# ANNUAL REPORT 2009

# SOUTH AMERICAN BIOLOGICAL CONTROL LABORATORY

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

Reporting period: July 2008 to June 2009

PRELIMINARY RESULTS
NOT FOR PUBLICATION

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## Introduction

This report summarizes the scientific, academic and miscellaneous activity at SABCL from July 1, 2008 to June 30, 2009, including major work on the following targets: Waterhyacinth (*Eichhornia crassipes*), Brazilian peppertree (*Schinus terebinthifolius*), balloon vine (*Cardiospermum grandiflorum*), Madeira vine (*Anredera cordifolia*), Brazilian waterweed (*Egeria densa*), water primrose (*Ludwigia hexapetala*), Lippia (*Phyla canescens*), parkinsonia (*Parkinsonia aculeata*), water lettuce (*Pistia stratiotes*), imported fire ants (S*olenopsis invicta, S. richteri*), glassy-winged sharpshooter (*Homalodisca vitripennis*), cactus moth (*Cactoblastis cactorum*), and little fire ant (*Wasmannia auropunctata*). Opportunistic work not reported here was conducted on fanwort (*Cabomba* spp.); genetic studies on alligator weed (*Alternanthera philoxeroides*) are in progress at the University of Buenos Aires.

SABCL has worked in close contact with the ARS counterparts, R. Vander Meer, S. Porter, D. Oi, S. Valles, D. Shoemaker (CMAVE, Gainesville, FL); T. Center, P. Tipping, G. Wheeler, M. Rayamajhi (IPRL, Ft. Lauderdale, FL); R. Carruthers, L. Anderson, B. Grewel (EIWRU, Albany and Davis, CA); J. de León, J. Goolsby (BIRU, Weslaco, TX); S. Hight (CMAVE, Tallahassee, FL); J. Carpenter (CPMR, Tifton, GA), and P. Follett (PBARC, Hilo, HI). Their support has been essential for the current SABCL program.

Important extramural support was provided by M. Julien, S. Schooler, T. Heard (CSIRO, Australia), A. McConnachie, D. Simelane, A. Fourie, M. Hill (PPRI and Rhodes Univ., South Africa), J. Heraty (U. of California, Riverside), G. Ervin and C. Brooks (Mississippi State University, Starkville).

In Argentina, SABCL interacted with: N. Cabrera, A. Marino de Remes Lenicov and S. Paradell (U. of La Plata); E. Virla (PROIMI, Tucumán), G. Valladares (U. of Córdoba); E. Greizerstein (U. of Buenos Aires); G. Traversa and F. Anderson (U. of Bahía Blanca), F. Cuezzo (Instituto Lillo, Tucumán); Conservation Land Trust; and A. Marvaldi (IADIZA, Mendoza).

## Location

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## Mission and Support

The main mission and objectives of SABCL are: (1) Search for natural enemies (NE) of weeds and insect pests, (2) Study the basic biology, ecology, and evaluate the specificity of those NE, (3) Ship selected agents to the United States or other countries, (4) Communicate and publish the results of the investigations, (5) Provide logistic support to USDA researchers and other scientists conducting investigations on biological control in Argentina, and (6) Liaison with agricultural research agencies and universities for detecting areas of potential cooperative work.

Since July 2008, the SABCL has been supervised by Dr. Daniel Strickman, Acting Director of the OBCLs. Local support is given by the American Embassy in Buenos Aires, including the Foreign Agriculture Service and David Mergen, Agricultural Counsellor. Current ARS projects have a counterpart in the US designated by National Program Leader Dr. Kevin Hackett.

**Staff**

Juan Briano: Director, Project Leader, jabriano@speedy.com.ar
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Master of Science, Entomology, University of Florida (1993)

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Laura Varone: Research Technician, lauvarone@speedy.com.ar
Licentiate in Biological Sciences, UBA (2004)

Cristian Otamendi: Gardener and maintenance assistant, caotamendi@hotmail.com

Liliana Cruz: Janitor

**Interns**

The following UBA interns assisted during the reporting period: M. Oleiro, F. Palottini, M. Cardo, S. Cabrera, M. Telesnicki, M. Guala, M. Manteca, G. Elsesser, L. Ramírez, M. Dalto, C. Rodríguez, M. Parisi, and M. Moore.

**Publications (July 2008-June 2009)**

**Manuscripts published**

1- **Sosa, A.**, E. Greizerstein, **M. Cardo, M. Telesnicki**, and M. Julien. **2008.** The evolutionary history of an invasive species: alligator weed, *Alternanthera philoxeroides*. In Proceedings of the XII International Symposium of Biological Control of Weeds. (Eds. Julien, Sforza, Bon, Evans Hatcher, Hinz, Rector). pp.: 443-450 CAB International Walingford, UK

2- **Sosa**, **A.**, M. Traversa, R. Delhey, M. Kiehr, **M. Cardo** and M. Julien. **2008.** Biological control of lippia (*Phyla canescens*): surveys for the plant and its natural enemies in Argentina. In Proceedings of the XII International Symposium of Biological Control of Weeds. (Eds. Julien, Sforza, Bon, Evans Hatcher, Hinz, Rector). pp.: 211-215. CAB International Walingford, UK

3- Toepfer S., **G. Cabrera-Walsh**, A. Eben, R. Alvarez – Zagoya, T. Haye, F. Zhang, U. Kuhlmann. **2008.** A critical evaluation of host ranges of parasitoids of the subtribe Diabroticina (Coleoptera: Chrysomelidae: Galerucinae: Luperini) using field and laboratory host records. Biocontrol Science and Technology 18: 483 – 504.

4- Triapitsyn., S. V. **Logarzo**, G. A. de León J. H. and E. G. Virla. **2008.** A new *Gonatocerus* (Hymenoptera: Mymaridae) from Argentina, with taxonomic notes and molecular data on the *G. tuberculifemur* species complex. Zootaxa 1949: 1–29.

5- **Mc Kay**, **F. Oleiro, M. Cabrera Walsh, G. Gandolfo**, D. Cuda, J. and Wheeler, G. **2009**. Natural enemies of Brazilian peppertree (Sapindales: Anacardiaceae) from Argentina: their possible use for biological control in the USA. Florida Entomologist 92: 292-303.

6- Oi, D., S. Porter, S. Valles, **J. Briano, and L. Calcaterra**. **2009.** *Pseudacteon* decapitating flies (Diptera: Phoridae): Are they potential vectors of the fire ant pathogens *Kneallhazia* (=*Thelohania*) *solenopsae* (Microsporidia: Thelohaniidae) and *Vaimorpha invictae* (Microsporidia: Burenellidae)? Biological Control 48: 310-315.

7- Schooler S., **G. Cabrera Walsh**, M. Julien. **2009.** The ecology and biological control of *Cabomba caroliniana*. In, Biological Control of Tropical Weeds using Arthropods, ed. R. Muniappan, G. V. P. Reddy, and A. Raman. Cambridge University Press. 480p.

8- Toepfer S., T. Haye, M. Erlandson, M. Goettel, J.G. Lundgren, R.G. Kleespies, D.C. Weber, **G. Cabrera Walsh**, A. Peters, R.-U. Ehlers, H. Strasser, D. Moore, J. Jackson, S. Keller, S. Vidal, U. Kuhlmann. **2009**. A review of the natural enemies of beetles in the subtribe Diabroticina (Coleoptera: Chrysomelidae): Implications for sustainable pest management. Biocontrol Science and Technology 19: 1 – 65

9- **Varone, L. and J. Briano. 2009.** Bionomics of *Orasema simplex* (Hymenoptera: Eucharitidae), a parasitoid of *Solenopsis* fire ants (Hymenoptera: Formicidae) in Argentina. Biological Control 48: 204-209.

10- Virla, E., Luft Albarracín E., Triapitsyn S., Viggiani, G. and **Logarzo, G. 2009.** Description and biological traits of a new species of *Paracentrobia* (Hymenoptera: Trichogrammatidae), an egg parasitoid of the sharpshooter *Tapajosa rubromarginata* (Hemiptera: Cicadellidae) in Argentina. Studies on Neotropical Fauna and Environment 44: 47-53.

**Manuscripts in press**

1- Díaz N.B., F.E. Gallardo, A.L. Gaddi, **G. Cabrera Walsh.** Description of a new genus and species of Eucoilinae (Hymenoptera: Cynipoidea: Figitidae) parasitoid of Ephydridae (Diptera). Annals of the Entomological Society of America.

2- Kula R.R., J.J. Martinez, **G. Cabrera Walsh.** Supplement to revision of New World Chaenusa Haliday sensu lato (Hymenoptera: Braconidae: Alysiinae). Proceedings of the Entomological Society of Washington.

3- Virla, E. G.; **Logarzo, G. A.**; and Paradell, S. L. Occurrence of the tamarix leafhopper, *Opsius stactogalus* Fieber (Hemiptera: Cicadellidae), in Argentina. Journal of Insect Science.

**Meetings, Seminars, Workshops, and Training**

1- 5th International Weed Science Congress, Vancouver, Canada, Jun 2008 (Not reported in 2008). Presentation of the works: **1)** Surveys on alligator weed (*Alternanthera philoxeroides*) in Argentina and Bolivia: rust and other fungal pathogens as possible biological control agents (Traversa, **Sosa**, Kiehr, Delhey and Julien), and **2)** The potential for biological control of Lippia, *Phyla cansescens* (Julien, **Sosa**, Traversa, Xu, Fatemi and Greizerstein).

2- Ant course. Fernández Yepes Biological Station, Henri Pittier National Park, Aragua, Venezuela, Aug 9-19, 2008; organized by the California Academy of Sciences and Museum of Comparative Zoology. **Calcaterra** took this course as part of his Doctoral program.

3- Polymerase Chain Reaction (PCR). Sep 2-9, 2008. **Varone, Briano and Calcaterra** were trained on the PCR technique at SABCL by Dr. S. Valles, ARS-CMAVE. Equipment, supplies and reagents were provided by CMAVE and 2 offices at SABCL were remodeled accordingly.

4- VII Argentine Congress of Entomology. Huerta Grande, Córdoba. Oct 21-24, 2008. Presentation of the works: **1)** The ecological basis of biological control, 1st Southern Cone Workshop on Biological Control (talk by **Cabrera Walsh**); **2)** Desarrollo larval de *Cactoblastis cactorum* (Berg) (Lep: Pyralidae) sobre diferentes especies de *Opuntia* y éxito en la entrada al hospedador (poster by **Varone, Logarzo, Manteca**); **3)** Tablas de vida de *Cactoblastis cactorum* (Berg) (Lep: Pyralidae) en cultivos de tuna en Argentina (poster by **Logarzo, Varone, Briano**, Lobos, Ruiz, **Guala, Palottini**, Hight); **4)** Relevamiento de parasitoides de *Cactoblastis cactorum* (Berg) (Lep: Pyralidae) en Argentina (poster by **Palottini, Guala, Varone, Logarzo**); **5)** Diversidad de hormigas en la Reserva Natural Iberá: el mayor humedal de Argentina" (poster by **Calcaterra**, Cuezzo, **S. Cabrera, Briano**); **6)** Estructura y funcionamiento de ensambles de hormigas en un área natural protegida de Buenos Aires" (poster by **S. Cabrera, Calcaterra**); **7)** Dos nuevas species de *Megamelus* en América del sur (Hemiptera: Fulgoromorpha: Delphacidae) (poster by Mariani, **Sosa**, Foieri, Remes Lenicov); **8)** Impacto, por separado y en combinación, de *Taosa* sp. (Hem.: Dictyopharidae) y *Megamelus scutellaris* (Hem.: Delphacidae) sobre el crecimiento del camalote *Eichhornia crassipes* (Pontederiaceae) (poster by **Sacco, Cabrera Walsh, Hernández, Sosa, Cardo, Elsesser**); **9)** Evaluación de parámetros demográficos de un agente de control biológico con respecto al grado de ploidía y la composición genómica de una planta invasiva (poster by **Telesnicki, Sosa**, Greizerstein, Julien); **10)** Insectos asociados con especies de *Ludwigia* (Onagraceae) en el Noreste de Argentina (poster by **Hernández, Cabrera Walsh, Sacco, Elsesser**); **11)** Biología y gama de plantas hospederas de *Tecmessa elegans* (Lepidoptera: Notodontidae), un candidato para el control biológico de *Schinus terebinthifolius* (Anacardiaceae) (poster by **Oleiro, Mc Kay, Cabrera Walsh**).

5- Lecture at the School of Natural and Exact Sciences, University of Buenos Aires, Oct 22, 2008. **Cabrera Walsh** talked on the "Basis and principles of biological control" as part of the Population Ecology Course.

6- CDFA Pierce's Disease Control Program Research Symposium, Dec 15-18, 2008, San Diego, CA. Presentation of the works: **1)** Identifying the species of mymaridae reared in Argentina and Mexico for potential introduction to California against the glassy-winged sharpshooter; and **2)** Preparing and submitting for publication a pictorial annotated key to the ater-group species of

*Gonatocerus*, egg parasitoids of the Proconiine sharpshooters in the neotropical region (Triapitsyn, **Logarzo**, Huber, de León, Virla and Aquino).

7- Introduction to Experimental Ecology. Mar 9-14, 2009. Department of Ecology, Genetics and Evolution, School of Natural Sciences, Univ. of Buenos Aires. **Varone** took this course as part of her Doctoral program.

8- USDA-ARS-Exotic and Invasive Weeds Research Unit, Albany, CA, Mar 16-20, 2009. **Briano** visited premises, field sites infested with aquatic weeds, and discussed projects and funding with R. Carruthers, L. Anderson, and B. Grewel.

9- USDA-ARS Overseas Biological Control Laboratories Workshop, Mar 21-22, 2009, Portland, OR. **Briano** co-participated in the discussions of administrative models, evaluation of the labs, long term function, shipping challenges, connection with stakeholders, expansion of mission and function, and planning for the strategic plan for 2010-2015.

10- Regional Meeting on Biological Control of Arthropods and Weeds, Mar 22-23, 2009, Portland, OR. **Briano** presented the seminar "Current program at the USDA-ARS-SABCL in Argentina".

11- Southeastern Ecology and Evolution Conference, Gainesville, FL Mar 27-29, 2009. Presentation of the work "Worldwide distribution of the mitochondrial diversity in the red fire ant *Solenopsis invicta*" (Ascunce, Yang, Wu, Shih, Oakey, McCubbin, **Calcaterra**, Shoemaker).

12- Imported Fire Ant and Invasive Ant Conference, Apr 6-9, 2009. Presentation of the poster "Presence of the fire ant pathogen *Kneallhazia solenopsae* in fire ant decapitating phorid flies" (Oi, Porter, Valles, **Briano, Calcaterra**).

13- Archbold Tropical Research and Education Center, Springfield, Dominica. May 11-30, 2009. **Varone** co-participated in the study of *Orasema* spp., parasitoids of the little fire ant, *Wasmannia auropunctata*. The research was supported by Dr. J. Heraty, UCA-Riverside. Several sites with *O. costaricensis* or *O. minutissima* were located to clarify their interaction with *W. auropunctata*.

14- Program Analyst Workshop, Beltsville, MD, May 2009. **Bugliani** attended to discuss changes in OSQR, Handbook, ARIS, 421, 115s and publications. ADOs and PAs attended conference call. Communication among different levels/areas was stressed. Bugliani met also with FMD staff.

15- II Meeting on Diseases and Pests of Greenhouse Crops, School of Agriculture and Forest Sciences, University of La Plata, Argentina. Jun 3-5, 2009. Presentation of the works **1)** The Neotropical Section of the IOBC (Paes Bueno and **Cabrera Walsh**) and **2)** Potential for Commercial Biological Control in Argentina (Zapater, Botto and **Cabrera Walsh**).

**Miscellaneous activity**

1- Evaluation of Doctoral projects. **Briano** evaluated the programs for the School of Natural Sciences, University of La Plata, Argentina, entitled: 1) Biology and biocontrol of *Dichroplus* *elongatus* (Orthoptera: Acrididae: Melamophinae), an agricultural pest in Argentina (Jul 2008); and 2) Diversity and pathogenicity of entomopathogenic fungi of "yerba mate", *Ilex paraguayensis*, in Misiones province (Aug 2008).

2- National Agency for the Promotion of Science, Technology, and Innovation. Ministry of Education, Science and Technology, Argentina. **Briano** was part of the Evaluation Committee of the project entitled "Arácnidos de la Reserva de Biosfera Delta del Paraná, San Fernando, Buenos Aires: un estudio integrado para su conservación (Aug 2008).

3- Productivity awards. On Sep 30, 2008, during the Annual Award Ceremony at the American Embassy-Buenos Aires, **Sosa** and **Calcaterra** received a certificate/cash award for their high productivity in terms of scientific publications and contributions to science.

4- Graduation of SABCL intern **F. Palottini**, Dec 1, 2008. **Logarzo** directed her program on Biological Sciences at the University of Buenos Aires and **Sosa** was part of the graduation committee. The thesis entitled "Functional response of *Gonatocerus* sp. "clade 1" (Hymenoptera: Mymaridae) near *tuberculifemur* (Ogloblin), candidate for the biological control of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in the United States".

5- Meeting at Monsanto headquarters in Argentina, Dec 17, 2008. **Cabrera Walsh** co-participated in a meeting with G. Head, T. Vaughn, G. Videla, and F. Mattioli to discuss methods for predicting corn rootworm (*Diabrotica speciosa*) damage on maize in South America.

6- Peer review for The Florida Entomologist. **Briano** reviewed the article "Biology and behavior of *Carmenta theobromae* (Lepidoptera: Sesiidae)" by Morillo et al. (Dec 2008).

7- Peer review for RIA Journal, INTA Argentina. **Briano** reviewed the article "The pathogen *Paranosema locustae* (Microsporidia) in the Pampa region: geographic distribution, a new host, and susceptibility of adult locusts in the laboratory" by Lange et al. (Feb 2009).

8- Meeting at Monsanto experimental station, Pergamino, Argentina, Apr 10, 2009. **Cabrera Walsh** co-participated in a corn rootworm (*Diabrotica speciosa*) damage evaluation in an experimental maize plot.

9- Graduation of SABCL intern **M. Oleiro**, Apr 2009. **Mc Kay** directed her program on Biological Sciences at the University of Buenos Aires and **Sosa** was part of the graduation committee. The thesis entitled "Biology and host range of *Tecmessa elegans* (Lepidoptera: Notodontidae), a candidate for the biocontrol of *Schinus terebinthifolius* (Anacardiaceae)".

10- Meeting at the Secretariat of Agriculture, Argentina, Apr 15, 2009. **Briano** co-participated in a meeting with R. Patrouilleau (Undersecretary of Agriculture), V. Giusti (Director of Economy, Finances and Trade), R. Blasetti (Director of International Affairs), D. Mergen (FAS, Agriculture Counselor) and F. Pirovano (FAS, Agriculture Specialist). The main topics were (1) to introduce the SABCL and (2) to discuss potential cooperative agreements.

11- Memorandum of Understanding (MOU). In May 2009, **Briano** prepared the first draft of a MOU between the ARS and the Secretariat of Agriculture of Argentina. After minor revision by R. Moore and B. Gregor (ARS-Beltsville, MD), the MOU was translated into Spanish and delivered to the Secretariat (R. Blasetti) for their consideration.

12- Registration at the Secretariat of Environment, Argentina. In May 2009, **Briano** registered SABCL at the Secretariat as a research institution. This was a new requirement to obtain exportation permits for the natural enemies to be shipped to the US or other countries. Also, the signatures of **Cabrera** and **Logarzo** were authorized to request such permits.

13- Giant reed, *Arundo donax* (Poaceae). In Jun 2009, **Cabrera Walsh** collected leaf tips at 4 sites in Formosa, Córdoba, Chaco, and Santa Fe. Samples were brought to SABCL in small plastic bags with silica gel and were shipped to J. Goolsby at the ARS-BIRU, Weslaco TX, for DNA extraction and genotype comparison with plants currently invading Texas.

14- Meeting at INTA Castelar, Argentina. In Jun 2009, **Briano** met R. Lecuona, Director of the Institute of Microbiology and Agricultural Zoology to discuss potential agreements.

**<u>Visitors</u>**

1- Tim Heard, CSIRO Australia, and Ricardo Segura, CSIRO Mexico, Jul 3, 2008. They discussed the parkinsonia project at SABCL.

2- Steven Valles, ARS-CMAVE, Gainesville, FL. Sep 2-9, 2008. Valles gave PCR training.

3- Ivette Perez, Jorge Fisher, and Alejandro Colombo, USDA-APHIS-Buenos Aires, Sep 11, 2008. They visited the premises and Briano gave a presentation to show the SABCL mission.

4- Hugh Maginnis, US Office of Foreign Service Operations, Nov 5, 2008. Visited premises and discussed with Briano the interactions with the American Embassy in Buenos Aires.

5- Brenda Grewell, USDA-ARS Exotic and Invasive Weeds Research Unit, Nov 18-20, 2008. She discussed progress on the *Ludwigia* project with Hernández and Cabrera Walsh.

6- Gary Ervin and Christopher Brooks, Department of Biological Sciences, Mississippi State University, Dec 10-19, 2008. Collected *C. cactorum* and hosts for molecular analysis.

7- Mic Julien, CSIRO European Laboratory, Jan 12-23, 2009. Julien, Sosa and Cabrera discussed projects. Sosa, Julien and Traversa surveyed Buenos Aires, La Pampa and Mendoza Provinces to collect insects for rearing and host specificity studies.

8- Stephen Hight, ARS-CMAVE, Tallahassee, FL, and Jim Carpenter, ARS-CPMRU, Tifton, GA, Feb 24-Mar 9, 2009. Surveyed natural *Opuntia* hosts and natural enemies of *C. cactorum*.

9- Andries Fourie, David Simelane and Khethani Mawela, PPRI, South Africa, Mar 18-Apr 5, 2009. They and Mc Kay surveyed northern Argentina for natural enemies of Balloon vine.

# WATERHYACINTH

by

M. Cristina Hernández, Willie Cabrera Walsh, Alejandro Sosa and Gerardo Elsesser

Cooperators: Ted Center, Phil Tipping and Min Rayamajhi, ARS-IPRL, Ft. Lauderdale, FL; Ray Carruthers, ARS-EIWRU, Albany, CA; Martin Hill, Rhodes Univ., South Africa.

**Abstract**

The effect of larvae of *Thrypticus truncatus* Bickel & Hernández (Diptera, Dolichopodidae) in waterhyacinth petioles was evaluated. No major damage was detected. The new *Taosa* sp. under study is now *Taosa impictifrons* Remes Lenicov; its damage evaluation on waterhyacinth revealed a significant reduction in the canopy height, dry biomass and ramet production. Studies on *Megamelus scutellaris* Berg and *T. impictifrons* showed that they are phloem feeders and block the vascular tissues with saliva deposits. Effect of *M. scutellaris* nymphs was tested on Waterhyacinth; no major damage was detected. Clean cultures of *Neochetina bruchi* Hustache and *N. echhorniae* Warner were attempted but failed.

**Studies on *Thrypticus truncatus***

**Damage on waterhyacinth.** *Objective*: Record the effect of larvae on biomass and morphology. *Methodology.* Damage tests of were successfully performed by comparing the plants growth in polycarbonate cylinders placed amidst a thick waterhyacinth canopy in canvass pools (350 l) with fertilized water. The canopy conditions are essential for the flies to reproduce. Twelve cylinders (six treatments and six controls) were used per pool (Fig. 1) with one plant of similar size and equal number of leaves and 10 females and 6-10 males. Effect on the height of the canopy, petiole length, and dry biomass was measured after 20 days of the insect's last molt. The test was finished after a month of the last released of flies once the four initial petioles began to decay, and the new ones developed. *Results*: The mean number of mines was 16.8/plant. Many larvae were not found although their mines were well developed. Test and control plants produced new and decayed petioles similarly. The mean biomass was 4.66 ± 3.08 g vs. 3.79 ± 2.79 in the control. The mean petiole length was 38.59 ± 12.47 cm vs. 33.98 ± 11.73 cm in the control (Fig. 2). Larvae of *Thrypticus* were growing on the 4 initial leaves exposed to the flies. In test and control plants, 4-5 new leaves developed during the trial period. No major damage was detected.

**Studies on *Taosa impictifrons.***

**Taxonomy**. After a taxonomic revision conducted by Dr. Ana M. de Remes Lenicov (La Plata Museum), the new *Taosa* under study (*Taosa inexacta* in previous reports) is now *Taosa impictifrons* Remes Lenicov. The publication of the description and biology is in progress.

**Rearing method.** In January 2009, a new attempt to rear *T. impictifrons* was done releasing 80 adults in a pool (1,2x1,4x0,3m) with well developed (fertilized) waterhyacinth plants. The pool was caged (1,8x1,8x1,8m) in the yard. *Results*: Adults did not reproduce; they survived around two weeks, feeding normally.

