” compared with a control (solvent), whereas tomato volatiles were as attractive as control. Also we tested if methanolic extracts from the surface of cabbage leaves affected larvae’s behavior. It was noted that larvae remained significantly more time in a filter paper impregnated with the leave extract than on a filter paper impregnated with solvent. Neonate larvae of *C. decolora* were able to detect volatile compounds obtained from the leaves of host plants and the surface of the leaves of cabbage, affecting their behavior. These results provide information about some of the key stimuli involved in host search and host selection in a generalist herbivorous insect’s behavior.

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Behavioral and Toxicological Effects of *Schinus molle* (Anacardiaceae) Essential Oils on the Egg Parasitoid *Trissolcus basal* (Hymenoptera: Scelinoidea)

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Essential oil of *Schinus molle*, a native plant of South America, has showed potential as botanical insecticide against stink bugs. In this work were evaluated their toxicological and behavioral effects on the parasitoid *Trissolcus basal*, a biological control agent of stink bugs. Essential oils were obtained via hydrodistillation from fresh leaves. Insecticide activity in *T. basal* adults was evaluated in direct contact and volatile fraction exposition bioassays. Behavioral effects of volatile fraction were evaluated in olfactometer bioassays. To evaluate the residual toxicity females of the parasitoids were exposed to paper oil residues of 1, 3 and 7 days. *S. molle* essential oil show a high contact toxicity (100% mortality in all the doses evaluated). The toxicity of volatile fractions was 75.6 $\mu\text{g ml}^{-1}$ and was similar to that observed for the brown stink bug nymphs. In addition this fraction not shows repellent effects on *T. basal* females. The contact toxicity have a residual effect for at least 3 days; after this period the acute toxicity and the sub-lethal effects, as diminution of parasitism potential, became non significant.

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Bioguided Isolation of an Antifungal Compound from *Trichocline reptans* (Wedd.) Hieron (Asteraceae)

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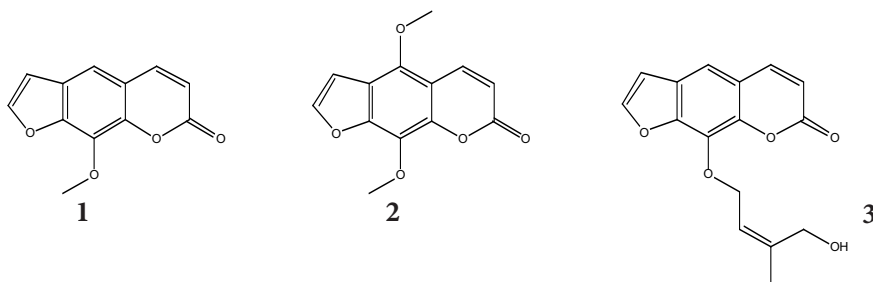
Previously, in a screening of 100 native plants, we have found that the ethanolic extract of *Trichocline reptans* was the most effective in the inhibition of *Fusarium verticillioides* (MIC bioautography on TLC= 0.03mg/spot). The active principle, isolated by column chromatography guided by antifungal assays, resulted to be Xanthotoxin (**1**). This compound showed a MIC liquid media= 0.1mg/ml against *F. verticillioides*.

In that chromatographic separation, two other coumarins **2** and **3**, were also isolated, and taking into account that the *T. reptans* extract also showed antifeedant and germination inhibition activity, these three compounds were also assayed for these activities.

The extract of *T. reptans* showed 97% inhibition of feeding of *Spodoptera frugiperda* (L II) at 500µg/cm², with an effective concentration 50 (CE₅₀) of 95.5µg/cm². Compound **1** and **3**, afforded CE₅₀ of 34.4 and 19.4µg/cm², respectively, against the same insect.

As germination inhibitor, *T. reptans* extract showed 100% germination inhibition (at 5mg/ml) of *Panicum miliaceum* (monocotyledonous) and *Raphanus sativus* (dicotyledonous) with CE₅₀ of 0.24 and 0.45mg/ml, respectively. The assay of **1** and **3** against *P. miliaceum* resulted in a CE₅₀ of 0.15 and > 0.75mg/ml, respectively, while no inhibition was observed for these two compounds against *R. sativus* up to 0.75mg/ml.

These results demonstrate that the ethanolic extract of *T. reptans* have different pesticidal activities being the antifungal one the most relevant. It is also shows that coumarin **1** is the only antifungal compound and it together with **3**, are also involved in the antifeedant and germination inhibitory activities of *T. reptans*.



Toxicity of Essential Oil from Leaves of *Schinus molle* var. *areira* (Anacardiaceae) against *Sitobion avenae* (Homoptera: Aphididae)

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The grain aphid *Sitobion avenae* is an important pest of numerous species of Poaceae, including all the cereals and pasture grasses of temperate climates. As well as the direct damage caused to cereals by feeding on the developing ears, *S. avenae* is an efficient vector of BYDV (especially strains BYDV-PAV and BYDV-MAV) both within and between crops [1]. Generally, the grain aphid is controlled with synthetic insecticides that caused several known issues. Therefore, it is important to look for alternative control methods such as botanical insecticides. The objective of the present study was to evaluate the contact toxicity produced by essential oil from leaves of *S. molle* var. *areira* on *S. avenae* apterae adults. The toxicity of the essential oil was measured in the laboratory using the immersion method described by [2]. The essential oil was prepared by hydrodistillation using a Clevenger type apparatus for 3 h. The oils were dried over anhydrous sodium sulphate and stored at 4°C under N₂. Stock solutions of test material were prepared by dissolving essential oil in 1 ml of a solution of ethanol and Tween 20% (0.012:10). The concentrations evaluated ranging from 25 to 75 mg/ml. Adult mortalities were determined 24 h after treatment. All treatments were replicated three times. The LC₅₀ value was calculated by probit analysis using the MicroProbit analysis program version 3.0. The LC₅₀ value obtained was 30.71 mg/ml. These result show that the essential oil from leaves of *S. molle* var. *areira* could be considered as a natural alternative in the control of *Sitobion avenae* on field crops.

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Effect of Volatiles Released by Maize and Bean Plants Damaged by the Brown Stink Bug *Euschistus heros* in the Foraging Behavior of the Egg Parasitoid *Telenomus podisi*

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Plants release volatiles (herbivorous induced plant volatiles - HIPVs) in response to damage provoked by herbivores, and these volatiles can act on the behavior of natural enemies. In the tri-trophic system soybean-stink bug-egg parasitoids the damage provoked by *Euschistus heros* (Hemiptera: Pentatomidae) induced the HIPVs production that attract the parasitoid *Telenomus podisi* (Hymenoptera: Scelionidae) [1]. As the HIPVs can change quantitatively and qualitatively between plant species, cultivars and are depending also of the herbivores species causing damage we tested the hypotheses that others plants than soybean may have differential attraction to *T. podisi* after the damage by the brown stink bug *E. heros*. To test this hypothesis we select two cultivated plants frequently attacked by *E. heros*, maize and dried bean. In each plant species was placed three virgin females of *E. heros*, and they were allowed to feed for 72 hours, plants undamaged were used as control. The response of the parasitoid to herbivory damage and undamaged plants was evaluated using “Y” tube olfactometer. The parasitoid showed a preferential response toward herbivory damaged compared to undamaged maize plants. On the other hand, in dried bean *T. podisi* did not show preference between damaged or undamaged dried beans plants. Studies are being conducted to evaluate the composition of HIPVs blends in order to obtain a total comprehension of this differential response.

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Conditioning Honeybees *Apis mellifera* (Hymenoptera: Apidae) to a Synthetic Floral Scent for Improving Foraging Towards Apple (*Malus domestica*) Trees

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Most of the floral cues experienced by honeybees at the field can be transferred and learned via social interactions among several nest mates inside the hive. Associative olfactory memories established within the colony can affect preferences of foragers for floral scents even several days after acquisition and can guide foraging to specific food sources [1]. This is relevant within agriculture settings since honeybees are excellent crop pollinators. With the aim to promote honeybee foraging to apple (*Malus domestica*) trees, a typical insect-pollinated crop, we developed a synthetic mixture (SM) formed by a few volatile compounds of its floral odor that accounted for a great proportion of the headspace extract [2]. In a laboratory experiment, honeybees generalized SM with the natural odor of apple flowers during classical conditioning assays of proboscis extension. In the field, SM-treated colonies (fed with SM-scented sucrose solution) showed higher levels of activity than the untreated ones (fed with unscented sucrose solution) during the apple blooming period. This occurred even though the offering of the SM was performed four days before starting the apple flowering. Present results suggest that specific memories of synthetic mixture established within the treated hives could bias foraging preferences towards apple crops, opening the possibility to improve the hive management during the pollination service of this crop.

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0This study was supported by funds from CONICET, UBACYT and ANPCYT grants to WMF. Also a Guggenheim Fellowship supports WMF.

Triterpenoids from *Melia azedarach* Fruits: are they Potential Anti-Insect Agents?

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Carmen Elisa Díaz-Hernández³, Ricardo Guillermo Alvarez³, Carmen Rossini¹

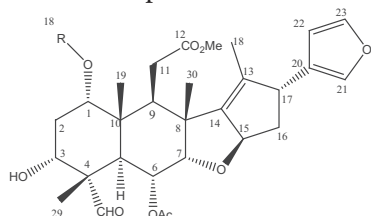
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Melia azedarach L. (Meliaceae) is native from Asia, but has been naturalized worldwide. Several structurally complex bioactive compounds have been isolated from Asian specimens. This study aimed to determine the anti-insect activity of organic extracts of *M. azedarach* fruits from Uruguayan populations, and to structurally elucidate the active compounds. Systematic fractionation of ethanolic extracts led to a triterpenoid-rich fraction, with strong activity against different insect models. For larvae of the generalist *Spodoptera littoralis* (Lepidoptera: Noctuidae), such fraction showed complete feeding deterrence in a choice experiment (100 %, N=5), but no toxic effect by oral cannulation ($p > 0.5$ one-way ANOVA, N=18). When tested against the adults of the specialist *Epilachna paenulata* (Coleoptera: Coccinellidae), the fraction also showed a strong deterrent effect (100 %, $p=0,002$, Wilcoxon Rank Test, N=15). Besides, the same fraction showed settling inhibition activity on *Myzus persicae* and *Ropalosiphum padi* (Homoptera: Aphididae) ($p=0,003$, N=18 and $p=0,023$, N=17 respectively, Wilcoxon Rank Test). Such fraction showed no contact toxicity against adults of *Nezara viridula* (Hemiptera: Pentatomidae), a common soybean pest ($p=0,58$, ANOVA, N=5). Three compounds previously reported from this species: ohchinolal (1), 1-O-detigloyl-1-O-cinnamoylohchinolal (2) and 1-O-detigloyl-1-O-benzoylohchinolal (3), never evaluated against those insect species, were isolated from that fraction. Until now, just 2 was tested, showing activity, neither by contact nor by oral cannulation. Presently we are working on the elucidation of four other compounds from the same fraction, which NMR data will be presented. On the whole, a triterpenoid fraction from the ethanolic extract of fruits of *M. azedarach* has been evaluated on its anti-insect activity, showing variable potential against different insect pest models, emphasizing the value of testing potential biocontrol products against different insect targets. One of the components of such fraction proved to be inactive on its own, indicating perhaps synergistic effect of the isolated compounds.



(1) R = Tig: ohchinolal

(2) R = Cyn: 1-O-detigloyl-1-O-cinnamoylohchinolal

(3) R = Bz: 1-O-detigloyl-1-O-benzoylohchinolal

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Histochemical Study of the Midgut in Resistant *Anticarsia gemmatalis* (Hübner) (Lepidoptera:Noctuidae) Larvae to Nucleopolyhedrovirus AgMNPV, Fed with Genotypes of Soybean Resistant to Insects

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This study aimed verify the chemical composition of the midgut of resistant *A. gemmatalis* larvae to its nucleopolyhedrovirus, treated with soybean genotypes with different concentrations of rutin and genistin The caterpillars, obtained at the Embrapa/Soja (Londrina-PR-Brazil), were fed with the following genotypes “in natura”: BRS257 (control), BR16, Dowling, PI229358, PI227687 and IAC100. The midguts were collected, fixed and processed for detection of acids and neutral polysaccharides (glycogen/PAS), proteins and lipids. The midgut of larvae fed on genotypes IAC100 and PI227687 showed strongest PAS positivity in columnar cells, as observed in control treatment, indicating the presence of neutral polysaccharides, particularly glycogen; only the genotype PI229358 caused moderate reaction to PAS in the goblet cells. To total proteins technique, the columnar cells showed a weak positive staining with moderate response to treatment with PI227687; the goblet cells were positive to the genotypes PI229358 and IAC100. There was a positive reaction for lipids in the columnar cells with PI 229358, IAC 100 and PI 227687. No reaction was detected for acid carbohydrates in the epithelial cells of the insects fed with any of the genotypes. We conclude that the rutin and genistin from resistant genotypes alters the chemical composition of the midgut epithelium in resistant *A. gemmatalis* larvae, changing the synthesis of compounds related to the digestion, being directly implicated in decreasing of the resistance of the insect to the AgMNPV after feeding on the resistant plants.

Synthetic Mixes of Floral Volatiles Used as Tools to Guide Honey Bees to Specific Crops

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Honeybees *Apis mellifera* are important pollen vectors for many crop plants. In some crops, productivity is ensured only through the pollination service of this insect species. Beekeepers use to condition honeybee colonies by feeding them with syrups containing crushed flowers of the species that pretend to be pollinated. Despite the use of this procedure little is known about the process that guide bees to the flowers of crop plants or about the dynamic and extent to which floral scent contributes to pollinator attraction in agricultural settings. Recent studies show that insect pollinators need only a few volatile compounds to recognize a specific floral scent. Based on this idea, we developed synthetic mixes of volatile compounds that bees confound with the natural floral fragrances of some specific crop plants. Our hypothesis is that memories established by the offering of synthetic-mix odor within the colonies (i) are used to orientate pioneer foraging bees while search for food nearby the crop field; and (ii) facilitate the transference of crop-related information through recruiting mechanisms such as dances. As an example, we will show evidences at the levels of cognition, orientation and navigation that suggest a prompt foraging task in sunflower crops, a fact that opens the possibility for an improved efficiency in pollination and crop yield.

