# Partial Report (1st. Year)



Partial Report (1st Year) of activities developed by the National Institute of Science and Technology in Agricultural Semiochemicals, with the participation of the Escola Superior de Agricultura Luiz de Queiroz – ESALQ/USP (Main Office), Federal University of Paraná – UFPR, Federal University of Viçosa – UFV, and the Federal University of Alagoas – UFAL.

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# Partial Report (1st Year)

# National Institute of Science and Technology of Agricultural Semiochemicals

CNPq-Fapesp



# Participating Institutions:











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# National Institute of Science and Technology of Agricultural Semiochemicals

CNPq-Fapesp

### **Institute Main Office**

Escola Superior de Agricultura Luiz de Queiroz – ESALQ University of São Paulo – USP

### General coordination

Laboratory of Semiochemicals

Prof. Dr. José Roberto Postali Parra (Researcher Level 1A CNPq) Laboratory of Insect Biology Escola Superior de Agricultura Luiz de Queiroz – ESALQ University of São Paulo – USP Piracicaba – SP

# Associated Laboratories and respective researchers responsible

Laboratory of Chemical Ecology and Insect Behavior
Escola Superior de Agricultura Luiz de Queiroz – ESALQ
University of São Paulo – USP
Piracicaba – SP
Researcher responsible: Prof. Dr. José Maurício S. Bento (Researcher Level 1D CNPq)

Department of Chemistry
Federal University of Paraná – UFPR
Curitiba – PR
Researcher responsible: Prof. Dr. Paulo Henrique G. Zarbin (Researcher Level 2 CNPq)

Laboratory for Research in Natural Resources - LPqRN
Institute of Chemistry and Biotechnology
Federal University of Alagoas
Maceió – AL
Researcher responsible:
Prof. Dr. Antônio Euzébio Goulart Santana (Researcher Level 1D CNPq)

Laboratory of Semiochemicals and Insect Behavior
Department of Animal Biology Animal/Entomology
Federal University of Viçosa – UFV
Viçosa – MG
Researcher responsible: Prof. Dr. Eraldo Lima (Researcher Level 2 CNPq)



# 1. Managing Committee - Meetings held and decisions made

Considering that the INCT of Agricultural Semiochemicals has 1 Main Office (2 Laboratories) and another 3 Associated Laboratories well distributed geographically (São Paulo, Minas Gerais, Paraná and Alagoas), the composition of the Managing Committee is restricted to the five leaders of these laboratories:

José Roberto Postali Parra, Esalq/USP (Coordinator)
José Mauricio Simões Bento, Esalq/USP
Paulo Henrique Gorgatti Zarbin, UFPR
Eraldo Lima, UFV
Antonio Euzébio Goulart Santana, UFAL

# 1.1. Meetings

5th- 6th March, 2009 – Piracicaba-SP 1st-4th December, 2009 – Viçosa-MG 22nd March, 2010 – Piracicaba-SP

### 1.2. Decisions

In the meetings, the direction of the research was discussed as well as the distribution of funds, always attending everyone's needs and with the aim of creating a research network.

At the same time, online and even personal contact was maintained with the respective laboratories constituting the INCT.

In the case of ESALQ/USP, the Dean's Office contributed an amount equal to that given by CNPq/Fapesp with the contraction of 3 employees: 1 university level technician (specialist in gas chromatography), 1 mid-level technician (insect rearing), and 1 secretary who should manage the INCT laboratory financial expenses and logistics.



# 2. Cooperative activities between participating groups of the INCT

# 2.1. Student exchanges from the ESALQ/USP to the UFPR:

- (i) Doctoral student Cristiane Nardi, ESALQ/USP visited the UFPR twice in 2009 to: -Studies of identification and electroantennography of the pheromones of *Diabrotica speciosa*;
- (ii) Doctoral student Newton Cavalcanti de Noronha Junior, ESALQ/USP went twice to the UFPR, once in 2009 and once in 2010 for: Induction of volatile compounds in *Murraya paniculata* (L.) Jack and their effect on the psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae);
- (iii) Doctoral student Nancy Barreto Triana, ESALQ/USP visited the UFPR with the aim of complementing her study with: Sexual and reproductive behavior of *Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae);
- (iv) Doctoral student Maria Fernanada Gomes Villalba Peñaflor, ESALQ/USP made a visit in 2009 to the UFPR to identify the compounds of her project: - Induction of volatiles in corn plants by oviposition of the fall armyworm Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) (1 trip in 2009);
- (v) Post-doctoral student Rejane Cristina Roppa Kuss Roggia, ESALQ/USP travelled twice to the UFPR in 2010, for identification and electroantennography of *Spodoptera eridania* and *S. frugiperda*.

# 2.2. Student exchanges from the UFPR to the ESALQ/USP:

- (i) Doctoral student Angela Maria Palacio Cortés of the UFPR made 3 trips to the ESALQ/USP in 2009, with the aim of adding to her project with: Genetic variation in *Diatraea saccharalis* and a reinvestigation of its sexual pheromone;
- (ii) Doctoral student Camila Borges da Cruz Martins, UFPR, travelled once to the ESALQ/USP in 2009 for training in insect behavior for her thesis project: Study of the chemical ecology of the rice water weevil, *Oryzophagus oryzae* (Coleoptera: Curculionidae);
- (iii) Masters student Diogo Montes Vidal, UFPR, travelled to the ESALQ/USP in 2009 for training in insect behavior.

# 2.3. Student exchanges from the UFAL to UFPR:

- (i) Henrique Fonseca Goulart, UFAL travelled to the UFPR in 2009 for training in electroantennography
- (ii) Rose Paula Cavalcante Mendonça, UFAL travelled to the UFPR in 2009 for training in electroantennography;
- (iii) Victor Lopes de Abreu Lima, UFAL travelled to the UFPR in 2009 for training in electroantennography;

# 2.4. Student Exchange from the UFV to the UFPR:



(i) In 2009, the Semiochemical Laboratory of the UFPR accepted the Masters student, Carla Cristina Marques Arce, of the Semiochemicals and Insect Behavior Laboratory of the UFV for analyses of volatile compounds in tomato. These volatiles were collected from tomato plants infested or not infested with the nematode *Meloidogyne javanica* with the aim of quantifying the alterations in volatile compounds of the aerial part induced by herbivory. While in this laboratory, the student made preliminary electroantennography tests with volatile compounds from tomatoes and antennae of *Tuta absoluta*.

# 3. Cooperative activities between the INCT's and other institutions (companies, ONGs, government institutions, etc)

- 3.1. International Institutions linked to the area of Chemical Ecology:
- (i) Penn State University-USA;
- (ii) Max Planck Institute of Chemical Ecology-Germany;
- (iii) University of California-Davis-USA;
- (iv) University of Neuchatel-Switzerland;
- (v) University of Tsukuba-Japan;
- (vi) Instituto Valenciano de Investigaciones Agrarias-Spain;
- (vii) Rothamested Chemical Ecology Research Group-U.K.;
- (vii) University of Alicante (Spain).