**Damage tests.** *Objective*: Evaluate the damage of *T. impictifrons* on petiole length, number of stolons and dry biomass. *Methodology*: (see Annual Report 2007). *Damage test with 20 nymphs:* To finish the trials, 5 replicates were carried out; the results confirmed that the insects significantly reduce the canopy height, dry biomass and ramet production (Fig. 3 and 4).

**Feeding behavior of *T. impictifrons* and *M. scutellaris*** *Objective*: Study conducted by Dr. M. E. Brentassi, La Plata Museum, to know if they exploit the same sap source. *Results*: Compound microscopy and staining techniques revealed the pathway of the suck-sap structures to the source of sap (Fig. 5). Both insects feed on the same tissues, but they use different parts of the plant to settle. Stylet-sheaths observed in both species were mostly branched, ending preferably in phloem tissues, and more often with intracellular pathway. The sclerenchyma associated with vascular bundles was avoided by *T. impictifrons* but it was crossed by *M. scutellaris*. In both species the stylet exploration was interrupted in the aerenchyma of the leaves. The predominantly intracellular stylet penetration caused cellular lyses and a considerable blockage of the vascular tissues with saliva deposits was observed.

**Feeding impact of *M. scutellaris*.** *Objective*: Evaluate the effect on growing and dry biomass. *Methodology*: Two floating cages were placed in a 350-liters canvass pool with soil in the bottom and filled up with fertilized water, in the greenhouse. Two Waterhyacinth plants of similar size and equal number of leaves were assigned at random to each cage. Trials were conducted in July and October 08 with 10-20 and 20-60 nymphs, respectively (5 replicates) until adult emergence. *Results*: No major damage was detected (Fig. 6). Surprisingly, the height of petioles increased in the 20-nymph treatment ($t=2.67$, $df=486$, $P=0.0081$) (Fig. 7).

***Neochetina bruchi* and *N. echhorniae* rearing.** *Objective:* to obtain cultures free of parasites (Nematodes, Microsporidia, etc) to be shipped to ARS-EIWRU, CA. *Methodology:* In February 2009, 137 *N. bruchi* and 145 *N. eichhorniae* were collected in Otamendi, Buenos Aires Prov. in the Paraná river delta (34º04’98’’S; 58º48’59’’W). The adults were placed in cages with clean waterhyacinth plants; plants were checked periodically for eggs. The eggs collected were surface sterilized with 0.05% hypochlorite solution and stored on wet paper in a Petri dish at 25ºC. The newly hatched larvae were placed in punctures in the petioles of waterhyacinth (DeLoach and Cordo 1976). *Results*: Larvae were transferred as follows: 90 *N. bruchi* (5/ plant), 55 *N. bruchi* (2/plant) and 36 *N. eichhorniae* (2/plant). Each group of plants was placed in pools with fertilized water in the garden. After 4 months (approximately the period for 1 generation), none larvae, pupae or adults were recovered. One possible explanation of the failure might be the shallow pools used that would hinder the pupation.

**Field Trips**

-**Nov.25-Dec. 7, 2008:** To Formosa Prov. by W. Cabrera Walsh.

- Several trips in Buenos Aires Prov., to Otamendi, by W. Cabrera Walsh.

**Future plans**

-*Thrypticus truncatus* damage and non choice tests continued, simulating natural situations.

-To obtain cultures of *Neochetina bruchi* and *N. eihhorniae*.

**Relevant accomplishment**

The finding that *M. scutellaris* and *T. impictifrons* are phloem feeders using different niches.

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**DeLoach, C. J. and H. A. Cordo 1976.** Life cycle and biology of *Neocheetina bruchi*, a weevil attacking waterhyacinth in Argentina, with notes on *N. echhorniae*. Ann. Entom. Soc. Am. 69(4): 643-652.



**Fig. 1.** *Thrypticus truncatus* damage test design. The cylinders with waterhyacinth plants were assigned at random to the treatment and control.

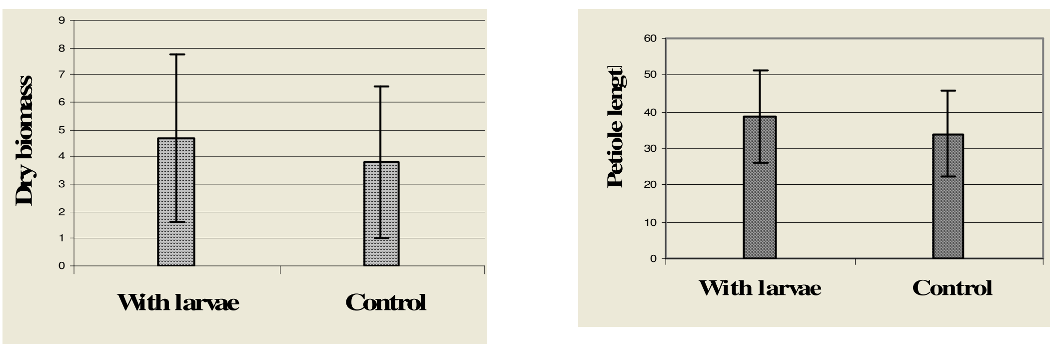


**Fig. 2.** Average and SD of the biomass and petiole length of waterhyacinth plants in the *T. truncatus* damage test. Six replications.

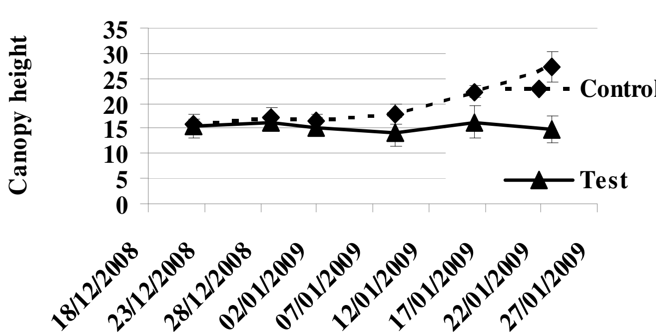


**Fig. 3:** *Taosa impictifrons*. Damage tests results for canopy height with 20 nymphs.

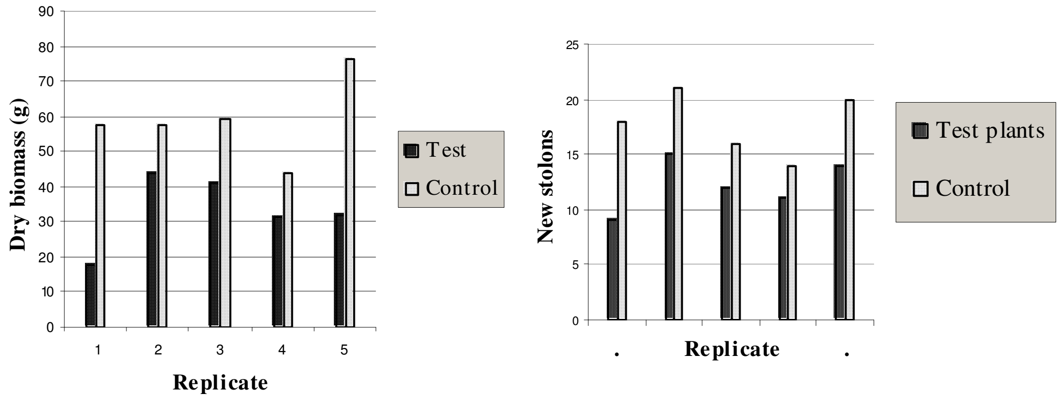


**Fig. 4:** *Taosa impictifrons*. Effect on the dry biomass and petiole length of 20 nymphs feeding on waterhyacinth.

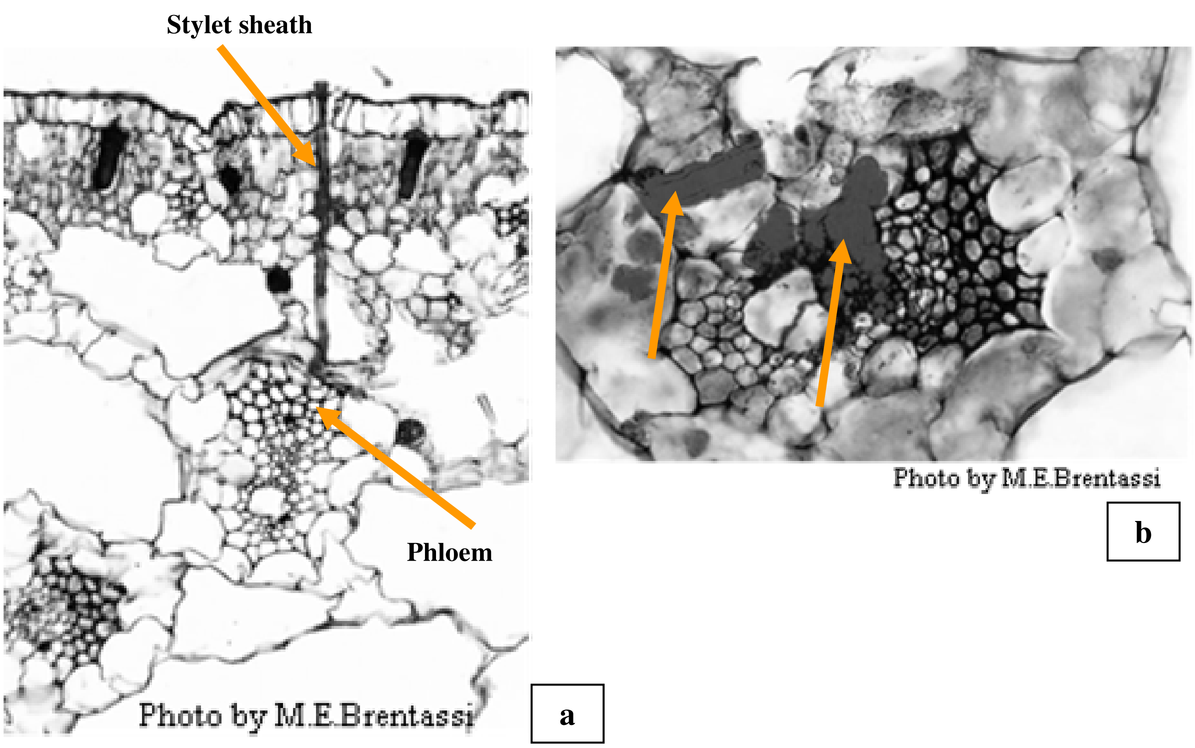


**Fig. 5. a**. Salivary pathway of *Megamelus scutellaris.* **b.** Salivary deposits of *Taosa impictifrons* in vascular tissue.

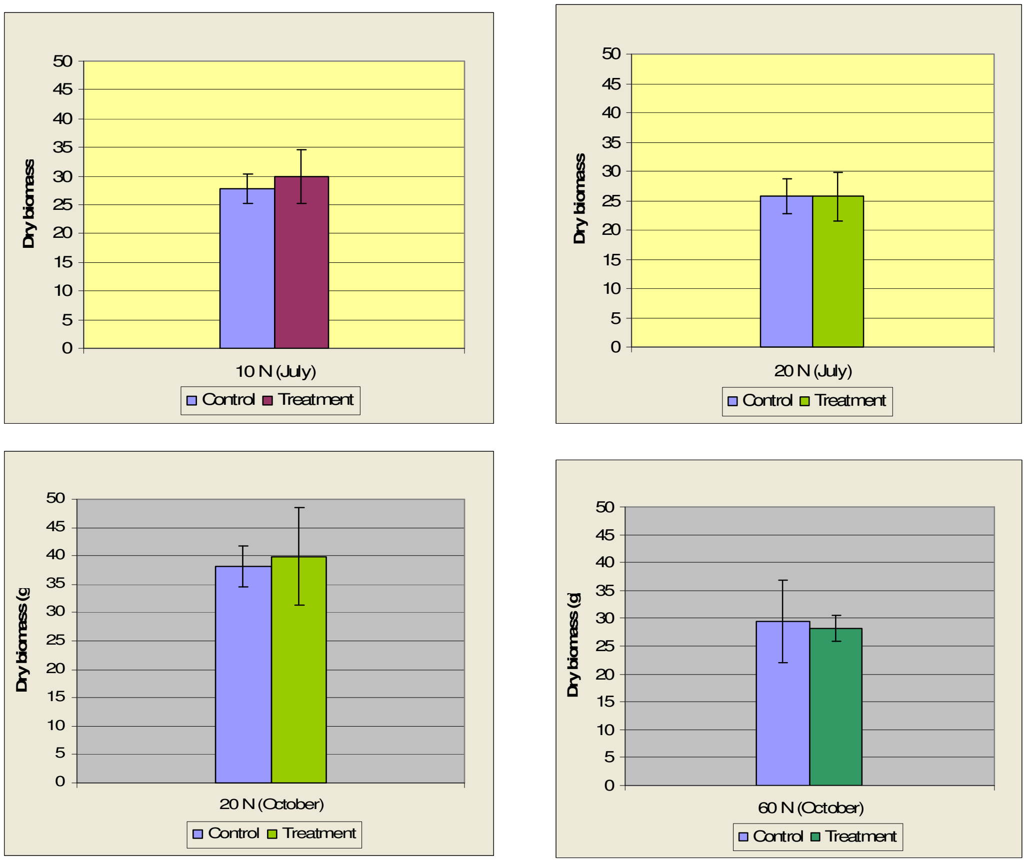


**Fig. 6.** *Megamellus scutellaris* effect on the waterhyacinth biomass.

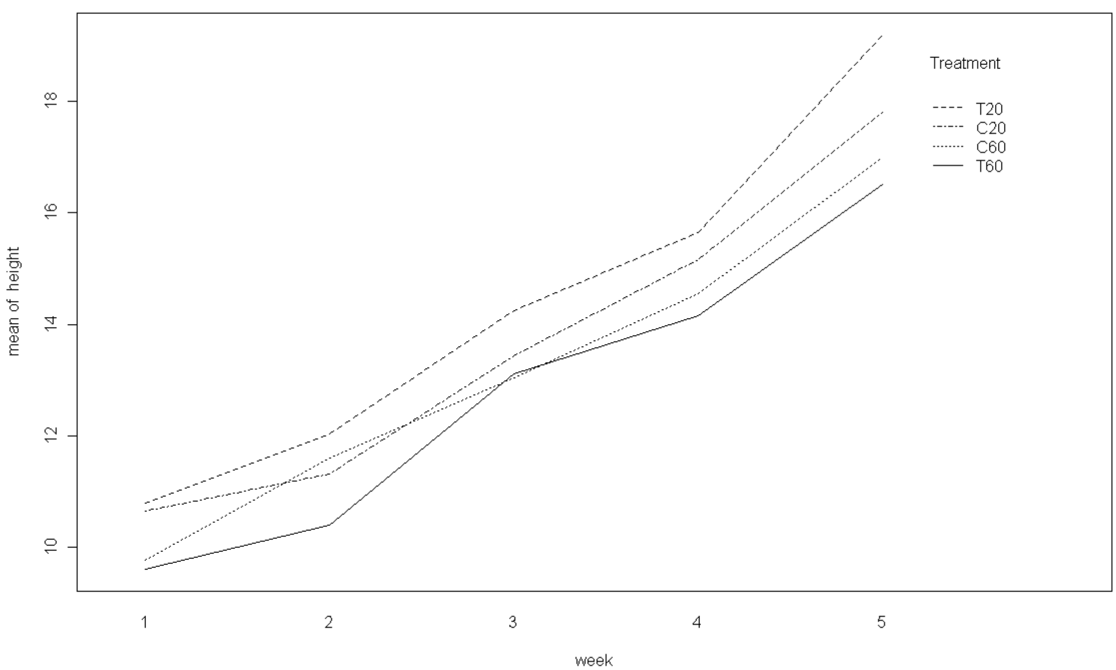


**Fig. 7.** Growing of WH (length of petioles) for treatments with *Megamelus* (T20: 20 initial nymphs, T60: 60 initial nymphs) and their respective controls (C20, C60).

# BRAZILIAN PEPPERTREE

by

Fernando Mc Kay

Cooperator: Greg Wheeler, ARS-IPRL, Fort Lauderdale, FL.

**Abstract**

During the reporting period, studies were focused on the seasonal occurrence and field host specificity of the blotch leaf miner (Lepidoptera: Gracillariidae), one of the natural enemies of Brazilian peppertree (BP) found in Argentina. Four field surveys were conducted between June 2008 and May 2009. *Seasonal occurrence.* The highest and the lowest number of leaflets with mines were recorded in September 2008 and May 2009, respectively. *Field host specificity.* Blotch mines were found on leaves of BP and 4 other Anacardiaceae: *Astronium balansae*, *Schinus lentiscifolius*, *S. longifolius* and *S. weinmannifolius*. No damage was found on leaves of *Lithrea molleoides* and *Schinus molle*. *New findings.* A leaf-galling midge, *Contarinia* sp. (Diptera: Cecidomyiidae) was found on BP. Unidentified Gracillariidae blotch leaf miners (*Eucosmophora* ? spp.) were found developing on the abaxial face of leaflets of BP, *S. weinmannifolius* and *S. lentiscifolius*. Stem-galling wasps, (Hymenoptera: Braconidae?) were found on BP.

The BP project started at SABCL in June 2004. For general information and background of the project, see previous Annual Reports and/or visit www.usda-sabcl.org.

## Materials and methods

**Seasonal occurrence and field host specificity.** *Foliage sampling.* Four foliage samplings were conducted from June 2008 to May 2009. *S. terebinthifolius* and other native Anacardiaceae were sampled in 10 sites along 150 km in north-eastern Argentina (Corrientes and Misiones provinces) (Fig. 1). At each site, a 10-liter bucket of foliage from 10 different plants was sampled. For each species, the number of leaflets contained in the bucket was counted. Samples were kept in plastic bags and brought to the laboratory. Under the stereomicroscope we quantified the number of leaflets with empty and active (with the larva inside) blotch mines.

## Results

**Seasonal occurrence.** The highest and the lowest number of leaflets with blotch leaf mines on BP were observed in September and May, respectively (Fig. 2). Although active leaf mines of different developmental stages are present all the year round, large numbers of synchronize active leaf mines were mostly found during the winter months.

**Field host specificity.** Gracillariidae blotch mines were found mainly on BP (primary natural host) and also on *A. balansae, S. lentiscifolius, S. longifolius*, and *S. weinmannifolius* secondary hosts). No blotches were found on *Lithrea molleoides* or *Schinus molle*. Considering previous lab results and field host specificity studies, *L. molleoides* would not be a natural host. Although *S. molle* hold very few eggs under no-choice tests, the fact that no mines were ever found in the field, would indicate that this species could act as host only under very conservative conditions. Unfortunately, recent host specificity studies conducted at the quarantine facility of the IPRL, revealed that this moth laid eggs and developed mines on North American Anacardiaceae (*Rhus* spp.), constituting a too wide host range for this biocontrol agent.

**New findings**

- Unidentified blotch leaf miners were found on the abaxial face of leaflets of and BP, *S. lentiscifolius* and *S. weinmannifolius*. (Fig. 3-5).
- A leaf-galling midge *Contarinia* sp. (Diptera: Cecidomyiidae) was found on a few leaflets of BP in Santo Tomé, Corrientes, Argentina. Further identification of this species is in progress (Dr. Raymond Gagné, USDA-ARS Systematic Entomology Laboratory) (Fig. 6).
- Stem-galling wasps (Hymenoptera: Braconidae?) were found on branches of BP in Misiones, Argentina (Fig. 7). As soon as adult specimens emerge, specimens will be sent to taxonomists to confirm the identity of this species.

**Field trips to survey for BP natural enemies and to collect Anacardiaceae for cultivation**

June 9-15, 2008. Corrientes and Misiones (Mc Kay and Oleiro).
September 19-24, 2008. Corrientes and Misiones (McKay and Oleiro).
December 1-7, 2008. Corrientes and Misiones (Mc Kay).
May 4-8, 2009. Corrientes and Misiones (Mc Kay).

**Significant accomplishments**

- Progress made on field host specificity of the Gracillariidae blotch leaf miner.
- Additional natural enemies found on BP and other Anacardiaceae.

**Future plans**

- *Blotch leaf miner*. Complete laboratory host specificity studies on Argentinean native Anacardiaceae (oviposition no-choice tests).
- *Stem-galling Hymenoptera*. Attempt laboratory rearing. Conduct biology and field host specificity studies.
- Conduct additional exploration in Argentina and Brazil for natural enemies

<u>**References Cited**</u>

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**Cuda, J. P., J. L. Gillmore, J. C. Medal, And J. H. Pedrosa-Macedo.** Biology and mass rearing of *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae), an approved biological control agent of Brazilian peppertree, *Schinus terebinthifolius* (Sapindales: Anacardiaceae). Florida Entomol., in press.

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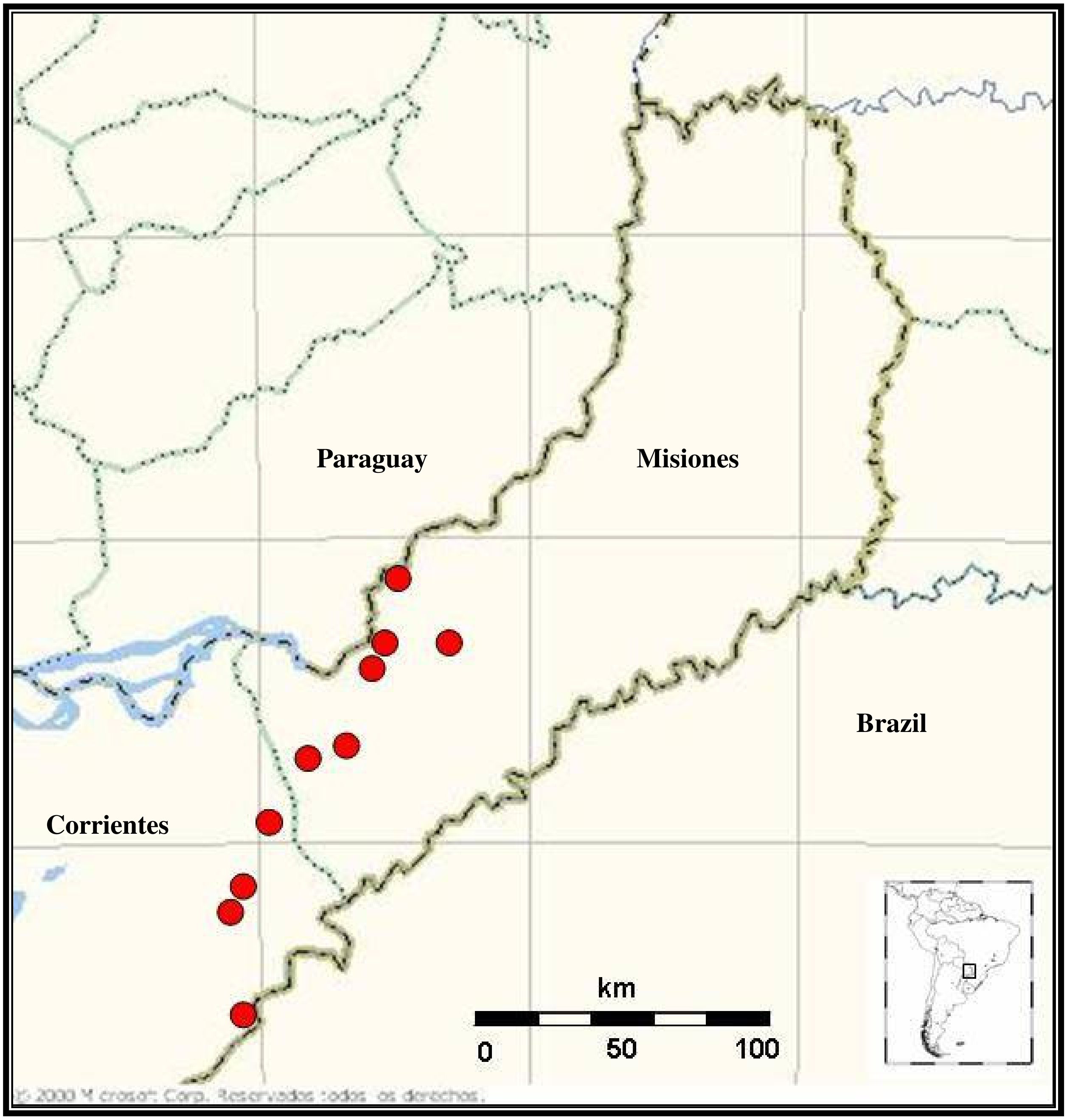