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Host Selection in the Willow Sawfly *Nematus oligospilus* and Secondary Metabolites in *Salix* spp

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The willow sawfly *Nematus oligospilus* (Hymenoptera: Tenthredinidae) is a major pest of willows. It causes severe defoliations in different species from the genus *Salix* [1]. Here, we evaluated host selection considering different *Salix* genotypes in field and lab experiments. Since the capacity of herbivores is frequently related to the presence of secondary metabolites of the host plant [2], the phenolic derivatives profile of the different genotypes is being studied in order to correlate them with host selection behavior. During field experiments, 115 individuals or genotypes of *Salix* spp were evaluated. The highest level of damage by defoliation was recorded on *S. nigra*, while the lowest was recorded on *S. viminalis*. In the laboratory, the following genotypes were compared in multiple choice oviposition experiments: *S. babylonica* var *sacramenta*, *S. nigra* 4, *S. viminalis*, the hybrids *S. babylonica* x *S. alba* and *S. matsudana* x *S. alba* and ED1, obtained from the controlled crossing between (*S. babylonica* x *S. humboldtiana*) x *S. matsudana*. Multiple choice oviposition lab experiments showed that *S. nigra* was highly preferred, which suggests that the highest level of damage observed in the field can be related with a high oviposition preference. On the other hand, *S. viminalis* showed a low oviposition preference and relatively low fecundity (ie. number of mature eggs present in the female oviduct at the time of hatching), which could explain the low level of damage detected in the field for this genotype. Differences in the chromatographic profiles of the different genotypes are currently being evaluated by HPLC analysis. These differences will be correlated with their susceptibility, especially related to its phenolic derivatives composition. This information is a first step in an attempt to reveal mechanisms of host selection in the willow sawfly aiming to find an oviposition signal that can be useful for crop protection.

[1] Cerrillo T. 2009a. Jornadas de Salicáceas 2009. Argentina.

[2] Hadacek F. 2002. Critical Reviews in Plant Sciences, 21 (4): 273-322.

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Field Test Using Sex Pheromone of *Pseudococcus calceolariae* in Raspberry Crop

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The citrophilus mealybug *Pseudococcus calceolariae* (Maskell) is an agricultural pest with worldwide distribution on several economically important crops, such as citrus, avocado, berries, sugarcane, cocoa and grape. In Chile and New Zealand, chrysanthemyl 2-acetoxy-3-methylbutanoate was identified as the sex pheromone, by means of aerations of cohorts of virgin females [1].

The absolute configuration was determined as (1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropylmethyl (2*R*)-acetoxy-3-methylbutanoate and supported by selective attractiveness of the stereoisomer of chiral sex pheromone in field trapping experiments.

Using a randomized complete block design, in raspberry plantation (Nogales, Región de Valparaíso, Chile) was carried out the field test of racemic and chiral pheromone. Traps baited with chrysanthemyl 2-acetoxy-3-methylbutanoate, chrysanthemyl (2*R*)-acetoxy-3-methylbutanoate caught similar numbers of males to traps baited with pure sex pheromone. Therefore, the (2*S*)-esters had neither an attractive nor an inhibitory effect. Dose response experiments were done with four different treatments (1, 10, 100, 1000 µg) of racemic pheromone. The highest numbers of males were caught with the 1000 µg loading, which trapped 3647 males, while 76 males were captured for control traps. The longevity of lures was assayed for eight weeks, with 100 µg of chrysanthemyl 2-acetoxy-3-methylbutanoate. During this field test > 16.000 males were caught.

In conclusion, no natural stereoisomers of the pheromone were not inhibitory in field trapping test, and therefore a mixture of chrysanthemyl 2-acetoxy-3-methylbutanoate could be used in IPM program.

[1] Chrysanthemyl 2-acetoxy-3-methylbutanoate: the sex pheromone of the citrophilous mealybug, *Pseudococcus calceolariae*. Tetrahedron Letters 51(7), 1075-1078.

Acknowledgements: This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT 11060527). M.F. Flores thanks the Comisión Nacional de Investigación Científica y Tecnológica (Conicyt) for doctoral fellowship.

Biosynthetic Precursors, Temporal Emission and Localization Site of Pheromones in Ambrosia Beetle *Megaplatypus mutatus*

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Megaplatypus mutatus, an Ambrosia beetle (Platygodidae) native to South America, is a main forest pest that attacks live standing trees, affecting commercial poplar and broadleaf plantations. Attack is initiated by pioneer males selecting a host tree to build a short nuptial gallery, from which they attract females using a sexual pheromone. In previous work we identified volatile emission as composed by (+) 6-methyl-5-hepten-2-ol ((+)-sulcatol), 6-methyl-5-hepten-2-one (sulcatone) and 3-pentanol. Behavioral bioassays in olfactometer and electroantennogram recordings confirmed an attractive response for the three chemicals specifically attracting females. In the present study, we determined the sites of production of the pheromones within males, the temporal pattern of pheromone emission during gallery initiation and establishment and the possible biosynthetic precursors for sulcatol and sulcatone.

(+)- Sulcatol, sulcatone and 3-pentanol were found only in proctodeum. Production of sulcatol is independent of its interaction with the host, although the amount produced significantly increases after 48hs of tunnel excavating. Exposition of living males to synthetic vapors of sulcatol, sulcatone, geraniol and citral showed that sulcatone and citral increase sulcatol production and consequently can be used as a precursors for sulcatol biosynthesis. We collected male volatile emissions daily during the hours of peak flight by using a specific polar microextraction phase and the extract was analyzed by GC-MS. Sulcatol and 3-pentanol production was detected 1-2 days after gallery initiation with maximal production between 5-12 days. Sulcatone production was noted during the same period although only in trace quantities. Both pheromones were present in male emissions from three different host species between days 2-12 after gallery initiation, but sulcatone was always present in very low concentrations. The temporal patterns of sulcatol and sulcatone production or storage in male *M. mutatus* corresponded to the temporal patterns of emission.

[1] Journal of Economic Entomology 98:1506–1509

[2] Journal of Chemical Ecology 34:1446-1451

This work was supported by the Agencia Nacional de Promoción Científica y Técnica (PICT 2005, N° 38313, Ecología química de la plaga forestal *Megaplatypus mutatus* y su aplicación a estrategias de control de bajo impacto ambiental a través del uso de feromonas y kairomonas).

Volatile Secretions as Chemical Signals in *Ulomoides dermestoides* (Coleoptera:Tenebrinidae)

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The Oriental tenebrionid *Ulomoides dermestoides* has been introduced in the country as alternative medicine for treatment of cancer and respiratory diseases. Unrestrained use of this beetle led to its dispersal and it has been detected infesting stored wheat grains in Argentina. We investigated the effect of entomopathogenic fungal infection on beetle's colony fitness and chemical signals.

The volatile organic compounds (VOC) released by *U. dermestoides* males and females separately set in sealed vials were analyzed by head space solid phase microextraction (HS-SPME) coupled to capillary gas chromatography-mass spectrometry (CGC-MS). We also used CGC-MS to analyze their epicuticular hydrocarbons.

The effect of entomopathogenic fungal infection on VOC secretion and on colony development after insect immersion for 6 s in a suspension of *Beauveria bassiana* strain GHA (1×10^9 conidia/ml) was also studied.

A pattern similar to that reported in other tenebrionids was detected [1]. Methyl-1,4-benzoquinone (MBQ), ethyl-1,4-benzoquinone (EBQ), 1-pentadecene (C15:1) and 1-tridecene (C13:1) were the major components of the volatile blend (> 90%); no sex dimorphism was detected. However, when insects were let walk on papers, HS-SPME analyses of the papers showed terpenoid-like components in males but not in females, in addition to the other VOC. These papers were only attractive to females in a two-choice experimental arena.

After fungal infection, the relative amount of each component was unaltered, but the total amount of volatile secretion dropped to 5% of that detected in healthy beetles. The development of the infected colony was strongly altered 3 months after treatment with *B. bassiana*, showing a significant reduction in insect progeny. These results suggest that after infection events take place, diminished amounts of defense secretion may be a physiologic clue for behavioral changes in infected beetles.

In addition to the large amounts of hydrocarbons in the VOC, saturated, unsaturated, and branched structures with chain lengths ranging from 13 to 43 carbons were also detected. n-pentacosane (C25:0) and 9,11-pentacosadiene (9,11-C25:2) were the most abundant components, representing more than 40% of the cuticular hydrocarbons. No significant differences between males and females were detected in the major hydrocarbon components.

[1] M.L. Villaverde et al. Comp Biochem Phys B 2009, 154: 381-386.

Enantioselective Response of Olfactory Receptors of the Mosquito *Aedes aegypti* to Major Components of *Eucalyptus grandis* x *Eucalyptus camaldulensis* Essential Oils

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Dengue is an acute viral infection transmitted by mosquitoes *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). Mosquito control by ethnobotanical products is an important activity in studies of dengue. Crude botanical extracts, essential oils and others plants components are studied and used in different areas of insect pests control including the application as insecticides and mosquito repellents.

Previous studies of our laboratory [1] established the composition of the interspecific hybrid *Eucalyptus grandis* x *Eucalyptus camaldulensis* essential oil by gas chromatography-mass spectrometry, determining the presence of 1,8-cineol at a rate of 49.65%, α - pinene in 30.65%, α -terpineol in 4.05%, *p*-cymene in 2.77%, *trans*- pinocarveol in 2.75%, linalool acetate in 1.43%, borneol in 1.42% and β - pinene at 1% as main components.

Chromatographic conditions with chiral column were optimized for the separation of optical isomers of major components of *E. grandis* x *E. camaldulensis* essential oil. Enantiomeric composition was determined for components with optical activity: α - pinene, β - pinene, *trans*-pinocarveol and α -terpineol.

Gas chromatography coupled to electroantennographic detection (GC-EAD) was used in *Ae. aegypti* to determine if any of the major components of essential oil under study elicit a signal in mosquito antennae. The results showed electrophysiological activity for 1,8-cineol and the (-) - α - pinene isomer.

Electroantennography (EAG) technique was performed to confirm electrophysiological activity of the major components of the essential oil under study. Studies of electrophysiological activity of individual components of the oil are presented here.

[1] Lucia A, Licastro S, Zerba E and Masuh H (2008). Yield, chemical composition and bioactivity of essential oils from twelve species of *Eucalyptus* on *Aedes aegypti* (L.). *Entomologia experimentalis et applicata* 129(1): 107-114.

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Evaluation of a New Variant of the BG-Sentinel Mosquito Trap to Monitor Disease-Vector Mosquitoes

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The mosquito *Aedes aegypti* is a vector of Dengue. Monitoring of mosquitoes in the field is an important aspect of an efficient disease-control strategy. Mosquito-monitoring methods include egg-sampling using 'ovitrap' and adult-sampling using adult-traps. In recent years, a novel adult-trap (BG-Sentinel™, Biogents AG, Regensburg) had been developed that has since proven to be especially attractive for *A. aegypti* and related species, when compared to other traps. The BG-Sentinel produces an upward air current and artificial human skin odors given off from a dispenser (BG-Lure™). This lure does not require CO₂, a mosquito attractant, which is impractical or expensive for field use. The aim of this work was the evaluation of a new, improved and more cost-efficient variant of the BG-Sentinel, named the Mosquitito™. To test the efficiency of Mosquitito, two positive controls were used: the BG-Sentinel and the Mosquitaire™, another and more rugged plastic version of the trap. All three trap types used the BG-Lure. For testing, one trap of each type (3 in total) was placed in an urban house garden in Diamante, Entre Ríos, Argentina, and the mosquitoes captured by each type was recorded. Experiments were carried out in parallel in two different gardens, and the position of the traps was rotated after each sampling day. Ovitrap previously distributed all over the town had indicated a maximal density of *A. aegypti* eggs near the gardens used. Preliminary data indicate that Mosquitito was the most efficient trap. This device caught 63 % of a total of 960 mosquitoes trapped while the others trapped less than 20 % each. However, only less than 3% of the total mosquitoes trapped were *A. aegypti*. The low *A. aegypti* capture rate could be due to different reasons, including the timing of the sampling time window (i.e., the end of the mosquito season).

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Assessment of Allelopathic Potential of *Baccharis dracunculifolia* DC in Laboratory and Field Conditions

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The current tendency is to find eco-friendly solutions to minimize the perceived hazardous impacts from herbicides and insecticides in agriculture production. Allelopathy is defined as a beneficial or detrimental effect from a donor plant to the recipient by chemical pathway.^[1] *Baccharis dracunculifolia* DC., (Asteraceae), found in the southern region of South America, is an invasive and colonizing species characteristic of the area. In a plantation of *B. dracunculifolia*, a noticeable decrease in diversity and development of the spontaneous species was observed, mostly in monocot species, when compared to the control plots. Phytotoxic studies were carried out to determine the in vitro toxicity of the essential oils on monocot and dicot species. The results showed a 90% reduction of hypocotyl growth for *Lolium multiflorum* and 60% for *Lactuca sativa* at the concentration of 1mg/mL of the essential oil. A high percentage of compounds with phytotoxic effects, such as limonene, α and β -pinene were found through analysis of the essential oil by gas chromatography. The field experiments showed that inhibition under *B. dracunculifolia* was not due to an effect of shading nor to nutrient competition. The reduction of *L. multiflorum* germination under *B. dracunculifolia* was higher than 90%. In the soil under the plantation a high concentration of total phenolics was measured. Phenolic compounds have been reported as having phytotoxic activity and could play a key role in many allelopathic interactions.^[2] These results indicate that allelopathic activity of *B. dracunculifolia* may be explained through a combination of phytotoxic effects, which include emission of volatile compounds and the accumulation of phenolics in the soil profile.

[1] Rice, 1984. Allelopathy (Second ed), Academic Press Inc., Orlando, FL (1984) p. 422.

[2] Inderjit, 1996. Plant phenolics in allelopathy. Bot. Rev. 62, 182-202.

Calling Behavior and Chemical Communication of Cocoa Pod Borers, *Carmenta theobromae* Busk and *Carmenta foraseminis* Eichlin (Lepidoptera: Sesiidae)

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Two Synanthedonini species, *Carmenta theobromae* and *C. foraseminis* (Lepidoptera: Sesiidae) are cocoa pest insects in Venezuela. At the moment, pest control is only carried out using cultural methods, so it is necessary to look for other control strategies, like traps baited with sex pheromone, which had been successfully used in others sesiids for monitoring and control. In the present work we studied the calling behavior and identified a putative female sex pheromone of these sesiids. Larvae and pupae were reared individually and maintaining in a room at 25°C, 63% RH and 12:12 (L:D)-h photoperiod. Female calling behavior and daily activity of adults kept in observation chambers were registered during 72h. Female calling behavior of *C. foraseminis* occurs the same day after emergence, with mean at 1200, while *C. theobromae* has a pronounced calling behavior peak at 1730 hours. This behavior remains at least until the third day in the both sesiids. During the calling behavior, the female behavioral repertory is different between these species. Also, female and male abdominal tip extracts were prepared and analyzed by GC-MS equipped with polar and non-polar capillary columns. Two exclusive components were found in extracts of *C. theobromae* female abdominal tip, and identified as *Z,E*-3,13-octadecadienyl acetate and *Z,E*-3,13-octadecadienol, reported for typically compounds of sesiid sex pheromones. The average ratio of these two components was about 28:1. In extracts of *C. foraseminis* females two exclusive compounds were also found. These compounds were 3,13-octadecadien-1-ol and its acetate, and to confirm the isomerism of them, assays with synthetic compounds are in progress.