### 3.2. Brazilian Institutions

- (i) Federal University of Ceará UFC;
- (ii) Federal University of Minas Gerais UFMG;
- (iii) Empresa Brasileira de Pesquisa Agropecuária Embrapa;
- (iv) Chemical Institute of Unicamp;
- (v) Federal University of Pelotas UFPel;
- (vi) Agricultural Research Organization of Rio Grande do Norte S/A EMPARN.

# 3.3. Companies

- (i) Citrus Defence Foundation Fundecitrus;
- (ii) Biocontrole (pheromones);
- (iii) Bug Biological Agents (commercialization of biological control agents);
- (iv) Sugarcane Technology Centre CTC.

# 4. Principal technical-scientific results

# 4.1. Joint Publications during the period under review



- Altesor, P.; Rossini, C.; Zarbin, P.H.G.; Gonzales, A. 2009. Sex pheromone of the bud borer *Epinotia aporema*: Chemical identification and male behavior response. **Journal of Chemical Ecology**, 35: 349-354, 2009.
- Ambrogi, B.G.; Fonseca, M.G.; Coracini, M. D. A.; Zarbin, P.H.G. 2009. Calling behaviour and male response towards sex pheromone of poplar moth *Condylorrhiza vestigialis* (Lepidoptera: Crambidae). **Journal of Pest Science**, 82: 55-60.
- Ambrogi, B.G.; Vidal Dm; Zarbin, P.H.G.; Rosado-Neto, G.H. 2009. Feromônios de agregação em Curculionidae (Insecta: Coleoptera) e sua implicação taxonômica. **Química Nova**, 32: 2151-2158.
- Bento, J.M.S.; Nardi, C. 2009. Bioecologia e nutrição *vs* ecologia química: as interações multitróficas mediadas por sinais químicos. In: Antonio R. Panizzi; José R.P. Parra. (Org.). **Bioecologia e nutrição de insetos: Base para o manejo integrado de pragas**. 1 ed. Brasília: Embrapa Informação Tecnológica, p. 277-296.
- Bergmann, J.; Gonzales, A.; Zarbin, P.H.G. 2009. Insect Pheromone Research in South America. **Journal of the Brazilian Chemical Society**, 20: 1206-1219.
- Bergmann, J.; Villar, J.; Zarbin, P.H.G. 2009. Synthesis of pheromones: Highlights from 2005-2007. **Current Organic Chemistry**, 13: 683-719.
- Cock, M.J.W.; van Lenteren, J.C.; Brodeur, J.; Barrat, B.I.P.; Bigler, F.; Bolckmans, K.; Cônsoli, F.L.; Haas, F.; Mason, P.G.; Parra, J.R.P. 2009. The use and Exchange of biological control agents for food and agriculture. FAO **Background Study No.** 47, FAO, Rome: 88p.
- Cock, M.J.W.; van Lenteren, J.C.; Brodeur, J.; Barrat, B.I.P.; Bigler, F.; Bolckmans, K.; Cônsoli, F.L.; Haas, F.; Mason, P.G.; Parra, J.R.P. 2010. Do new Access and Benefit Sharing procedures under the Convention on Biological Diversity threaten the future of biological control? BioControl (Dordrecht), 55: 199-218.
- Fonseca, M.G.; Zarbin, P.H.G. 2009. Mating behaviour and evidence for sex-specific pheromones in *Hedypathes betulinus* (Coleoptera: Cerambycidae: Lamiinae). **Journal of Applied Entomology**, 133: 695-701.
- Janssen, A.; Grosman, A.H.; Cordeiro, E.G.; de Brito, E.F.; Fonseca, J.O.; Colares, F.; Pallini, A.; Lima, E.R.; Sabelis, M.W. 2009. Context-dependent fitness effects of behavioral manipulation by a parasitoid. **Behavioral Ecology**, 20: 1-4.
- Lima, Eraldo R.; McNeil, Jeremy N. 2009. Female sex pheromones in the host races and hybrids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). **Chemoecology**, 19: 29-36.



- Nava, D.E.; Gomez-Torres, M.L.; Rodrigues, M.D.; Bento, J.M.S.; Haddad, M.L.; Parra, J.R.P. 2010. The effects of host, geographic origin, and gender on the thermal requirements of *Diaphorina citri* (Hemiptera: Psyllidae). **Environmental Entomology**, 39(2):678-684.
- Panizzi, A.R.; Parra, J.R.P. 2009. A biecologia e a nutrição de insetos como base para o manejo integrado de pragas. **Bioecologia e nutrição de insetos: base para o manejo integrado de pragas**. 1 ed. Brasília -DF: EMBRAPA/CNPq, p. 1107-1139.
- Parra-Pedrazzoli, A.L.; Leal, W.S.; Vilela, E.F.; Mendonça, M.C.; Bento, J.M.S. 2009. Synthetic sex pheromone of citrus leafminer in Brazlian citrus groves. **Pesquisa Agropecuária Brasileira** 44: 676-680.
- Parra, J.R.P.; Lopes, J.R.S.; Gómez Torres, M.L.; Nava, D.E.; Paiva, P.E.B. 2010. Bioecologia do vetor *Diaphorina citri* e transmissão de bactérias associadas ao 'Huanglongbing' (HLB). Revista Laranja (In Press)
- Pontes, W.J.T.; Lima, E.R.; Cunha, E.G.; Andrade, P.M.; Lôbo, A.P.; Barros, R. 2010. Physical and chemical cues affect oviposition by *Neoleucinodes elegantalis*. **Physiological Entomology** (Published Online: Feb 25 2010. DOI: 10.1111/j.1365-3032.2010.00720.x).
- Zarbin, P. H. G.; Villar, J.; Marchi, I.; Bergmann, J.; Oliveira, A.R.M. 2009. Synthesis of pheromones: Highlights from 2002-2004. **Current Organic Chemistry**, 13: 299-338.
- Zarbin, P.H.G.; Rodrigues, M.A.C.M.; Lima, E.R. 2009. Feromônios de insetos: Tecnologias e desafios para uma agricultura competitiva no Brasil. **Química Nova**, 32: 722-731.

# 4.2. Summary of results obtained with the species proposed in INCT's research plan

Olfactive responses of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to the volatiles of the plant, *Murraya paniculata* (l.) Jack (Rutaceae) and the conspecifics

In the last few years, world citrus has been suffering severe economic losses due to the disease 'Huanglongbing' or HLB, caused by the bacteria *Candidatus* Liberibacter spp., which are transmitted by the psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Besides citrus, jasmine plant *Murraya paniculata* (L.) Jack



(Rutaceae) is mentioned to be a preferential host of all the *D. citri* stages. For this reason, the hypothesis that volatiles released by this plant could play a role in attraction and in the sexual partner encounter of D. citri was investigated. Thus, the effects of volatiles released from induced and non-induced *M. paniculata* plants, as well as from conspecifics, to *D. citri adults*, were assessed in the laboratory using a Y-tube olfactometer arms with controlled air flow (400 mL.min 1). The treatments consisted of volatiles released by: (i) Healthy plants; (ii) healthy plants + 20 females; (iii) healthy plants + 20 males; (iv) induced plants during 24 hours with 20 females; (v) induced plants during 24 hours with 20 males; (vi) 20 females; (vii) 20 males; and (viii) clean air (control). Each treatment was compared with the control, and subsequently the ones that statistically differed from the control were compared between themselves. Males and females were tested individually to each treatment, with 20 replicates. The bioassays were performed during photophase at 25°C and a 70% relative humidity. The results were analyzed through a chi-square test ( $P \le 0.05$ ). Males and females responded distinctly. The females were attracted to the treatments containing volatiles of jasmine plant, *M.* paniculata (i, ii, and iv) except when they were associated with males (iii and v). On the other hand, males were only attracted to volatiles from females (ii and vi). These results suggest that volatiles from the jasmine plant, M. paniculta, or even a possible sexual attractant, may be involved in the differential attractiveness between males and females of *D. citri*.