**Figure 1.** Gracillariidae blotch leaf miner sampling sites.

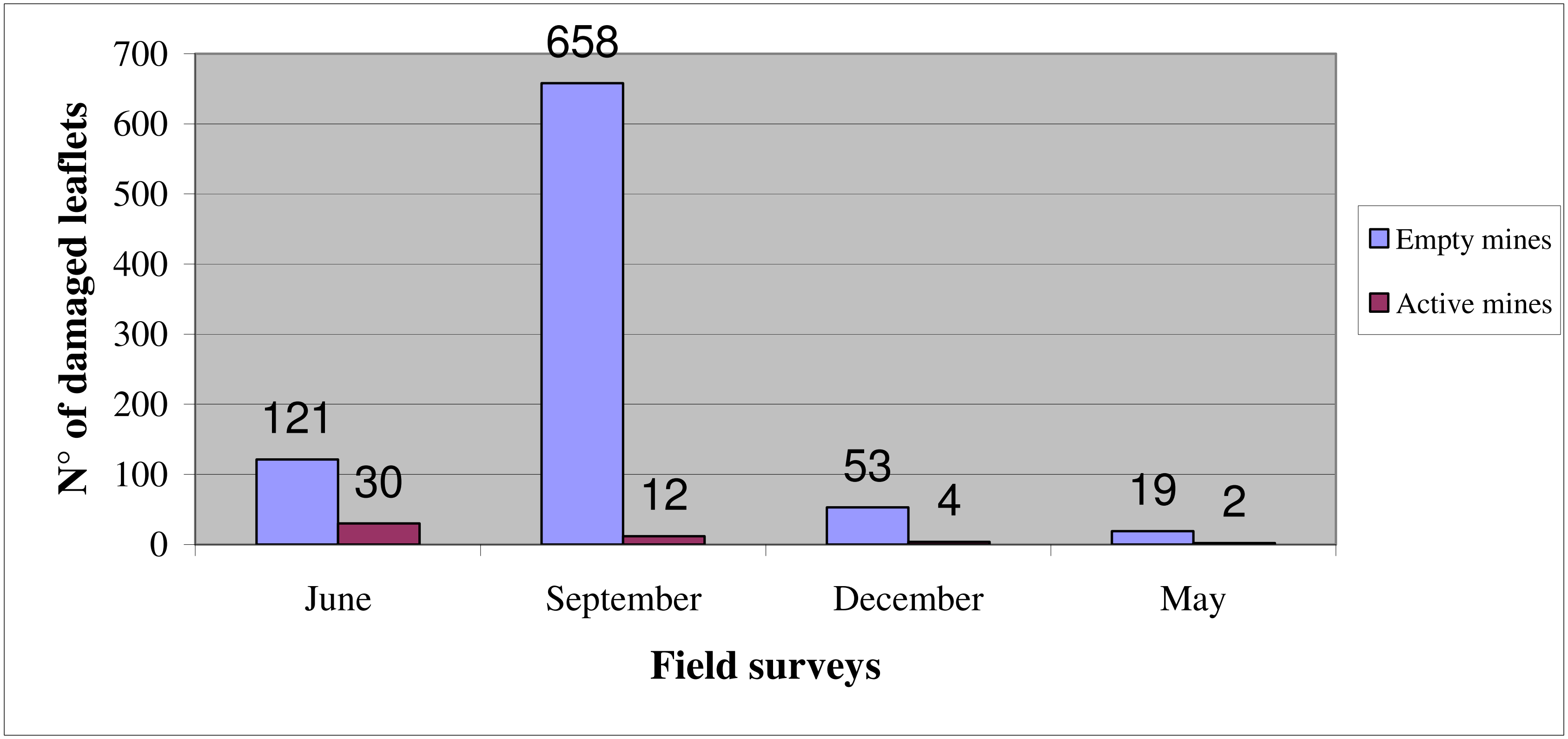


**Figure 2.** Gracillariidae blotch leaf miner seasonal occurrence on BP. June 2008 and May 2009 field surveys.

**3**

**4**

**5**

**6**

**7**

**Figures 3-7. 3-5-** Unidentified leaf mines on BP, *Schinus lentiscifolius* and *S. weinmannifolius* leaflets.
**6-** Leaf galls produced by *Cotarinia* sp., (Diptera:Cecidomyiidae) on BP leaflets.
**7-** Hymenoptera stem galls on BP branches.

# BALLOON VINE and MADEIRA VINE

by

Fernando Mc Kay

Cooperators: Andrew McConnachie, PPRI Hilton, Liamé Van Der Westhuizen and David Simelane, PPRI Pretoria, and Andries Fourie, PPRI Stellenbosch, South Africa.

## Abstract

**Balloon vine.** *Interspersion open-field tests*: the fungus *Phyllacora rimulosa* (Phyllachoraceae: Phyllachorales) was found infesting three *Cardiospermum* spp., the rust *Puccinia arechavaletae* (Uredinales: Puccianiaceae) was found infesting only *Cardiospermum grandiflorum* and *C. halicacabum*. The insects *Lisseurytomella flava* (Eulophidae: Tetrastichinae) and *Chlorostrymon simaethis sarita* (Lepidoptera: Lycaenidae) were found on three *Cardiospermum* species, which agrees with field surveys in northern Argentina. *Moodnopsis* n. sp. near *perangusta* (Lepidoptera: Pyralidae) was found feeding on *C. grandiflorum* and *C. corindum*, while *Cissoanthonomus tuberculipennis* (Coleoptera: Curculionidae) and *Gargaphia* sp. (Hemiptera: Tingitidae) were found only on *C. grandiflorum*. **Madeira vine.** *Loxomorpha cambogialis* Guenee (Lepidoptera: Crambidae), a new leaf-feeding moth was found in Misiones province, Argentina.

## Background information

**Balloon vine.** In January 2005, the ARC-Plant Protection Research Institute, Pretoria, South Africa, and the SABCL initiated a cooperative research program for the biological control of Balloon vine, *Cardiospermum grandiflorum* Sw., in South Africa. For further background on this weed see Annual Reports 2004-08 and www.usda-sabcl.org.

**Madeira vine.** *Anredera cordifolia* (Tenore) Steenis, is a perennial climber Native to southern South America, occurring in Bolivia, Paraguay, southern Brazil, Uruguay and northern Argentina (Brickell and Zuk 1997, Wagner *et al.* 1999). Extensively cultivated in tropical gardens of the world as an ornamental plant, *A. cordifolia cordifolia* has become a serious environmental weed in Australia, South Africa, Hawaii, New Zealand and other Pacific Islands (Nagata 1995, Timmins and Reid 2000). The biology and host specificity of two leaf-feeding flea beetles, *Phenrica* sp. 2 and *Plectonycha correntina* (Chrysomelidae), have been studies as possible biological control agents for Madeira vine (van der Westhuizen 2006; Cagnotti et al. 2007). This latter species is being considered for release in Australia (Bill Palmer pers. comm.).

Official research on this weed will be initiated after the signature of the new contract with PPRI. In the meantime, some opportunistic work was conducted. The main objectives of this project will include: collection and shipment of host specific natural enemies; distribution and phenology of *A. cordifolia* and the associated insects.

**Research conducted**

**Balloon vine**

***Interspersion open-field test.*** Two 8x10m field plots, 40 km apart, were set up in Misiones province in April 2008, 10 to 300 m away from natural populations of the weed and the agent. In each plot, 4 plants of *Cardiospermum grandiflorum*, *C. halicacabum* var *microcarpum* and *C. corindum* were randomly assigned (Fig. 1). The plots were visited in September and December 2008, and in March and May 2009; plants were examined for the presence of insects.

**Results**

Results are summarized in Tables 1 and 2. In plot 1, the fungal pathogen *Phyllacora rimulosa* was found infesting the three *Cardiospermum* species while the rust *Puccinia arechavaletae*, only *C. grandiflorum* and *C. halicacabum*. The phytophagous insects *Lisseurytomella flava* and *Chlorostrymon simaethis sarita* were found infesting the three *Cardiospermum* species, which is in correspondence with field surveys conducted throughout northern Argentina. *Moodnopsis* n. sp. near *perangusta* was found feeding on *C. grandiflorum* and *C. corindum*, while *C. tuberculipennis* and *Gargaphia* sp. were found only on *C. grandiflorum*.

Although plots 1 and 2 were set up at the same time (April 2008), plants in plot 2 were severely retarded by frosts. This could be one of the reasons for the fewer natural enemies found. So far, results indicate that *Gargaphia* sp. and *C. tuberculipennis* are the only natural enemies found exclusively on Balloon vine plants.

**Madeira vine**

In November 2008, adults of *Plectonycha correntina* were found and reared on *Anredera cordifolia* in the SABCL garden. In January 2009, 15 $F_1$-adults were shipped to quarantine facilities at PPRI Pretoria.

In December 2008, larvae of a leaf-feeding moth were found near Posadas, Misiones province, Argentina (Fig. 2). Adult specimens were tentatively identified as *Loxomorpha cambogialis* Guenee (Lepidoptera: Crambidae) (Alma Solís, SEL, ARS-USDA). Unfortunately, no specimens of *Phenrica* sp. 2 were found.

**Significant accomplishments**

- The open-field tests have provided valuable host specificity information.
- The discovery of a new natural enemy on Madeira vine.

**Exploratory trips to survey for Emerging weeds natural enemies**

August 18-24, 2008. Salta, Jujuy, Formosa. (Mc Kay).
Septermber 19-24, 2008. Corrientes and Misiones. (Mc Kay).
December 1-7, 2008. Misiones (Mc Kay).
March 18-April 5, 2009. Salta, Jujuy, Formosa, Chaco, Corrientes and Misiones (Mc Kay, Fourie and Simelane).
May 4-8, 2009. Misiones. (Mc Kay).

**Shipments to PPRI, South Africa**

Insects (and permit numbers): January 2009:
- 15 *Plectonycha correntina* (FAUNA 34456 SENASA DNPV 1032/08)

Insects (and permit numbers): April 2009:
-300 *Cochylis campuloclinium* (FAUNA DFS Nº 5590/09 SENASA DNPV Nº 188/09)
-65 *Anthonomus santacruzi* (FAUNA DFS Nº 5174/09 SENASA DNPV Nº 178/09)
-20 *Cissoanthonomus* sp. (FAUNA DFS Nº 5175/09 SENASA DNPV Nº 179/09)
-60 *Gargaphia* sp. (FAUNA DFS Nº 5172/09 SENASA DNPV Nº 181/09)
-10 *Contarinia* sp. (Permits FAUNA DFS Nº 5173/09 SENASA DNPV Nº 180/09).
Plant material (and permit numbers):
-*Serjania glabrata* (FLORA N° 06328 Phytosanitary N° 01104035)
-*Serjania marginata* (FLORA N°06325 Phytosanitary N° 01104036)
-*Cardiospermum halicacabum* (FLORA N° 06326 Phytosanitary N° 01104037)
-*Urvillea chacoensis* (FLORA N° 06323 Phytosanitary N° 01104034)
-*Serjania meridionalis* (FLORA N° 06327 Phytosanitary N° 01104039)
-*Cardiospermum grandiflorum* (FLORA N° 04489 Phytosanitary N° 01103190)
-*Cardiospermum corundum* (FLORA N° 06324 Phytosanitary N° 01104038).

## Future Plans

***Balloon vine***

- Continued examination of field plots to evaluate specificity of *C. tuberculipennis* and other natural enemies under natural conditions.

***Madeira vine***

- Collect and ship host specific natural enemies upon request of cooperators.
- Study the distribution, phenology and natural enemies.

***Pompom weed***

- Shipments of natural enemies to PPRI facilities upon request of the cooperators.

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**Figure 1 and 2. 1-** Experimental field plot with *Cardiospermum* species in Misiones province, Argentina. **2-** Larva of leaf-tying moth on Madeira vine.

**Table 1.** Presence of Balloon vine natural enemies in field plot 1.

| Dates | Field plot 1 | | |
|---|---|---|---|
| | *C. grandiflorum* | *C. corundum* | *C. halicacabum* var. *microcarpum* |
| September 2008 | -*Phyllacora rimulosa*<br>-*Puccinia arechavaletae*<br>-*Cissoanthonomus tuberculipennis*<br>-*Lisseurytomella flava*<br>-*Chlorostrymon simaethis sarita* | -*Phyllacora rimulosa*<br>-neither flowers nor fruits were present on any of the plants of the plot. | -*Phyllacora rimulosa*<br>-*Lisseurytomella flava*<br>-*Chlorostrymon simaethis sarita* |
| December 2008 | -*Phyllacora rimulosa*<br>-*Gargaphia* sp.<br>-no fruits available | -*Phyllacora rimulosa*<br>-no fruits available | -*Phyllacora rimulosa*<br>-*Puccinia arechavaletae*<br>-*Chlorostrymon simaethis sarita* |
| March 2009 | -*Phyllacora rimulosa*<br>-*Puccinia arechavaletae*<br>-*Gargaphia* sp. | -*Moodnopsis* n. sp. near *perangusta*<br>-*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* | -no plants available |
| May 2009 | -*Cissoanthonomus tuberculipennis*<br>-*Moodnopsis* n. sp. near *perangusta*<br>-*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* | -*Moodnopsis* n. sp. near *perangusta*<br>-*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* | -no plants available |

**Table 2.** Presence of Balloon vine natural enemies in field plot 2.

| Dates | Field plot 2 | | |
|---|---|---|---|
| | *C. grandiflorum* | *C. corundum* | *C. halicacabum* var. *microcarpum* |
| September 2008 | -small vegetating plants | -small vegetative plants | -small vegetative plants |
| December 2008 | -small vegetating plants | -small vegetative plants | -small vegetative plants |
| March 2009 | -*Puccinia arechavaletae* | -*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* | -*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* |
| May 2009 | -*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* | -*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* | -*Chlorostrymon simaethis sarita*<br>-*Lisseurytomella flava* |

# BRAZILIAN WATERWEED

by

Willie Cabrera Walsh and Magalí Dalto

Cooperators: Ray Carruthers, John Herr & Lars Anderson, ARS-EIWRU, Albany and Davis, CA

## Introduction

*Egeria densa* Planchon (Brazilian elodea or Brazilian waterweed) is a South American submerged perennial in the Hydrocharitaceae that has become a weed in North America, Australia, New Zealand, South Africa, and parts of Asia and Europe. It crowds out other plant species by forming dense stands, negatively affecting the native biota, as well as water sports, fishing, navigation, delivery of irrigation water, and hydropower production. The larva of *Hydrellia* nv. sp. (Diptera: Ephydridae), feeds on the mesophyl, producing chlorosis of whole whorls, and mining the stem in between them. Under laboratory conditions a single gravid female can cause the defoliation of a whole stem. Under field conditions in Argentina, damage can also be very high, in spite of several mortality factors (see Annual Reports 2007 and 2008).

## Material & Methods

No-choice feeding tests were performed on *Hydrellia* with 20 aquatic plant species. The subject plants were exposed to first instars of *Hydrellia* that were forced to leave the leaf and search for food. For this, one or more leaves, as needed, of *E. densa* with *Hydrellia* eggs, 20 per replicate, were pinned with minuten to the stem of the test plant. In laboratory conditions this procedure had an almost 100% success rate when practiced on host plants.

Also, gravid females were released inside small containers with the test plants, and allowed to lay their eggs at will. This test was performed so as to minimize the effect of starting feeding – however briefly- on the host plant. The test plants selected were all the available species within the Hydrocharitaceae, all the submerged aquatic plants that co-occur with *E. densa* in its known native range in Argentina, and several aquatics with floating foliage that have significant leaf areas physically available to the fly's aquatic larvae, i.e. the underside of the leaves break the water surface (Table 1).

The test plants that sustained any degree of *Hydrellia* development were further tested for preference compared to *E. densa* in a series of double choice tests. We evaluated oviposition preference, feeding preference as expressed by the proportion of larvae that migrated from the test plants to the natural host, and suitability of the test plant as expressed by survival rates and development time compared to the natural host. Gravid females showed little oviposition specificity in previous tests, laying their eggs on several plant species or on the container wall, however, while the need to relieve an egg load may be urgent enough for a gravid female to lay their eggs anywhere, this does not mean that oviposition preference can play an important role in specificity in the field.

The natural damage caused by *Hydrellia*, and seasonal abundance in the field were followed every month, counting the number of larvae and pupae, estimating number of damaged tips, and proportion of damaged whorls from an *E. densa* patch in Otamendi, Buenos Aires province, located 50 km from the SABCL. For this, fifty 30-cm *E. densa* tips are collected every month.

Also, an estimation of the field immature mortality and its causes, were estimated from the field samples. We accounted for parasitized and diseased larvae and pupae. Thus, direct observation allowed us to attribute mortality to diseased, parasitized, and unaccountable immatures.

Finally, the list of presumed generalist natural enemies of immature stages was extended by exposing a known number of *Hydrellia* larvae to different predators: fish (*Jenynsia* spp.), predatory leeches, Dytiscidae (Coleoptera) larvae, Gyrinidae adults, and dragonfly larvae. Their predation potential was evaluated under starvation conditions (no other food source was available to the predators) by comparing the total number of adults emerged from test jars and controls.

**Results**

The laboratory host range of *Hydrellia* nv. sp. resulted limited to the *Egeria-Elodea* subgroup within the Hydrocharitaceae (Table 1). In the field, however, it has only been found on *E. densa*. Also, larvae showed a preference for *E. densa* in a double choice test, as oviposition was far greater on its natural host than on the test plants, most larvae migrated from the test plants to its natural host, *E. densa,* when incubated together. Also, development success from egg to adult was highest on *E. densa*, and development time was shortest (Table 1).

In the field *Hydrellia* seems to be present year round in every stage, according to our monthly samples that cover September 2006 to June 2009 (Fig. 1). Between 90% and 100% (average 98.6%) of the plants showed *Hydrellia* feeding damage. The flies are most abundant during Summer and Autumn in general terms, and population slumps were observed mostly from Winter to Spring (Fig. 1). However, these tendencies do not always hold true, and other factors seem to affect fly density, i.e, water level variations, since it has been observed that when the water levels rise drastically in our collection patches we find fewer larvae in the ensuing samples.

As for the natural enemies of *Hydrellia* nv. sp., we have found three specific natural enemies: 1) a pupal parasitoid, *Hydrelliaeucoila* sp., gen. & sp. nov (Hym.: Eucoilidae), 2) a larval parasitoid, *Chaenusa* sp. nov. (Hym.: Braconidae), and 3) a pathogenic fungus, *Lecanicillium* sp. (*Fungi Imperfecti*). All these natural enemies were described and are currently awaiting publication. The *Hydrellia* average mortality attributable to these specific natural enemies is 13.7%. However many unspecific mortality factors may yet be unaccounted for. Of the generalist predators tested so far, only leeches appear to cause some degree of mortality on *Hydrellia* larvae/pupae in starvation conditions. Also, other mortality is thus far unaccounted, specifically: egg mortality, which is around 7% in the laboratory, may be higher in the field due to predation and disease; and adult mortality to generalist predators.

**Relevant Accomplishments**

- Host range testing was completed on several plant species of the same family, others in the same subclass, and yet other unrelated species that can be exposed to the plant.
- Advanced identification and description of *Hydrellia* natural enemies

**Field Trips**

-**Nov 25-Dec 7, 2008:** To Formosa Prov.

-**Dec 3-6, 2008:** To Formosa and Corrientes Prov.
-**Mar 5-10, 2009:** To Chaco and Corrientes Prov.
-**June 8- 14, 2009:** To Formosa, Cordoba, Chaco and Santa Fe Prov.
- Monthly day trips to Otamendi, Buenos Aires Prov.

**Future plans**
- Ship laboratory specimens to quarantine facilities in ARS Albany, clean of pathogens and parasitoids, to continue host range tests in the US.
- Continue gathering field and experimental data for climate matching (determine the fly's seasonal and geographical potential distribution in the US) and potential impact on the weed in its introduced range.

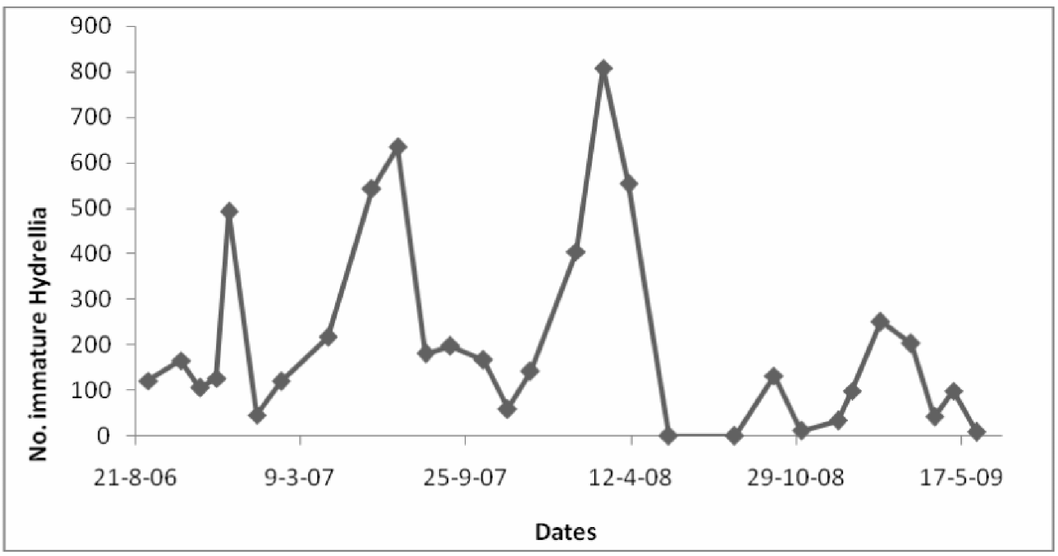


Figure 1. Abundance of *Hydrellia* sp. throughout the seasons at Otamendi, Buenos Aires.

**Table 1. List of aquatic plant species tested for of *Hydrellia* nv. sp. host range**

| Test plant | Feeding | Development | Emergence (days, 25ºC) | % egg to adult | Oviposition preference (test plant/*E. densa*)x100 |
|---|---|---|---|---|---|
| ALISMATIDAE | | | | | |
| HYDROCHARITACEAE | | | | | |
| *Egeria densa* Planchon | y | y | 30 | 79.5 | 100 |
| *Egeria naias* Planchon | y | y | 27 | 36.5 | 23 |
| *Elodea callitrichoides* Caspary | y | y | 42 | 23 | 9.6 |
| *Naias guadalupensis* Morong | y | n | n/a | 0.1 | 0 |
| *Limnobium laevigatum* (Humboldt & Bonpland ex Willdenow) Heine | n | n | | | |
| *Vallisneria americana* Michaux | n | n | | | |
| *Vallisneria spiralis* L. | n | n | | | |
| ALISMATACEAE | | | | | |
| *Sagittaria subulata* Buchenau | n | n | | | |
| POTAMOGETONACEAE | | | | | |
| *Potamogeton gayi* Bennett | n | n | | | |
| *Potamogeton pusillus* L. | n | n | | | |
| *Potamogeton illinoensis* (Hicken) Horn af Ratzien | n | n | | | |
| *Potamogeton spirilliformis* Hagstrom | n | n | | | |
| MAGNOLIIDAE | | | | | |
| CABOMBACEAE | | | | | |
| *Cabomba caroliniana* Gray | n | n | | | |
| CERATOPHYLLACEAE | | | | | |
| *Ceratophyllum demersum* L. | n | n | | | |
| ROSIDAE | | | | | |
| HALORAGACEAE | | | | | |
| *Myriophyllum aquaticum* (Vellozo) Verdcourt | n | n | | | |
| ASTERIDAE | | | | | |
| LENTIBULARIACEAE | | | | | |
| *Utricularia foliosa* L. | n | n | | | |
| MENYANTHACEAE | | | | | |
| *Nymphoides indica* (L.) Kuntze | n | n | | | |
| LEMNACEAE - *Lemna* sp. | n | n | | | |
| AZOLLACEAE - *Azolla* sp. | n | n | | | |
| CHARACEAE - *Chara* sp. | n | n | | | |

# WATER PRIMROSE

by

M. Cristina Hernández, Willie Cabrera, and Gerardo Elsesser

Cooperators: Brenda Grewell and Ray Carruthers, ARS-EIWRU, Albany and Davis, CA.

**Abstract**

The insects reared from *Ludwigia hexapetala* from different sites include 4 species with leaf feeding larvae, 3-4 with larvae that feed on young leaves and meristems, 7 with stem-miner larvae, 1 with fruit-feeder larvae, 1 of cell content feeders (thrips) and sap feeders; 9 of these species have defoliating adults. Biological information on most of them was obtained. Non choice feeding tests carried out with a leaf tier larvae (Lepidoptera: Tortricidae) showed its development on *L. grandiflora*, *L. leptocarpa*, *L. elegans*, *Myriophyllum aquaticum*, and *Alternanthera philloxeroides*. It was discarded for further studies. The chromosome numbers of several *Ludwigia* spp. were obtained.