[1] J. Econ. Entomol. 97: 344-352.

[2] Biosci. Biotechn. Biochem. 69:508-516.

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Changes in Concentration of Isoflavonoids after *cis*-Jasmone Spraying in the Leaves of Two Soybean Cultivars

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cis-Jasmone is a naturally-occurring compound in plants that activates direct and indirect defence in model and crop plants^{1,2}. Exogenous application of *cis*-jasmone increases flavonoid concentrations in soybean and affects negatively *Anticarsia gemmatalis* weight gain. Here, foliar isoflavonoid concentrations were evaluated after *cis*-jasmone spraying on soybean cultivars BRS 134 and IAC 100, grown at Embrapa Soybean greenhouse facilities. At V₃ development stage, plants were sprayed with water, Tween 20 + water or *cis*-jasmone + tween 20 + water. For flavonoid extraction, leaves were collected 12, 24, 48, 96, and 120h after spraying, and immediately frozen in liquid nitrogen, ground in a mortar and extracted in MeOH 90% or EtOH 80% + HCl (0.001 M). Isoflavonoid concentrations were estimated by HPLC analysis. The extracts obtained from leaves of both genotypes of 24 h *cis*-jasmone treated plants presented higher quantities of daidzin, malonyl-daidzin, glycitin, malonyl-glycitin, glycitein and genistin, when compared to water and Tween 20 + water treated plants. Daidzein was present in high concentration, in both cultivars, 24 h and 48 h after *cis*-jasmone spraying, but no longer detected in samples collected 120 h after treatment. Coumesterol was detected, but only from samples collected 48 h after spraying. It is possible that such increases may be related to the decrease in daidzein concentration, considering that this compound is involved in coumesterol synthesis. Comparing solvent efficiencies, EtOH 80% + HCl (0.001 M) presented higher extraction capacity than MeOH 90%; coumesterol concentration in ethanolic extracts was approximately twice as high as in methanolic extracts. Our results indicated that *cis*-jasmone induces the production of non-volatile compounds in soybean such as isoflavonoids, and that EtOH 80% + HCl (0.001M) extraction is more efficient when compared to MeOH 90%.

[1] Bruce, T.J. et al. (2008). *Proceedings of National Academy of Science* 105, 4553-4558.

[2] Moraes, M.C.B. et al. (2009) *Entomologia Experimentalis et Applicata*, 131, 178-188.

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A Baiting Trap Using Host Odours for *Triatoma infestans* (Hemiptera, Reduviidae: Triatominae)

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The haematophagous bug *Triatoma infestans* is the main vector of Chagas disease in Latin America. These insects exploit different sensory cues released by their hosts to locate a blood source including CO₂ and L-lactic acid (L-Lac). It is known that L-lac did not evoke an attractive response when it was offered alone. However, a marked attractive response was observed when L-Lac was offered together with subthreshold amounts of CO₂, evincing a synergism effect between both odours [1]. The aim of this work was to design a device capable to detect/capture triatomines using cues related to hosts. We conducted a dual choice test in which one container was placed in an experimental arena with the attractant and the other one without it. The stimuli tested were chicken feathers, yeast, CO₂, CO₂ plus L-Lac. An alive mouse was used as positive control. In each assay, one insect was released in an experimental arena, for 45 minutes (N=20). The total number of insects trapped for each stimulus was recorded. Results showed that insects were not trapped using chicken feathers. The device with yeast trapped 55% of the total insects assayed. 90% of the insects were trapped by the device which released CO₂. When the effectiveness of the device with CO₂ was compared with the positive control, 16.7% of the total insects were trapped. The effectiveness of the device with CO₂ plus L-Lac increased to 37.5% of insects trapped when it was compared with the positive control. The device with inorganic CO₂ plus L-Lac seem to be better than the other sources tested. The device is innocuous, low cost and can trap insects alive, however more efforts are necessary to increment its effectiveness for further tests under seminatural and natural conditions. In fact, it may be useful as a tool for the detection/capture of triatomines.

[1] Barrozo, R. B. and Lazzari, C. R. 2004. The Response of the Blood-sucking Bug *Triatoma infestans* to Carbon Dioxide and Host Odours. *Chem. Senses* 29:319-329.

Cuticular Lipids Mediate Mate Recognition in Triatomines

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Triatomine bugs (Hemiptera, Reduviidae) are vectors of the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease. Cuticular lipids (CL) are known to participate in insect intraspecific chemical communication as contact cues; e.g. in aggregation and sexual behavior. In triatomines, CL include a blend of hydrocarbons, free and esterified fatty acids and fatty alcohols, together with minor components. CL extracts or selected fatty acid components of *Triatoma infestans* -a major Chagas disease vector- were shown to trigger aggregation and arrestment behavior [1]; however no reports on their role in sex-related events are yet available in triatomines. Here we report CL contribution to contact sex recognition both in *T. infestans* and in secondary vector species of *T. sordida* subcomplex. *Chemical composition:* Male and female CL were solvent-extracted and analyzed by capillary gas chromatography (CGC) coupled to mass spectrometry (MS). Alternatively, CL were extracted by solid phase microextraction (SPME) either by direct contact with a fiber, or from the head space of heated intact insects. The hydrocarbon fractions were analyzed by CGC-MS. *Bioassays:* CL were evaluated for their role on the copulatory behavior. Male copulation attempts were quantized in the presence of freeze-killed females, either intact, deprived of their CL, or reconstituted with a female extract; the response of males of *T. sordida* subcomplex against heptacosane (*n*-C27) was also evaluated. *T. sordida*, *T. garciabesi* and *T. guasayana* showed significantly larger amounts of *n*-C27 in females compared to males (2-6 times), with smaller variation in other hydrocarbon components. On the contrary, no sex dimorphism was evident in the major lipid fractions of *T. infestans*. Contact cues are necessary in mate recognition by males of all the species tested; the cuticular recognition cues are removed by hexane wash. Males contact females with their antenna and legs prior to elicit mating attempts. Recently freeze-killed females were attractive to males (80-100% mating attempts); no response was detected when males were exposed to hexane-washed females. The attraction was partially recovered when the lipid extract was reloaded on the female dorsal surface. In the species of *T. sordida* subcomplex, the hydrocarbon fraction elicited similar response than that of the whole lipid extract; a like response was also obtained with 5 equivalents of *n*-C27, suggesting the participation of other hydrocarbon components. We conclude that cuticular lipids trigger mate

recognition in triatomines, probably involving the participation of contact chemoreceptors. The identity of the sex chemical signal seems to be species specific in triatomines. Further studies are ongoing to detect sex dimorphism in minor cuticle lipid fractions in *T. infestans*.

[1] Parasites & Vectors 2009, 2:8

Semiochemicals Perceived by the Guatemalan Moth *Tecia solanivora* (Povolny) - Control Opportunity for Storage Pest?

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For insects with potato as a host for feeding, mating and ovipositing, information, which can improve the survival the progeny and the whole population, is crucial. Potato *Solanum tuberosum* elicits volatile compounds that resemble the status of the plant and hence mediate the quality and the phenological stage of the tissue, to the surrounding air.

Females of Guatemalan moth *Tecia solanivora* use volatile compounds released from potato for host finding and egg-laying. Electrophysiological recordings (GC-EAD) of the female moth antenna revealed detection of several compounds emitted from foliage, flowers and tubers. Through further electrophysiological assay (EAG) a dose-response relationship of the antennal active compounds was made. Antennae from 2-3 day old mated female were tested for 22 compounds in four concentrations. The compounds that enhanced the response caused of the level of exposure, was selected for further assays. The compounds benzaldehyde, β -caryophyllene, decanal, (*E,E*)- α -farnesene, (*E*)- β -farnesene, methyl phenylacetate, 6-methyl-5-hepten-2-one, β -myrcene, nonanal, 3-octanone, 1-octanol, 1-octen-3-ol and 2-phenylethanol showed a dos-response effect while α -caryophyllene, d-elemene, germacrene D, tetradecanal and sabinene did not.

To gain further knowledge about antennal active potato odours and the behavioural information mediate by them, an attraction assay in olfactometer is now conducted. Preparatory assay in semi-field was made with floral compounds to test trap type and concentration levels. One floral compound, methyl phenylacetate showed promising results and additional test are now preformed with blends of the selected potato compounds. Trap baited with the blends are placed in potato storage in controlled environment in Corpoica, Colombia, to evaluate lure attractiveness to males and females moth released in the compartments. Investigations of the combination of the antennal active potato volatiles will assess the synergistic effect between compounds.

Isolation and Identification of Kairomones from *Lycopersicon esculentum*, and their Activity on the Tomato Fruit Borer *Neoleucinodes elegantalis*

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Tomato crops have a significant economic importance in Venezuela as well as in other Latin American countries. The presence of pests is the usual risk factor that should be kept under control in agriculture, and one of the most common in tomato is the fruit borer *Neoleucinodes elegantalis* (Lepidoptera: Crambidae). Although the sex pheromone communication system has been already studied in *N. elegantalis*, it is necessary to find out more about the insect-plant chemical communication mechanism. The main objective in this research is to study the possible presence of tomato fruit kairomones, and their possible use in pest management. Volatile organic compounds released from non-ripened tomato fruits were isolated through HS-SPME using a 85 μm Carboxen/PDMS fiber, and analyzed by GC-MS. Nine monoterpenes were identified as potential constituents of this kairomone, and their biological activity were tested using wind tunnel bioassays. After comparing results from experiments using blends of these compounds and non-ripened fruits, we obtained that the original blend of compounds and the mixture of two monoterpenes (β -pinene and *p*-cymene) are more attractive than green tomato fruits. According to these results, it is possible to use a synthetic kairomonal blend as an alternative to improve management and control programs already developed for the tomato fruit borer *N. elegantalis*.

Semiochemicals of *Neomegalotomus Simplex* (Hemiptera: Alydidae)

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Neomegalotomus simplex (= *parvus*) is the principal alydid bug in several crops in Brazil and other South American countries, damaging dried beans, green beans, soybeans and other legumes. Adults and nymphs suck the seed and may transmit fungal disease, resulting in reduced seed quality which, in soybeans, is the main type of damage. This work focused on identification and characterization of semiochemicals produced by *N. simplex* that may be useful in IPM programs. Extracts of volatile semiochemicals from adult females and males were obtained by dissection of the metathoracic gland complex characteristic of the heteropteran bugs and by air entrainment and analysed by GC-FID, GC-MS and GC-EAG. Biological activity of the gland and air-entrainment extracts was evaluated in olfactometer bioassays. The metathoracic gland reservoirs of males and females contain the typical defensive compounds similar to those of alydid and pentatomid stink bugs. Additionally, the paired lateral accessory glands (LG) of the metathoracic gland complex contained five female-specific compounds and two male-specific compounds. GC-EAG analysis showed that male antennae responded to five compounds from LG and air-entrainment extracts, and the higher response was obtained for three female specific compounds. Bioassays showed attraction of males to live females and to air-entrainment extracts of females. These results suggest that *N. simplex* females produce a pheromone attractive to males. Bioassays with specific blends and individual compounds produced by females are being conducted to establish the composition of this sex pheromone.

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Effects of the Flavonoids Rutin and Genistin in the Midgut of *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae) Larvae: Histochemical Study

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In this study we verified the chemical components of the epithelial cells of the midgut of *Anticarsia gemmatalis* larvae after the ingestion of soybean genotypes resistant to insects. The caterpillars, obtained at Embrapa/Soja (Londrina-PR-Brazil), were fed with genotypes that present different concentrations of rutin and genistin: BRS 257 (control), BR16 (0,0016mg/g genistin), Dowling (0.0429 mg/g genistin), PI229358 (0.0212–0.0136 mg/g de rutin/genistin), IAC100 (0.0972–0.0142 mg/g de rutin/genistin) e PI227687 (0,3682-0,0122mg/g de rutin/genistin). The midguts were collected, fixed and processed for histochemical analyses to detection of acids and neutral polysaccharides, proteins and lipids. The neutral polysaccharides were visualized specially in columnar cells, being the greatest staining observed in insects fed with the control genotype (BRS257). After diastase, it was detected glycogen in epithelial cells of larvae fed with IAC100. For total proteins technique, we observed positive labeling in columnar, goblet and regenerative cells for almost all genotypes, being the goblet cells the most reactive cellular type in insects treated with Dowling, similar to results detected in the control genotype. No labeling was visualized for acid carbohydrates and lipids. We conclude that the columnar cells of midgut in *A. gemmatalis* larvae presents neutral carbohydrates, especially those structural, while the proteins were detected in goblet cells. Besides, the different concentration of the phenolic compounds rutin and genistin in the resistant genotypes were responsible by the changes in chemical composition of midgut epithelial cells, which could affect the digestive process.

Differential Response of Soybean Plants to the Herbivory of Two Species of Stink Bugs (Hemiptera: Pentatomidae) and their Influence in the Foraging Behavior of Egg Parasitoids (Hymenoptera: Scelionidae)

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In response to herbivory attack, plants produce herbivory induced plant volatiles (HIPVs) that could be used as cues by parasitoids when they are foraging for hosts. It is known that the HIPVs show variations related to plant species and varieties and herbivorous species causing damage. For the tri-trophic systems soybean-stink bug-egg parasitoids Moraes et al.^[1] showed that the parasitoid *Telenomus podisi* responded selectively to volatile from plants where the brown stink bug *Euschistus heros* fed. This work had the objective to test the hypothesis that HIPVs of soybean by different species of stink bugs (*E. heros* or *Nezara viridula*) are different and could implicate in a differential behavior response of the parasitoids *Trissolcus basalidis* and *Telenomus podisi*. It would be expected that if soybean induced defenses are dependent of the herbivore species, the parasitoids could respond differentially and in relation to the attack of their preferential hosts (*E. heros* for *T. podisi* and *N. viridula* for *T. basalidis*). The volatiles from plants undamaged and damaged by each or both herbivores were collected using air-entrainment each 24 h and lasted 7 days. The plants extracts were analyzed by GC and GC-MS. Plants damaged as described above were used in olfactometer bioassays to test the influence of the volatiles in the foraging behavior of the parasitoids. Preliminary results showed quantitative and qualitative differences in HIPVs of soybean plants herbivore damaged when compared to undamaged soybean and responses of the parasitoids dependent on the identity of the herbivore causing the damage.