Olfactive responses of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to the volatiles of *Citrus sinensis* (l.) Osbeck (Rutaceae) plants infected with *Candidatus* Liberibacter asiaticus

'Huanglongbing' or HLB is currently considered the most serious and devastating disease of citrus, having as its causal agent the fastidious bacteria, *Candidatus* Liberibacter spp., exclusively transmitted by the sucking insect *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). With the advance of the HLB in the main regions of citrus in the world, control of the disease, as well as of the insect vector,



have become a great challenge to citrus management. Thus, with the aim of better understanding the insect-plant and bacterial interactions, the hypothesis that *D.* citri is able to recognize volatile compounds from Citrus sinensis (L.) Osbeck plants infected by *Candidatus* Liberibacter asiaticus was put forward. The olfactory responses of *D. citri* were measured in a Y-tube olfactometer, allowing the psyllids to freely choose the odors. The presence of *Ca*. L. asiaticus in treated plants was confirmed by molecular biology techniques. The experiments were started only when plants became symptomatic. The plants used in the tests were *C. sinensis*, variety Pêra, grafted onto *Citrus limonia* (L.) Osbeck. In these studies, *D. citri* males and females were individually exposed to the following treatments: (i) Healthy plants, (ii) plants infected by Ca. L. asiaticus, and (iii) clean air. The proportion of choices of *D. citri* to volatiles from infected plants *versus* healthy plants was significant, with 79% of male responses, and 71% of female responses to infected plants (chi-square test,  $P \le 0.05$ ). The results provide evidence that *D. citri* is not only able to distinguish, but is also attracted to volatiles induced by infection of bacteria Ca. L. asiaticus in citrus. This information opens up new perspectives in the search for attractive compounds for *D. citri*, which may be employed in the integrated pest management of citrus.

Effect of volatiles from guavas, *Psidium guajava* l. (Myrtaceae) in the localization of *Citrus limonia* (l.) Osbeck (Rutaceae) plants by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)

Recent studies carried out in Vietnam, Southeast Asia, showed that intercropping of citrus and guava plants, *Psidium guajava* L. (Myrtaceae) could reduce the infestation of the Asian citrus psyllid, *Diaphorina citri* Kuwayma (Hemiptera: Psyllidae). This psyllid is the vector of 'Huanglongbing', a very important bacterial disease in citrus. According to these studies, volatiles from guava plants could hypothetically be repellent to *D. citri*. Therefore, the aim of this study was to evaluate the olfactory response of *D. citri* to the volatiles of *P. guajava* plants. Two experimental steps were established asking the following questions: (1) Do *P. guajava* volatiles alter *D. citri* behavior?; and (2) in which way do these volatiles



affect D. citri? To answer (1) a Y-tube olfactometer was used with the following treatments: (i) volatiles of either Citrus limonia (L.) Osbeck (Rutaceae); or of (ii) volatiles of *P. guajava*a plants; (iii) a combination of both volatiles; and (iv) control (clean air). To answer (2) a 4-way-olfactometer setup was used with the following treatments: (i) P. guajava volatiles; and (ii) control (clean air). Males and females of *D. citri* were tested in each bioassay. The time spent in an odor field, as well as the total number of choices for an odor field, were recorded. The results showed an alteration of *D. citri* behavior after contact with the volatiles of either *P. guajava* or *C. limonia*. Compared to the control (clean air), individuals preferred odor fields containing citrus volatiles (total number of choices = 78.3%,  $P \le 0.01$  chi-square). Interestingly, when offered P. guajava alone and a mixture of P. guajava and C. *limonia* volatiles, compared to the control, in the Y-tube olfactometer we observed no response by either sex to the same parameters. When testing *D. citri* to volatiles in the 4-way olfactometer setup, individuals significantly avoided guava plant volatiles (total number of choices = 22.2%,  $P \le 0.01$  chi-square). Our results strongly suggest that *P. guajava* leaves contain volatiles acting as effective repellents against *D. citri*.

# The Effects of Host, Geographic Origin, and Gender on the Thermal Requirements of *Diaphorina citri* (Hemiptera: Psyllidae)

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is the vector of the bacteria that causes citrus greening and is considered one of the world's most important citrus diseases. We examined how host, geographic region, and gender affect the thermal requirements of *D. citri*. The insects were reared in climate chambers at constant temperatures of 18, 20, 22, 25, 28, 30, and 32 ± 1°C, 70 ± 10% RH, and a 14 h photophase. Host plants for *D. citri* included orange (*Citrus sinensis* [Rutaceae]) varieties Pêra and Natal, the rootstock, Rungpur lime (*C. limonia* [Rutaceae]) and the natural host, Orange jessamine (*Murraya paniculata* [Rutaceae]). To study the influence of geographic origin on thermal requirements, we studied *D. citri* populations from Piracicaba, SP (warmer region) and Itapetininga, SP (cooler region). The duration and survival of the development stages and the duration of



the total development (egg-adult) did not differ significantly on the different hosts, but it did vary with temperature. Nymphs of *D. citri* reared on the different hosts have the same thermal requirements. The thermal requirements for this species collected from the two climate regions were identical; males and females also had the same thermal requirements.

Olfactory Response of the fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), and its specialist endoparasitoid *Campoletis flavicincta* Ashmead (Hymenoptera: Ichneumonidae) to Herbivore-induced Volatiles emitted by Maize.