## Introduction

*Ludwigia hexapetala* (Hooker & Arn.) Zardini et al. (Onagraceae, Sec. Oligospermum) shares the Oligospermum section with 8 spp. and 4 sub spp. (Wagner et al. 2007), among them, *L. hexapetala, L. grandiflora* (Michaux) Zardini et al., *L. hookeri* (Micheli) Hara, *L. helminthorrhiza* (Martius) Hara and *L. peploides* (Kunth) Raven and the subspecies *peploides*, *montevidensis* and *glabrescens* can be found in Argentina.

## Materials and Methods

**Plant taxonomy.** To obtain a reliable character for the identification of local *Ludwigia* spp. in the Oligospermum section, chromosome counts were carried out by Dr. Eduardo Greizerstein, Cytogenetic and Evolution Department, School of Natural Sciences, University of Buenos Aires, with samples of roots provided by SABCL.

**Weevil taxonomy.** The weevils reared from the samples on Ludwigia species were identified by Dr. Adriana E. Marvaldi, Instituto Argentino de Investigaciones de Zonas Áridas, Mendoza Province, Argentina.

**Field surveys.** As in previous years (see Annual Reports 2007-2008), surveys were carried out in north-east of Argentina sampling on *Ludwigia* species in predetermined plots. Stems containing insects in different stadia were analyzed and the immatures incubated until adult emergence. The observations focused on diversity of species, damage, stem miner larvae and particular marks to recognize the different species of miner larvae by the oviposition scars.

## Results

**Plant taxonomy.** The chromosome counts revealed the following numbers: *L. hexapetala* 2n = 80 chromosomes, for sites in Corrientes (plots CO SEQ.008; CO GOY.011), Santa Fe (plots SF GUS.025), and Buenos Aires (plots BA OTA); *L. grandiflora*, 2n = 48, for sites in Chaco and Formosa (plots CH 960.022; FO CAN); *Ludwigia* sp. 2n = 64, for a site in Entre Rios (octoploid hybrid according with Zardini et al.1991) (plot ER GUA.005). Counts from other sites are in progress.

**Insects associated with *L. hexapetala*.** Insect reared from *L. hexapetala* from different sites and biological observations are listed in Table 1 and shown in Figures 1 and 2. This information is a preliminary estimation of the field populations obtained using sub-samples of each plot.

The external oviposition marks of the 6-7 *Tyloderma* spp. showed significant differences that might be used as taxonomic tools (Fig.3). Three cultures were started with *Merocnemus binotatus* (Boheman), *Tyloderma* sp. (*aeneotinctum* group) and *Tyloderma longisquameum* Wibmer.

**Preliminary no choice feeding tests**

**Defoliator larvae of Tortricidae (Lepidoptera).** The larvae developed on *Ludwigia grandiflora*, *L. leptocarpa*, *L. elegans*, *Myriophyllum aquaticum*, *Alternanthera philloxeroides*. Due to the wide host range, it was discarded for further studies.

***Tyloderma* small weevils.** Plants tested were *L. leptocarpa*, *L. elegans*, *L. grandiflora*, and *L. hexapetala* as control. Five adult weevils were used per test plant. In addition two specimens were left in complete starvation (no food or water). The weevils fed on all the species tested. Because the specimens in starvation survived 45 days, the trial was canceled.

**Field trips**

-**Nov.25-Dec. 7, 2008:** To Santa Fé, Chaco, Formosa and Corrientes Prov. (Cabrera Walsh).
-**Mar. 5-7, 2009**: To Santa Fé, Chaco, Corrientes and Entre Ríos Prov. (Cabrera Walsh).
-Several one-day trips to Otamendi and Mercedes, BA (Cabrera Walsh).

**Future plans**

- To continue the exploration for insects associated with *Ludwigia* species.
- To increase the number of *Ludwigia* spp. cultivated at SABCL for choice tests.
- To continue the evaluation of natural enemies.

**Relevant accomplishment**

- The discovery of a great diversity of insects associated with *L. hexapetala*, mainly within the *Tyloderma* complex.
- A rearing method for these weevils was developed.

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**Table 1: Species reared from *Ludwigia hexapetala*. (E, egg. L, larva. P, pupa. Ad, adult.) Sites in blue= *hexapetala* confirmed**

| | Host tissue: | Species | wpt | Observations |
|---|---|---|---|---|
| **Larvae** | Leaf feeders | Tortricidae (Lepidoptera) | SF GUS, CH 960 | Leaf tier. Eggs in long stripe mass on the leaves. Larvae black head and greenish body, very agile. Specificity: low. In no-choice, full development on *Ludwigia elegans, L. grandiflora, L. leptocarpa,Myriophyllum aquaticum, Alternanthera phylloxeroides.* |
| | | Sphyngidae (Lepidoptera) | SF GUS, | Defoliator. Young larvae green that change to reddish in middle age L, and full grown L with transverse black, red and white stripes.<br>Specificity: ? Two L with no choice didn't fed on *Myriophyllum* and *Alternantera*. |
| | | Noctuidae (Lepidoptera) | CO-ITU, | Defoliator. Black larvae. Not frequent. |
| | | Lysathia sp. (Col., Curculionidae) | CO ITU, | Defoliator L and A<br>Specificity: Found in L. leptocarpa, L. bonariensis (008bis) |
| | Young leaves and axilar meristems | Pterophoridae (Lepidoptera) | CH-960 | Feed on young leaves and use them as protection. L and P with dorsal hairs similar to the hairs in the plants.<br>Feb08,Mar09.<br>Specifity: ?. |
| | | *Auleutes bosqui* (Col.,Curculionid.)<br>*Auleutes* sp dark CH960,CO GOY<br>*Auleutes* sp black CO CAB | CH 960<br>SF GUS,<br>CO ITU,<br>BA BRA,<br>CO CON,<br>COGOY,<br>COCAB | L bore through the buds killing them. A, defoliator.<br><br>Specificity: low. Found in *L. elegans*. |
| | Stem miners | Microlepidoptera 1<br>" 2 | CO CAB<br>CO SEQ | L dig helicoidal mines under the epidermis in the stems.<br>Damage: ?<br>Specificity: ? |
| | | *Merocnemus binotatus (Boheman)* | SF GUS,<br>CO VCU,<br>CO BV1? | E in oval scar with fiber in one edge. L digs in the medulla. Could be associated with apical damage. Total span life around 45 days. |
| | | *Tyloderma sp. (aeneotinctum* group of Wibmer 1989) | SF GUS,<br>CH SAL,<br>CH 960 | E in oval scar with fibers in the middle. L digs in the medulla. P last 8-10 days. Ad remain inside the mine several days, after that cuts a circular exit. |
| | | *Tyloderma affine* Wibmer | CH SAL,<br>CH 960? | E in oval scar with fibers in the middle and L digs initially one round mine, subepidermical, after that it goes to the vascular medulla. |
| | | *Tyloderma* med. back light brown | SF GUS,<br>CH SAL,<br>CH 960, | E in irregular slit, enlarged in one end. L digs in the medulla. P last 7-8 days. |
| | | *Tyloderma longisquameum* Wibmer | CH SAL,<br>CH 960,<br>CO CAR,<br>CO GOY | E laid in axilar bud and L digs in the medulla. P last 7-8 days. |
| | Fruit feeders | *Tyloderma nigromaculatum* Hustache | CH 960,<br>CH SAL,<br><br>CO CAR,<br>CO GOY | Egg at sepal base, the whole development goes inside the fruit but several seeds remain undamaged. |

Continuation **Table 1: Species reared from *Ludwigia hexapetala*. (E, egg. L, larva. P, pupa. Ad, adult.) Sites in blue= *hexapetala* confirmed**

| | | | | |
|---|---|---|---|---|
| **Adults** | Cell content feeders | Thrips | SF GUS, CH 960, CO ITU, CO BV1, CO CAR, CO GOY, CO SEQ | Eggs are laid on the surface of the leaves. All nymphs and Ad feed on the young leaves, apical and axilar. They produce death cell-spots  and even the death of the buds. |
| | Sap feeders | Fulgoroideae (Cabeza achatada) | SF GUS, CO SEQ | |
| | | Delphacidae 1 | COSEQ 008 | Dark spot in base of tegmina. |
| | | Delphacidae 2 | BA OTA | The eggs inserted in the stems produce the bend of it. White nymphs. |
| | | Delphacidae 3 | 028 | Yellow nymphs and adults dark brown with a transverse white line in the edge of the tegmina, braquipterous. |
| | Defoliators | All the adults Coleoptera (described as larvae) | | |

**Survey Nov.25-Dec.8, 2008** | **Survey Mar. 4-8, 2009**

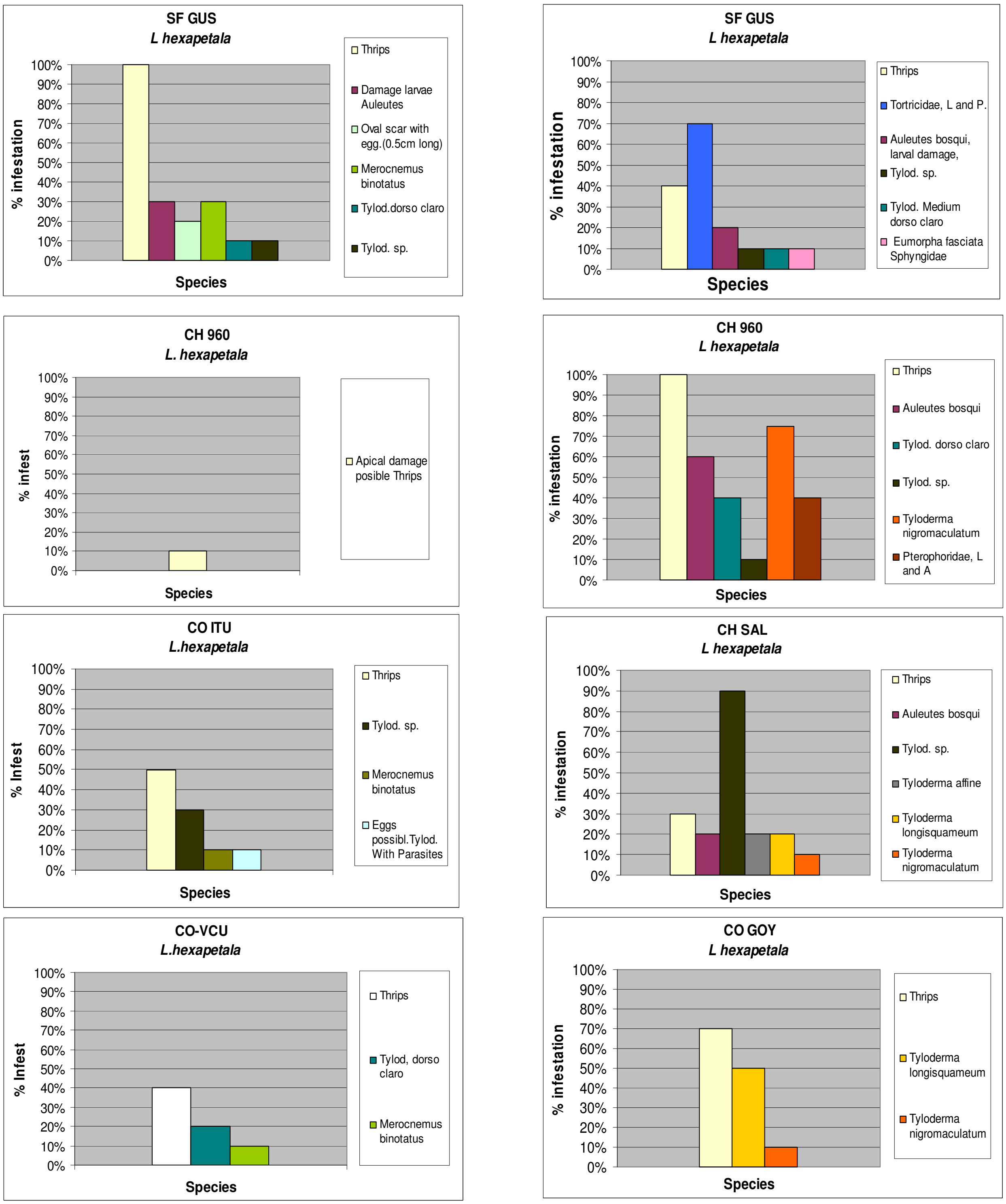



**Fig. 1:** Main insect species (%) associated with *Ludwigia hexapetala* in the pre-established sites in the Santa Fe, Chaco and Corrientes Province.

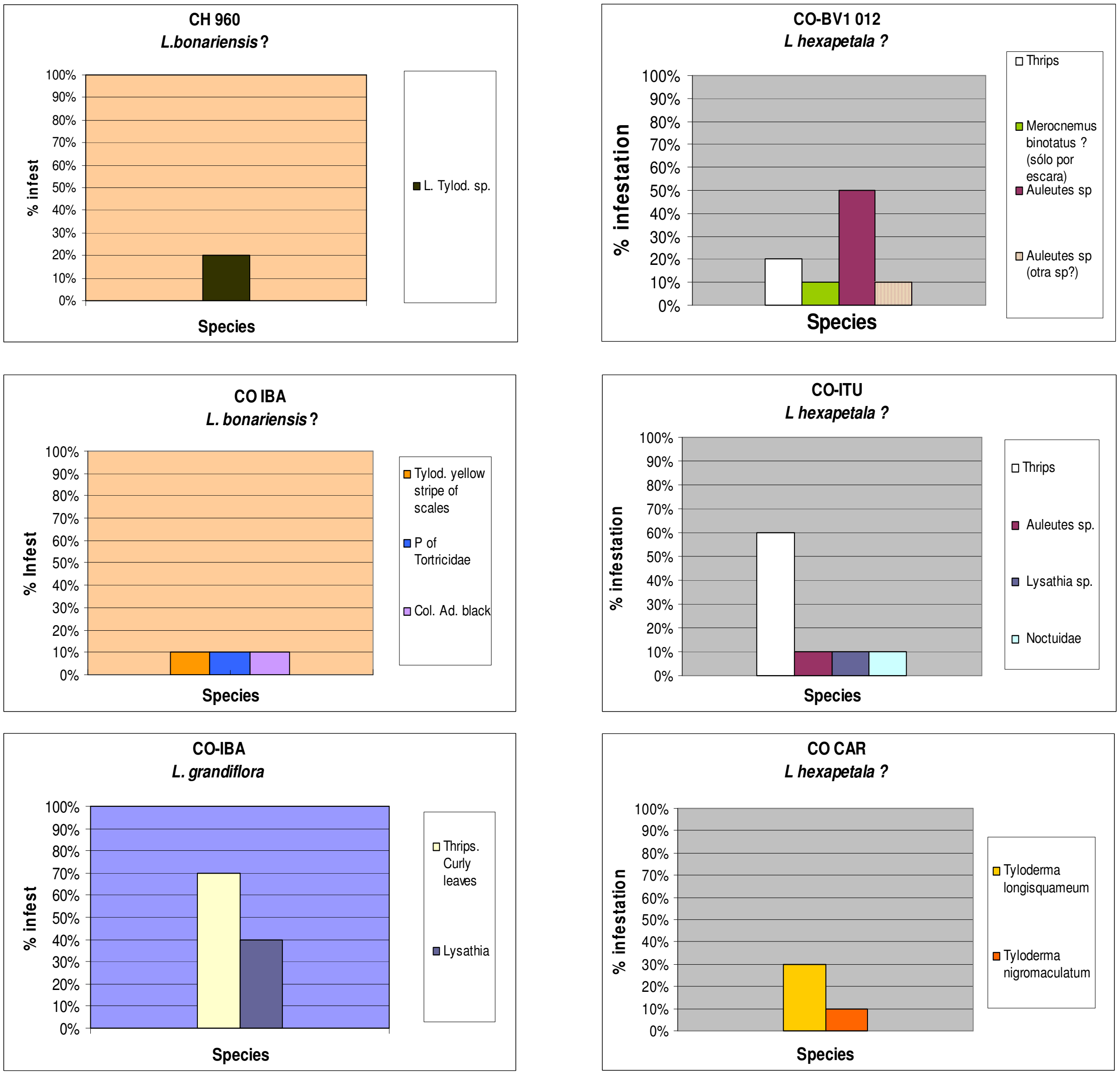
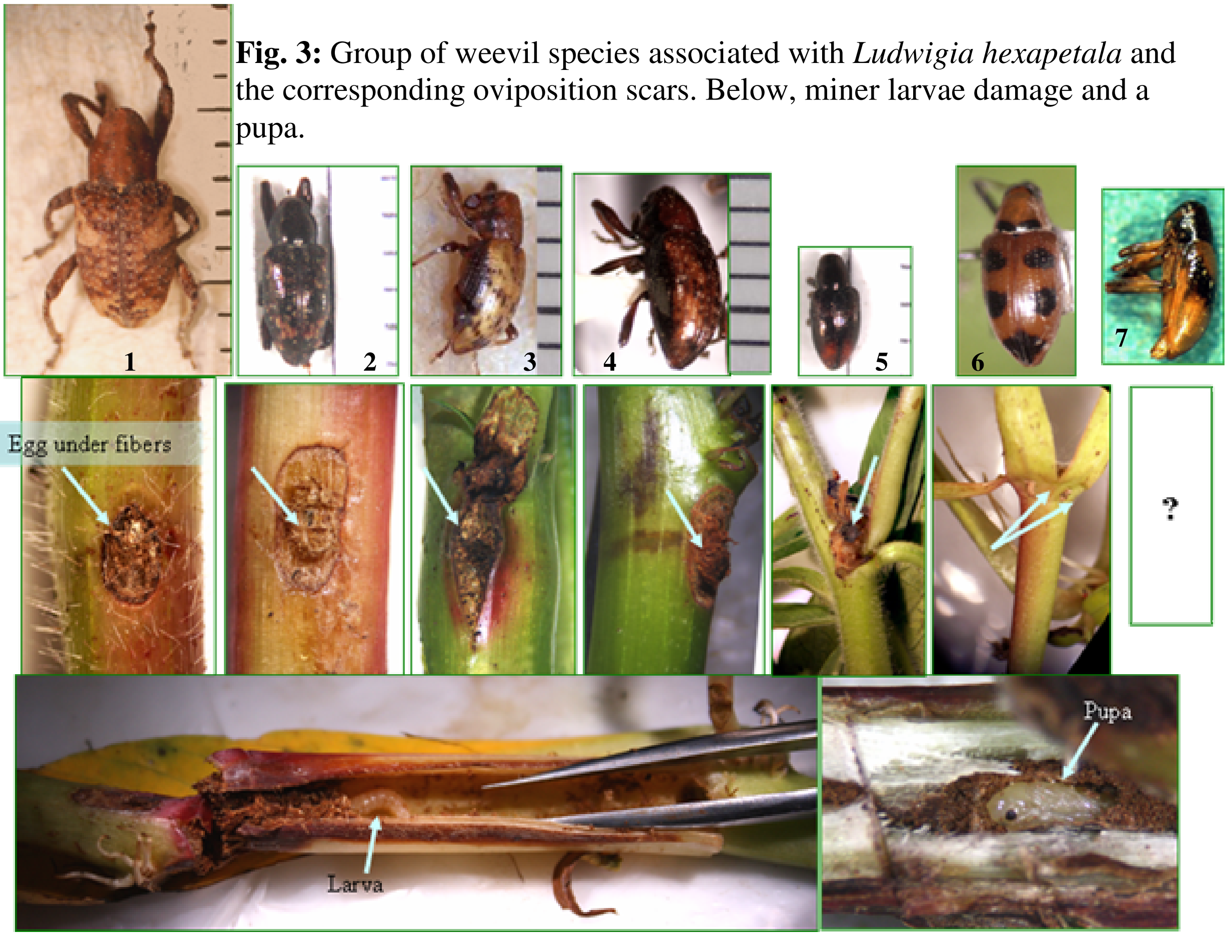



**Fig. 2:** Main insect species (%) associated with *Ludwigia bonariensis* and *L. grandiflora* in the pre-established sites in Chaco and Corrientes Province. The graphics of *L. hexapetala* are made with unconfirmed identification of the plant species.

**Fig. 3:** Group of weevil species associated with *Ludwigia hexapetala* and the corresponding oviposition scars. Below, miner larvae damage and a pupa.




1. *Merocnemus binotatus* (Boheman); 2. *Tyloderma* sp. *(aeneotinctum* group of Wibmer); 3. *Tyloderma* sp. med. back light-brown; 4. *Tyloderma affine* Wibmer; 5. *Tyloderma longisquameum* Wibmer; 6. *Tyloderma nigromaculatum* Hustache. 7.*Tyloderma elongatum* Wibmer

# LIPPIA

by

Alejandro Sosa, Cristian Rodriguez and Marta Telesnicki

Cooperator: Mic Julien-CSIRO Entomology, Indooroopilly, Australia

**Abstract**
The native range of *Phyla* sp. has been spanned through surveys in Argentina, adding new information about plant taxonomy and its natural enemies. New information about rearing methods and preliminary host specificity of *Kuschelina bergi* and *Longitarsus* spp. are reported.

***Phyla*, surveys, taxonomical studies, and its herbivore diversity**
We extended the exploration in Argentina for the weed distribution and natural enemies. New information on the plant taxonomy is being provided by Dr. N. O'Leary (Instituto de Botánica Darwinion), conducting a systematic study of *Phyla*. So far she confirmed that *P. nodiflora* and *P. fruticosa* (both referred as *P. reptans* in previous reports) are native of Argentina. She is investigating if *P. canescens* and *P. nodiflora* are two "forms" of the same species. Molecular studies are being replicated in Australia with plant material collected from herbarium specimens.

The richness of natural enemies was visually compared by sampled-based species accumulation curves (Gotelli and Cowell 2001). We entered occurrence data in a two-way matrix (species x sample) and used the EstimateS 8.0 software (Colwell 2006). Curves were obtained after 1000 randomizations. Indexes were selected to estimate the number of species expected to occur.

We found 64-90% of the expected arthropod richness (Table 1). The indexes showed different plateau in the accumulation curves (Fig. 1).

**Biology of *Kuschelina bergi***
Pupation substrate. The adequate pupation conditions are crucial for a successful lab rearing. A study of the substrate requirements and humidity was conducted. We collected third-instar larvae from a lab culture and put them individually in small containers (N=180) with sand and soil as two different substrates and a control with no substrate. Two treatments were considered per substrate, moist and dry. The assessments of substrates were based on the emerged/not emerged adults from the containers. The data were analysed using generalised linear models, logistic regression with over-dispersion accounted for through the use of quasi binomial error variances.

Host specificity
**a. Adult feeding, no-choice.** Adults from a lab culture were kept individually in cages with one of the Verbenaceae *Phyla canescens*, *P. nodiflora*, *P. fruticosa*, *Lantana megapotamica*, *Glandularia platensis*, *G. tenera*, and *Duranta* sp. The plant part exposed was the apical portion of one stem with leaves. Six replicates were considered (n=42). Plants were exposed for one week and consumption of foliar area (consumed/offered) was measured and compared. The leaf area was estimated using ImageJ 1.4g from photographs of the leaves before and after the experiment. One way ANOVA followed by Tukey HSD was used to compare means.

**b. Larval feeding, no-choice.** Starvation test to study the larval development on the plants used above. We followed the development of newly hatch larvae on each plant. One larva per container (6 replicates; n=42) with moist tissue paper and a leaf of the plant species being tested. The data were analysed using GLM, using emerged/not emerged with binomial error distribution.
**c. Maternal and pre-adult effect (in progress).** To predict the risk of *K. bergi* on *P. nodiflora* in Australia, following McLean et al. (2009) we are testing if the maternal and pre-adult host plant influence the fitness and behaviour of *Kuschelina* progeny. We initiated "lines" consisting on 6 groups of 10 newly emerged larvae from a laboratory culture. Each line was randomly assigned to cages with one plant of *P. cansecens*, *P. nodiflora* or *P. fruticosa*. The emerged adults were counted, sexed and a couple placed in plastic containers in a rearing chamber to evaluate fertility and fecundity. The ensuing larvae from each line were separated to study (1) the feeding preference and (2) the larval development on *Phyla* spp. in relation to the original line. The emerged adults were treated as above to study fecundity and fertility. The ensuing larvae were separated for preference and larval development. The preference, survivorship, and fecundity of the last generation will be compared considering the host plant of the parental generation. Statistical analysis was performed using R (version 2.9.0; http://www.r-project.org)

Results

Pupation substrate. Emergence of *K. bergi* adults varied with the type and condition of substrate (Fig. 2). The control treatment showed the lowest emergence regardless of moist level (t=-2.845, $P$= 0.00498). Similar results were observed for dry sand. The addition of moisture increased pupation (t=-3.707, $P$=0.00021) in the sand and control but slightly decreased in soil. The highest adult emergence was observed in soil, slightly higher in dry soil and similar to moist sand (t=3.668, $P$=0.000245). With this information we improved the rearing methods; as a result, we developed a lab culture of 500 individuals after the third generation from the rearing of 40 adults.