^[1]Moraes, M. C. B.; Laumann, R. A.; Sujii, E. R; Pires, C.; Borges, M. *Entomologia Experimentalis et Applicata*, 115:227-237, 2005.

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Forager Distribution in Nectar Sources and Sugar Acceptance Threshold within an Ant Colony

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Chemical control in social insects has the restriction to use only alimentary baits. Almost all the urban ants collect sugar solutions as an important part of their diet. Thus, sugar is a common bait used in ant control. Some ant species present a wide range in worker sizes and foragers of different sizes participate in nectar collection. Different worker sizes involve diverse restrictions and limitations regarding intake respect to solutions of different concentrations. We analyzed how forager sizes relate with colony's foraging organization and some of the underlying mechanisms that might operate.

In field experiments, we found that workers of different sizes collecting for nectar are distributed according to sources productivity. Larger ants forage on nectar that is more concentrated or produced at higher flows. For laboratory experiments, we firstly developed a new protocol to record sugar acceptance threshold (SAT), which represents the lowest sucrose concentration at which an ant can distinguish between water and sucrose eliciting a licking response. It allows detecting differences in SAT under carefully controlled conditions using a wide range of concentrations in harnessed ants of different sizes. Results showed that large ants had a higher threshold than small ants when the colony was not sugar starved, but when the colony was longer starved, both small and large ants presented the same low threshold for sugar acceptance. These physiological variations between ants of different sizes can operate as a differential trigger in forager recruitment contributing to the distribution found in the field among nectar sources, which leads to a greater overall system efficiency.

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Chemical Characterization of the Volatile Emissions from the Clone *Populus x canadensis* 'Conti 12' Associated with *Megaplatypus mutatus* Chapuis Attack

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Megaplatypus mutatus (= *Platypus mutatus*) (Chapuis) (Coleoptera, Platypodidae) is an ambrosia beetle native to South America. The galleries weaken the tree trunks, causing them severe stem-breakage and mortality in commercial poplar plantations. The attack is initiated by pioneer males selecting a host tree to build a short nuptial gallery, from which they attract females using a sexual pheromone. The preference of attack by *M. mutatus* has been correlated with the increasing diameter.

In this work we explore the possibility that differential susceptibility of individual plants could be associated with volatiles emitted. With this aim we collected and analyzed volatiles emitted by wood-bark of both attacked and non attacked plants during *M. mutatus* flying season. Furthermore, we tested the biological activity of selected compounds in the volatile emissions with a walking behavioral assay. The comparison of the volatile profiles of attacked and non attacked trees showed both qualitative and quantitative differences.

The attacked plants, unlike the non-attacked ones showed the following compounds: a long chain aldehyde of rt:20.57, α -ylangene, δ -cadinene, α -gurjunene and β -cubebene, while β -sesquiphelandrene and β -chamigrene were detected in non attacked plants but not in attacked ones. The results showed that α -Copaene is the common component of all the samples analyzed, its concentration being increased in attacked individuals. Behavioral bioassays analysis showed that males *M. mutatus* are attracted to α -copaene, while females are not. The relative increase of α -copaene in attacked individuals and the positive behavioral answer of males to it, could suggest that this compound may play a role in the orientation of the pioneer male towards the most suitable host.

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Food's Odor Learning in *Tetragonisca angustula* Stingless Bee

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Tetragonisca angustula stingless bees present a wide-spread distribution in Latin America from southern Mexico to the northern part of Argentina. They have been catalogued as poor recruiters and as solitary foragers who, in their orientation towards a food source, use chemical cues left by co-specifics and visual cues of co-specifics or landmarks but lack sophisticated communication strategies to inform a food source's location. To assess the relevance of the food's odor in their foraging strategies we investigated their ability to learn the association between odors and reward (sugar solution), and the effect on learning of previous encounters with scented food either inside the hive or during foraging. During food choice experiments, when the odor associated with the food had been encountered by the bee in a previous foraging trip at the feeding site, their choice was biased to that odor. However when the scented food was placed inside the nest where the contact with the odor depended on the food circulation dynamics of the colony their choice was not altered. To individually assess their olfactory learning abilities we performed a differential olfactory conditioning of proboscis extension response (PER). The individuals did not show significant discrimination levels unless they had previously ingested scented food. Only then they were able to learn the association of food to a specific odor. From our results it appears that *T. angustula* bees can effectively learn the food's odor and use that information in their search for food, and that they rely more on their own individual prior experiences than on the information provided by nest mates.

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Effect of Resistant and Susceptible Soybean Cultivars on the Nymphal Development, Fecundity and Mortality of *Euschistus heros* (Hemiptera: Pentatomidae)

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The association of resistant varieties and biological control has great interest and potential to be used for pest management. The aims of this work were to evaluate the effect of cultivars Silvânia (susceptible to stink bug attack), Dowling e IAC100 (resistant) on *E. heros* nymph development and to investigate the influence of the flavonoids on the resistance [1]. Nymphs of stink bug were placed in Petri dishes on a diet of soybean pods and observed daily. After adult emergence, they were weight and then put in pairs for mating. The nymph mortality, the adult longevity, fecundity and the eggs fertility were evaluated for each cultivar. To quantify the flavonoid compounds, extracts of immature seeds (cv Silvânia and Dowling) undamaged and damage by herbivory, were analysed using high performance liquid chromatography (HPLC). The nymphs reared on cv Dowling did not complete their biological cycle. Survivorship curves of immatures in cv. Sylvania and IAC100, analysed by Kaplan-Meier Survival Distribution, did not show significant difference. The medium weight of adults on others cultivars was not different. The medium male longevity on cv IAC100 was 11.0 days, while on cv Silvânia was 35.8 days; for females was 13.4 days on cv IAC100 and 40.6 days on cv Silvânia. The female fecundity (105.7 eggs/female) and egg fertility (66.3 nymphs) on cv Silvânia were higher than on cv IAC100 (10.2 eggs/female e 5.8 nymphs). Total amount of flavonoids compounds was higher in cv Silvânia than in cv Dowling, but did not differ when compared between herbivory damage and undamaged plants in the same cultivar. These results suggest that the cv IAC100 and Dowling present direct defense (constitutive) against *E. heros*, and that the herbivory damage did not induce flavonoids production.

[1] Piubelli, G. C., Hoffmann-Campo, C. B., Moscardi, F., Miyakubo, S. H., Oliveira, M. C. N. 2005. Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis*? *J. Chem. Ecol.* 31: 1509-1525.

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Floral Volatiles from South American Alliaceae

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The Alliaceae is a widely distributed monocot family, mostly formed by perennial herbaceous plants that thrive well in arid environments. The representative genus is *Allium*, exclusive of the Northern hemisphere, to which belong edible species such as onion, garlic and leek. In South America, the Alliaceae are represented by *Tulbaghia*, *Prototulbaghia* and thirteen endemic genera of the Gilliesieae y Allieae tribes. The Gilliesieae are the most specialized in floral biology, with several species lacking nectarines and presenting osmophores as odor glands. Such characteristic suggests a mechanism of deceptive pollination, similar to the orchid's family. A preliminary study of the volatile chemical profile in floral scents of species belonging to this tribe may, shed light on such pollination strategy.

In this study, we present GC-MS analyses of volatile organic compounds (VOCs) emitted by four endemic South American species of Alliaceae, cultivated in controlled conditions: *Gethyum atropurpureum* (Gilliesieae), *Leucocoryne coquimbensis* (Allieae), *Nothoscordum ostenii* (Allieae, Inodorum's section) and *Nothoscordum sp.* (Allieae, Nothoscordum's section). *G. atropurpureum* showed a very complex profile, with more of 90 compounds characterized, and the presence of high amounts of saturated aldehydes (10-16 carbon atoms). *L. coquimbensis* presented smaller VOC diversity, including mostly esters of benzil alcohol, elemicin and several green-leaf volatiles. *Nothoscordum ostenii* emitted quite a different profile than that of *Nothoscordum sp.*, which may be relevant as a chemotaxonomic character, since they belong to different sections. The first emitted low amounts of VOCs, mostly composed of monoterpenes, and the second showed large quantities of benzenoid derivatives such as methyl benzoate, methyl salicylate and benzaldehyde. These differences in VOCs may be related to differences in the pollination strategies of these closely related species, since the Inodorum section flowers at night, while the Nothoscordum section is diurnal.

Phytotoxic Activity of Extracts and Eremophilane from *Senecio filaginoides* var *filaginoides*

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Most secondary compounds play an important defensive role against herbivores and other plants, this fact could be useful as a strategy for novel pesticides with less environmental effects. *Senecio* species (Asteraceae) are widely distributed, and their principal chemical constituents are furanoeremophilanes, eremophilanolides, pyrrolizidine alkaloids and volatile terpens.

Phytotoxic assays against *Lycopersicum esculentum* (tomato) were performed with a crude ethanolic extract (E.E.), an alkaloidal extract (A.E.) and 9-oxofuranoeremophilane (Compound 1), all obtained from the aerial parts. E.E was evaluated at 20, 50 and 100 mg/ml; A.E. and compound 1 at 0.1, 0.3 and 0.5 mg/ml. Percentage of Inhibition (germination and radicle length) is calculated as $I\% = 100 \times (C - T) / C$ where C is the control and T the treatments. Negative values mean promotion of germination or radicle growth.

The A.E. had much greater effect and significantly inhibited germination and radicle growth. Compound 1, to a lesser extent, had an inhibition effect on germination, but promoted seedling and radicle growth at the lowest concentration. The E.E. had similar effect on germination, but required higher concentrations and radicle length was also promoted. The difference in the effect could be explained by the different structure of eremophilanes and pyrrolizidine alkaloids, meanwhile in the ethanolic extract antagonist or synergist effects could be responsible.

[1] J.Agric.Food Chem. 55 (2007), 10656-10663.

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Activity of Extracts and Fractions from *Anacardium humile* in the Nest of Leaf-Cutting Ants

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The leaf-cutting ants are known by the power of destruction of a large number of plant species and the economic damage caused in agriculture. They cause serious losses in Brazilian agriculture and forests, where the main economic targets are species of eucalyptus and pine. The symbiotic relationship between leaf-cutting ants the fungus (*Leucoagaricus gongylophorus*) is essential for their survival [1], thus, inhibition of the fungus growth may be a relevant method to ant control. The *Anacardium* have been widely used for therapeutic purposes in various diseases. The present study evaluated the biological action of the *A. humile* in *Atta sexdens rubropilosa* and their symbiotic fungus. The ethanolic extract of the leaves (AHF), quercetrine and quercetine were assayed in nest of leaf-cutting ants. The extract AHF and quercetrine showed insecticidal activity on *A. sexdens rubropilosa*, but only low inhibitory activity on *L. gongylophorus*. The ants survival median (S_{50}) were in the 4th, 3th and 13th (2000 $\mu\text{g.mL}^{-1}$, 2000 $\mu\text{g.mL}^{-1}$ and 100 $\mu\text{g.mL}^{-1}$) for AHF, quercetrine and quercetine, respectively, compared with the controls with median of 16 days. The extract AHF inhibited the symbiotic fungus growth in 17 % at concentration of 1000 $\mu\text{g.mL}^{-1}$, quercetrine and quercetine inhibited 26 % and 27 % (100 $\mu\text{g.mL}^{-1}$), respectively, when compared with the control.

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Acknowledgements: FAPESP, CNPq, CAPES, INCT

Defenses of Tropical Freshwater Macrophytes Against Herbivory

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Herbivory show strong impacts on ecosystem and can be important in controlling the structural properties and dynamics of freshwater macrophytes communities. Defense mechanisms against predation in plants are know for marine and terrestrial ecosystem, but few studies are conducted with freshwater macrophyte. The aim of this study was investigate the role of secondary metabolites of 12 freshwater macrophytes of Parque Nacional da Restinga de Jurubatiba (Rio de Janeiro, Brazil) against *Biomphalaria sp.* The assays were conducted with crude extract and reconstituted plants (specimens whose overall plant architecture was scrapped). The secondary metabolites profile of organisms was analyzed by Thin Layer Chromatography (TLC). Our results showed that 9 of the 12 species studied were deterrents against herbivory ($p < 0,05$, statistic test accordin according each experimen). Although most of the extracts were deterrent, the crude extract of *Ceratopteris thalictroides* was the only one to be able to inducing the consumer ($p = 0,01$, Wilcoxon, $N = 21$). Among all species chemically defended, *Potamogeton montevidensis*, *Xyris brevifolia*, *Eleocharis interstincta* and *Paepalanthus sp* showed greatest defense potential against herbivory. The TLC analysis indicated presence of phenolics and terpenoids - known to act as defenses against herbivores - correlated positively with those more deterrent. Tests using reconstituted plants showed that despite the mischaracterization of their architectures, the chemically defended species *E. azurea*, *P. montevidensis* e *Utricularia gibba* maintained its defensive potential, with reduced rates of consumption ($p = 0,001$ ANOVA, $N = 30$). However, the species *Typha domingensis* and *X. brerifolia* that showed defensive properties, were the most consumed ($p = 0,001$ ANOVA, $N = 30$), pointed that despite the production of chemical defenses, other factors such as nutritional content may be involved in preference and consumption by the herbivore. Our results suggest that freshwater macrophytes can show deterrent properties and significantly influence on the food herbivores preference interfering in ecological relationships on ecosystems in which they are inserted.

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Attraction of Virgin *Aedes (Stegomyia) aegypti* (Linnaeus 1762) to the Odor of Conspecific Adults and to Hexanic Extracts of Adults

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It was proposed early 1950's that male *Aedes aegypti* locate females conspecific by wing beating sound [1]. Recent studies reported the possible existence of sex pheromone [2]. However, there is a lack of behavioral studies on the role of conspecific chemical communication. The aim of this study was to evaluate olfactory responses of virgin male and female *Ae. aegypti* mosquitoes to extracts and odors of conspecific insects. The mosquitoes were reared in an insectary (27 ± 2 ° C, 75-80% RH, 12L:12D), separated by sex in the pupal stage and kept in separate rooms. The responses of groups of *A. aegypti* of 5-10 days old were evaluated in a horizontal double choice olfactometer with 10 insects/test. The olfactory stimuli were: (1) Conspecific mosquito odors: consisted of an airflow system passing through insects coupled to the olfactometer (n=15). (2) Hexanic extracts of virgin insects (400 males and 400 females) consisted of filter paper impregnated with 50µl of extract and the control (hexane) (n = 3) and the responses of male and female virgins with and without blood meal were measured. The results of conspecific mosquito odors showed that males were attracted to both sexes (p <0.05, *t* - Test) and the presence of virgin females was an important stimulus for flight activation (p <0.05). Females were not attracted by any conspecific mosquito odor, except the males odors that stimulated their flight activation (p <0.05). Males were attracted to extracts of conspecific males and remained flying. Females without blood meal were attracted to extracts of both sexes (p <0.05) whereas blood fed females were attracted to male extracts (p <0.05). We conclude the existence of pheromone in *Ae. aegypti* and we are currently identifying them for possible use in mosquito traps for surveillance.