Plants recognize and produce specific volatile substances that attract parasitoids after herbivore attack, characterizing a process known as indirect defense. However, the ability of these plants in processing and releasing novel volatile compounds elicited by a non-host herbivore attack has been poorly explored. Therefore, the current study aimed to investigate the effect of volatiles emitted by maize plants being attack by the diamondback moth, *Plutella xylostella*, which is not a host, to those emitted by a common host, the fall armyworm, Spodoptera *frugiperda*, on the behavioral response of these insects and their respective parasitoids *Apanteles piceotrichosus* and *Campoletis flavicincta*. The bioassays with the parasitoids were conducted during photophase and they were exposed to volatiles from undamaged maize, with volatiles released at time intervals 0-1, 5-6 and 24-25h after mechanical damage or simulated herbivory during photophase, and volatiles released at the time interval 5-6h after these same treatments in scotophase. The bioassays with the moths were carried out in scotophase and they were exposed to volatiles from undamaged maize, with volatiles released at the time interval 5-6h after mechanical damage or simulated herbivory during photophase, and volatiles released at time intervals 0-1 and 24-25h after these same treatments in scotophase. Additionally, for *P. xylostella* volatiles from undamaged kale were tested while for A. piceotrichosus volatiles from undamaged kale, mechanically damaged and *P. xylostella* caterpillar damaged kale were tested. *C. flavicincta* females were attracted to volatiles emitted by the maize plants at the



interval of 5-6h after the treatment with the S. frugiperda regurgitate in scotophase. Curiously, they were not attracted to volatiles released at the same time interval after the induction elicited by the regurgitate in the photophase. S. frugiperda mated females were attracted by volatiles from undamaged plants and volatiles released at time intervals of 5-6 and 24-25h after the mechanical damage or treated with the regurgitate of this herbivore. Nevertheless, they preferred the volatiles from undamaged maize to those plants treated with the regurgitate during photophase. These results demonstrated that these insects are able to distinguish among the volatile blends present in their natural habitat where there is a tritrophic relationship: maize (host plant) – S. frugiperda (herbivore) – C. *flavicincta* (parasitoid). In the same way, in the relationship: kale (host plant)- P. xylostella (herbivore) – A. piceotrichosus (parasitoid), P. xylostella females were attracted by the volatiles of undamaged kale and the A. piceotrichosus females were attracted to volatiles emitted by caterpillar-damaged kale. In the case of simulating a new relationship, maize (non-host plant) – P. xylostella (herbivore) – C. flavicincta (parasitoid), A. piceotrichosus females and P. xylostella were not able to respond to undamaged maize, mechanically damaged and mechanically damaged+ P. xylostella regurgitate. The determination of these mechanisms can aid in better understanding the evolutionary context of plants and insects and for obtaining new advances in pest management and biological pest control.

# Wing polymorphism in the Cydnidae: morphological and behavioral studies on adult *Scaptocoris carvalhoi* Becker and *S. castanea* (Perty) (Hemiptera: Cydnidae)

Among soil pests, the brown root stinkbug has increased its population substantially in various crops. This insect is subterranean and the adults disperse by swarming at the beginning of the rainy season. During these swarms part of the population leaves the soil, for *Scaptocoris carvalhoi*, and part stays due to wing polymorphism. However for *S. castanea* the population has long wings and the population generally flies away. The analysis of covariance between the data of the hind wing length and body length demonstrate the formation of two distinct



groups for S. carvalhoi, classified into brachypterus and macropterus. In the present study, it was found that males and females with short wings showed an RPC less than 0.58, while the macropterous adults showed an RPC above 0.58. There were significant differences between brachypterus and macropterus individuals regarding wing mobility and flight reactions. The morphological standard shown by adults of S. castanea is visually characterized into individuals with long wings (macropterus), which have long front and hind wings, passing the apex of the abdomen. Wing mobility and wing reactions of adult *S. castanea* were similar. Among the individuals present at the swarming, 66% moved their wings during the biotrials, while 65% of those collected in the soil showed this behavior. This study gives the description of the morphological characters of the immature stages and the eggs of *S. carvalhoi*. The importance of a premature identification of insects and the growing need to do morphological and biological studies of the immature stages of the Heteroptera of the Superfamily Pentatomoidea has been emphasized recently by many authors although it has been long recognized that this favors finding solutions for the many entomological problems which exist.

# Synthetic sex pheromone of the citrus leaf miner in Brazilian citrus groves

The objective of this study was to determine the best conditions for using the synthetic sex pheromone of *Phyllocnistis citrella* Stainton for monitoring this species in citrus groves in northeastern Brazil. Pheromone doses (0.0, 0.1, 1, 10 and 100 µg) and longevity (1, 15, 29, 43 and 57-day-old lures) and trap height (0.5, 1.5 and 2.5 m), color (green, red, and white) and model influence on *P. citrella* males capture were evaluated. The doses of 10 and 100 µg of the synthetic sex pheromone – a 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal – attracted the greatest number of *P. citrella* males. Traps baited with these two dosages continued to capture *P. citrella* males at a comparable rate for over eight weeks in citrus groves. Although there was no significant decrease in activity of either dosage until 57 days of exposure to the environment, as time passed, the higher dose attracted significantly more *P. citrella* males than the lower dose. There were no significant differences in male capture in traps with synthetic



sex pheromone placed at 1.5 and 2.5 m height, which gave the better results. Trap color and model did not affect male capture.

# Sexual behavior of the coffee berry borer, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae)

The study of sexual behavior in insects includes all the events which occur from pair encounter by means of courtship until separation after mating. These studies are important and represent the first step in research which aims to elucidate the pheromones of target species. Little is known of the sexual behavior of the coffee berry borer, Hypothenemus hampei (Ferrari), which is the most important coffee pest in the world. Researchers have focused on the use of traps based on alcohols (e.g. ethanol and methanol) to manage this pest but results are conflicting. For that reason, the aim of the present study was to investigate the sexual behavior of *H*. hampei, which will support subsequent studies on pheromone evaluation in this species. The daily mating rhythm of *H. hampei* occurred at any time during the 24hour cycle. Although at low rates, 60% of paired adults mated at least once when they were 0-24 hours old, reaching 100% in older pairs. The highest sexual activity was observed at ages of 48-72 and 72-96 hours, with the occurrence in the latter being  $6.9 \pm 0.47$  matings/pair with a mean duration of  $2.04 \pm 0.13$  minutes each. The behavior pattern shown was similar to other groups of Curculionidae, with premating, mating and postmating. The results also indicated the presence of a pheromone produced by the males that mediates mating. The fact that the H. hampei females copulate again after they leave the fruits, opens up new perspectives for investigating the viability of using this pheromone in managing this pest.

# Sexual and reproductive behavior of *Sphenophorus levis* Vaurie, 1978 (Coleoptera: Curculionidae) in sugarcane

With the aim of obtaining basic information on the bioecology and behavior of *Sphenophorus levis* Vaurie, different bioassays were carried out in order to:



(i)understand its sexual and reproductive behavior; ii) obtain and test the biological activity of its natural pheromone under laboratory and field conditions; iii)verify the capture efficiency of the synthetic aggregation pheromone under field conditions; iv)determine the most efficient type of traps for capture and management of the adults; and evaluate the radius of action of the current traps. The results showed that *S. levis* mates any time of the day, has multiple mating and the mates between the ages of 14 and 35 days presented the highest mating frequencies of 76 to 88%, respectively. The mean duration for the first mating was 5.75 ± 4.22 h, and the total mating time varied between 1 to 17 h. The preoviposition period for females between 14 and 35 days, was  $5.52 \pm 4.53$  to  $10.90 \pm$ 5.37 days. The number of eggs/female was 0.25 to 7.80, with viability of between 33 and 63%. Females of *S. levis* with advanced ages between 85 and 210 days were receptive to mating (66%), oviposited between 4.50 to 7.09 eggs with a viability between 49 and 79%. Males and females of *S. levis* with capacity to mate presented typical frequency behavior to effect mating. The pre-mating duration varied from 2 to 5 hours. Mating was long and lasted between 7 and 13 hours. The post-mating duration lasted between 1.31 to 1.41 hours. Sexual behavior associated with mating was influenced by visual contact between the couples and the physical among conspecifics. Bioassays in a "Y" tube olfactometer demonstrated that the males were responsive to sugarcane volatiles; sugarcane + males; and sugarcane + females; while the females were stimulated at least by sugarcane + males. The males responded to the natural extract of male + sugarcane; while the females to the extract of males + sugarcane, extract of females and extract of females + sugarcane. The isomer (S)-2-methyl-4-octanol generated behavioral activity of both sexes of *S. levis*. In the field, the evaluation of the attraction of natural extracts and the synthetic compounds (isomer S; R and racemic mixture of S-R), showed differences in the capture of females and totals of adults of S. levis between the isomer R and the control; however, it was not different in other treatments. The capture of wild *S. levis* adults in the different traps tested differed between the control ('bait CTC') and the tested baits/traps (models 'galão', 'funil', and 'moleque da bananeira'). The recapture of marked adults of S. levis was low and corresponded to 2% of the liberated insects within the longest distance of 5 m (10