Host specificity.
**a- Adults feeding, no choice.** After a week all adults survived on all test plants. However, feeding was only observed on *Phyla* spp. The highest consumption was on *P. canescens* with 20% followed by *P. nodiflora* and *P. fruticosa* with 10% (F = 21.449, df = 2,15, $P$<0.05) (Fig. 3).
**b- Larval feeding, no choice.** Development of larvae to pupae and to adults occurred only on *Phyla* spp.; differences were not significant (t=5.98e-17, $P$>0.05) (Fig. 4). These results revealed that *K. bergi* is restricted to *Phyla* species.
**c. Maternal and pre-adult effect.** So far, adults have emerged from the three plants; although ~80% emerged from *P. canescens* and only ~50% from the other *Phyla*. Observations suggest that feeding was also higher on *P. canescens*. The experiment is in progress.

***Longitarsus spp.***
The insect coloration is quite uniform with dark brown body and amber legs, with the typical developed metafemur of Alticinae used to jump, making its capture difficult in the field. It has long moniliform antennae, as long as its elytra. Despite the uniformity, sometimes we found darker individuals with different esternites. Cryptic species might be present. As for *K. bergi*, females are larger than males (Furth 2007) and show vaginal papillae. Tentatively, we are considering the lighter species (northern distribution) as *Longitarsus* sp. 1 and the darker one (southern distribution) *Longitarsus* sp. 2.

Host specificity. We tested the feeding behaviour of field collected adults of *Longitarsus* sp.1 with the methodology and test plants used for the adult feeding no choice of *K. bergi*. We measured the consumption rate (ANOVA, linear model package from R) and the number of feeding marks (holes) (GLM, binomial error structure).

Results

A significant preference was found for *P. canescens* and *Glandularia* (F=5.1529, df=5, 25; $R^2$= 0.5075, *P*=0.0022). The consumption rate was higher in *P. canescens* (t=4.119, *P*=0.000365) and in *Glandularia* (t=3.828, *P*= 0.000770). On the other hand, *P. canescens* (Z=2.571,*P*= 0.0101) and *P. fruticosa* (Z=2.427, *P*= 0.0152 ) showed more feeding marks (Fig. 5).

**Relevant accomplishment**

The significant improvement in the rearing methods of *K. bergi*.

**Future Plans**

1. Conclude host specificity studies of *K. bergi*.
2. Conduct bionomic and host specificity studies of *Longitarsus* spp.

**Travel**

-Jul 2008: Santiago del Estero, Tucumán, Salta and Jujuy to collect *Longitarsus* sp. and pathogens (Sosa, Telesnicki, Traversa).
-Oct 2008: Buenos Aires Province, to collect *K. bergi* (Sosa and Telesnicki).
-Jan 2009: Buenos Aires, La Pampa, Mendoza, and San Luis Provinces to collect *Longitarsus* sp., *K. bergi*, pathogens and plant material for DNA extraction (Sosa, Julien, Traversa).
-Feb 2009: Santiago del Estero, Tucumán, Salta, Jujuy, Formosa, Chaco, Corrientes, Misiones, and Entre Ríos, to collect *Longitarsus* and plant material (Sosa, Telesnicki).
-Apr 2009: Santiago del Estero, Tucumán, Salta, Jujuy, Formosa, Chaco, and Entre Ríos, to collect *Longitarsus*, pathogens and plant material (Sosa, Rodriguez, Traversa).

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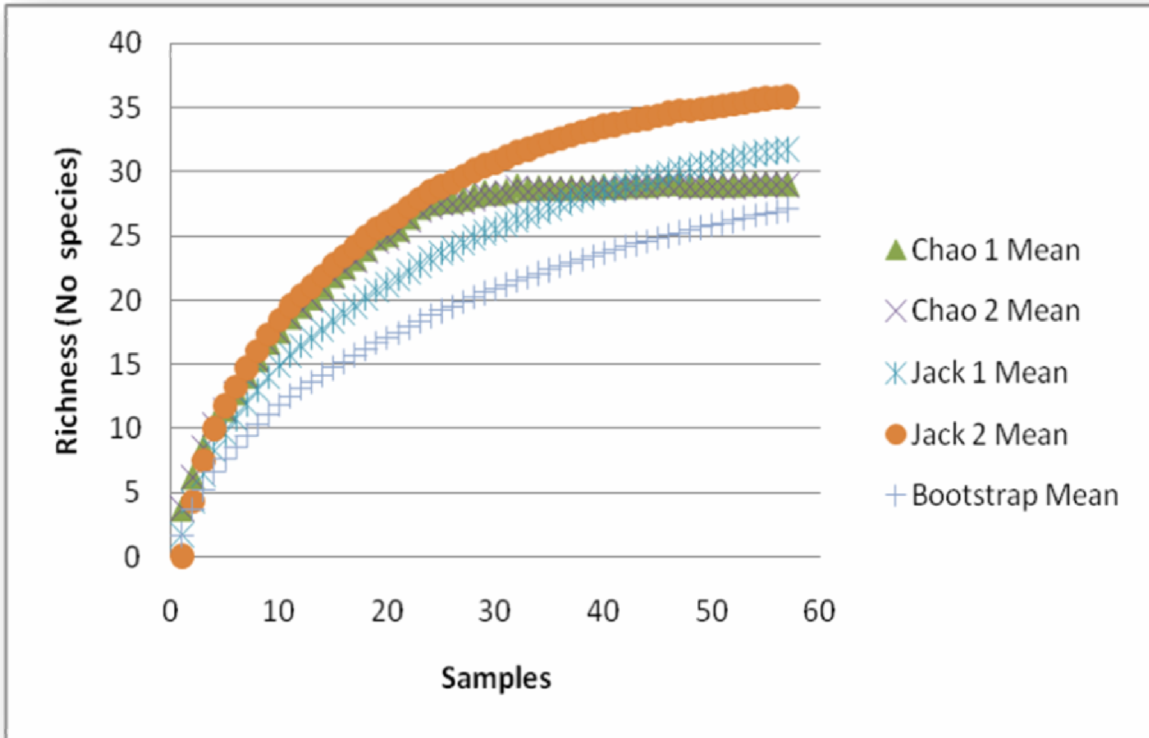


Fig.1. Species accumulation curves from exploration in Argentina, using different richness estimators.

Table 1. Richness from 5 estimators of explored regions in Argentina

| | Chao 1 | Chao 2 | Jack 1 | Jack 2 | Bootstrap |
|---|---|---|---|---|---|
| No samples | 57 | 57 | 57 | 57 | 57 |
| No. Expected Species | 29 | 28.89 | 31.84 | 35.79 | 27.08 |
| No. Observed Species | 23 | 23 | 23 | 23 | 23 |
| % Estimated Richness | 79.3 | 79.6 | 72.4 | 64.3 | 90 |

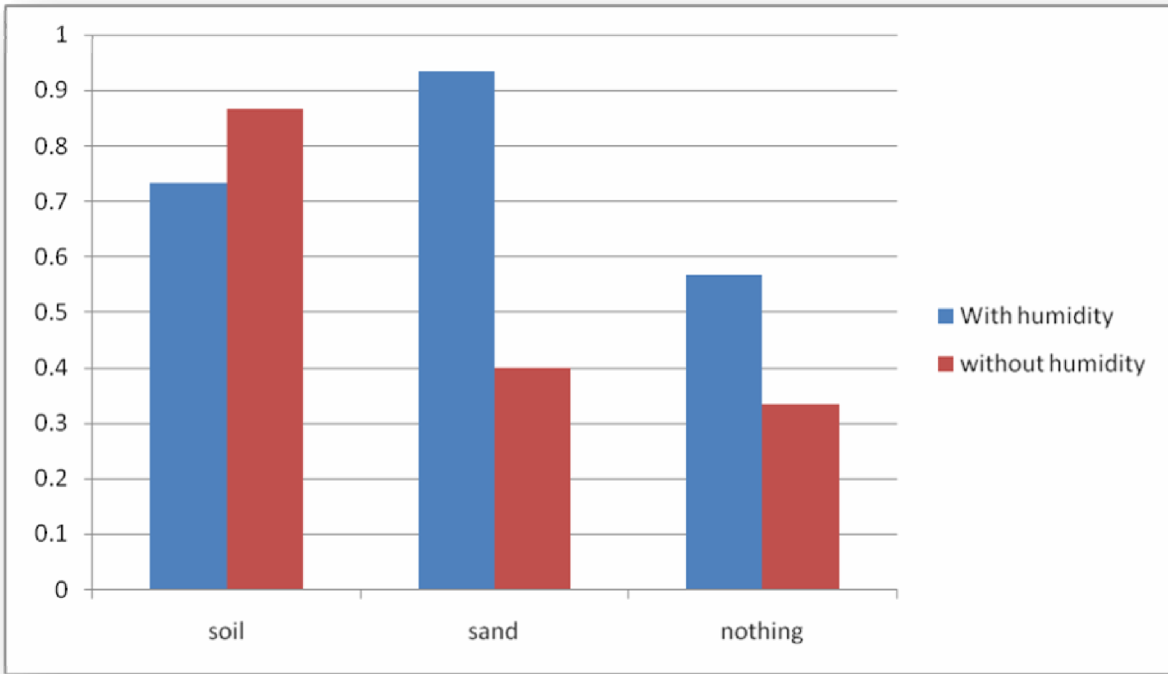


Fig. 2. The proportion of adults of *K. bergi* that emerged from three different substrates (soil, sand and no substrate) and two moisture conditions.

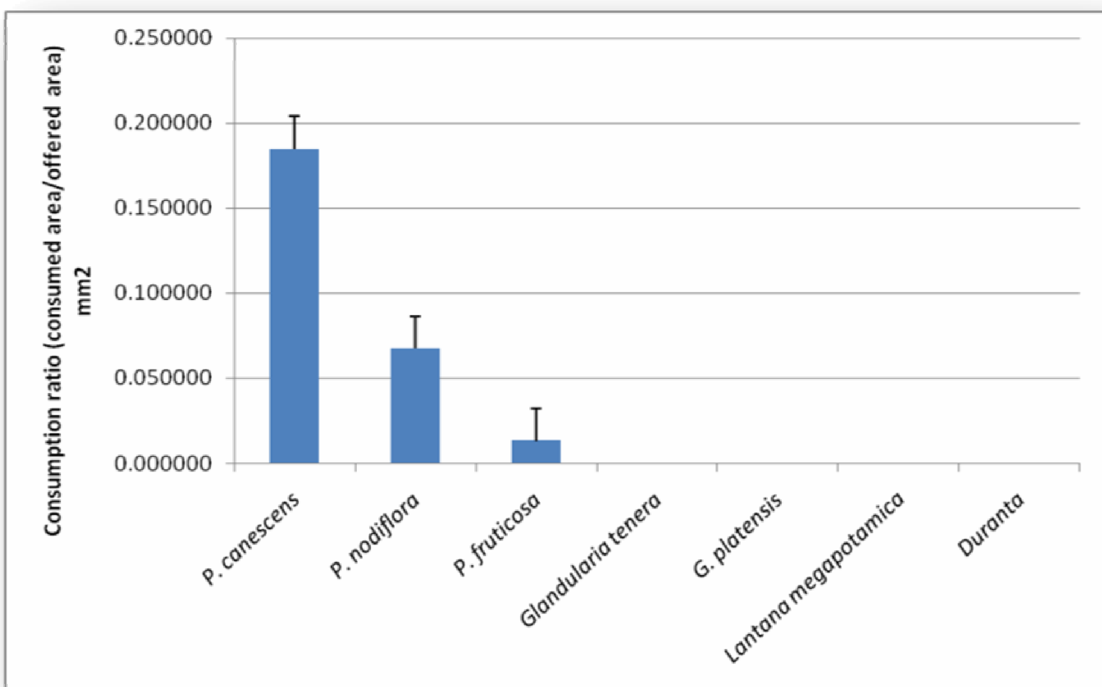


Fig. 3 .Adult feeding no choice. Consumption of *K. bergi* adults on Verbenaceae species. The segments in bars represent 1 SE.

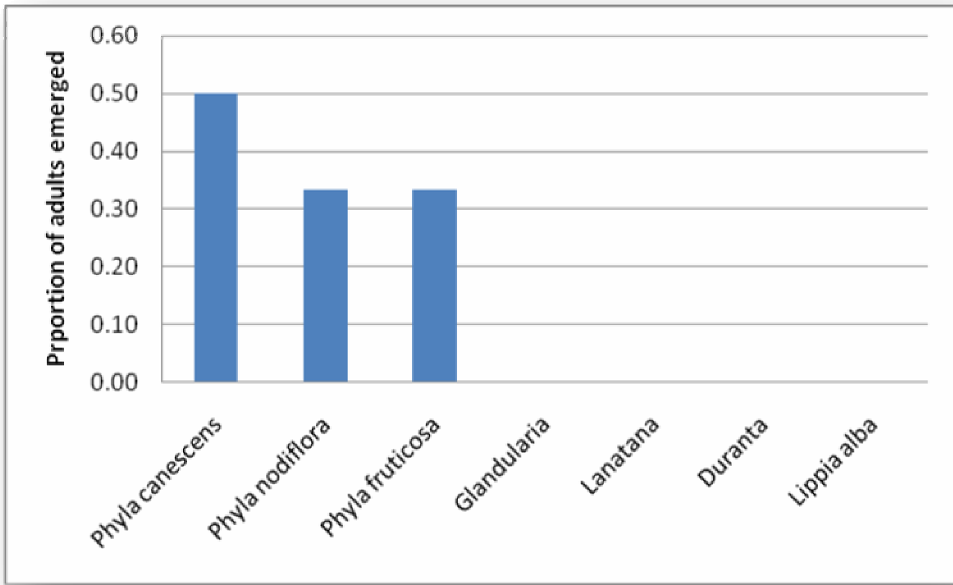


Fig. 4. Adults of *Longitarsus* sp.1 emerged from the non chocie test.

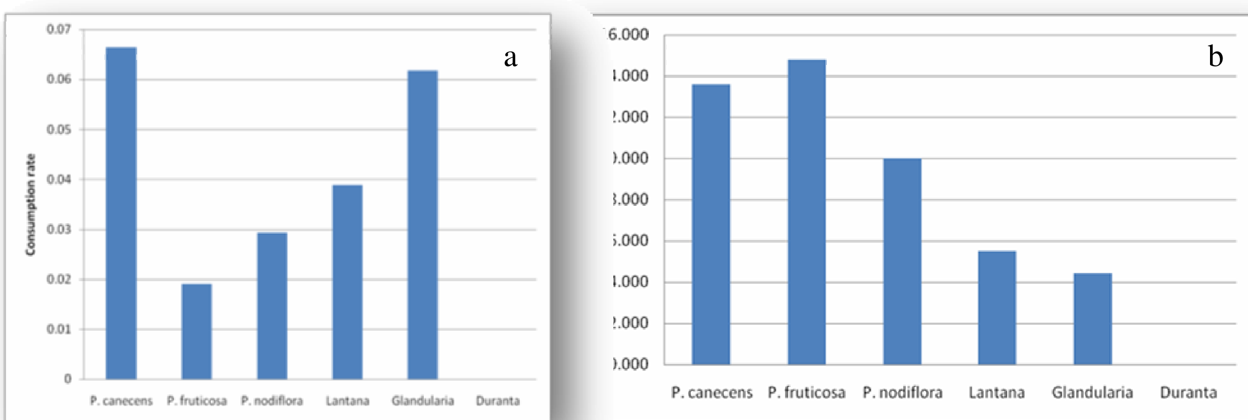


Fig. 5. Feeding preference of *Longitarsus* sp.1. In a non choice experiment. a-Bars represent the consumption rate (leaf area consumed/leaf area offered).b- Estimation through the number of feeding marks produced by an insect in a week.

# WATER LETTUCE

by

Willie Cabrera Walsh and Magalí Dalto

Cooperators: Phil Tipping and Ted Center, ARS-IPRL, Ft. Lauderdale, FL.

## Introduction

Water lettuce, *Pistia stratiotes*, is a serious weed in several countries, and can restrict water flow in irrigation and flood control canals, as well as interfere with recreational activities and hydroelectric operations. However in Argentina it is only considered a nuisance plant on occasions, mostly in recreational or park ponds. Waterlettuce inhabits oxbows, ponds, canals, and slow-flowing streams, throughout the Paraná-Uruguay basin. The rosettes are perennial in the north, but behave mostly as annuals in more temperate zones, exhibiting seasonal growth in the provinces of Buenos Aires, and southern Entre Ríos and Santa Fe, with high rosette densities during Summer and Autumn, and low densities during Winter and Spring. Several natural enemies have been studied in the past (Cordo et al. 1978).

The SABCL has been assigned to study the potential for biocontrol of this weed in cooperation with the IPRL, Ft. Lauderdale, FL. This is not the first time this weed has been studied at our laboratory, since a great deal of work was done in the 70s and late 90s. One weevil species, *Neohydronomus affinis*, was released in several parts of the world, including the USA. Another agent, the polyphagous moth *Samea multiplicalis*, is also present in the USA, although it feeds on several water ferns as well as water lettuce. Both of these species may be referred to for their role on *Pistia*, but no further work will be done on them for now.

## Material and Methods

Surveys on *P. stratiotes* consist in collecting samples of roughly the same volume (250-litre bags, ca. 5.2kg, drained weight) along the Paraná basin. The objective is to assess the number of species that feed on this plant, and their geographical and seasonal distribution. We have started water-lettuce cultures in canvass pools and tanks, in walk in cages and greenhouses. Weevils collected are placed on clean plants in 3 to 5-litre jars with fertilized water, to obtain eggs. These will be surface sterilized to obtain clean colonies. Specimens of *Ochetina bruchi* and *N. affinis* are recorded but discarded, in the first case for being a polyphagous species.

Insect infestations in the field are being evaluated on a volumetric basis, i.e. every sample is roughly of the same fresh weight. This method was considered better than a per-plant calculation due to marked seasonal and latitudinal size variations. Most insects have been collected with collapsible Berlese funnels that have a plastic bag with a small waterlettuce plant fixed to the mouth, or by sinking the plants placed inside open net bags in tanks. One or two plants are left floating in the tanks for the emerging weevils to climb on to, thus concentrating the catch from dozens of plants on only one (Cordo et al. 1978).

**Results**

*Pistia* is rarely seen in large lakes or fast flowing rivers. In general terms, it is most abundant in the north and western parts of the Paraná basin of Argentina (Fig. 1), areas with marked rain seasons, with temporary or highly fluctuating water bodies. *Pistia* does not do well where waterhyacinth is abundant, possibly due to competitive displacement through shading. Conversely, waterhyacinth is rare in the temporary watercourses where waterlettuce prospers. Not surprisingly, the most abundant collections of *Pistia* insects (during the current period and the past efforts) come from these environments in the provinces of Formosa, Corrientes and Chaco, and similar environments in Buenos Aires. Recent drought, however, have reduced drastically the available water lettuce environments.

So far, the Berlese funnels seem more efficient, yielding more insects from the samples, but the collection is spread throughout 2-3 weeks, whereas the sinking method works in 2-3 days. A total of 16 sites have been sampled so far, one of them (PIrt9, 40 km from the SABCL) is sampled regularly every month since last spring. Results are presented in table 1, where we provide collection sites and dates, and the number and identification of the different weevil species. The most common herbivore on waterlettuce has been *Samea multiplicalis* (Lep.: Crambidae), but being a polyphagous species, and present in the U.S. already, we are not rearing or computing them. Other insects, like the larvae of the detritivorous flies *Palpada* sp. (Dipt.: Syrphidae) and *Hedriodiscus* sp. (Dipt.: Statiomyidae) tend to invade leaves damaged by *Samea*, apparently increasing the damage mechanically, but cannot be considered suitable *Pistia* biocontrol agents.

Of the specific organisms, *N. affinis* is by far the most common species (Table 1). Of the species currently known for *Pistia*, we are interested in the weevils *Pistiacola cretatus*, *Argentinorhynchus breyeri, A. bruchi*, and *A. squamosus* (Fig. 2). What little is known of them indicates they are specific insects (Cordo et al. 1978, 1981, Cordo & Sosa 2000). Furthermore, the *Argentinorhynchus* species apparently share the plant up to a certain point, finishing their development in different organs. Specifically, it appears that *A. squamosus* larvae feed in the stem of the plant, while the other species prefer the crown and stolons. The larvae can be told apart quite easily by their morphology (Fig. 3). So far we have not been able to establish a laboratory colony, partly because collections of these species are quite recent, and late in the season, and partly because adults of these species seem to be rather rare (table 1). However, we collected several larvae (table 1), which we have placed on plants for them to finish development. The larvae that drop off the plants are placed in plastic container lined with a moist sterile peat moss/river bed mixture to try and obtain pupae. Previous publications state that the larvae do not pupate in water (Cordo et al. 1978, Cordo & Sosa 2000).

Our collection data suggest that *P. cretatus* is distributed throughout the Argentine range of the plant, while the *Argentinorhynchus* spp. may be limited to the tropical areas only (Table 1). This may be due to the fact that these species appear to need well developed plants to mature, which are rare in its temperate/subtropical range due to the disappearance of the plant in winter. Three yet unidentified weevil species were collected in samples from the north. However, being very rare, we suspect they may be casual visitors from other plants. They have been sent for identification already.

**Field Trips**

-**Nov 25-Dec 7, 2008:** To Formosa Prov.

-**Dec 3-6, 2008:** To Formosa and Corrientes Prov.
-**Mar 5-10, 2009:** To Chaco and Corrientes Prov.
-**June 8- 14, 2009:** To Formosa, Cordoba, Chaco and Santa Fe Prov.
- Several trips in Buenos Aires Prov., to Otamendi, by W. Cabrera Walsh.

**Future plans**

- Determine geographical and seasonal distribution of the waterlettuce weevils, and study their biology in the field and the laboratory
- To obtain colonies of *Pistiacola cretatus* and *Argentinorhynchus* spp. for host testing and shipment to quarantine in the US

**Relevant accomplishment**

This is a new project that is in its beginnings and there is not much to report yet. However, we have been able to collect every species we plan to work with, and keep living colonies at the laboratory.