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Acknowledgement: CNPQ

Behavioural Response of the Blood-Sucking Bug *Triatoma infestans* Larvae to Volatiles Released by Disturbed Adults

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Triatoma infestans adults possess two pairs of exocrine glands, metasternal and Brindley's glands. According to previous reports, one of them or both could release their volatiles under different contexts such as alarm, defense or sexual behaviour. Apparently, both types of glands discharge their contents during alarm and defense context, after mechanical disturbance [1]. Isobutyric acid might induce different behavioural effects as a function of concentration [2] and is the main compound released by Brindley's glands when adults are disturbed. Using a locomotion compensator, we analysed the orientation response of fourth-instar larvae evoked by airstreams loaded with volatiles released by mechanically disturbed adults or different dose of isobutyric acid (0.001, 0.01, 0.1, 1, 10, 100, 1000 and 10000 μg). The mean walking angle (α) displayed by each insect along the experimental time was computed and subsequently, for every experimental group a mean angle (α_m) and the length of the resultant mean vector (r) were calculated. Besides, an orientation index was calculated. In our results larvae exposed to odours from disturbed adults exhibited an oriented response walking away from the source of the volatiles. This result supports a previous hypothesis which proposes that the blend released by disturbed adults constitutes an alarm pheromone. On the other hand, preliminary results for isobutyric acid bioassays showed variable orientation behaviour in response to different concentrations tested. We propose a possible biological role of this compound within the intra-specific chemical communication.

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Divergent Strategy for the Synthesis of Chiral γ -Lactone-Core Insect Pheromones. Synthetic Approaches

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Insect pheromones are valuable tools for pest management programs, due to the key roles they play in intraspecific chemical communication. Often these semiochemicals are chiral compounds in which their stereochemical purity is essential for activity. Thus, developing stereoselective strategies for their synthesis is still a challenging task for synthetic organic chemistry. Herein we report a divergent strategy for the synthesis of chiral γ -lactone-cores of a variety of insect aggregation pheromones [1].

The key steps in the designed synthetic route are: a Johnson-Claisen rearrangement [2] leading to a γ -unsaturated ester, and a Sharpless Asymmetric Dihydroxylation (SADH) [3], developing the corresponding chiral γ -hydroxyesters in an enantiomerically pure form (Figure 1). Cyclization of these compounds will be performed in order to obtain the desired (*R*)- and (*S*)- γ -lactones. By further functionalization with the corresponding side chains, the different compounds of interest will be achieved (Figure 2).

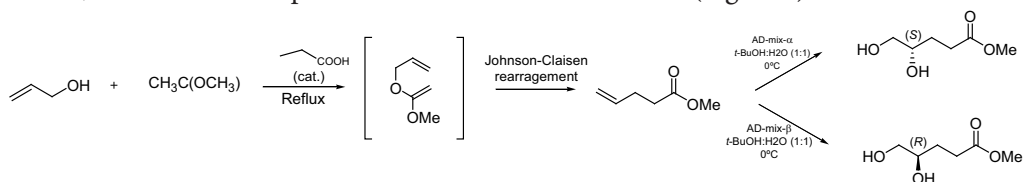


Figure 1

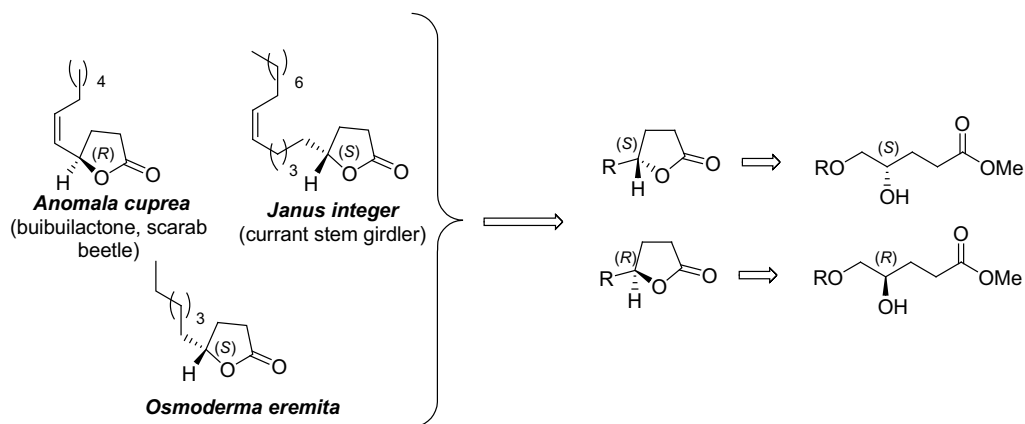


Figure 2

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Support for this work from PEDECIBA (Programa de Desarrollo de Ciencias Básicas) and ANII (Agencia Nacional de Investigación e Innovación) is gratefully acknowledged.

Biological Activity of Essential Oils on *Bemisia tabaci*

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Bemisia tabaci is a polyphagous pest insect very difficult to control specially on greenhouse horticultural crops. It is a significant pest in lettuce causing leaf speckling or yellowing. Heavy infestations in early stages of lettuce crops result in poor growth. Synthetic products such as nicotinoid and pyrethroid insecticides are mainly used to control whiteflies generally producing toxic residues, human health problems and pesticide resistance. Essential oils constitute a natural source for pest and disease management strategies and cause no risk to either the environment or human health [1] [2].

The objective of this work was to evaluate repellence and mortality effect of whitefly attacking greenhouse lettuce using essential oils of *Cymbopogon citratus* Stapf (lemmon grass), *Laurus nobilis* L. (laurel) and a mixture of both.

Repellence effect was assayed on randomly selected lettuce plants (*Lactuca sativa* L.) grown in greenhouse receiving three different treatments and a control (each one repeated five times). Mortality was tested using a laboratory technique using glass flasks in which adult whiteflies were placed and received same treatments and repetitions. Aqueous solutions containing 5% essential oils and emulsifier were pulverized for both assays. Results were subjected to statistical analysis of variance and Tukey test.

Highly significant differences were found for the three treatments respect to the control in both tests. The solution applied with mixture of both essential oils presented a better behaviour in the control of whitefly although statistically there were no significant differences comparing with essential oils separately.

Results may confirm that these essential oils mainly blended can be used as an alternative in integrated pest management

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A New Strategy of Storing Chemical Defenses in the Harvestmen *Iporangaia pustulosa* and *Neosadocus maximus* (Arachnida: Opiliones: Laniatores)

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Opiliones or harvestmen are found in all continents and are the third major group of arachnids with 6000 known species. They have a chemical defense mechanism that involves secreting volatile compounds through two lateral glands [1]. In the suborder Laniatores these secretions contain mixtures of benzoquinones and alkylated phenols isomers, whereas in the suborder Eupnoi the secretions contain alcohols, naphthoquinones and other acyclic short chain substances [1]. We studied the secretions of *Iporangaia pustulosa* and *Neosadocus maximus* (both Laniatores) using GC-MS and ¹H and ¹³C NMR spectroscopy. The major constituents of their secretions and their relative abundances are in Table 1. The presence of compounds 1-hepten-3-one (**1**) and 1-(6-butyl-3,4-dihydro-2H-pyran-2yl)-pentanone (**2**) have already been reported in the literature by Hara *et al* [2]. Using the secretion of several individuals, however, we were able to characterize compound (**2**) as a dimer of (**1**). A Diels-Alder mechanism was proposed for the dimerization. Racemic (**2**) was obtained synthetically at high temperature and pressure, and the spectral data are identical to the natural product. The identity was also confirmed by co-elution in GC-MS of the synthetic dimer (**2**) with the component in secretion of *Neosadocus maximus* and *Iporangaia pustulosa*. For the first time a pyranyl ketone as (**2**) was reported in harvestmen contributing for identification of the Opiliones chemistry, as well as for understanding storing strategies of volatile compounds. Ketones (**1**) and (**2**) are present in 11 among the 22 species studied by Hara *et al* [2] and this dimerization phenomena can occur also in other species of harvestmen containing vinyl ketones.

Table 1. Composition of harvestmen secretions (GC-MS).

| Compd. | <i>I.pustulosa</i> | <i>N.maximus</i> |
|---------------|--------------------|------------------|
| 1 | 79.0% | 80.4% |
| 2 | 13.6% | 15.8% |
| others | 7.4% | 3.8% |

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Characterization And Evaluation of Phytotoxic Potential of New Clerodane Isolated of Foliar Exudates of *Blakiella bartsiiifolia* (S. F. Blake) Cuatrec.

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Blakiella bartsiiifolia (Asteraceae), an endemic plant that has colonized the desertic Andean Paramus, presents numerous glandular trichomes in its foliar surface and stem. In these aerial parts were detected flavonoids, terpenes and terpenoids, among others. One of these secondary metabolites isolated and identified by different chromatographic and spectroscopic techniques (MS, IR, ¹H y ¹³C-RMN, COSY, HSQC, HMBC, NOESY) was the clerodane denominated 15,16-dihidroxi-3,13(Z)-clerodadien-20-oico acid (blakiefólico acid 1). diterpene's alelopatic potential was determinated as the phytotoxic activity (% germination, root's length) on different species of monocots [*Allium cepa* L. (onion)] and dicots [*Lactuca sativa* (lettuce)]. It showed a inhibitor effect on germination and growing for both species.

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Spatial Distribution of Oviposition in *Rhodnius prolixus* and the Possible Cues Involved

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Oviposition aggregation pheromones are found in a variety of insects. In some cases this pheromone is on the eggs themselves [1]. *Rhodnius prolixus*, an important vector of Chagas' disease in the north of South America and a classical model of insect physiology, stick their eggs to substrates. It has already been shown that oviposition substrates modulate female's fecundity [2], but it is not clear if there are any chemical or mechanical cues mediating the preference on a particular oviposition substrate or behavior. We tested whether *R. prolixus* females lay their eggs with any particular spatial distribution, and the possible cues mediating that distribution. Along 13 days, one or three females were kept in a cylindrical container with filter paper in each base, which was divided in eight equal regions and serve as oviposition substrate. Experimental groups were containers: without any chemical mark, with two regions marked with an extract of 200 eggs washed in 300 μ l of di-chloromethane (DCM) and with only the vehicle (control). The number of eggs in each region was counted and the degree of aggregation was analyzed. Preliminary results show that, there is spatial aggregation of eggs, but it does not seem to be mediated by a chemical cue in DCM extracts, since there were no significant differences in the number of eggs laid in the marked and unmarked regions. Conversely, the proportion of eggs laid on the edges between the filter paper and the container were significantly higher than those laid in the central area. These results do not support the hypothesis of chemical cues guiding aggregation oviposition pattern and suggest that mechanical cues are involved. Spatial aggregation of laid eggs together with a temporal synchronicity of hatching could benefit *R. prolixus* reproductive success by reducing the risk of predation and deleterious effects of dehydration.

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Profiling Gland Secretions from Pampas Deer

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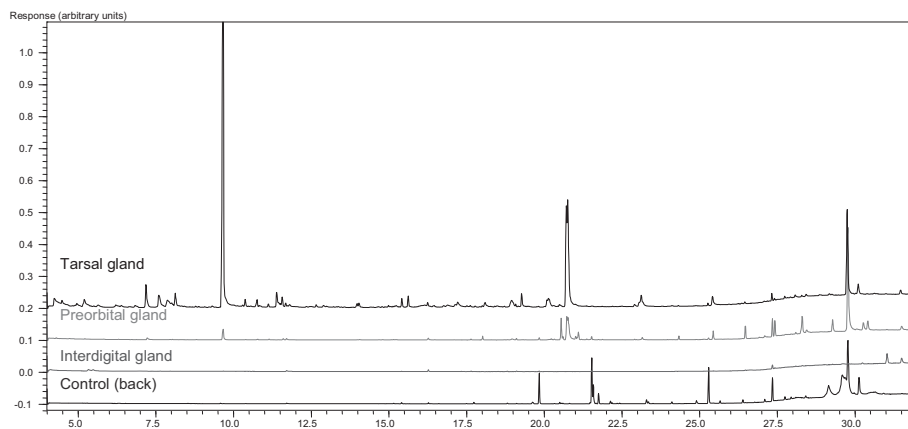
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Pampas deer (*Ozotoceros bezoarticus*), an emblematic species of the Southern Cone grasslands, is in serious risk of extinction. Nowadays, the main semicaptive population (*ca.* 80 individuals), is sited in Uruguay (Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar-34°3' S, 55°1' W). We have previously reported pampas deer marking behavior, and its variation in relation to age and season. Gland secretions have great importance in intraspecific communication, triggering many social interactions (agonistic or maternal behaviors, alarm calls, territorial marking, etc.). Moreover, as olfactory clues have important direct effects on mammalian communication -and in particular on ungulate reproductive behavior-, we have characterized the main compounds of the cutaneous glands (preorbital, tarsal, and interdigital) as possible candidates of scent marking pheromones.

Two groups of males, one of 6 adults (4-6 years old) and one of 6 yearlings (1.5 years old) were studied during 13 months, to typify marking behavior. During this time, all males were anaesthetized every 3 months to obtain samples from glands. Secretions were then extracted as analyzed by GC/MS for volatiles. Preliminary chemical characterization was made on the basis of the spectral data and retention indexes. Whereas mercaptanes were the major compounds within tarsal volatiles, sterols were the main ones in the preorbital secretion. In the case of interdigital samples, their volatile profiles showed the same kind of compounds but in much lower amounts. We have also found that the volatiles extracted from the cutaneous glands showed distinctive profiles (Figure 1), a crucial attribute to postulate pheromone function.

Figure 1: Typical GC/MS traces of the secretions extracted from a Pampas deer adult.



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Inhibition Electron Transport Chain by Natural Products from *Ruta graveolens* L.