adults). In general, these results add to the knowledge of *S. levis* sexual and reproductive behavior and are useful for future programs of integrated management of this pest.

# Sexual behavior of *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae)

In this study, we showed that the mating behavior of *Diabrotica speciosa* had well-defined sequences of premating, mating and postmating. The first mating occurred between the third and seventh day after emergence, being predominant in four-day-old insects. Sexual activity was higher between the end of the photophase (18-20h) and the first half of scotophase (20-2h). No mating behavior was observed between 4 and 8 hours. During seven days of observation, the majority of couples mated only once, although 30% of adults mated 2 to 4 times. *D. speciosa* males were attracted to virgin females, as well as to their natural extracts. Behavioral observations are presented in this study and allow us to conclude that the attraction of males is due to a sex pheromone produced by *D. speciosa* females.

# Behavioral and electroantennographic response of male *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) to the sexual attraction pheromone

In this study, we investigated the daily rhythm of *Diabrotica speciosa* male responses to the sexual pheromone of females of different ages under laboratory conditions. *D. speciosa* females of 3 to 15 days old were attractive to males while newly-emerged females seem not to produce sexual pheromone. Males responded to the pheromone at different times of the day. GC-MS analysis revealed that females produce six chemical volatiles that are distinct from those produced by males, with one major and five minor compounds. In GC-EAD, these compounds caused electrophysiological responses in male antennae, with four of these responses being stronger. The current study provides an important contribution to studies about *D. speciosa* chemical ecology, mainly in regard to the chemical identification of sexual pheromones.



# Contact sexual pheromone of *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae): behavioral evidence

To evaluate the hypothesis that a contact sexual pheromone occurs in *D. speciosa*, we investigated male biological activity towards natural extracts of cuticular compounds from other males and virgin females (1 day old) and sexually mature females (6 days old) at doses of 1, 2 and 3 insect equivalents in different solvents (ketone, hexane, chloroform and dichloromethane). Biological activity of the sexual pheromone was also compared with cuticular compounds. The ketone was the solvent that provided higher response frequencies. *D. speciosa* males displayed a typical courtship behavior when in contact with female extracts which did not happen when male extracts were tested. Although immature female extracts stimulated some males to mate, the response frequency was low and it did not differ from the control. Regarding mature female extracts, doses that were more efficient in stimulating mating attempts were 2 and 3 female equivalents, which also showed more time spent in relation to 1- female-equivalent extract. Sexual pheromone did not show contact action when applied by itself. The chromatographic profile of cuticular compounds demonstrated the presence of distinct compounds in males and females. The current study reveals that *D.* speciosa females produce cuticular compounds that act as a contact sexual pheromone, besides a volatile pheromone for sexual attraction.

Effect of foliar, root and simultaneous herbivory in corn on the host selection behavior for oviposition in *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae)

In the current study, we investigated the hypothesis that gravid *D. speciosa* females use volatiles derived from healthy maize plants and herbivore-induced plants under attack by their conspecific immatures and adults in host selection for oviposition. In order to verify this, the response of *D. speciosa* females to volatiles



was quantified using the following treatments: (i) healthy plants; (ii) plants induced by root herbivory (damaged by larvae); (iii) plants induced by leaf herbivory (damaged by adults); and (iv) plants induced by simultaneous herbivory (damaged by larvae and adults). Furthermore, we assessed if females were able to identify conditions of competition and host adequacy to offspring by means of chemical cues emitted by these plants. The results indicated that the presence of *D. speciosa* conspecifics on maize plants triggered the emission of volatiles which influenced host selection for oviposition. Gravid females avoided plants induced by larval attack (root herbivory). The same effect was observed in plants induced by simultaneous herbivory (root+leaf), however, only at time intervals prior to 6 hours. The leaf herbivory induction, separately, did not influence the host selection behavior of females. The weight gain was higher in larvae kept on healthy plants than on plants induced by root or simultaneous herbivory, suggesting that female preference is related to its offspring performance.

# Bioecological studies of the giant sugarcane borer, (*Telchin licus*) (Drury, 1773) (Lepidoptera: Castniidae) with the aim of synthesizing a species pheromone

The objective of the research was to calculate the lower thermal development threshold or base temperature and the thermal demands of the egg phase of *Telchin licus* to store it at its base temperature with the aim of obtaining larvae when the pest cannot be found in the field in southeast Brazil. Recently-laid eggs of *T. licus* were kept in Petri dishes (10 cm diameter x 2.0 cm high), lined with filter paper, and placed in climate cabinets with seven different regulated temperatures: 18, 20, 22, 25, 28, 30 e 32°C, RH of 70 ± 10% and a photophase of 14 h. The incubation period and egg viability were evaluated for each of the seven temperatures. The base temperature (Tb) and the thermal constant (K) for the egg phase of *T. licus*, respectively 10.3 °C and 192 DD (degree days). It was concluded that the ideal thermal band for laboratory studies for the embryonic phase of *T. licus* is situated between 22 and 28°C, the temperature band to which the egg should be transferred after being stored at the base temperature of 10.3°C. The continuous maintenance of the insect in the laboratory is fundamental for



semiochemical studies. An artificial diet composed of proteins, vitamins, mineral salts, carbohydrates, lipids and sterols has allowed this insect to be reared although some adjustments are still necessary so that the laboratory insect is comparable to the field insect.