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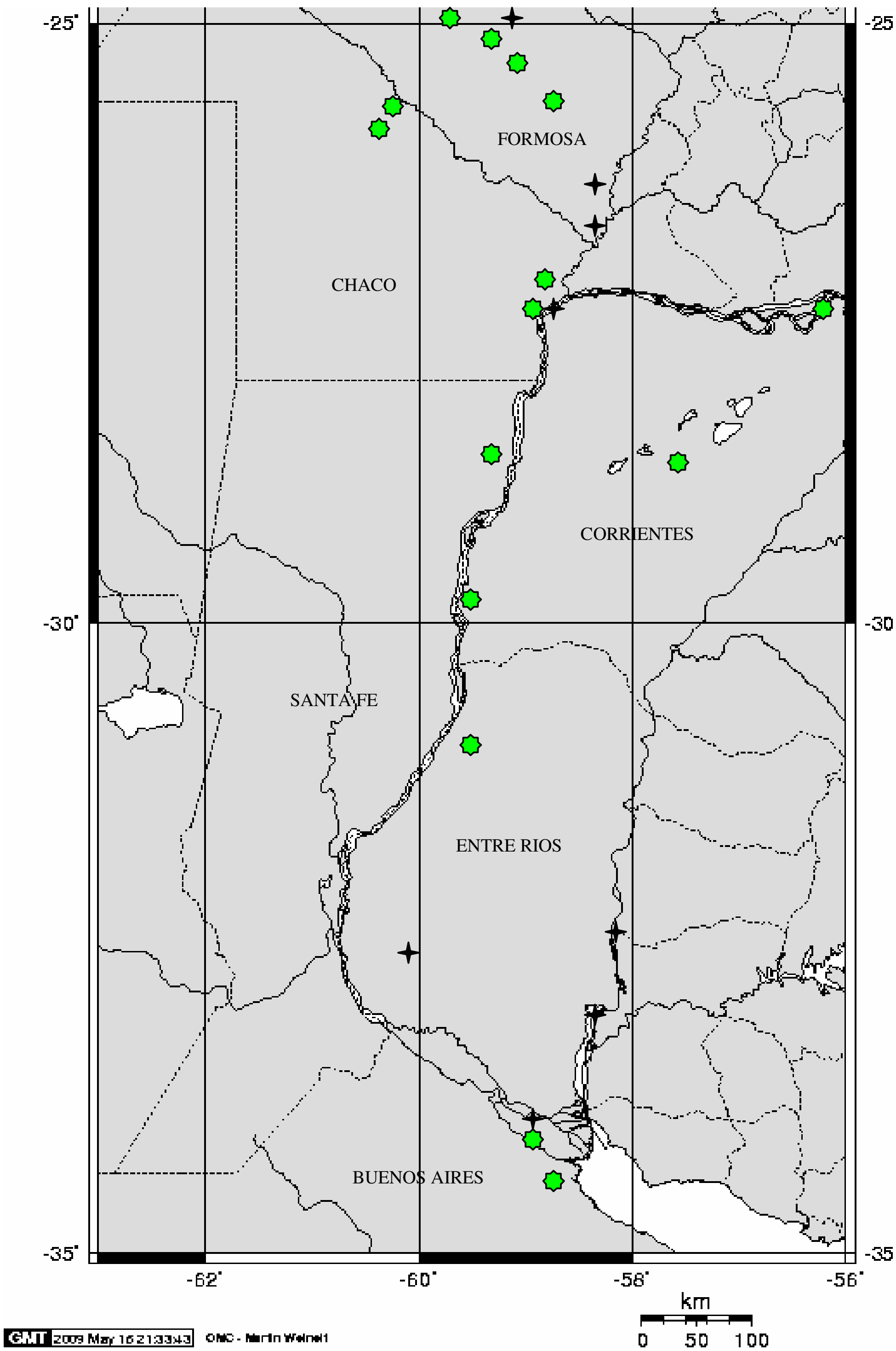
**Table 1.** Relative abundance of water lettuce weevils (Number of individuals found)

| SITE | Site code | Date | *A. breyeri* larvae | *A. breyeri* | *A. bruchi* larvae | *A. bruchi* | *A. squamosus* larvae | *A. squamosus* | *Neohydronomus* | *Pistiacola* | *Ochetina* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Otamendi | PI004 | 08-feb | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 0 | 1 |
| Entre Ríos, St Elena | PI012 | 05-mar | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 4 |
| Chaco, Cerrito | PI001 | 08-mar | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Chaco, Resistencia | LU020 | 09-mar | 0 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 2 |
| Santa Fe, Ceibalito | PI002 | 09-mar | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Otamendi | LUHTWI | 28-mar | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 3 |
| Corrientes, Rincon | PI005 | 03-abr | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 2 |
| Corrientes, Ibera | PI006 | 20-abr | 0 | 0 | 0 | 0 | 0 | 0 | 117 | 22 | 46 |
| Otamendi | LUHTWI | 27-abr | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 |
| Rt9, Escobar | PIrt9 | 27-abr | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 3 | 5 |
| Rt9, Escobar | PIrt9 | 05-jun | 0 | 0 | 0 | 0 | 0 | 0 | 216 | 5 | 4 |
| Chaco, Fn. Lavalle | PI007 | 10-jun | 18 | 0 | 10 | 0 | 0 | 0 | 4 | 1 | 0 |
| Chaco, Fn. Lavalle 2 | PI008 | 10-jun | 4 | 0 | 0 | 1 | 3 | 0 | 21 | 3 | 5 |
| Fsa, Bdo. Estrella | PI009 | 10-jun | 2 | 0 | 2 | 0 | 0 | 0 | 26 | 2 | 14 |
| Fsa, Fontana | PI010 | 11-jun | 9 | 0 | 0 | 0 | 4 | 0 | 18 | 4 | 13 |
| Fsa, Palo Santo | PI003 | 11-jun | 76 | 0 | 5 | 0 | 4 | 0 | 2 | 1 | 7 |
| Chaco, Resistencia | PI011 | 11-jun | 12 | 4 | 4 | 0 | 4 | 0 | 13 | 5 | 11 |
| TOTALS | | | 121 | 6 | 7 | 1 | 2 | 0 | 557 | 46 | 118 |

Figure 1. *Pistia* sites surveyed since March 2008 in Argentina.




Large, “pure” waterlettuce patches

Pure waterhyacinth populations

Figure 2. *Argentinorhynchus* adults showing characteristic scale patterns

Figure 3. *Argentinorhynchus* spp larvae showing characteristic features.

Fourth instars of *Argentinorhynchus*. From left to right: *A. squamosus, A. breyeri*, and *A. bruchi*. Note the flat terminal segments of *A. bruchi*, and *A. breyeri*.

Terminal segment of *Argentinorhynchus* larvae: left, *A. bruchi*; right *A. breyeri*

# PARKINSONIA

by

Fernando Mc Kay, Alejandro Sosa and Marcelo Parisi

Cooperator: Tim Heard, CSIRO Entomology, Indooroopilly, Australia

## Abstract

During the reporting period, efforts were focused on searching and studying leaf-feeding Geometridae moths and other natural enemies of *Parkinsonia aculeata* in Argentina. Nine exploratory trips were made from August 2008 to June 2009 in central-northern Argentina. ***Eueupithecia cisplatensis***: this leaf-feeding moth was found in 7 provinces of Central-Northern Argentina. *Morphology*. Differences between male and female adults were found on body sizes and antennae. *Biology:* developmental period from egg to adult emergence was ca. 23 days. *Natural enemies*. Two species of *Conura* (Hymenoptera: Chalcidoidea) were obtained from cocoons. *Laboratory host specificity*: preliminary larval survival no-choice tests show a strong preference for *P. aculeata*. No larval development was registered on any of the tested species. ***Additional natural enemies***. A stem-galling midge, *Neolasioptera* sp. (Diptera: Cecidomyiidae), the leaf-feeding moth *Melipotis acontioides* (Lepidoptera: Noctuidae) and three different Geometridae were also found on *Parkinsonia*.

## Introduction

*Parkinsonia aculeata* (Caesalpinioideae), native to the Americas, has a pan-tropical distribution following introduction as an ornamental, hedging, fodder and shade tree (Woods 1992, Hawkins and Harris 1998, Wagner *et al*. 1999, PIER 2000, Hawkins 2001). In Australia it appears to have been planted mainly as an ornamental and shade tree. The detrimental effects of *P. aculeata* in Australia includes its propensity to form dense, thorny, impenetrable thickets along drainage lines, depressions, ephemeral wetlands and, to a lesser extent, uplands. Chemical and mechanical control work of *P. aculeata* was commenced by 1940. Investigations into the potential biological control of *P. aculeata* began in 1983 (Woods 1986). Two insects from the Sonoran Desert Region (southern USA and Mexico), *Rhinacloa callicrates* Herring (a sap-sucking mirid) and *Mimosestes ulkei* (Horn) (a seed-feeding bruchid) were released in Queensland in 1993 (Julien and Griffith 1998) and the Northern Territory in 1989 (Donnelly 2000) and 1994 (Flanagan *et al*. 1996) respectively. A third insect from Argentina, the seed-feeding bruchid, *Penthobruchus germaini* Pic., was released in Australia in 1995 (Briano *et al*. 2002, van Klinken 2005, van Klinken *et al*. 2008). Existing agents therefore do not appear to be having a significant impact, and new potential agents are unlikely to be found in the United States or northern Mexico. Recent genetic studies indicating very old dispersal events of *P. aculeata* in South America (Hawkins *et al*. 2007), stimulated interest in survey work in this area. Previous native-range surveys conducted in Argentina and Paraguay by ARS researchers revealed the existence of two prospective geometrid moths, *Eueupithecia cisplatensis* (Prout) and *Euacidalia* sp. In September 2008, a research cooperation agreement was signed with the CSIRO Entomology, Australia, to study the prevalence and host specificity of leaf-feeding Geometridae moths. One of the candidates prioritized for further studies is *Eueupithecia cisplatensis* Prout. In the present report we include updated results of larval survival no-choice tests and preliminary results of the field host specificity. We also present additional natural enemies of *Parkinsonia*. For general information visit www.usda-sabcl.org

## Materials and methods

Nine exploratory trips were made from August 2008 to June 2009 in central-northern Argentina. A survey of *P. aculeata* populations was conducted along the main roads of Buenos Aires, Corrientes, Entre Ríos, Formosa, Salta and Santa Fe provinces. Plants were sampled mostly by visual inspection, hand collection and beating. Immature insects were held in plastic containers and provided fresh leaves until the emergence of adults. Voucher specimens of plants and insects collected are maintained at the SABCL.

Laboratory host specificity of Geometridae

*No-choice larval survival tests.* No-choice larval survival was evaluated on 10 spp. of Fabaceae, Subfam. Caesalpinoideae (5 genera) and Mimosoideae (2 genera) (Table 1). Plants were selected on the basis of taxonomic relatedness to *P. aculeata* and availability. Experiments were carried out in controlled environmental chambers (25±2°C: 60-80% RH; 16:8 L:D). In each replicate, 10 newly emerged larvae were placed in 0.7-liter plastic containers with perforated lids and moist tissue paper. The larvae were fed with bouquets of freshly excised leaves with their petioles inserted in small recipients filled with water. The bouquets were replaced every 48-72 hours as needed. Feeding damage and larval mortality were recorded daily until adult emergence. The insects for the tests were the F1 of larvae collected in La Plata, 60km south Buenos Aires in November 2008 and February 2009.

Field host specificity

On field trips conducted through Salta, Formosa and Corrientes provinces, *P. aculeata*, *P. praecox* and *A. caven* coexisting plants were searched for Geometridae larvae by beating.

## Results

*Systematic status***.** Based on specimens collected in different localities (Fig. 1), Geometridae specialist Dr. Axel Hausmann (München, Germany), identified the leaf-feeding moth as *Eueupithecia cisplatensis* (Lepidoptera: Geometridae). *Adult morphology*. In general, females are bigger than males, with a wider abdomen (Fig. 2). Sexual dimorphism is also present on the morphology of the antennae: pectinate in the male and simple in the female (Figs.3-4). *Biology.* Brown cylindrical eggs are usually laid individually on the leaflets (Fig. 5). Body colour of larvae changes progressively from light brown-greenish in the early instars to green-purple in the later instars (Fig. 6) mimicking *P. aculeata* leaf rachises and young shoots. As larvae develop, they eat most of the leaflets and parts of the rachises. Larvae that were fed bouquets of leaves of *P. aculeata* inside plastic jars containing wet tissue paper in the bottom spin a silk cocoon either on the jar lid or in the tissue paper. The shortest developmental period from egg to adult emergence was ca. 23 days. The duration of the stages was approximately: 6 days for eggs, 11 days for larvae, and 7 days for pupae. *Natural enemies*. Two species of *Conura* (Hymenoptera: Chalcidoidea) were obtained from cocoons. Further identification is in progress (Daniel Aquino, Facultad de Ciencias Naturales y Museo, La Plata, Argentina). *Laboratory host specificity.* No-choice larval survival tests. *Eueupithecia cisplatensis* showed a strong preference for *P. aculeata*

(Table 1).The larvae exposed to other species died after 2-6 days. On the other hand, only 50% of the larvae reared on *P. aculeata* reached the pupal stage (Table 1). However, considering that the survival of *E. cisplatensis* on *P. aculeata* is rather low, larval development will be evaluated under different conditions (*e.g.* insect rearing sleeve wrapped around branches of potted plants or potted plants in outdoor cages). *Field host specificity.* Larvae were found feeding on *P. aculeata* and *P. praecox.*

*Additional natural enemies*
-*Neolasioptera* sp. (probably a new species) (Diptera: Cecidomyiidae) (Fig. 7). This stem galling-midge was found only on *Parkinsonia* in Salta and Formosa provinces. Further identification and description will be conducted by Dr. Raymond Gagné (ARS Systematic Entomology Laboratory). Its rearing is being attempted. Five adults were confined into a rearing sleeve wrapped around a branch of *Parkinsonia* potted plant. Adults were alive for a week. The plant will be monitored for galls formation.
-*Melipotis acontioides* (Lepidoptera: Noctuidae). Larvae of this leaf-feeding moth were found in several sites in northern Argentina (Fig. 1). It is reported defoliating *P. aculeata* in the Sonora desert region of the United States and Mexico (Woods 1992) and the ornamental *Delonix regia* in Florida and the West Indies (Watson 1945). Host-specificity tests have shown that *P. aculeata* is the preferred host (Woods, unpubl.). So far in Argentina, *M. acontioides* has been found feeding on *P. aculeata*, *P. praecox* and *Acacia caven*.
-Other 3 species of Geometridae, Ennominae, moths were found on *P. aculeata* (Figs. 8-10).

## Significant accomplishments

- Finding, rearing and testing of *Eueupithecia cisplatensis*
- Finding other prospective natural enemies

### Field trips to survey for Parkinsonia natural enemies

August 18-24, 2008. Salta and Formosa provinces (Mc Kay).
November 24, 2008. Road N° 11, 21 km S La Plata, Buenos Aires province (McKay and Sosa).
December 1-7, 2008. Entre Ríos and Corrientes (Mc Kay).
January 2009. Salta and Formosa provinces (Sosa).
February 2009: Chaco, Corrientes and Formosa provinces (Sosa).
March 18-April 5, 2009: Salta Province (Mc Kay).
March 8-9, 2009: Chaco, Corrientes and Santa Fé provinces (Cabrera Walsh).
March 28-Arpil 3, 2009: Salta, Santiago del Estero and Tucumán (Sosa).
June 8-12, 2009: Corrientes, Chaco and Formosa (Cabrera Walsh).

## Future plans

-*Eueupithecia cisplatensis:* Continue studying the phenology, distribution and biology; conduct additional larval development no-choice tests; evaluate larval survival; conduct field surveys on native Fabaceae in *P. aculeata* natural distribution area.
-Conduct additional exploration for new natural enemies.

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**Table 1.** *Eueupithecia cisplatensis*: Preliminary larval survival no-choice test.

| Tested plants | N° of replicates | % pupation | % adult emergence |
|---|---|---|---|
| **Fabaceae** | | | |
| Caesalpinoideae | | | |
| *Bahuinia candicans* | 6 | 0 | 0 |
| *Caesalpinia gillesii* | 6 | 0 | 0 |
| *Gleditsia amorphoides* | 7 | 0 | 0 |
| ***Parkinsonia aculeata*** | **5** | **50** | **44** |
| *Peltophorum dubium* | 6 | 0 | 0 |
| *Senna corimbosa* | 6 | 0 | 0 |
| *Senna spectabilis* | 6 | 0 | 0 |
| *Mimosoideae* | | | |
| *Acacia caven* | 8 | 0 | 0 |
| *Acacia visco* | 5 | 0 | 0 |
| *Prosopis alba* | 5 | 0 | 0 |

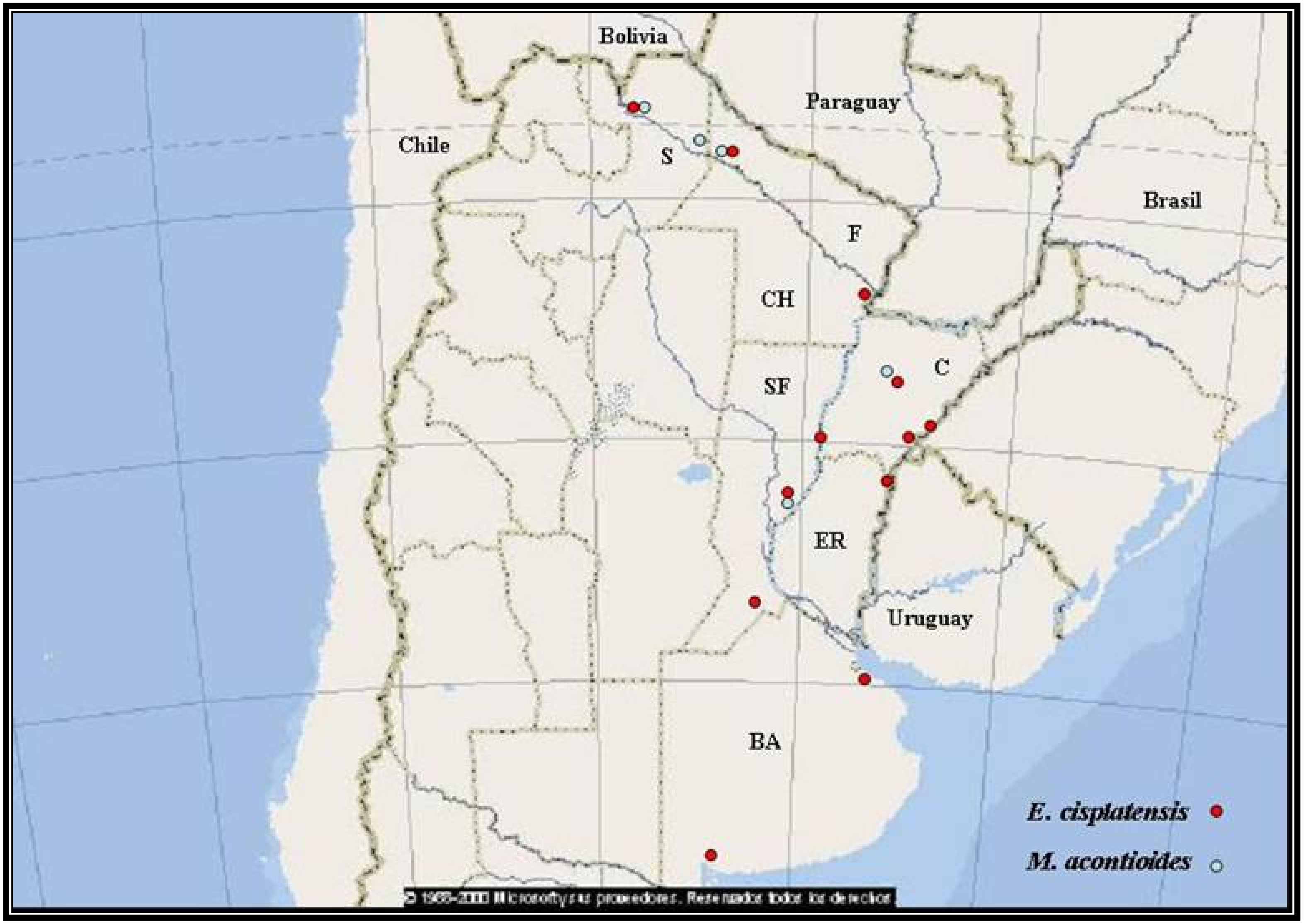


**Figure 1.** Collecting sites of *Eueupithecia cisplatnesis* and *Melipotis acontioides* in Argentina. BA, Buenos Aires; CH, Chaco; C, Corrientes; ER, Entre Ríos; F, Formosa; S, Salta; SF, Santa Fe.


**2**

**3**

**Figures 2-4.** ***Eueupithecia cisplatensis*.** 2- Female and male adults. 3- Female antenna. 4- Male antenna.

**4**

**5**

**6**

**7**

**Figures 5 - 7. 5 & 6-*Eueupithecia cisplatensis*. 5-** Eggs. **6-** Mature larvae. **7-** Stem galls on *P. aculeata* caused by *Neolasioptera* sp. (Diptera: Cecidomyiidae) and detail of larvae cells within the gall.

8

9a

9b

10

**Figures 8 - 10-** Geometridae moths found on *Parkinsonia aculeata*. **8-** *Physocleora* sp. **9-** *Herbita cf praeditaria or Prochoerodes* sp. **10-** *Macaria* sp.

# IMPORTED FIRE ANTS

by

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Cooperators: R. Vander Meer, S. Porter, D. Oi, S. Valles, and D. Shoemaker,
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The Imported Fire Ant Project started at SABCL in 1988 to evaluate natural enemies of native fire ants as candidates for the biocontrol of *Solenopsis invicta* Buren and *S. richteri* Forel in the US. For information on the project, see previous Annual Reports and www.usda-sabcl.org.

## Microsporidia

In November 2008, 30 *S. invicta* colonies were collected in the area of San Javier, Santa Fe Province, for *Vairimorpha invictae*. The colonies were excavated and brought to the lab in dusted buckets. Workers from each colony were examined under a phase-contrast microscope for the presence of spores of the microsporidium. Eleven infected colonies were shipped to CMAVE.

## Viruses

**Surveys.** In November 2008, a survey was conducted in Santa Fe, Entre Ríos and Corrientes provinces; 97 *S. invicta* colonies were sampled in 13 sites by plunging 20-ml scintillation vials into the nests. Ants were kept alive and transported to the laboratory. RNA was extracted from 10-15 ants of each colony following the Trizol method (according to Invitrogen®´s instructions). Subsequently, cDNA was synthesized and two polymerase chain reactions (PCR) were conducted to detect the presence of the positive-strand RNA viruses *S. invicta* virus 1, 2 and 3 (SINV-1, SINV-2, and SINV-3). First, three separate reactions were carried out with three specific oligonucleotide primers (Valles and Hashimoto, 2009; Valles et al., 2004; Valles et al., 2007) for each of the 97 samples. Secondly, a multiplex PCR method was conducted using the three primers simultaneously in each sample. The multiplex reaction allowed the comparison with the virus screening conducted by Steven Valles (CMAVE) in ants collected around Gainesville, FL.

**Results.** From the 97 colonies, 30 (30.9%) were infected as follows: 22 with SINV-1 (22.7%), 11 with SINV-2 (11.3%) and 6 with SINV-3 (6.2%, Table 1); 24 colonies showed the presence of a single virus and six a combination of two/three (Table 2). When using the multiplex method, also 30 colonies were found infected but with a different virus combination (Table 3). Thus, SINV-1 was present in 16 colonies (16.4%), SINV-2 in 10 (10.3%) and SINV-3 in 4 (4.1%). The difference might be the consequence of some RNA degradation affecting the method sensitivity.

## Studies on local fire ants

We studied the effect of habitat and livestock grazing on the structure and composition of terrestrial ants in grasslands, savannas, forests, and floating islands of the Iberá Nature Reserve (INR) in northeastern Argentina. We also investigated the specific grazing response of *S. invicta* to confirm whether or not this ant is more abundant in disturbed (grazed) areas, as has been reported for its introduced range in North America. We used pitfall and bait traps, manual collections, mini-Winkler and Berlese extractors on the ground, vegetation, and litter strata.

**Results.** A total of 94 species in 30 genera were captured, all native from Argentina, being the highest number reported for a protected area of Argentina. The richest genus was *Pheidole* with 23 species and the most common species was *Solenopsis invicta* especially in habitats dominated by grasses (Table 4). The savanna was almost twice as rich in species as the grassland (Fig. 1); however, grazing did not affect species richness (Fig. 2). The grassland showed the highest number of shared species with other habitats. The Forest showed the lowest richness and diversity of ground-dwelling ants, but many species were exclusive, promoting differentiation in species and functional composition (Fig. 3). Half of floating islands species were exclusive. Generalized myrmicines were predominant in all habitats, dominating both grazed and non grazed habitats (Fig. 4). Ant species in grazed sites were less equitably distributed; consequently, they were dominated by a lower number of species with higher populations (Table 4). Because of this, mean biomass was two-fold higher in the grassland grazed sites (Fig. 5). *Solenopsis invicta* was the most frequently captured (61.4%) grouping both grazed and non-grazed sites and numerically dominant species but *Camponotus punctulatus punctulatus* showed the highest biomass. The occurrence and abundance of the red imported fire ant were favored by grazing only in grassland situations. Our findings indicate that 1) habitat specialization could be an important factor determining the organization of ant assemblages in Iberá and 2) extensive grazing in the INR has a low effect on overall structure and composition of the ant assemblages in subtropical habitats, but a high impact on specific functional groups or taxa promoting occurrences and substitutions of some groups and more abundant populations.

**Future plans**

- Phylogenetic (and population genetic) studies of fire ants and *Pseudacteon* flies.
- Phylogeographical and population genetic studies of *Solenopsis daguerrei.*
- Annual monitoring of disturbed (grazing and fire) and non-disturbed red fire ant populations.
- Survey for the presence and prevalence in Argentina of a DNA virus (*Densovirus*) in *S. invicta.*
- Collection and shipping of fire ant colonies and natural enemies to CMAVE.

**Field Trips**

1) Sep 15-20, 2008. To Iberá, to continue ecological studies (Calcaterra, S. Cabrera, Ramírez).
2) Oct 20-23, 2008. To Córdoba, to attend VII Congreso Argentino de Entomología (Calcaterra, S. Cabrera).
3) Nov 9-15, 2008. To Santa Fe, Corrientes and Entre Ríos to survey fire ant colonies for viruses and microsporidia (Varone and Ramírez).
4) Apr 14-19, 2009. To Iberá, to conclude ecological studies (Calcaterra, Ramírez).
5) Jun 30, 2009. To Zárate-Ramallo (BA) to collect *S. richteri* infected with *K. solenopsae* for DNA studies. Due to a severe drought, colonies were not found (Briano).

**Shipments**

December 9, 2008: 11 *S. invicta* colonies infected with *V. invictae* to David Oi (ARS-CMAVE).