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It is well known that plants compete with other plants for light, water and nutrients and have evolved complex strategies during evolution to cope with this problem. The production and accumulation of secondary compounds, which inhibit the germination or the development of other plants, is one way to enhance the fitness of a plant producing them. The molecular targets of secondary compounds involved in allelopathic interaction include membrane stability, enzymes, electron transport chains, photosynthesis among others [1, 2]. Three natural products were isolated from the plant *Ruta graveolens* and their effects on electron transport chain in thylakoids were tested. Isoimperatorin (1) and Chalepentin (2), furanocoumarins, inhibited ATP synthesis; and the alkaloid arborinine (3), inhibited electron flow (phosphorylating and uncoupled) from water to methylviologen (MV) in freshly lysed spinach thylakoids. Compounds 1 and 2 inhibited ATP synthesis with I_{50} of 59.8 μM and 73.2 μM respectively being considered potent inhibitors. ATP synthesis coupled to electron flow from water to MV was determined titrimetrically. The effect of 3 non-cyclic electron transport rates showed significant results. This compound inhibited the flow electron phosphorylating and uncoupled with I_{50} of 11.6 and 9.8 μM respectively. The mechanism of action on electron transport are in development.

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Repellent Activity of Extracts from Fruits of *Schinus molle* var. *areira* (Anacardiaceae) Against Antennectomized Adults of *Blattella germanica* (Dyctioptera: Blattellidae)

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Blattella germanica (L.) is an important medical insect pest because of their cosmopolitan occurrence and abundance in homes and other buildings as potential carriers of faecal pathogens and major source of allergens [1]. Although the use of synthetic insecticidal is really advantageous for the control of urban pests at short periods, many problems emerge from their utilization after longer periods. As a consequence, it is important to look for alternative control methods. In the current study we tested the repellent effect of hexanic and ethanolic extracts from fruits of *Schinus molle* var. *areira* on antennectomized males of *B. germanica*, in order to determinate the probably location of the chemoreceptors involved in this behavioral response. A 9 cm round filter paper was cut in half. One side was treated with 0.5 ml of the test compound solution, and the other side was treated with either hexane or ethanol, depending upon which solvent was used to dissolve the test material. The papers were allowed to dry for 1 h before being placed in a 10 cm petri dish arena. Ten antennectomized (remove the antennae at the scape) and nonantennectomized insects were released in the center of each Petri dish, and their distribution was recorded 24 h later. Repellence percentages were calculated for each extract. Comparison was performed by using a paired *t*-test. The repellency decreased a 34% for the hexanic extract and a 50% for the ethanolic extract on antennectomized insects. Similar results were obtained by [2]. These results suggest that the chemoreceptors involved in this behavioral response are probably located on the antennae of *B. germanica* and that hexanic and ethanolic extracts from fruits of *S. molle* var. *areira* had volatile compounds that generated an olfactory response on this urban pest.

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Bioactivity of Essential Oil from Leaves of *Schinus molle* var. *areira* (Anacardiaceae) Against *Rhopalosiphum padi* (Homoptera:Aphididae)

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Rhopalosiphum padi, the bird cherry oat aphid, is one of the numerous of economically important aphids of winter cereals in Argentine [1]. This pest damages the host by direct feeding and by transmitting barley yellow dwarf virus (BYDV) [2]. Limiting the bird cherry oat aphid infestations can prevent damage, reduce incidence of BYDV and sustain yield of small grains. These infestations may be limited by various control strategies including host plant resistance, chemical, biological and cultural control [3]. The main objective of this study was to evaluate the contact toxicity produced by essential oil from leaves of *S. molle* var. *areira* on *R. padi* apterae adults. The toxicity of the essential oil was measured in the laboratory using the immersion method described by [4]. The essential oil was prepared by hydrodistillation using a Clevenger type apparatus for 3 h. The oils were dried over anhydrous sodium sulphate and stored at 4°C under N₂. Stock solutions of test material were prepared by dissolving essential oil in 1 ml of a solution of ethanol and Tween 20% (0.012:10). The concentrations evaluated ranging from 25 to 75 mg/ml. Adult mortalities were determined 24 h after treatment. All treatments were replicated three times. The LC₅₀ value was calculated by probit analysis using the MicroProbit analysis program version 3.0. The LC₅₀ value obtained was 14.05 mg/ml. These results showed that the essential oil from *S. molle* var. *areira* could be used to the management of populations of *Rhopalosiphum padi*.

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Evaluation of the Efficiency of Pheromone Formulations Used to Control *Metamasius hemipterus* (Linnaeus, 1758) and *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) in Agroecosystem Coconut (*Cocos nucifera*) in Neópolis - Sergipe

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The culture of *Cocos nucifera* aiming the production of coconut has been increasing production as an alternative sustainable and economically viable, with the advantages for early cut, high productivity and good fruit quality. Among the pests that damage the crop stand out weevils: *Metamasius hemipterus* L. that despite being considered a minor pest has been increasing more among the palms and *Rhynchophorus palmarum* L., and beyond the direct damages may facilitate entry of pathogenic microorganisms in plants. The aim of this study was to test the efficiency of seventeen pheromone formulations used to control these pests in coconut agroecosystem. The experiment was conducted during November 23 to December 23, in an area with 25,000 coconut plants, located in the Plateau de Neópolis, distant 121 km from Aracajú state of Sergipe. The seventeen formulations were used in quintuplicata randomly arranged with 100 meters away from each other with a total of 85 closed traps. In the traps were placed 04 stalks of sugar cane with 10 cm size and cut lengthwise. The traps were checked weekly and the data analysis showed that the 4-methyl-5-nonanol and 2 - methyl-4-heptanol when formulated 1:1 was more efficient only for *M. hemipterus* and the formulation of 6-methyl-2-hepten-4 -ol did not show preferency.

Aristolochic Acid Content in Peruvian *Battus* Swallowtails (Lepidoptera: Papilionidae: Troidinii)

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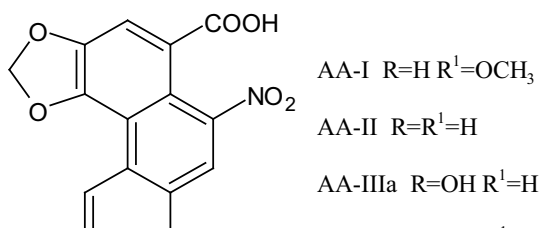
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Larvae of the genus *Battus* (Papilionidae, Troidini) are able to sequester from their host-plants aristolochic acids that remain in their body during their lifetime, and are transferred to their progeny once they reach adulthood. Thus, aristolochic acids are essential for the defence and oviposition behaviour in this genus of butterflies [1,2].

We explored the sequestration of aristolochic acids (AAs) in male adults tissues (body and wings extracts) of: *Battus polydamas polydamas* (Linnaeus, 1785), *B. maydes chlorodamas* (Guenée, 1872), *B. maydes plinius* (Weymer, 1890), *B. streckerianus* (Honrath, 1884) and *B. crassus* (Cramer, 1777) from Tingo María and Rodríguez de Mendoza (Perú). The methanolic extracts were quantitative analyzed by HPLC-DAD in order to obtain information of the distribution of phenolic and non-phenolic AAs in these butterflies.

The composition of AAs was very different among the species, with higher concentration in the body than in the wings tissues. With the exception of *B. maydes chlorodamas*, aristolochic acid-I (AA-I) was found as the mayor component in all the other species, some of them also contain AA-IIIa and AA-IVa, and AA-II, was only found in minute amounts in *B. crassus*. Non-phenolic AAs generally show higher toxic activities than phenolic AAs (AA-IIIa and AA-IVa) [3], in particular, AAI is among the most toxic aristolochic acid. AAI is the most abundant AA both in wings and body extracts, thus supporting the idea of a defensive role of non-phenolic AAs.



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Volatiles from Uruguayan Honeys in Relation to their Botanical Origin

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Apis mellifera bees produce honey using compounds from flowers and other plant structures, and even from secretions of sucking insects. The origin of these compounds gives the honey certain physicochemical and organoleptic characteristics, such as appearance, aroma and flavor, which can be used to distinguish the botanical origin of the honey.

In Uruguay, honeys produced in major amounts are mainly from *Lotus* spp., *Eucalyptus* spp. and native Uruguayan forest. Less common botanical sources are white and red clover, sunflower, citrus and soybean. The aim of this study was to characterize the head-space volatiles from honeys of different botanical origins, in order to establish a chemical database to characterize commercial honey.

We performed a palynological analysis of each honey to know the floral origin. Head-space volatiles were collected by solid-phase microextraction (SPME) using a carboxen-polidimethylsiloxane (C/PDMS) fiber, and characterized by GC-MS and retention index data. Floral terpenic compounds and anthropogenic substituted benzene were identified in all honey samples. Differences in the composition of honey head-space volatiles were observed, which can be used as chemical markers of botanical origin.

Identification of the Sex Pheromone of the Stink Bug *Agroecus griseus* Dallas (Hemiptera: Pentatomidae)

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Stink bugs are among the main pests in the world and a promising biorational form to do its control in agriculture is by sex pheromone traps. Thus, volatiles compounds released from both sexes of the stink bug *Agroecus griseus* were collected through the aeration process and extracted with hexane. The gas-chromatographic analysis comparison of these extracts made it possible to observe the presence of a male-specific compound, which caused electrophysiological activity for females antennae when tested with GC-EAD. The identity of this compound was determined by GC-MS and micro-derivatizations as the methyl 2,6,10-trimethyltridecanoate, which was previously identified as the sex pheromone of the pentatomid *Euschistus heros* (F.) [1]. The confirmation of this structure was performed by coinjection of the extract with synthetic standard. Behavioral tests using a Y-Tube olfactometer showed that females are attracted to both natural extract and the synthetic pheromone making it possible to confirm the biological activity of the male-specific compound to this species. Finally, studies were performed on the emission pattern of pheromone, showing that there was apparently a preference for the release of this pheromone during the photophase period.

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Two Methods for Extraction of Volatile Compounds from the Biocontrol Agent *Trichoderma viride*

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Species of *Trichoderma* genus produce volatile and non-volatile secondary metabolites active against several plant-pathogenic fungi. One of the most abundant volatile compounds released by these fungi is 6-pentyl- α -pyrone (6PAP) which has been studied for its activity against a wide range of plant-pathogenic fungi, such as *Rhizoctonia solani* and *Fusarium oxysporum* [1]. The capture of volatile compounds released by the biocontrol agent may be obtained using solvent and/or solvent-free methods. The objective of this study was to evaluate two methods to extract volatile compounds from *Trichoderma viride* which could be used in the identification of bioactive volatiles of *Trichoderma* isolates. Dynamic (Purge & Trap, P&T) and static (solid-phase microextraction, SPME) headspace methods were used to extract VOCs from *T. viride*. Porapak Q and a polydimethylsiloxane coated fused-silica fiber (PDMS) were used as adsorbent matrix, respectively. Volatiles captured by both methods were analyzed by gas chromatography/mass spectrometry (GC-MS). Using both methods was possible to extract 6PAP but different relative areas were obtained, $43.9 \pm 34.2\%$ and $96.7 \pm 0.6\%$ by means of P&T and SPME methods respectively. The numbers of extracted compounds by P&T and SPME methods were 42 and 12, respectively. 6PAP was the only compound extracted by both methods. Unlike P&T, SPME showed good reproducibility. A standard deviation lower than 0.65% was obtained with the PDMS fiber (SPME), whereas values over to 34% were obtained with Porapak Q (P&T). In conclusion, SPME could be considered a simple, rapid and efficient method to extract VOCs released by *Trichoderma* spp., especially 6PAP. These advantages are determined by the simplicity of sampling, solventless extraction, selectivity of fiber coating and thermal desorption. P&T was suitable for the extraction of volatile and semi-volatile compounds and for conserving an extract that could be used in assays related to their biological activity.

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Comparison of Different Fibers for Solid-Phase Microextraction of Fungal Volatile Metabolites

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A suitable technique for the capture and analysis of fungal volatile metabolites is the headspace solid phase microextraction (SPME) used in combination with gas chromatography–mass spectrometry (GC-MS), allowing a fast detection of volatile compounds without the use of organic solvents. The use of this technique for trapping volatiles from fungi is increasing. Different kind of volatile compounds can be captured depending of the chemical composition of the SPME fiber. The aim of this study was to compare different SPME fibers used for extracting volatile compounds from a saprophytic fungus. Four SPME fiber types Polydimethylsiloxane (PDMS, 100 μm), Polyacrylate (PA, 85 μm), Carboxen/PDMS (CAR/PDMS, 75 μm), Polydimethyl-siloxane/Divinylbenzene (PDMS/DVB, 65 μm) were evaluated in terms of their efficiency in extracting volatile metabolites emitted by *Schizophyllum commune* grown in potato dextrose agar (PDA). Preliminary results have shown that *S. commune* release inhibitory volatile metabolites against some plant pathogen fungi, such as *Botrytis cinerea* (not published data). Different classes of volatile compounds were extracted, such as alcohols, esters, thio-esters and sulphur-derivate compounds. Esters were the most abundant compounds extracted by the tested fibers excepting PA. PDMS/DVB fiber favored the extraction of esters (62.3%) and thio-esters (25.6%), whereas the profile of volatile compounds extracted using PA fiber showed the highest proportions of alcohols (75.7%). Relative area of esters diminished to 27.7% in CAR/PDMS, while thio-esters reached 64.9%. The relative area of esters and thio-esters extracted by PDMS fiber were 38.4% and 21.6%, respectively. Volatile esters have been reported by their high inhibitory activity against a wide range of pathogenic microorganisms [1]. Our results showed that fibers containing PDMS were suitable to collect this kind of compounds, contrasting PA fiber which was suitable to extract mainly alcohols.

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Orientation and Communication in Meliponine Bees: Semiochemicals Used During Defence and Aggression

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Meliponines ('stingless bees') represent highly rewarding research objects to study the chemical ecology and communication of insect societies possessing a flying worker caste. Among the worldwide more than 400 species one can find a great variety of communication and defensive strategies. I studied the behaviour and chemical ecology of sympatrically occurring neotropical species to identify the chemical signals and cues important for the intra- and intercolonial communicative interactions both on intra- and interspecific level.