# Chemical ecology study of *Hedypathes betulinus* (Klug, 1825) (Coleoptera: Cerambycidae: Lamiinae)

The objectives of this study were to evaluate the mating behavior and the evidence of pheromone produced by *Hedypathes betulinus*; to identify male-specific compounds and the biological activity of synthetic compounds alone or combined with host plant volatiles; and determine the location of male sex pheromone glands and study the pheromone biosynthesis. The mating behavior shows that male recognition by females may be mediated by a male-produced pheromone, and that males recognize females by a contact pheromone. Behavioral responses of H. betulinus males and females in a Y olfactometer to volatiles released from males and females confirmed the presence of a male-specific pheromone, since females were significantly attracted to male extracts. In a plastic arena, 70% of males attempted to mate with dead-washed extract-treated females, suggesting that these extracts contained contact pheromones. Analysis of male and female volatiles by gas chromatography (GC) showed the presence of three male-specific compounds, at a ratio of 91.40: 7.90: 0.70. These compounds were identified as a (R)-(-)-(E)-(-)-(E)-(-)-(E)-(-)-(E)-(-)6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) and (R)-(-)-(E)-6,10dimethyl-5,9-undecadien-2-ol (minor compounds). Females were attracted to the principal racemic compound combined with host plant volatile or to the ternary racemic mixture combined or not with host plant volatiles. These findings opened a new perspective for pheromone use in the integrated management of *H. betulinus* in Paraguayan tea. Pheromone biosynthesis occurs in glands located in the prothorax and appears to be released by small pores distributed through the prothorax. By using deuterated precursors it was demonstrated that the main



pheromone component of *H. betulinus* is biosynthesized from geranyl acetone. These results are initial studies about the chemical ecology of *H. betulinus*.

Aggregation pheromone of *Sternechus subsignatus* Boheman, 1836 (Coleoptera: Curculionidae): evidence, structural identification and evaluation of behavioral activity

The soybean stalk weevil, Sternechus subsignatus Boheman, 1836 (Coleoptera: Curculionidae), is being considered a key soybean pest, mainly in south Brazil and West Bahia state. Its control is difficult because the immature stages develop inside plant stems, limiting insecticide action. Thus, the use of pheromones to manage this insect is very promising. The objectives of this study were to identify the aggregation pheromone, verify the behavioral activity of S. subsignatus and evaluate adult response to synthetic compounds in the laboratory and field. Male and female volatiles of S. subsignatus were collected by aeration and initially used to verify the presence of specific compounds of each sex. Later, the volatiles were collected in the presence and absence of the food source to evaluate the effect of the host plant. The periodicity of emission of these compounds was evaluated by collecting the volatiles during photophase and scotophase and subsequently, extractions were made every two hours during the photophase. The behavioral response of both sexes to the different treatments was evaluated in the laboratory using a Y olfactometer. Both sexes were significantly attracted to the host plant and to the male extractions associated with the host plant, demonstrating that the communication between *S. subsignatus* is mediated by an aggregation pheromone and plant volatiles. Seven male-specific compounds were detected by chromatographic analyses, in the proportion of 9.7:2.7:7.1:41.4:0.2:1.6:37.3 respectively, providing a chemical support to the behavioral data. It was found that the liberation of these compounds depends on the presence of the host plant and occurs during the photophase, with a peak from 4 to 6h after its start, and this is also the peak of greatest activity of the insect in the field. The (E)-2-(3,3)dimethylciclohexidilene)ethanol is the main component and the chemical structures of the minor components were revealed from mass spectrometer



analyses and derived compounds, such as: 2-((1R,2S)-1-methyl-2-(prop-1-en-2-il)ciclobutil) ethanol (grandisol),  $\gamma$ -isogeraniol, (Z)-2-(3,3-dimethylciclohexidilene) ethanol, (Z)- and (E)-2-(3,3-dimethylciclohexidilene) accetaldehyde, and the acid(E)-2-(3,3-dimethylciclohexidilene) accetate, which is described for the first time as a natural product. Gas chromatography analyses using quiral columns showed that the natural stereoisomer of grandisol is the (1R,2S). The principal component was attractive by itself in the laboratory. Field experiments using pitfall traps were done to verify the attraction of the compounds. The traps were inefficient in capturing insects, possibly due to the chemical composition in the liberators, demonstrating the need for new tests.

Variation of mitochondrial DNA and the sexual pheromone of *Diatraea* saccharalis (Fab., 1794) (Lepidoptera: Crambidae) as a function of the locality.

The main components of the sexual pheromone of *Diatraea saccharalis* are (9*Z*,11*E*)-hexadecadienal and (*Z*)-hexadec-11-enal, which were identified and quantified in 4 Brazilian populations and one from Colombia through analyses involving GC-EAD, GC-MS and GC. Three different relationships were observed between the compounds mentioned previously, 9:1, 3:1 and 6:1. The concentration of the main component, (9*Z*,11*E*)-hexadecadienal, varied between 21.9 and 6.8 ng/gland and between 6.5 and 1.7 ng/gland for the minor component, (*Z*)-hexadec-11-enal. Twenty-five sequences of COII of *D. saccharalis* were analyzed showing a low inter- and intra-variation, as well as eleven haplotypes, with the most frequent one represented by specimens from the states of São Paulo, Paraná, and Pernambuco. The specimens from Colombia showed the greatest genetic diversity. More than geographic proximity, the values for the genetic variability between the specimens coincided with the values obtained in the analyses of the sexual pheromone extracts. These results suggest that the relationship of the two components of the sexual pheromone should be redefined taking into



consideration the genetic variability and the geographic origin of this species as a way to monitor or control it through the sexual confusion technique.

# Obtaining the sordidin pheromone

This objective was reached in this period of the first year. Technical-scientific results included the synthesis of the sordidin pheromone and the scaling up of the process. The route for commercial synthesis is ready. Now, studies on the liberators, stability of the molecule in the field and the formulation of an efficient bait to attract the pest need to be done. The details of the synthesis have already been obtained.

## Obtaining the pheromone of *Metamasius hemipterus*

The four constituents of the pheromone were obtained and with a pilot scale production and field tests, show excellent insect capture. Specific formulations for *Metamasius hemipterus* and others for *Rhynchophorus palmarum* and others for both species were prepared. The results show a selective capture.

## Interactions between Capsicum annuum and Aphis gossypi

The susceptibility of varieties of the plant, *Capsicum annuum* to the aphid, *Aphis gossypi* was evaluated and the chemical constituents responsible for these relationships, determined. The evaluation of cis-jasmone as an inductor of resistance to phytophagous insects and the action on natural enemies was also determined. This action will be studied in the field after the electroantennograph results.

# Interaction between Vigna unguiculata and Aphis craccivora

The action of volatiles on the attraction of phytophagous insects was studied, the semiochemicals involved determined and the induction of resistance by the action



of cis-jasmone studied. The action on natural enemies in the field will be studied in greater detail after the electroantennograph results.

# Resistance of cotton genotypes (*Gossypium hirsutum* L.) to *Aphis gossypii* and the induction of resistance by cis-jasmone

The resistance to aphids, the semiochemicals involved and the action of cisjasmone as an inductor of resistance to phytophagous insects was studied. The field evaluation on natural enemies was adversely affected by meteorological problems and a new experiment will be set up in the field to measure this.

# Recognition and acceptance of the host (*Neoleucinodes elegantalis* x *Solanum lycopersicon*)

The recognition and acceptance of a suitable host plant by phytophagous insects requires the integration of visual, physical and chemical cues. The host cues that a specialist insect integrates to optimize its decisions for oviposition and if these cues are used in a specific way, were investigated. The study also determined if the small tomato borer, Neoleucinodes elegantalis (Guenée) (Lepidoptera: Crambidae), an important tomato pest in Brazil, shows a preference for ovipositing in places which differ in physical and/or chemical characteristics. When isopor spheres are supplied as artificial fruits, *N. elegantalis* deposits significantly more eggs on corrugated fruits compared to smooth ones. The hexanic extract of fruits applied to artificial fruits strongly stimulates oviposition. Physical and chemical cues also affect female oviposition when offered together. Besides this, some parts of the artificial fruits are preferred, independently of the presence of chemical stimuli. Both the physical and chemical cues affect oviposition decisions and, therefore, this pest depends on stimuli from different sensorial modes. The Entomology Masters student, Hernane Dias Araújo, will develop an evaluation of the semiochemicals involved in the relationship between tomato fruits and *N*. elegantalis in his Masters thesis.