**Relevant Accomplishments**

- Detection of three RNA viruses infecting *S. invicta* in Argentina.
- The highest number of ant species (94) was reported for a protected area of Argentina.

**Table 1.** *Solenopsis invicta* colonies infected with SINV-1, SINV-2 and/or SINV-3 in Argentina according to simple PCR.

| Province and site | Coordinates S | Coordinates W | Collection date | No. Collected colonies | Positive colonies with SINV-1 | SINV-2 | SINV-3 |
|---|---|---|---|---|---|---|---|
| **Corrientes** | | | | | | | |
| EBCo | 27º 33´ 10,4´´ | 58º 40´ 46,3´´ | 14-Nov-08 | 9 | 4 | 2 | |
| 3 de Abril | 28º 19´ 33,2´´ | 58º 48´ 07,9´´ | 14-Nov-08 | 4 | 3 | 1 | 2 |
| Santa Lucía | 28º 54 54,1´ | 59º 00´ 02,1´ | 14-Nov-08 | 6 | | 3 | |
| Esquina | 30º 00´ 06,7´´ | 59º 31´ 47,7´´ | 14-Nov-08 | 5 | 1 | 1 | |
| **Entre Ríos** | | | | | | | |
| Mojones | 31º 36´ 05,2´´ | 59º 11´ 50,6´´ | 15-Nov-08 | 9 | 1 | | |
| Mansilla | 32º 29´ 0,23´´ | 59º 17´ 42,4´´ | 15-Nov-08 | 6 | 1 | 1 | |
| **Santa Fe** | | | | | | | |
| Florencia | 28º 01´ 38,9´´ | 59º 13´ 30,3´´ | 13-Nov-08 | 1 | | | |
| Reconquista | 28º 54´ 46,2´´ | 59º 33´ 24,8´´ | 13-Nov-08 | 6 | 1 | 1 | |
| El Ceibo | 29º 50´ 9,9´´ | 59º 48´27,0´´ | 13-Nov-08 | 8 | 3 | | |
| San Javier | 30º 31´07,1´´ | 60º 01´01,9´´ | 12-Nov-08 | 18 | 5 | 1 | 4 |
| Calchines | 31º 31´ 22,8´´ | 60º 28´ 54,4´´ | 12-Nov-08 | 8 | 2 | 1 | |
| Landeta | 32º 01´37,3´´ | 61º 52´37,9´´ | 12-Nov-08 | 8 | | | |
| Centeno | 32º 16´ 33,3´´ | 61º 25´ 53,0´´ | 12-Nov-08 | 9 | 1 | | |
| | | | **Total** | **97** | **22** | **11** | **6** |

**Table 2.** Presence of SINV-1, SINV-2 and SINV-3 according to simple PCR.

| Virus combination | Colonies collected in Corrientes | Entre Ríos | Santa Fe | Total |
|---|---|---|---|---|
| 1 | 4 | 2 | 9 | 15 |
| 2 | 4 | 1 | 1 | 6 |
| 3 | 0 | 0 | 3 | 3 |
| 1+2 | 2 | 0 | 1 | 3 |
| 1+3 | 1 | 0 | 0 | 1 |
| 2+3 | 0 | 0 | 1 | 1 |
| 1+2+3 | 1 | 0 | 0 | 1 |
| No infection | 12 | 12 | 43 | 67 |
| **Total collected** | **24** | **15** | **58** | **97** |

**Table 3.** Presence of SINV-1, SINV-2 and SINV-3 according to multiplex PCR.

| Virus combination | Colonies collected in Corrientes | Entre Ríos | Santa Fe | Total |
|---|---|---|---|---|
| 1 | 6 | 2 | 4 | 12 |
| 2 | 4 | 1 | 2 | 7 |
| 3 | 0 | 0 | 1 | 1 |
| 1+2 | 1 | 0 | 1 | 2 |
| 1+3 | 1 | 0 | 1 | 2 |
| 2+3 | 0 | 0 | 1 | 1 |
| 1+2+3 | 0 | 0 | 0 | 0 |
| No infection | 12 | 12 | 48 | 72 |
| **Total collected** | **24** | **15** | **58** | **97** |

**Table 4.** Most abundant ant species captured in savanna and grassland habitats in Iberá.

| Species | No. (%) of samples | | | | |
|---|---|---|---|---|---|
| | Savanna | | Grassland | | Total |
| | Non-grazed | Grazed | Non-grazed | Grazed | |
| *Solenopsis invicta* | 16 (80) | 17 (94) | 3 (19) | 7 (44) | 43 (61) |
| *Pheidole laevinota* | 7 (35) | 9 (50) | 6 (38) | 2 (13) | 24 (34) |
| *Paratrechina fulva* | 7 (35) | 4 (22) | 5 (31) | 6 (38) | 22 (31) |
| *Pheidole aberrans* | 8 (40) | 9 (50) | 1 (6) | 1 (6) | 19 (27) |
| *Camponotus punctulatus punctulatus* | 1 (5) | 4 (22) | 6 (38) | 5 (31) | 16 (23) |
| *Pheidole obscurithorax* | 6 (30) | 8 (44) | 0 (0) | 0 (0) | 14 (20) |
| *Pheidole* sp. 1 | 4 (20) | 1 (6) | 6 (38) | 3 (19) | 14 (20) |
| *Camponotus punctulatus cruentus* | 6 (30) | 5 (28) | 0 (0) | 1 (6) | 12 (17) |
| *Crematogaster quadriformis* | 2 (10) | 2 (11) | 2 (13) | 4 (25) | 10 (14) |
| *Pheidole radoszkowskii* | 5 (25) | 4 (22) | 0 (0) | 1 (6) | 10 (14) |
| *Solenopsis* sp. 1 | 3 (15) | 6 (33) | 0 (0) | 0 (0) | 9 (13) |
| *Cyphomyrmex rimosus* | 5 (25) | 1 (6) | 2 (13) | 0 (0) | 8 (11) |
| *Brachymyrmex* sp. | 4 (20) | 1 (6) | 2 (13) | 0 (0) | 7 (10) |
| *Pheidole rudigenis* | 5 (25) | 2 (11) | 0 (0) | 0 (0) | 7 (10) |
| *Pheidole spininodis* | 4 (20) | 1 (6) | 1 (6) | 0 (0) | 6 (9) |
| *Gnamtogenys triangularis* | 4 (20) | 1 (6) | 0 (0) | 0 (0) | 5 (7) |
| *Atta vollenweideri* | 2 (10) | 1 (6) | 1 (6) | 0 (0) | 4 (6) |
| *Dorymyrmex staigeri platensis* | 0 (0) | 4 (22) | 0 (0) | 0 (0) | 4 (6) |
| *Pheidole rosula* | 1 (5) | 0 (0) | 0 (0) | 3 (19) | 4 (6) |
| *Pogonomyrmex cunicularius* | 0 (0) | 4 (22) | 0 (0) | 0 (0) | 4 (6) |
| *Solenopsis* sp. 2 | 0 (0) | 3 (17) | 0 (0) | 1 (6) | 4 (6) |
| *Solenopsis* sp. 3 | 3 (15) | 1 (6) | 0 (0) | 0 (0) | 4 (6) |

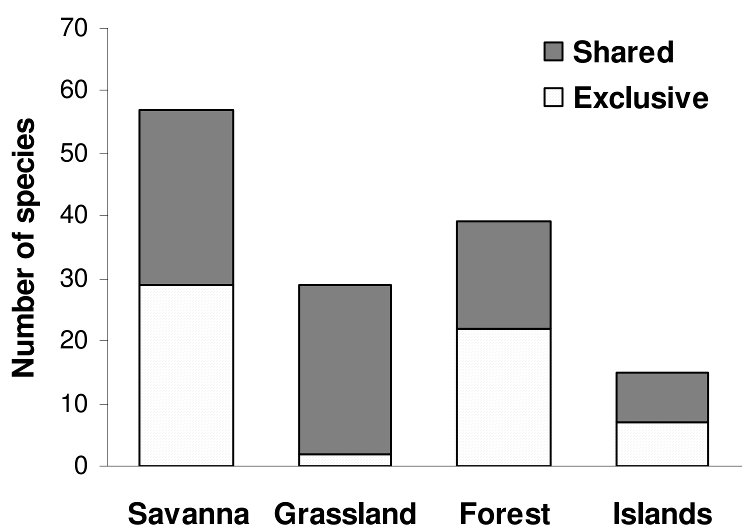


**Fig. 1.** Total number of ant species (shared + exclusive) in the four habitats surveyed in the Iberá Nature Reserve.


**Fig. 2.** Mean number (±SE) of species captured.

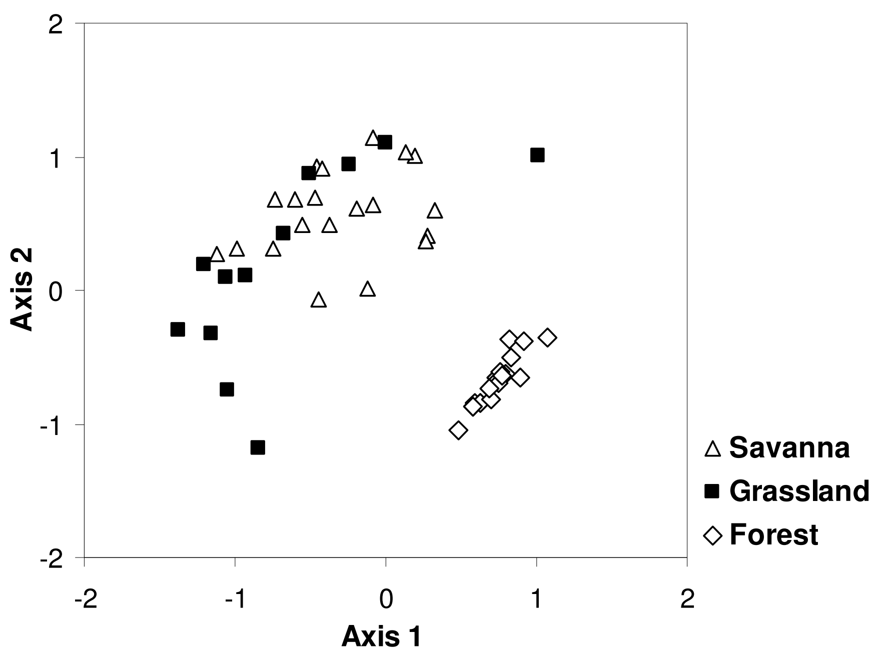


**Fig. 3.** Non-metric multidimensional scaling (MDS) ordination plot showing differences in ant species composition among sampling units (points) located in different habitats.

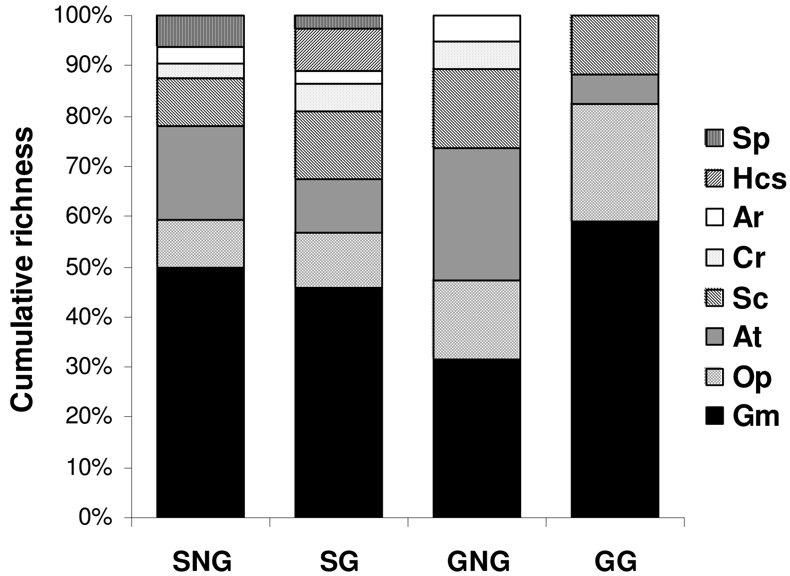


**Fig. 4.** Functional group profiles of the ants captured in savanna non-grazed (SNG), savanna grazed (SG), grassland non-grazed (GNG), and grassland grazed (GG). Data are proportions of total of species represented by each group: Op = Opportunists, Sp = Specialist predators, Sc = Subordinate camponotini, At = Attini, Cr = Cryptics, Gm = Generalized Myrmicinae, Ar = Arboreals, and Hcs = Hot-climate species.

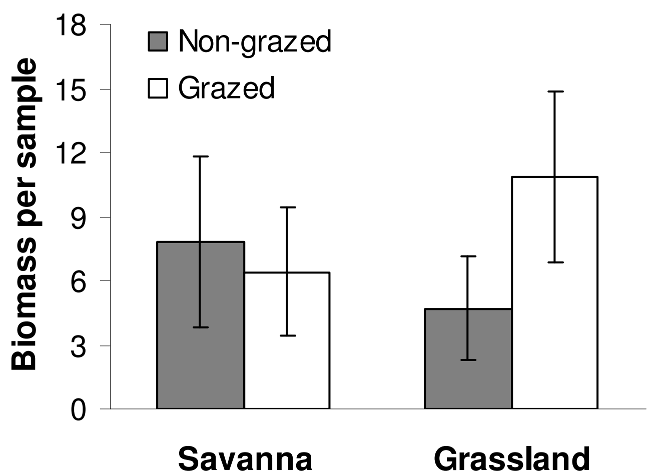


**Fig. 5.** Mean biomass (±SE) of the 24 more abundant species.

# GLASSY WINGED SHARPSHOOTER

by

Guillermo Logarzo

Cooperators: Jesse de León, BIRU, Weslaco, TX, Serguei Triapitsyn, University of California, Riverside.

Cooperator in Argentina: Eduardo Virla, Conicet-Proimi, San Miguel de Tucumán
Interns: Florencia Palottini and Mariel Guala

We continued with the reciprocal cross-breeding experiments with the remaining clades of *Gonatocerus tuberculifemur* and *G. metanotalis* to find out the status of the studied clades.

**Studies on the *G. metanotalis* complex.**
**Reciprocal cross studies.** We used the methodology for *G. tuberculifemur* (see Annual Report 2008). We conducted crosses between Clade 1 from Eldorado and Clade 2 from San Vicente, Misiones province. Unfortunately, reciprocal crossing with Clade 3 from El Manantial, Tucumán province was not possible due to lack of host eggs of *Tapajosa rubromarginata*.

**Results**. Clade 1 and Clade 2 were reproductively incompatible, producing 99% male offspring in the crosses ♂ Eldorado and ♀ San Vicente and 100% males in its reciprocal cross; whereas the controls (crosses between ♂ and ♀ from the same locality) produced male and female fertile offspring (Fig. 1).

**Studies on the *G. tuberculifemur* complex.**
Although more than 300 sentinel eggs were exposed, we failed to collect *G. tuberculifemur* clade X from Pucará, Neuquén province. It is suspected that was used for the original description of *G. tuberculifemur*.

## Field Trips

- Oct 25-Nov 2, 2008. To Córdoba to attend Argentina Meeting of Entomology and to Misiones to leave sentinel eggs (Logarzo, Palottini).
- Feb 15-20, 2009. To Mendoza and Neuquén to place sentinel eggs (Logarzo, Guala).

## Future Plans

The continuity of this project is uncertain.

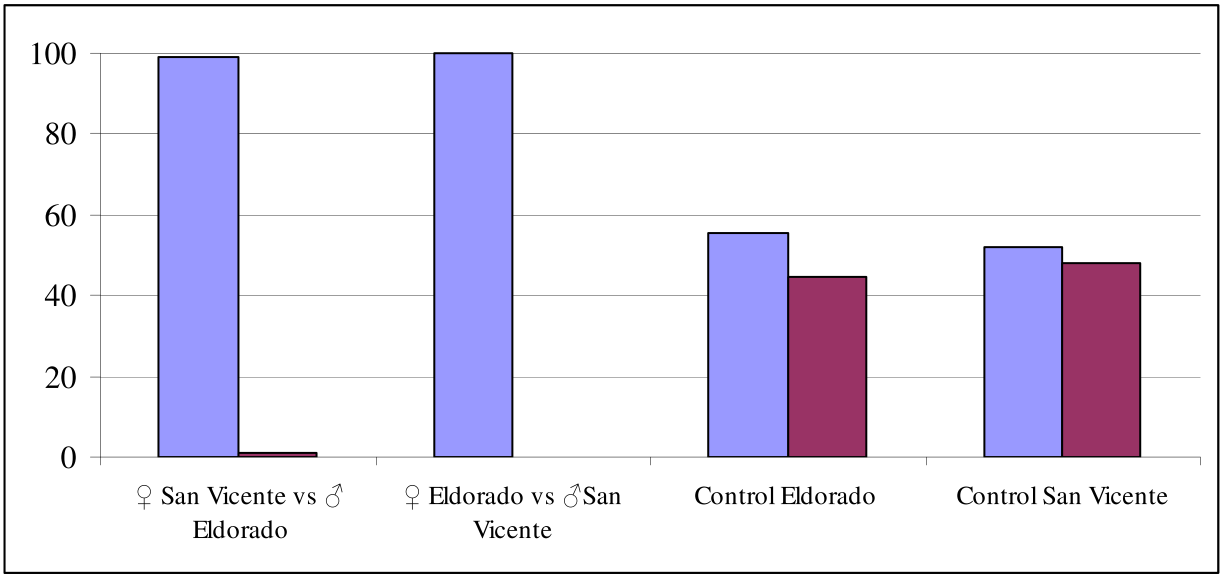


**Fig. 1.** Mean percentage of female and male offspring emerged from eggs parasitized by *G. metanotalis* females obtained from crosses of populations of Eldorado (Clade 1) and San Vicente (Clade 2), Misiones province.

# CACTUS MOTH


by

Guillermo Logarzo, Laura Varone and Juan Briano

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Cooperators in Argentina: Enrique Lobos, Facultad de Agronomía y Agroindustrias, UNSE. Darío Ruiz, Facultad de Ciencias Agropecuarias, UNC.

Assistants: Mariel Guala, Mariana Manteca Acosta, María Florencia Moore, Florencia Palottini, Alfredo Heredia, Matías López, José Diez and Diego Medina.



**Abstract**

Since August 2007, the SABCL has conducted life table studies and surveys of natural enemies to identify mortality factors and quantify their importance (See Annual Report 2008). Studies are being conducted on prickly pear fields and native *Opuntia* spp. The larval parasitoid *Apanteles alexanderi* (Braconidae) was found potentially important for its occurrence and impact. Two *Nosema* pathogens were found. Oviposition preference and larval performance of *C. cactorum* was evaluated on native and exotic potted *Opuntia* spp. in multiple choice and non-choice tests.


## Life tables

**Study sites. 1)** For Prickly pear cactus (*O. ficus-indica*): Quilino, Córdoba province (managed field) and Pampa Muyoj, Santiago del Estero province (unmanaged field); **2)** For *O. quimilo*: Quilino, 2-3 km from the above site; **3)** For *O. megapotamica*: Tanti, Córdoba and Nogoyá, Entre Ríos province. Life table studies on *O. ficus-indica* were conducted as stated in Annual Report 2008. The methodology used for native *Opuntia* spp. is similar, with some changes reported below.

**Data collection on native *Opuntia* spp. Eggs:** At each study site, 50-100 plants were used for each generation. All cladodes were checked weekly for eggsticks during oviposition period. The new eggsticks were numbered and labelled with plastic tags. The old ones were checked and egg mortality factors were recorded until all eggs hatched and all first instar larvae penetrated the cladodes. **Larva I-IV:** Attacked cladodes were sampled every 2 weeks from penetration of larvae until larvae left the cladode to pupate in the soil. In the laboratory, the collected larvae were reared until adult stage when mortality factors were recorded. **Larva V and pupa:** mortality factors were studied on 5-10 selected plants. On the base of each plant, the ground surface (2 m in diameter) was covered with fabric with the edges rolled to facilitate the pupation within the pleats. Since last-instar larvae started to fall off the cladodes, the fabric was checked weekly and mortality factors recorded. In addition, when larvae reached 4$^{th}$ instar, 10-15 selected cladodes were covered with fabric bags. The selected cladodes were those attacked by *C. cactorum* in which the initial number of eggs was known. For last instars and pupa, predation was calculated as the difference between field and laboratory survivorship. **Moth:** Adult survivorship was estimated using the ratio between the number of eggs laid in the study site of the following generation (obtained by sampling) and female fecundity (obtained by dissecting females, n=30).

**Preliminary Results**

**Prickly pear cultivation (*O. ficus-indica*).** In Pampa Muyoj (unmanaged field, plant size over 3 m height) and Quilino (managed field, plants about 1.2 m height) for the third generation, egg mortality was 21- 25% (Tables 1 & 2). Wind, rainfall, and predation (mostly by ants) were the main eggs mortality factors in the managed field while rain and wind were the main factors in the unmanaged farm. Larva mortality was higher in Pampa Muyoj (0.71) compared with the managed plantation in Quilino (0.57) (Tables 1 & 2). The impact of the larva mortality factors was different at each site. In the unmanaged cultivation, we observed high mortality during cladode penetration (0.21), in larvae II-IV, inside the cladode (0.30) and larvae V for predation when pupated in the soil (0.47). In the managed cultivation, the main larva mortality factor was predation of larva V when pupated in the soil (0.46). Mortality factors were impossible to discriminate inside cladodes. Pupae mortality also showed differences in both sites. In the managed cultivation predation (0.45) was the main mortality factor, while in the unmanaged cultivation, an unknown mortality factor, probably a disease, killed 32% of the pupae. Sex rates were 1:1, and fecundity was 173.9 and 112.4 eggs/female in Pampa Muyoj and Quilino, respectively.

***Opuntia megapotamica* and *O. quimilo.*** The main egg mortality factor was weather, 56% on *O. megapotamica* (Table 4) and 24% on *O. quimilo* (Table 3). During cladode penetration, 54% of larvae died in *O. quimilo* (by predation and plant mucilage) and 21% in *O. megapotamica*. In larvae V, predation, 0.94 and 0.84, and parasitism by *Apanteles alexanderi,* 0.09 and 0.11, were the main mortality factors for *O. quimilo* and *O. megapotamica* respectively. In *O. megapotamica* predation of pupa was 49% (Table 4), while in *O. quimilo* was almost negligible (Table 3).

**Survey for natural enemies**

**Arthropods.** Surveys were carried out in 11 provinces in north-central Argentina. Native and exotic damaged *Opuntia* spp. were sampled in 43 sites in road sides, natural areas, active and abandoned plantations and 40 eggsticks and 1,258 larvae were sampled.

**Diseases.** Most samples collected during the surveys for natural enemies of *C. cactorum* (see Annual Report 2008) were examined for diseases. From July to October 2008, 2,909 larvae were examined individually or in groups (n = 2-150) for the presence of microsporidia, mainly *Nosema* spp. The larvae were ground in water with a tissue grinder and a drop of the aqueous extract was examined under a phase-contrast microscope (400x) for the presence of spores. Some larvae were also Giemsa-stained for immature stages.

The prevalence of *Nosema* sp.2 was studied in cohorts from 4 field-collected eggs sticks from Quilino. A total of 26 pupae and 16 adults were individually examined as above for the presence of spores. In addition, 137 eggs were Giemsa-stained for the presence of immature stages of *Nosema*. The prevalence in larvae was estimated using the information from the field survey. Additional prevalence studies are in progress.

**Results**. The most frequent parasitoid was *Apanteles alexanderi* Brethes (Hymenoptera: Braconidae), collected in 34.9% of the sites. Other parasitoids were *Pseudochaeta* sp. (Diptera: Tachinidae) (7.0%) and *Podogaster* sp. (Hymenoptera: Ichneumonidae) (4.7%) (Table 5). The microscopic examination revealed the presence of two microsporidia, tentatively *Nosema* sp. 1 and 2, in 33% (10/30) of the sites in Santa Fe, Córdoba, Santiago del Estero, Tucumán and

Catamarca (Table 6). *Nosema* sp. 1 was more widely distributed. Identification of the microsporidia is pending but they are likely to be *Nosema cactoblastis* and *N. cactorum,* respectively. Preliminary contact has been made with Dr. D. Boucias, U. of Florida, Gainesville, for their identification by molecular techniques.

*Nosema* sp. 2 was present in 82-100% of the pupae and 75-100% of the moths. This high prevalence suggests the existence of important epizootics in *C. cactorum* populations that must be confirmed with more evaluations. Surprisingly, the infection was not seen in eggs. The overall prevalence in larvae ranged from 0.5 to 14%. Since most larvae were examined in groups, the actual prevalence will remain unknown until individual larvae from infected cohorts are examined.