Whereas the GCMS analysis of pure labial gland extracts mainly contained a small number of less volatile esters, the mandibular gland extracts in all tested species contained a variety of volatile substances. In all cases the majority of mandibular gland substances were moderately to highly volatile (volatility higher than that of 2-tridecanol). 2-heptanol was the only major compound (more than 10% of the sum of all detected peak areas) occurring in the two main study objects (*Trigona spinipes* and *Scaptotrigona*). *T. spinipes* had only one other major component (nonanal), while *S. aff. depilis* showed two additional ones (benzaldehyde and 2-tridecanone). The chemical composition of mandibular gland volatiles of *T. spinipes* differs substantially from that of *S. aff. depilis*. Bees consistently attacked a standardized target upon exposure to mandibular gland extracts at the nest entrance. In contrast to this finding, bees rarely attacked the same target when air, pure labial gland extract, pure hypopharyngeal gland extract or the solvent pentane had been released in the same way. The results consistently show that in all hitherto tested meliponines the mandibular glands were the only pheromone containing glands with an obvious potential to elicit aggressive or defensive behavior not only on intracolony and intraspecific, but also on interspecific level.

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Toxin Profile of *Alexandrium catenella* from the Chilean Coast as Determined by Liquid Chromatography with Fluorescence Detection and Liquid Chromatography Coupled with Tandem Mass Spectrometry

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The tetrahydropurine toxins associated with paralytic shellfish poisoning (PSP) are potent neurotoxins. These toxins are produced by marine dinoflagellates belonging to the genera *Alexandrium*, *Pyrodinium* and *Gymnodinium*, as well as by freshwater and brackish water cyanobacteria, such as *Aphanizomenon flos-aquae*, *Anabaena circinalis* and *Lyngbya wollei*. Suspension-feeding shellfish can be vectors of such phytoplanktonic toxins via accumulation in their tissues, but these toxins can also move directly through pelagic food chains, affecting zooplankton, fish, birds and marine mammals.

In Chile the presence of PSP is endemic in the southern part of the ocean littoral. In these Austral Regions, *Alexandrium catenella* has been reported as the main agent responsible for PSP toxin production and shellfish contamination [1]. The occurrence of *Alexandrium catenella* was first recorded in the Magellan strait in 1972 and since then its known range in Chilean waters has expanded from 55° 55'S to 44° 44'S. During the last three decades, several hundred people in Chile have suffered from PSP syndromes, and more than 25 humans have died after shellfish consumption; as a consequence, quarantines have frequently been imposed on shellfish collection, transportation and commercialization. Since 1991, PSP outbreaks have been recorded continuously in this geographical area. Several studies have been mainly devoted to the analysis of PSP toxins in shellfish, such as the Chilean blue mussel *Mytilus chilensis*, the striped mytilid (or Chilean ribbed mussel) *Aulacomya ater* (native name “cholga”), and two carnivorous gastropods, *Concholepas* (native name “loco”) and *Argobuccinum ranelliformes* (native name “caracol del sur”). Nevertheless, the toxin profile of the causative organisms in Chile has not been heretofore clearly identified and confirmed by advanced analytical methods.

This work reports the first confirmatory analysis of a Chilean strain of *Alexandrium catenella* simultaneously carried out by liquid chromatography with post-column derivatization followed by fluorescence detection (LC-FD) and with tandem mass spectrometry (LC-MS/MS). These data are compared with PSP toxin profiles reported from

mussels of the same temporal and geographical provenance, with respect to the processes of biotransformation and toxin metabolism in associated toxic shellfish.

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Formate Analogues as Antagonists of the Sex Pheromone of the Honeydew Moth, *Cryptoblabes gnidiella*: Field Evidence

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Cryptoblabes gnidiella Millière (Lepidoptera: Pyralidae) is an economically important exotic pest in vineyards of Southern Brazil and Uruguay. It was first detected in Uruguay in 1981, and its incidence in vineyards has increased during the past decade. The larvae develop and feed inside the grapes, causing direct damage and secondary infection by microorganisms, which in turn decrease yields and wine quality¹. The sex pheromone of *C. gnidiella* was identified as a mixture of (*Z*)-11-hexadecenal and (*Z*)-13-octadecenal, in a 1:1 ratio and it has been used to monitor populations in Israel. The development of mating disruption for this species may be hampered by the chemical instability of the natural pheromone. Aldehydes are unstable under field conditions, easily suffering chemical transformations such as oxidations and polymerizations. More stable and behaviorally active structural analogues of aldehyde pheromones are hence potentially useful for pest management². We previously studied the electrophysiological and behavioral response of males in wind tunnel to combinations of formate analogues, (*Z*)-9-tetradecenyl formate and (*Z*)-11-hexadecenyl formate, synthetic pheromone and calling females. In this study, we evaluated the male captures in field traps lured with sex pheromone and varying amounts of formates. We observed that male captures decreased showing a dose-response pattern, when different amount of formates were added to septa containing a fix amount of pheromone. Such decrease was already significant with a 5:1 pheromone:formates ratio. The formates act as sex pheromone antagonists and could be potentially useful as mating disruptants for *C. gnidiella* in commercial vineyards.

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Changes in the Repellent Behaviour of *Rhodnius prolixus* after Continuous Exposure to DEET

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N, N-diethyl-m-methylbenzamide (DEET) is the active ingredient of most insect repellents. Its efficacy has been proved in a number of insect species [1], however very few is known about its mode of action. In this work we study changes in the repellency of fifth instar nymphs of *Rhodnius prolixus* to DEET, after being continuously exposed to high concentrations of this repellent. A circular filter paper treated with a solution of DEET in acetone (2 mg/cm²) [2] was placed in a closed plastic container, where a nymph of *R. prolixus* was allowed to walk on the treated surface. Then, the repellency to DEET of each individual was tested in a circular arena divided into halves: one half was treated with DEET and the other one with acetone. Repellency was expressed as Repellency Coefficients (RC). Three variables were considered: the time of continuous exposure (1, 5, 10 or 20 minutes), the time after exposure (10, 20 and 30 min), and the test concentration of DEET. When the test concentration of DEET was lower than the exposure concentration, a decrease in the RCs was observed in insects exposed 5, 10 or 20 minutes compared with control groups (without continuous exposure to DEET); 1 minute exposure did not decrease the RC. The response to DEET was recovered 10 minutes after treatment in insects exposed 5 or 10 minutes; and 20 minutes after treatment when insect were exposed 20 minutes. No decrease in the RCs was observed when repellency was tested with a concentration of DEET higher than the concentration used in the treatments. The decrease of the repellency after continuous exposure of *R. prolixus* nymphs to the repellent and the fast recovery of the response suggest a sensory adaptation phenomenon. We propose a role of the sensory system, probably olfactory, in the detection of DEET.

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Age Signals in Cuticular Lipids of the Stingless Bee *Scaptotrigona aff. depilis* (Hymenoptera, Apidae, Meliponini)

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In social insects, the cuticle surface of each individual shows a unique signature, carrying species-specific cues, as well as cues about its nest origin, age, genetic lineage, caste, sex, reproductive status, and its function in the colony. Insects' cuticle is covered by a thin waterproof wax layer consisting of long chain lipids. These substances were originally used as protection against water loss and a pheromonal function evolved later [1]. The aim of this work is to investigate possible variations in chemical surface compounds of stingless bees *Scaptotrigona aff. depilis* according to their ages. For the tests, we collected four newly emerged workers, four young workers and four old workers, from two colonies. The collected bees were stored individually in small vials and killed by freezing. The epicuticular compounds were individually extracted in hexane for 5 min. The extracts were analyzed by a gas chromatograph coupled with a mass spectrometer equipment (Shimadzu QP2010). The most part of the compounds were hydrocarbons with chains between 21 and 33 carbon atoms. The most abundant substances in newly emerged workers were Z-nonacosene ($23,38 \pm 4,28$; i.e. mean of abundance relative \pm deviation standard) and heptacosane ($17,33 \pm 3,15$), for young workers, heptacosane ($24,40 \pm 6,73$) and Z-heptacosene ($20,22 \pm 7,86$) and for old workers Z-heptacosene ($22,94 \pm 8,56$) and pentacosane ($12,68 \pm 6,67$). Newly emerged individuals contained higher relative amounts of alkanes with shorter chains, such as heneicosane and tricosane, compared to older individuals. These findings are similar to what is known from *Frieseomelitta varia* [2]. The chemical analysis of the cuticular compounds of *S. aff. depilis* showed clear differences between the groups suggesting that these compounds might be a chemical cue that indicates the age of individual bees and probably its function in the colony.

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Taste Detection of Phytoecdysteroids by the Dog Tick *Rhipicephalus sanguineus*

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Ticks are blood feeding arthropods, quite important as vectors of many infectious pathogenic agents. Ecdysteroids (ES) are polyhydroxylated steroids that seem to occur in all arthropods classes. In insects they have a regulatory role controlling molting and reproduction and it is suspected that they play the same role in ticks. Phytoecdysteroids (PES) are ES analogues that occur in a certain proportion of plant species. They are usually considered as defensive compounds against insects, that can disrupt their hormonal levels and affect the growth and reproduction process. Several sensible insect species are able to detect PES by gustatory sensilla and avoid feeding in substrates PES-treated [1]. In this study we tried to verify the taste response of *Rhipicephalus sanguineus*, the brown dog tick, to seven different PES. Electrophysiological recordings were performed from contact chemoreceptors located on the tick chelicerae using a TastePROBE amplifier [2] Marion-Poll and Van der Pers, 1996). Series of recordings were done starting with 10^{-2} M KCl (the negative control, also used as electrolyte of nonconductive solutions) followed by ascending concentrations of PES (centesimal steps from 10^{-12} to 10^{-4} M) and by the positive control, a 10^{-3} M glutathione reduced solution. The stimulation had 2s of duration. There was no difference in spikes frequencies (SF) between the sexes, except to Innokosterone, which was more active in females. The SF elicited by Ecdysone, 20-Hydroxyecdysterone, Ponasterone A and Innokosterone were statistically similar to the negative control in all concentration and smaller than the positive control. Pterosterone and Makisterone elicited dose-dependent response, with SF higher than the negative control in all concentrations and statistically similar to the positive control at the threshold 10^{-4} M. Pinnatasterone elicited a dose-dependent response at the threshold 10^{-8} M with SF similar to the positive control at 10^{-6} M and 10^{-4} M. It is necessary to find a feeding assay to determine if the PES Pterosterone, Makisterone and Pinnatasterone act as feeding deterrents in *R. sanguineus*, as it is expected.

[1] Marion-Poll, F., Descoins, C. 2002. Taste detection of phytoecdysteroids in larvae of *Bombyx mori*, *Spodoptera littoralis* and *Ostrinia nubilalis*. *Journal of Insect Physiology*. 48:467-476.

[2] Marion-Poll, F., Van Der Pers, J.N.C., 1996. Un-filtered recordings from insect taste sensilla. *Entomologia Experimentalis et Applicata*, 80, 113-115

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Contact Toxicity of Essential Oils Against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* Motsch, Insects of Stored Products

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Plant essential oils have been recognized as an important natural source of pesticides and are considered to be an alternative to synthetic chemicals insecticides because essential oils reputedly pose little threat to the environment as well as to human health [1]. In stored-product insect pest control, essential oils may have numerous types of effects; they may have a fumigant activity, penetrate inside the insect body as contact insecticides, as repellents, act as anti-feedants or may affect some biological parameters such as growth rate. The contact toxicity of essential oils of *Tagetes terniflora* (Asteraceae), *Cymbopogon citratus* and *Elyonorus muticus* (Poaceae) against adults of *Tribolium castaneum* and *Sitophilus oryzae* L were determined in an impregnated paper assay. To determine toxicity using the filter paper assay, aliquots of 0.7 mL of the essential oils were applied to filter papers (Whatman N° 1, 9 cm diameter). The solvent (*n*-hexane) was allowed to evaporate for 10 min. Each filter paper was then placed inside a glass Petri dish with 10 insects per concentration, then were covered with a plastic tape with some holes to prevent them from escaping and kept at 29°C, 70%r.h and in darkness. Five independent replicates per concentration and control (without application of products) were performed. Mortality was recorded after 72 h. and the LC₅₀ (lethal concentration) values were calculated by Probit analysis 3.0 [2]. The essential oil of *T. terniflora* was found to be toxic to both *T. castaneum* and *S. oryzae* adults (CL₅₀ = 217.26 g/cm² and 146.58 g/cm² respectively). On the basis of LC₅₀, *S. oryzae* was significantly more susceptible than *T. castaneum*. In addition, the essential oil of *E. muticus* was more toxic than the essential oil of *C. citratus* against *S. oryzae* (LC₅₀ = 99.63 g/cm² and 435.41 g/cm² respectively). Our study demonstrates that the essential oils of *T. terniflora*, *C. citratus* and *E. muticus* had contact toxicity and could be used to control insects in stored products.

[1] Tripathi, A.; Upadhyay, S.; Bhuiyan, M. and Bhattacharya, P.A review on prospects of essential oils as biopesticide in insect-pest management. *Journal of Pharmacognosy and Phytotherapy*, 1(15):52-63.(2009).

[2] F.A.O. 1974. Boletín Fitosanitario de la FAO. Método provisional para gorgojos adultos importantes en cereales almacenados, con malatión o lindano. Método N° 15. FAO, 22:127-137. (1974).

Acknowledgements PGI-UNS 24/B142

Antifungal Capacity of Essential Oil from Different Chemotypes of *Artemisia* sp. (Asteraceae)

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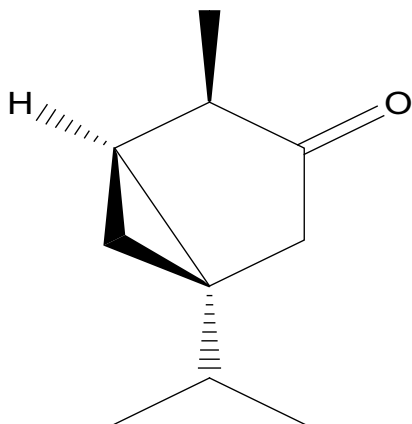
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Essential oils (EOs) have been the source of many potential botanical pesticides in the past. Currently, there is a worldwide trend towards the development of new strategies for pest and disease control that can be used in alternation and/or replacement of conventional pesticides, given the drawbacks arising from their intensive use: developing of resistance mechanisms, adverse effects in the ecosystem, etc. EOs as botanical pesticides are regarded as less toxic, given their natural origin and lower persistence in the environment. Antifungal activity of EOs from different families, including Asteraceae, has been reported. However, several independent studies have shown a variable degree of activity in EOs from the same species, as well as differential chemical composition; two facts that most probably correlate. In turn, variable chemical composition in EOs may be due to differences arising from the geographical origin. In this work, two EOs from one species of *Artemisia* were characterized in their chemistry and activity. The plants extracted were from Canelones-Uruguay and from Zaragoza-Spain. EOs from both samples varied chemically, being the most remarkable difference in composition, the absence in the latter of E-thujone (1). The antifungal activity of both EOs against two fungal plant pathogens, *Alternaria solani* (isolated from apple) and *Fusarium oxysporum* (isolated from onion) was studied by a bioassay in which only EO vapors are in contact with the mycelia. The inhibition of mycelium growth compared with a growth control [calculated as $100 - (\text{diameter treatment} / \text{control diameter}) \times 100$] was different from both EOs depending on doses applied. For both EOs, when $1.0 \mu\text{L}/\text{cm}^3$ was applied growth was completely inhibited. However at $0.5 \mu\text{L}/\text{cm}^3$, only the EO from Spain exhibited activity against both fungi, with *A. solani* (100% inhibition) being more susceptible than *F. oxysporum* (50% inhibition). These results may open the possibility of considering *Artemisia* as a candidate for companion planting practices.