# Tritrophic interactions between Solanum lycopersicon, Meloidogyne and Tuta absoluta

Plants develop in a complex, multi-trophic environment. Various studies show the interdependence of the population dynamics of organisms which live in the soil or on it and of natural enemies, mediated by defence responses of various plant organs (above and below the soil). However, most multi-trophic studies focus on interactions that occur above the soil and generally pay no attention to the fact that the organisms above and below the soil interact among themselves in complex ways.

Communities which live above the soil can be affected by both direct and indirect interactions according to the trophic chain of soil organisms. Feeding activities of this chain in the detritus stimulate nutrient cycling and absorption as well as plant performance, thus influencing the herbivores on the aerial plant parts. Most of the multi-trophic studies focus almost exclusively on interactions which occur above the soil and generally neglect the fact that that the organisms above and below the soil interact in very complex ways. Soil organisms intimately associated with plant roots have a potential to induce defences in the aerial part of the plant. These organisms can be mutualists, such as mycorrhizal fungi and nitrogen-fixing bacteria, or antagonists, such as pathogens and herbivores. The impact of herbivory on the root system and its effects on aerial plant parts was evaluated. It is presumed that when the roots are attacked, the plant activates the defence system of the aerial part through volatiles which modify herbivore behavior. Plants with roots infested by nematodes (*Meloidogyne* sp.) totally modify the physiology of the aerial part of the tomato (*S. lycopersicon*) and the oviposition of the tomato leaf miner (*Tuta absoluta*). The females of *T. absoluta* avoid ovipositing on nematode-infested plants during the first 10 days of infestation, when the levels of protease inhibitors have increased and reached their maximum values in the leaves. However, oviposition increased in plants with more than 20 days of infestation. These results show that herbivory of the root system affects the behavior of herbivores of the aerial system. Possible changes in the profile of plant volatiles of attacked and unattacked plants still needs to be investigated.



- 5. National and international events: presentation of research, organization of courses, seminars; lectures; roundtables
  - 5.1. Creation of the Latin American Chemical Ecology Association- ALAEQ

The INCT of Agricultural Semiochemicals played a fundamental role in the creation of the Latin American Association of Chemical Ecology – ALAEQ, founded in December 2009. Three researchers from the INCT participate in the Board Management. The ALAEQ currently has researchers from Uruguay (actual Head Office), Brazil, Argentina, Chile, Venezuela and Mexico. The internet site for the ALEQ has been created and can be accessed at:

<http://alaeg1.fg.edu.uy/index.html>

5.2. Creation of the Sociedade Brasileira de Ecologia Química.SBEQUIM

During the VI Brazilian Chemical Ecology Meeting was founded in December 2009 in Viçosa-MG

5.3. 1st. Latin American Meeting of Chemical Ecology

The INCT sponsors and promotes the **1st. Latin American Meeting of Chemical Ecology**, which will take place between October 17th-20th, 2010, in Uruguay. The internet site of the event can be accessed at:

< http://alaeq1.fq.edu.uy/index.html>

5.4. I Brazilian Meeting of Chemical Ecology – VI EBEQ

The INCT promoted the VI Brazilian Meeting of Chemical Ecology - VI EBEQ, 1st

- 4th, December, 2009, in Viçosa-MG. The president of the event, Prof. Eraldo Lima
- UFV, is one of the members of the INCT of Agricultural Semiochemicals. Various



Brazilian and international lecturers participated in the event with the participation of 120 researchers, teachers and students. Prof. Evaldo Ferreira Vilela was awarded the 1st National Prize for Chemical Ecology "Prof. José Tércio Barbosa Ferreira" for relevant services to Brazilian chemical ecology.

Information and summaries of the papers presented at the event can be accessed at:

http://www.insecta.ufv.br/ebeq/noticias.php

# 5.5. International events with the participation of INCT members

- International Society of Chemical Ecology, 23-27 August, 2009, Neuchatel-Switzerland:
- 5th Asia-Pacific Association of Chemical Ecologists (APACE) Conference with the theme "Exploring Diversity in Life's Workings: A Celebration of Chemical Ecology", 26-30 October, 2009, Honolulu, Hawaii, USA;
- XXXVI Colombian Entomological Society Meeting, June 29<sup>th</sup> to July 03<sup>rd</sup>,
   2009, Medelin, Colombia;
- International Workshop on *Tamarixia* species, USDA-APHIS, Mc Allen, Texas-USA, February 02-04<sup>th</sup>, 2010.

# 5.6. Brazilian events with the participation of INCT members

- VI Brazilian Meeting of Chemical Ecology VI EBEQ, 1-4 December, 2009, in Viçosa-MG;
- *III Workshop Northeast, North and Centre-west of Organic Synthesis*, 25-26 March, 2010, in Maceió-AL;
- Advances and perspectives for Science in Brazil, Latin America and the Caribbean. *Brazilian Academy of Sciences* (ABC), Rio de Janeiro-RJ;
- 4th edition of the Seminar on Technological Routes of Biotechnology, which included the presence of various INCTs, 11-13 November, 2009, in Ribeirão Preto-SP.



- *2nd Brazilian Conference on Natural Products*, 9-12 November, 2009, in São Pedro-SP.
- IX Regional Meeting of the SBBq Northeast, I Workshop Northeast of
   Biotechnology and the Latin American Symposium of Biochemical Teaching,
   05-12 December, 2008 UFC UEC, in Fortaleza CE;
- XI Brazilian Symposium on Biological Control, Bento Gonçalves-RS, June 01-05<sup>th</sup>, 2009.