E-thujone (1)

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Translocation of Isoquinoline Alkaloids to the Hemiparasite *Tristerix corymbosus* from Its Host, *Colletia spinosissima*

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Tristerix corymbosus (L.) Kuijt (Loranthaceae) is a stem hemiparasitic flowering species from the Andean region of South America where it grows with many different host-plant species. Translocation of alkaloids to *T. corymbosus* from its hosts was assessed by comparing the alkaloid content of *T. corymbosus* hosted by *Cryptocarya alba* (Molina) Looser (Lauraceae), and by *Colletia spinosissima* Gmel. (Rhamnaceae), two isoquinoline alkaloid-containing species [1,2].

Two alkaloids related to the quaternary 1-benzyltetrahydroisoquinoline alkaloids found in *C. spinosissima* [1] were isolated from *T. corymbosus* growing on *C. spinosissima*, suggesting biotransformation in the hemiparasite of translocated alkaloids from the host. On the other hand, no alkaloids were found in *T. corymbosus* growing on *C. alba* from which the 1-benzyltetrahydroisoquinoline alkaloids, (S)-reticuline has been isolated[2].

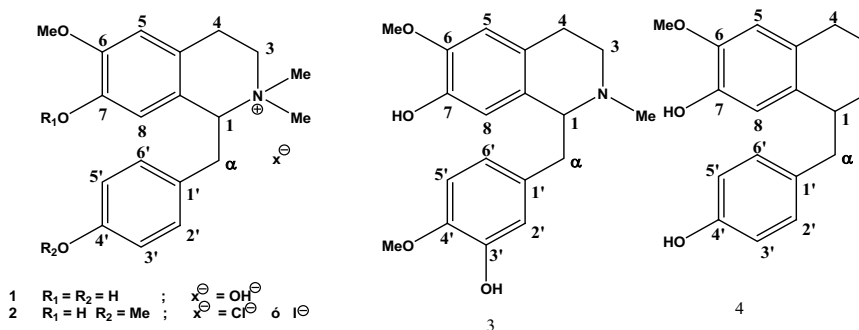


Fig. 1.-Alkaloids from *C. spinosissima* (1,2), *C. alba* (3) and *T. corymbosus* hosted on *C. spinosissima* (4)

[1] Sánchez E., Comin J. "Studies on argentine plants—XXIII: Quaternary bases from *Colletia spinosissima* Gmel" *Tetrahedron*, 1967, **23**, 1139-1143.

[2] Urzúa A., Torres R., Cassels B., "(+)-Reticulina en *Cryptocarya alba*" *Rev. Latinoamer Quím.*, 1975, **6**, 102- 104.

Acknowledgements: VRID Universidad de Santiago de Chile, Santiago Chile.

Study of the Allelopathic Potential of Compounds Isolated from *Oxylobus glanduliferus*

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Oxylobus Glanduliferus is a plant from the Asteraceae family, which is developed in parts of Central America and the Andes of Venezuela and Colombia. [1] In their leaf surface are present cell structures known as glandular trichomes which may contain compounds with phytotoxic activity. This work studied the glandular exudates (GE) derived from the aerial parts of *O. glanduliferus* to isolate compounds with phytotoxic activity of interest. Methodology chosen was biodirected isolation. The glandular exudates were obtained by immersing the plant material in DCM for 15 s in an ultrasonic bath. Later phytotoxicity bioassays of the GE were conducted on various species target; the species most affected were: *Cucumis sativa* (~ -50 % for shoot 's and root 's grow inhibition, 200 ppm) and *Allium cepa* (~ -45% for stem 's and root 's grow inhibition, 200 ppm). Then, the exudates were separated by vacuum column chromatography giving a total of 13 fractions, bioassays were performed again, and 5 of these fractions had relevant phytotoxic activity. These fractions were purified through chromatographic techniques, and two compounds were isolated: Labdan-8 α , 15-diol and 13-epi- labdan,-8 α 15-diol. The phytotoxic activity of these compounds was -48% (shoot) and -29% (root) in the case of Labdan-8 α , 15-diol for *Cucumis sativa* and -66% (shoot) and -53% (root) for *Allium cepa*. For 13-epi-Labdan-8 α , 15-diol inhibition values were -51% (stem) and -40% (root) for *Cucumis sativa* and -72% (stem) and -27% (root) for *Alliums cepa*. The results show that despite differ only in the setting of one of its carbon, the epimers have very differential phytotoxic activity. These results support the hypothesis that the compounds on the leaf surface can participate as a defense strategy (allelopathy).

[1] Amaro, J.M; Adrian, R.M (1982) Diterpenoides de *O. glanduliferus* (Schp. Bip) Gray. *Revista Latinoamericana de Química*. 13, 110-113

Field Test of the Semiochemical (*E*)-2-hexenal on Egg Parasitoids (Scelionidae: Hymenoptera) and its Application in Biological Control of Soybean Pests

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The volatile (*E*)-2-hexenal, a common green leaf volatile and also found in stink bug's defensive glands, can be used as kairomone by egg parasitoids [1]. We studied the influence of (*E*)-2-hexenal on the dynamics of host (stink bug's egg) - parasitoid (Scelionidae) relationships, to understand how applications of this compound act on the population dynamics of parasitoids and what's the impact on the population of their hosts. During the reproductive stage of the plant, large scale experiments were performed in three areas of soybean cultivation (conventional, organic, semiconvencional) in sets of nine plots (20 m x 20 m) distributed in each of three experimental units. A small-scale experiment was conducted with controlled infestation of stinkbugs in six cages (2,40 m x 1,80 m x 1,20 m) covered with canvas. Plots were distributed randomly into three groups: control (n-pentane), (*E*)-2-hexenal 4 mg (H1) and 10 mg (H2). Cages were placed randomly in the field with treatment (*E*)-2-hexenal 5 mg diluted in n-pentane or only n-pentane (control). Parasitoids were sampled weekly using yellow sticky traps (n=3/plot or cage) and the parasitism indexes monitored through sentinel eggs of *Euschistus heros* (n=150/plot or cage) reared in the laboratory. Stink bugs were monitored weekly with shake-cloth in each plot (n=10/week) or cage (n=4/week). Statistical analyses included PRC, GLM, χ^2 and repeated measures ANOVA. Overall, there was effect of treatment with (*E*)-2-hexenal on parasitoids, especially at a concentration of 4 mg in the final stages of culture, but the increase in parasitoid abundance wasn't associated with the occurrence and intensity of parasitism. The population incidence of stink bugs pest complex reduced throughout the experiment and was not altered by the release of the volatile. Other variables such as climate and precipitation should be considered to establish the effects of (*E*)-2-hexenal clearly.

[1] Laumann, R. A. et al. 2009. Journal of Chemical Ecology. 35:8-19.

Acknowledgements: CNPq, FAP-DF, EMBRAPA.

Activity of Extracts and Fractions of *Astronium graveolens* (Anacardiaceae) against *Leucoagaricus Gongylophorus* (Agaricaceae)

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The leaf-cutting ants are among the herbivore species considered pests due to the large amount of plant material to sustain the nest. Leaf-cutting ants have a mutualistic association with *Leucoagaricus gongylophorus*, it assimilates the plant material making it more palatable by the ants. Thus, inhibition of the symbiotic fungus can be considered a method for ants control [1]. *Astronium graveolens*, known popularly as guaritá, was repellent to the ant species *Atta laevigata* [2]. This work investigates the biological action of the species *Astronium graveolens* against the symbiotic fungus of leaf-cutting ants. The fungus was isolated from a nest of ants and maintained in laboratory conditions. The samples submitted test were incorporated into the culture medium and dissolved in distilled water. Then the test tubes with the medium / extract and Petri dishes were autoclaved and subsequently, the culture media / extract were poured into Petri dishes using laminar flow cabinet. After solidification of the culture medium, each Petri dish was inoculated at the center position with a disc of agar, 8 mm in diameter, previously colonized by the symbiotic fungus *L. gongylophorus* and kept incubating during 30 days. Each sample was prepared in three replicas. The extracts of the root (RV), stems / branches (CGV), twigs (GFV), partitions of ethyl acetate (RVACT) and partitions of hydroalcoholic (RVAQ) of *Astronium graveolens* plant were tested on the fungus *L. gongylophorus*. The extracts RV, CGV, GFV and the partitions RVACT and RVAQ inhibit 91%, 17%, 35%, 90% and 77%, respectively, the symbiotic fungus in a concentration of 1000 mg mL⁻¹ when compared with controls. Statistical analysis was performed using the Student t test unpaired.

[1] Chen, T. K. *et al.* . "A volatile leafcutter ant repellent from *Astronium graveolens*". *Naturwissenschaften*, **71**: 97-98, 1984.

[2] Silva, A. *et al.*. "Starch metabolism in *Leucoagaricus gongylophorus*, the symbiotic fungus of leaf-cutting ants." *Microbiol. Res.*, **161**: 299-303, 2006.

Acknowledgments: FAPESP, CNPq, CAPES, INCT

Chemical Investigation of Harvestmen Defensive Secretions (Arachnida: Opiliones: Laniatores)

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The arachnid order Opiliones (harvestmen) is characterized by the presence of a pair of scent glands in the lateral parts of the body. One of the defensive strategies of harvestmen consists in secreting strong smelling compounds whose chemical nature changes depending on the family¹. Therefore, the study of the chemical nature of harvestmen defensive secretions has great importance for the chemotaxonomy. In this work we analyzed the defensive secretions of 12 harvestman species of the suborder Laniatores using GC-MS. Most compound classes could be identified comparing their mass spectra with those in the Wiley 275 mass spectral database (Table 1). The components of the *Gonyleptes saprophilus* secretion was further characterized using ¹H and ¹³C NMR experiments. This secretion contains the α,β -unsaturated ketone 4-methylhex-1-en-3-one (**1**) and its dimer (**2**) (Figure 1).

Table 1. Classes of compounds found in harvestmen secretions

| Species | N | Ketones | Phenols | Quinones | Unknown |
|-------------------------------------|----|---------|---------|----------|---------|
| <i>Bourguyia hamata</i> | 22 | - | - | 97,8% | 2,2% |
| <i>Cadeadoius niger</i> | 5 | 100,0% | - | - | - |
| <i>Chavesincola inexpectabilis</i> | 31 | - | 4,6% | 95,4% | - |
| <i>Discocyrtus oliverioi</i> | 11 | - | - | 99,2% | 0,8% |
| <i>Gonyleptes gonyleptoides</i> | 9 | - | - | 98,7% | 1,3% |
| <i>Gonyleptes saprophilus</i> | 15 | 86,9% | - | - | 13,1% |
| <i>Metarthrodes longipes</i> | 3 | - | 97,2% | - | 2,8% |
| <i>Mischonyx cuspidatus</i> | 29 | 10,0% | - | 89,3% | 0,7% |
| <i>Pachylus</i> sp. | 24 | 1,1% | 1,1% | 95,9% | 1,9% |
| <i>Progonyleptoidellus striatus</i> | 10 | - | 100,0% | - | - |
| <i>Pseudotrogulus funebris</i> | 12 | 96,4% | - | - | 3,6% |
| <i>Sodreana sodreana</i> | 5 | 80,8% | - | - | 19,2% |

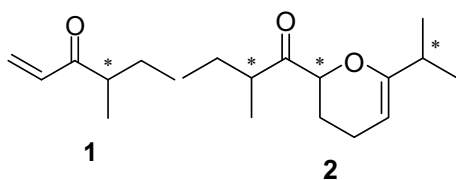


Figure 1. Structure of 4-methylhex-1-en-3-one (**1**) and its dimer (**2**)

[1] Gnaspini, P.; Hara, M.R. Defense mechanisms, In: Pinto-da-Rocha, R.; Machado, G.; Giribet, G. (Eds.) *Harvestmen: The Biology of Opiliones*; Harvard University Press: Cambridge, 2007; p. 374-399.

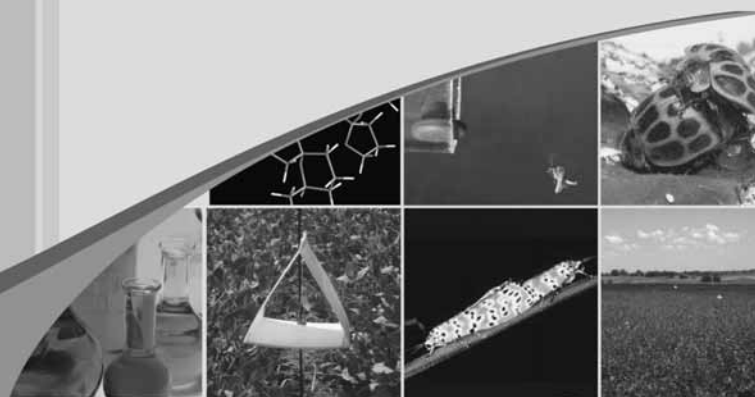
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1st Latin American Meeting of Chemical Ecology

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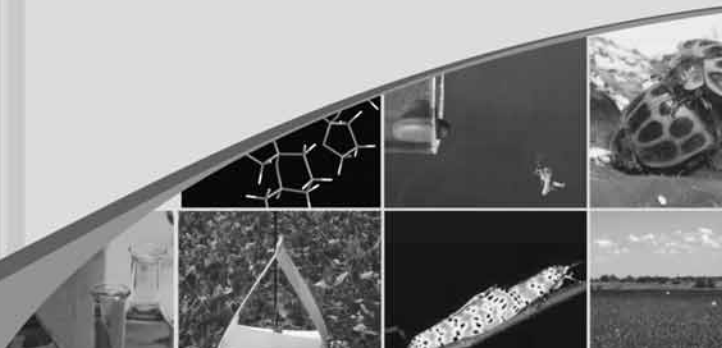
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