# 5.7. Lectures given by INCT members

- Lima, E.R. 2009. Integrating pheromone traps and geostatistics to monitor coffee leafminer in Brazil. 2009. – Chemical Ecology Group – Swedish University of Agricultural Sciences – Alnarp – Sweden;
- **Bento, J.M.S.** 2009. Advances in the use of plant volatiles in pest control, VI Brazilian Meeting of Chemical Ecology VI EBEQ, Viçosa-MG;
- **Bento, J.M.S.** 2009. INCT of Agricultural Semiochemicals, IV Seminar on Technological Routes of Biotechnology, Ribeirão Preto-SP;
- **Bento, J.M.S.** 2009. Joint use of pheromones and natural enemies for the control of insect pests: help or hindrance?, IX Ecology Congress of Brazil, São Lourenço-MG;
- Parra, J.R.P. 2010. Ecology and biology of *Tamarixia radiata* in Brazil, Mc
   Allen, Texas-USA;
- **Parra, J.R.P.** 2009. Advances and perspectives for Science in Brazil, Latin America and the Caribbean. Biological control and IPM in Brazil (Lecture given to the Brazilian Academy of Sciences RJ);
- Santana, A.E.G. 2009. Synthesis of pheromones for pest control. II
   Workshop in Organic Synthesis of the North-Northeast-UFRPE;
- **Santana, A.E.G.** 2009. Pheromones in Agricultural Pest Control. IX Brazilian Ecology Congress, São Lourenço-MG;
- **Santana, A.E.G.** 2009. Use of Pheromones in Pest Control. 1st Agricultural Seminar, IFAL/Campus Satuba, Satuba-AL;
- Santana, A.E.G. 2009. Use of Pheromones in Pest Control. Institutional



- Program of Starting Scholarships in Development and Innovation (PIBITI/CNPq), UFAL, Maceió-AL;
- **Santana, A.E.G.** 2009. Identification of Pheromones, State University of Alagoas, UNEAL, Arapiraca-AL;
- Zarbin, P.H.G. 2009. Pheromone Chemistry in the Semiochemical Laboratory of the UFPR, VI Brazilian Meeting of Chemical Ecology– VI EBEQ, Viçosa-MG;
- **Zarbin, P.H.G.** 2009. Male-specific compounds released by *Cratosomus flavofasciatus* (Coleoptera: Curculionidae): Chemical identification and temporal pattern of emission, Apace Chemical Ecology, Hawaii-USA;
- **Zarbin, P.H.G.** 2009. Potential use of pheromones to control of *Sternechus subsignatus* (Curculionidae) and *Hedypathes betulinus* (Cerambycidae), São Lourenço-MG;
- **Zarbin, P.H.G.** 2009. Present situation of the insect pheromones researches in South America, Bento Gonçalves-RS.

### 5.8. Courses

- Intensive course of "Mass production and commercialization of natural enemies as a strategy of integrated pest management (IPM), JRP Parra, July, 28th, 2009, Medelin, Colombia;
- Intensive course of Chemical Ecology, 5-10 December, 2009, administered by Prof. Jeremy N. McNeil of Western Ontario University Canada. In this course participated 15 students of the Postgraduate Program in Entomology of the UFV.

## 6. Activities for the formation and training of human resources

- 6.1. Two (2) Post-doctorates concluded
- Ana Lia Parra-Pedrazzoli Monitoring strategies for control of the citrus miner, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) using sexual pheromone;



 - Lucia Madalena Vivan – Wing polymorphism, dispersal capacity of Scaptocoris carvalhoi Becker and S. castanea (Perty) (Hemiptera: Cydnidae) and description of the immature stages of S. carvalhoi in Mato Grosso

# 6.2. Seven (7) Doctorates concluded

- Newton Cavalcanti de Noronha Jr. Effect of the conspecifics and volatiles of the plants *Murraya paniculata* (L.) Jack, *Psidium guajava* L. and *Citrus sinensis* (L.) Osbeck on the behavior of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae);
- Cristiane Nardi Olfactive stimuli involved in the sexual behavior and host selection of *Diabrotica speciosa* (Germar)(Coleoptera: Chrysomelidae);
- Nancy Barreto-Triana Sexual and reproductive behavior of Sphenophorus levis Vaurie (Coleoptera: Curculionidae) in sugarcane.;
- Marcy das Graças Fonseca Study of the chemical ecology of the Paraguayan tea borer, *Hedypathes betulinus* (Klug, 1825) (Coleoptera: Cerambycidae: Lamiinae);
- Bianca Giuliano Ambrogi Aggregation pheromone of *Sternechus subsignatus* Bohemam, 1836 (Coleoptera; Curculionidae): Evidence, structural identification and evaluation of behavioral activity;
- Wendel da Silva Pontes Chemical ecology and reproduction of Neoleucinodes elegantalis Guenée (Lepidoptera: Crambidae);
- Johnnatan Duarte de Freitas Synthesis of the Aggregation Pheromone of the banana weevil, *Cosmopolites sordidus* (German) (Coleoptera: Curculionidae)

## 6.3. Five (5) Masters concluded

- Weliton da Silva Dias Sexual behavior of the coffee borer, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae);
- André Gustavo Corrêa Signoretti Induction of volatiles in corn plants by a host, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and a



non-host, *Plutella xylostella* L. (Lepidoptera: Plutellidae) and its effect on these insects and their respective parasitoids;

- Lívia Maria Silvia Ataíde Behavioral evaluation of *Tuta absoluta* to resistant and susceptible tomato varieties;
- Silvana Aparecida de Souza Oviposition of *Ceratitis capitata* (Diptera: Tephritidae) in *Coffea arabica* and *Coffea canephora*;
- -Amanda Daniela Simões Photophase impact on the *Mahanarva fimbriolata* (Stal, 1854) (Hemiptera: Cercopidae) biology.

# 7. Perspectives and future steps

Since this is the first year of the INCT, some adjustments were necessary so that the Associated Laboratories could function harmoniously and dynamically. The collaboration of the financing agencies and the researchers involved in setting up the proposed network permitted considerable advances in research which have been demonstrated in the results obtained up to now. Such advances can only be verified by comparing the results presented here with the Plan of Objectives sent for evaluation and approval by the CNPq/Fapesp. Thus, apart from the modernization of the laboratories, which was adversely affected by bureaucratic problems and the availability of funds, all the items listed in The Plan of Objectives were reached or surpassed as follows:



Item	Objective	Results Obtained
Research		
Publications	6	18
Theses (Doctorate and Masters)	3	12 (7 Doctors and 5
		Masters)
Courses (operational training)	4	12
Formation of Human Resources		
Masters selected	6	9
Doctorates selected	10	21
Post-doctors	2	2
Scientific initiation	3	9
Transfer of Knowledge and Technology		
Early search of 1st patent	1	1
Scientific Education and Promotion		
Workshop	1	4
Promotion of Brazilian events	1	4
Promotion of international	1	3
events		

It is hoped that in the second year, with the bureaucratic problems and logistics resolved, including the availability of funds and the installation of imported equipment, that the INCT can evolve even more.

The present visibility of the INCT, domestic and international, should increase research exchanges in the laboratories and this was observed during the first year of activities.

The results obtained by the INCT are comparable to the research developed by the principal centres of Semiochemicals in the world and should result in solutions for problems which afflict Brazilian agribusiness. An example which could be cited as an example is the case of 'greening' in citrus, where research developed with its vector, the psyllid, *Diaphorina citri*, show that the volatiles studied in Brazil can solve this problem which threatens to destroy the Brazilian citrus industry. This



research is also about to result in a patent in collaboration with Fundecitrus and the Valencian Institute of Agrarian Research from Spain. Also, the results obtained for the sugarcane borer, *Diatraea saccharalis*, which have the aim of identifying and synthesizing a sexual pheromone for this pest for use is biological control for its control, demonstrates the advance of the research. However, among the objectives of the INCT of Agricultural Semiochemicals, the formation of a network and highly-trained human resources, can also contribute to the maintenance of quality and advances in Brazilian science.

Piracicaba, April 29th, 2010

Prof. Dr. José Roberto P. Parra

Coordinator