**Multiple choice I: Oviposition preference and larval performance**

Multiple choice oviposition experiments were conducted placing 10 different potted *Opuntia* sp. inside a 1.7 x 1.7m fine mesh cage, in a circular arrangement. Ten couples of *C. cactorum* were released in the center of the cage and, after 4 days, the eggsticks in each plant were counted. Ten replicates were considered. Initially, plants were grown from cladodes and moths came from the *O. ficus indica* plantation in Quilino. The native test plants were *Opuntia anacantha* Speg. *O. elata* Link & Otto ex Salm-Dyck*, O. megapotamica* Arechav*, O. quimilo* (Quimil)*, O. sulphurea* Gillies ex Salm-Dyck*, O. cardiosperma* K. Schum. and *O. arechavaletae* Spegazzini, and the exotic ones were *O. ficus indica, O. leucotricha* DC and *O.* robusta H.Wendland ex Pfeiffer. The size of each plant was estimated considering the foliar area of all cladodes calculated as an ellipse ($\pi.r^1.r^2$). The development of the eggsticks, larvae and pupae were followed until adult emergence. Adults were kept in alcohol for body dimensions and eggs counting.

**Results.** *Cactoblastis cactorum* showed a significant oviposition preference for the native *O. quimilo* (Fig. 1) (Friedman ANOVA Chi Sqr = 17.8; d.f. = 9, $p < 0.03$). However, the highest proportion of attacked plants was observed on the exotic *O. ficus indica* and *O. leucotricha* (Fig. 2)*,* which received the second highest number of eggs. On the other hand, no oviposition was observed on *O. anacantha* and the lowest number of eggs was on *O. elata*. Despite showing a moderate ovipisition, *O. robusta* showed the best larval performance and the highest percentage of pupation (Fig. 1). Also *O. cardiosperma* and *O. sulphurea* received a moderate number of eggs, but the larval performance was very poor. The number of eggs laid was not related with plant size. Adult body dimensions are being measured.

**Multiple choice II: Oviposition preference**

With the information provided in the MC I, a second test was conducted with the native *O. quimilo* and *O. sulphurea* (highly and moderately preferred, respectively), *O. megapotamica* and *O. elata* (poorly preferred) and the exotic *O. ficus indica* and *O. leucotricha*. The same protocol was followed, but the larval performance was not evaluated.

**Results.** A similar oviposition pattern was found with a clear preference for *O. quimilo* (Figs. 3 and 4) (Friedman ANOVA $\chi^2 = 12.02$; df= 5, $p < 0.03$), and *O. elata* receiving the lowest number of eggs. The distribution of eggs on the six species was similar for both experiments; females laid a total of 2,351 and 2,554 eggs in the MC I and II respectively. The largest difference was found in the oviposition on *O. megapotamica,* which was 3 times higher in MC II.

**Non choice: Larval performance**

Since not all the *Opuntia* spp. showed oviposition, non-choice trials were conducted to evaluate the larval performance feeding on different potted plants. One eggstick (30 eggs) was artificially glued on a cladode of each of the same 10 species used in the multiple choice tests. Three replicates were conducted. Penetration success and larval performance was evaluated following the development of the eggs, larvae and pupae until adult emergence.

**Results.** Most larvae of *C. cactorum* penetrated the cladodes; the highest percentage of pupation was observed on the three exotic species, *O. ficus indica, O. leucotricha* and *O. robusta* (Fig. 3). Larvae failed to develop on *O. sulphurea, O. arechavaletae* and *O. elata*. In the remaining *Opuntia* spp., larvae failed to penetrate and/or had a low larval survival.

**Field Trips**

- Jul 17-23, 2008. To Córdoba and Santiago del Estero, life table (Logarzo, Guala)
- Aug 22-23, 2008. To Entre Ríos, life table. (Logarzo, Palottini).
- Sep 1-9, 2008. Entre Ríos, Córdoba and Santiago del Estero, life table (Logarzo, Palottini).
- Nov 9-15, 2008. To Córdoba, life table (Ramirez, Varone).
- Dec 3, 2008. To Entre Ríos, life table. (Logarzo, Palottini).
- Dec 10-19, 2008. To NW Argentina, survey of NE (Varone, Logarzo, Ervin, Brooks)
- Feb 3-5, 2008. To Córdoba and Santiago del Estero, collect *Opuntia* spp. and life tables (Manteca, Varone).
- Feb 24-March 9, 2009. To NE Argentina, survey of NE (Varone, Logarzo, Hight, Carpenter).
- May 5-14, 2009. Entre Ríos, Córdoba and Santiago del Estero, life table (Logarzo, Guala).

**Future Plans**

- Continue life table studies on native *Opuntia* spp. in Nogoyá, Quilino, and Tanti.
- Confirm genetic identity of *C. cactorum*.
- Prevalence and impact of *Nosema* spp.
- Taxonomic identification of *Nosema* spp.

**Relevant accomplishments**

- The number of annual generations of the moth is influenced by the host plant.
- Best larval performance was not observed in those host plants preferred for oviposition
- Two *Nosema* spp. were found infecting *C. cactorum.*

**Table 1.** Life table for the third generation of *C. cactorum* on *Opuntia ficus-indica* in Pampa Muyoj, Santiago del Estero province.

| Stage | Mortality factors | lx | dx | qx | Sx | k-value |
|---|---|---|---|---|---|---|
| **Eggs** | | 62597 | | | | |
| | Infertility | | 4382 | 0.07 | 0.93 | 0.0726 |
| | Rain and wind | | 8732 | 0.15 | 0.85 | 0.1625 |
| **Larvae I** | | 49483 | | | | |
| | Failure cladode penetration | | 10391 | 0.21 | 0.79 | 0.2357 |
| **Larvae II-IV** | | 39092 | | | | |
| | Several factors inside cladode | | 11727 | 0.30 | 0.70 | 0.3567 |
| **Larvae V** | | 27364 | | | | |
| | Parasitoids | | 97 | 0.00 | 1.00 | 0.0000 |
| | Predation | | 12816 | 0.47 | 0.53 | 0.6349 |
| **Pupae** | | 14452 | | | | |
| | Predation | | 289 | 0.02 | 0.98 | 0.0202 |
| | Malformation | | 1700 | 0.12 | 0.88 | 0.1278 |
| | Non-emergence | | 3988 | 0.32 | 0.68 | 0.3857 |
| **Moths** | | 8475 | | | | |
| | Mortality & migration | | 7964 | 0.94 | 0.06 | |
| | Sex (51% ♀♀) | 343 | 168 | | | |
| **Adult females** | 173.9 eggs/♀ | 175 | 0 | | | |

**Table 2.** Life table for the third generation of *C. cactorum* on *Opuntia ficus-indica* in Quilino, Córdoba province.

| Stage | Mortality factors | lx | dx | qx | Sx | k-value |
|---|---|---|---|---|---|---|
| **Eggs** | | 74158 | | | | |
| | Infertility | | 3263 | 0.04 | 0.96 | 0.0450 |
| | Parasitoids | | 1418 | 0.02 | 0.98 | 0.0202 |
| | Predation | | 6948 | 0.10 | 0.90 | 0.1054 |
| | Rain and wind | | 6878 | 0.11 | 0.89 | 0.1165 |
| **Larvae I** | | 55651 | | | | |
| | Failure cladode penetration | | 5009 | 0.09 | 0.91 | 0.0943 |
| **Larvae II-IV** | | 50643 | | | | |
| | Several factors inside cladode | | 3039 | 0.06 | 0.94 | 0.0619 |
| **Larvae V** | | 47604 | | | | |
| | Parasitoids | | 3332 | 0.07 | 0.93 | 0.0726 |
| | Predation | | 20365 | 0.46 | 0.54 | 0.6162 |
| **Pupae** | | 23907 | | | | |
| | Predation | | 10758 | 0.45 | 0.55 | 0.5978 |
| | Malformation | | 920 | 0.07 | 0.93 | 0.0726 |
| **Moths** | | 12228 | | | | |
| | Mortality & migration | | 10762 | 0.88 | 0.12 | 2.1211 |
| | Sex (51.0% ♀♀) | 1466 | 718 | | | |
| **Adult females** | 112.4 eggs/♀ | 748 | | | | |
| **Eggs 1 st generation 2008** | | 84045 | | | | |

**Table 3.** Life table for the first generation of *C. cactorum* on the native *Opuntia quimilo* in Quilino, Córdoba province.

| Stage | Mortality factors | lx | dx | qx | Sx | k-value |
|---|---|---|---|---|---|---|
| **Eggs** | | 3758 | | | | |
| | Infertility | | 49 | 0.01 | 0.96 | 0.0408 |
| | Parasitoidism | | 9 | 0.00 | 0.96 | 0.0408 |
| | Predation | | 157 | 0.04 | 0.92 | 0.0853 |
| | Rain and wind | | 912 | 0.24 | 0.68 | 0.3922 |
| **Larvae I** | | 2631 | | | | |
| | Mucilage | | 643 | 0.24 | 0.68 | 0.3857 |
| | Predation | | 780 | 0.30 | 0.70 | 0.3567 |
| **Larvae II-IV** | | 1208 | | | | |
| | Several factors inside cladode | | 157 | 0.13 | 0.87 | 0.1393 |
| **Larvae V** | | 1051 | | 0.13 | 0.87 | 0.1393 |
| | Parasitoids | | 95 | 0.09 | 0.91 | 0.0943 |
| | Predation | | 898 | 0.85 | 0.15 | 2.8134 |
| **Pupae** | | 88 | | | | |
| | Malformation + other causes | | 14 | 0.16 | 0.02 | 3.7581 |
| **Moths** | | 67 | | 0.23 | 0.77 | 0.2657 |

**Table 4.** Life table for the first generation of *C. cactorum* on the native *Opuntia megapotamica* in Tanti, Córdoba province.

| Stage | Mortality factors | lx | dx | qx | Sx | k-value |
|---|---|---|---|---|---|---|
| **Eggs** | | 15658 | | | | |
| | Infertility | | 1446 | 0.09 | 0.91 | 0.0969 |
| | Parasitoids | | 79 | 0.01 | 0.99 | 0.0050 |
| | Predation | | 587 | 0.04 | 0.96 | 0.0382 |
| | Rain and wind | | 8768 | 0.56 | 0.44 | 0.8210 |
| **Larvae I-IV** | | 4778 | | | | |
| | Failure cladode penetration | | 1007 | 0.21 | 0.79 | 0.0665 |
| | Several factors inside cladode | | 467 | 0.10 | 0.90 | 0.0303 |
| **Larvae V** | | 3303 | | | | |
| | Parasitoids | | 374 | 0.11 | 0.89 | 0.0242 |
| | Predation | | 2783 | 0.84 | 0.16 | 0.1957 |
| **Pupae** | | 146 | | | | |
| | Predation | | 72 | 0.49 | 0.51 | 0.0046 |
| | Failure to pupate | | 1 | 0.01 | 0.99 | 0.0001 |
| | Malformation | | 1 | 0.01 | 0.99 | 0.0000 |
| | Non emerged | | 2 | 0.01 | 0.99 | 0.0001 |
| **Moths** | | 70 | | | | |
| | Mortality & migration | | | | | |
| | Sex (35.0% ♀♀) | | | | | |
| **Adult females** | 91 eggs/♀ | | | | | |
| Eggs 2 st generation | | | | | | |

**Table 5.** Survey of parasitoids of *C. cactorum* in its native range, Argentina.

| Province | *Opuntia* sampled sites | Sites with *Cactoblastis* | Stage sampled | Parasit. Sites (%) | Parasitoid |
|---|---|---|---|---|---|
| Entre Ríos | 17 | 9 | Egg | 0 (0) | |
| | | 6 | Larvae | 5 (83.3) | *Apanteles alexanderi* |
| Corrientes | 16 | 7 | Egg | 0 (0) | |
| | | 7 | Larvae | 2 (28.6) | *Apanteles alexanderi* |
| Chaco | 17 | 2 | Egg | 0 (0) | |
| | | 7 | Larvae | 2 (28.6) | *Apanteles alexanderi* |
| Formosa | 18 | 2 | Egg | 0 (0) | |
| | | 6 | Larvae | 1 (16.7) | *Pseudochaeta sp.* |
| Salta | 25 | 1 | Egg | 0 (0) | |
| | | 5 | Larvae | 3 (60) | *Apanteles alexanderi* |
| | | | | 1 (20) | *Podogaster* sp. |
| | | | | 2 (40) | *Pseudochaeta* sp. |
| Jujuy | 18 | 1 | Egg | 0 (0) | |
| | | 6 | Larvae | 0 (0) | |
| Tucumán | 3 | 1 | Egg | 0 (0) | |
| | | 3 | Larvae | 2 (66.7) | *Apanteles alexanderi* |
| Catamarca | 13 | 3 | Egg | 0 (0) | |
| | | 1 | Larvae | 0 (0) | |
| Córdoba | 1 | 1 | Egg | 0 (0) | |
| | | 0 | Larvae | 0 (0) | |
| La Rioja | 3 | 0 | Egg | 0 (0) | |
| | | 1 | Larvae | 1 (100) | *Apanteles alexanderi* |
| San Luis | 1 | 0 | Egg | 0 (0) | |
| | | 1 | Larvae | 1 (100) | *Podogaster* sp. |

**Table 6.** Survey for *Nosema* spp. infecting *C. cactorum* in north central Argentina

| **Collecting site Province and locality** | **Collecting date** | **Nº larvae examined** | **Infection found** |
|---|---|---|---|
| **Santa Fe** | | | |
| Rt. 34 km 158 | 29-Jan-08 | 100 | *Nosema* sp. 1 |
| Rt. 34 km 275 | 23-Feb-08 | 20 | *Nosema* sp. 1 |
| **Córdoba** | | | |
| Villa Quilino | 21-Apr-08 | 3 | *Nosema* sp. 2 |
| Dean Funes | 26-Feb-08 | 5 | *Nosema* sp. 1 |
| | | 50 | *Nosema* sp. 1 & 2 |
| | | 50 | *Nosema* sp. 2 |
| Trompa de Elefante | 27-Feb-08 | 150 | *Nosema* sp. 1 |
| **Santiago del Estero** | | | |
| Rt. 34 km 485 | 11-Apr-08 | 50 | *Nosema* sp. 1 |
| **Tucumán** | | | |
| Aguilares | 25-Feb-08 | 5 | *Nosema* sp. 1 |
| La Cocha | 25-Feb-08 | 1 | *Nosema* sp. 2 |
| **Catamarca** | | | |
| Rt. 38 | 26-Feb-08 | 20 | *Nosema* sp. 1 |
| Miraflores | 26-Feb-08 | 35 | *Nosema* sp. 1 |
| Others | several | 2,420 | Infection free |
| | | | |
| TOTAL | | **2,909** | |

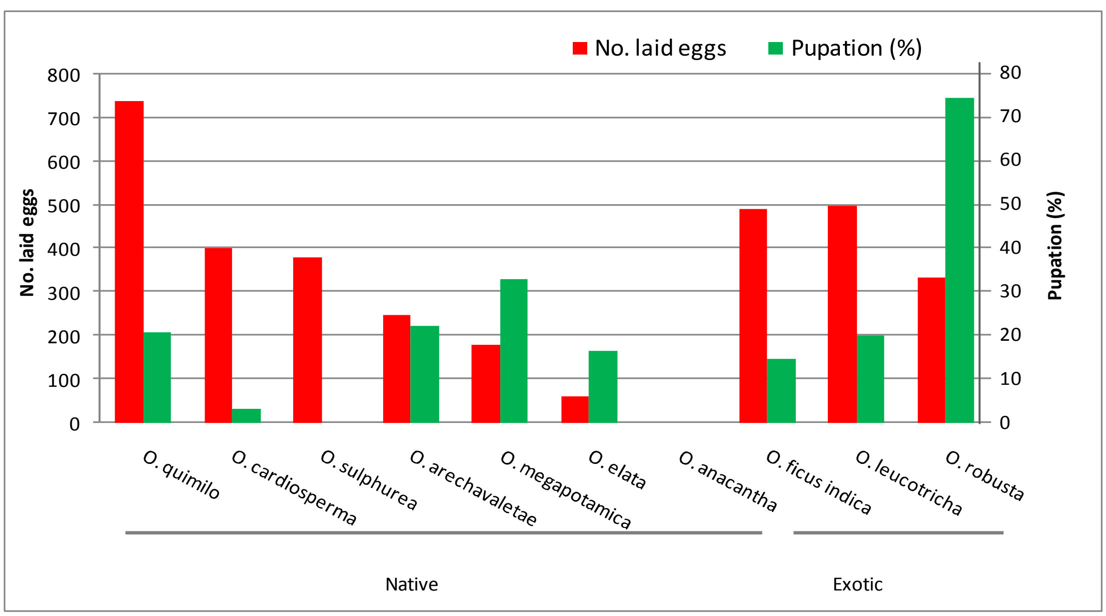


Fig. 1. Multiple choice experiment I: number of eggs of *C. cactorum* laid and percentage of eggs that emerged, penetrated, fed and reached the pupal stage on *Opuntia* spp.

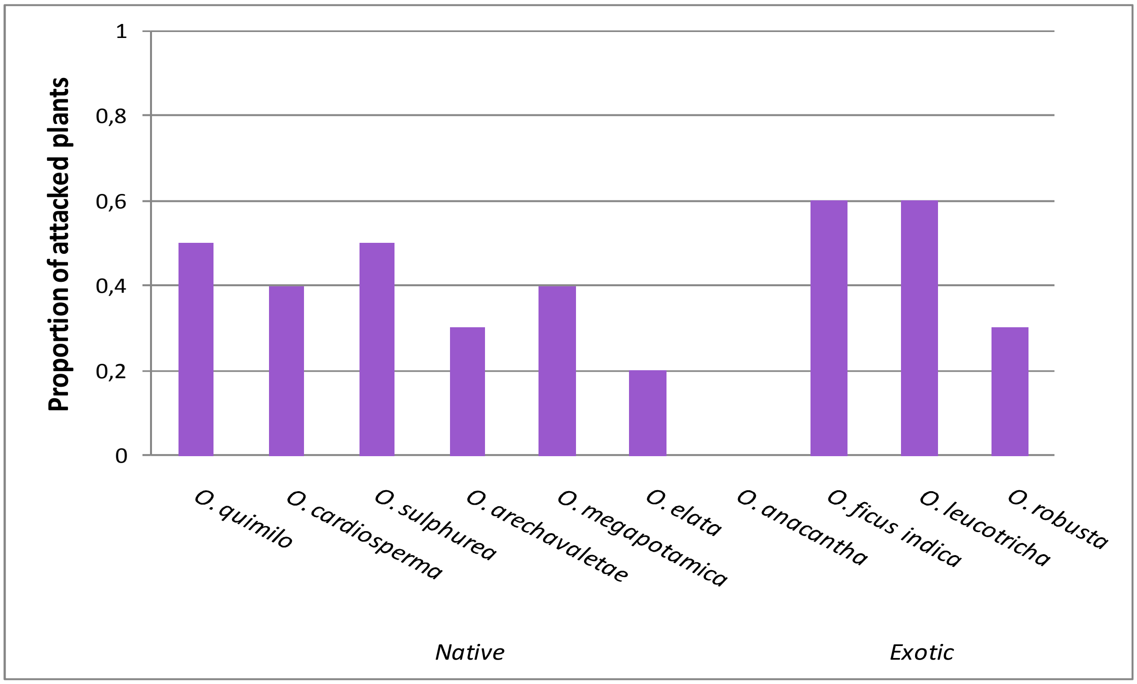


Fig. 2. Proportion of attacked plants in the multiple choice experiment I (N = 10).

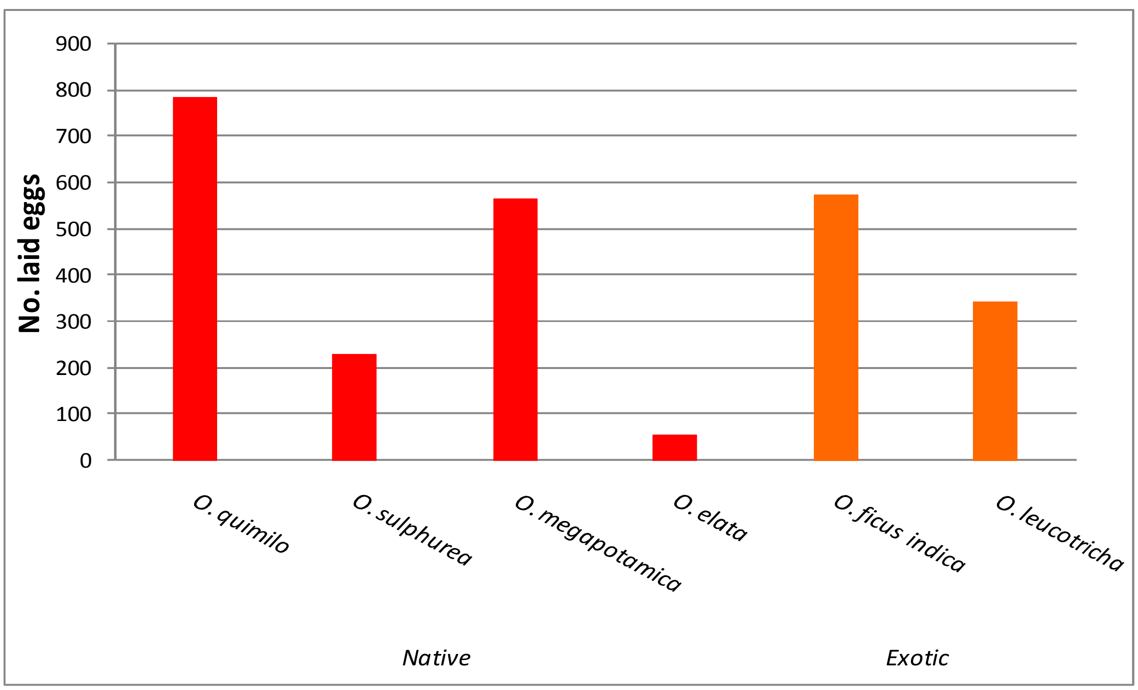


Fig. 3. Multiple choice experiment II: number of eggs of *C. cactorum* laid on *Opuntia* spp.

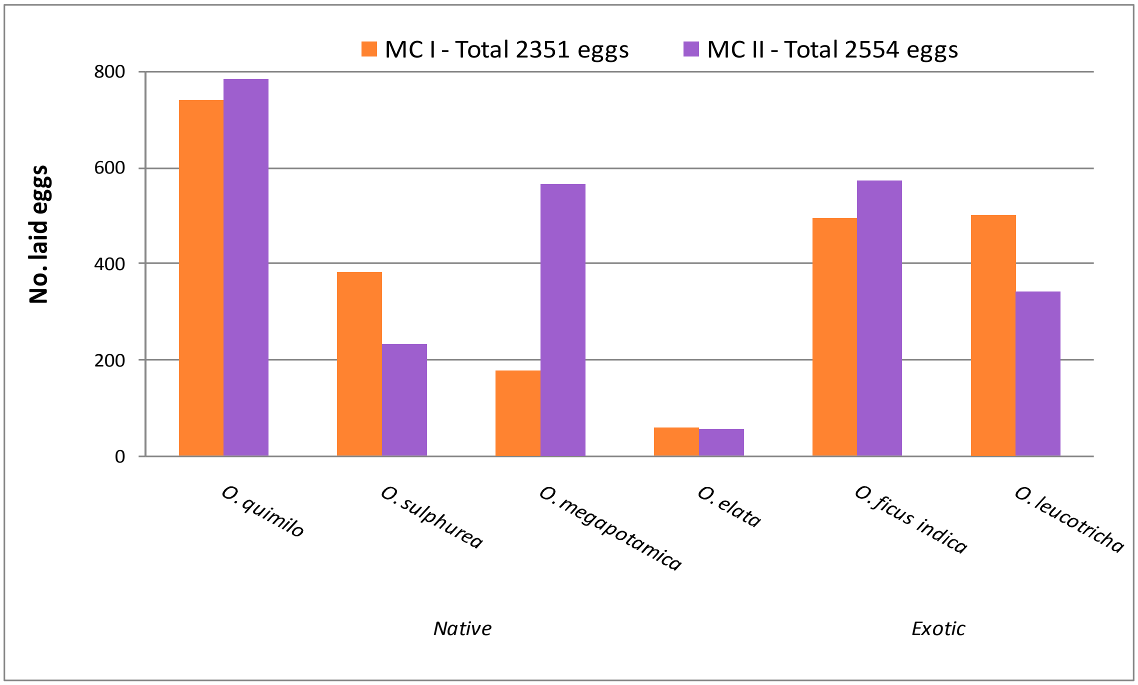


Fig. 4. Comparison of number of eggs laid on *Opuntia* spp. in two multiple choice experiments: MC I with 10 *Opuntia* spp. and MC II with 6.

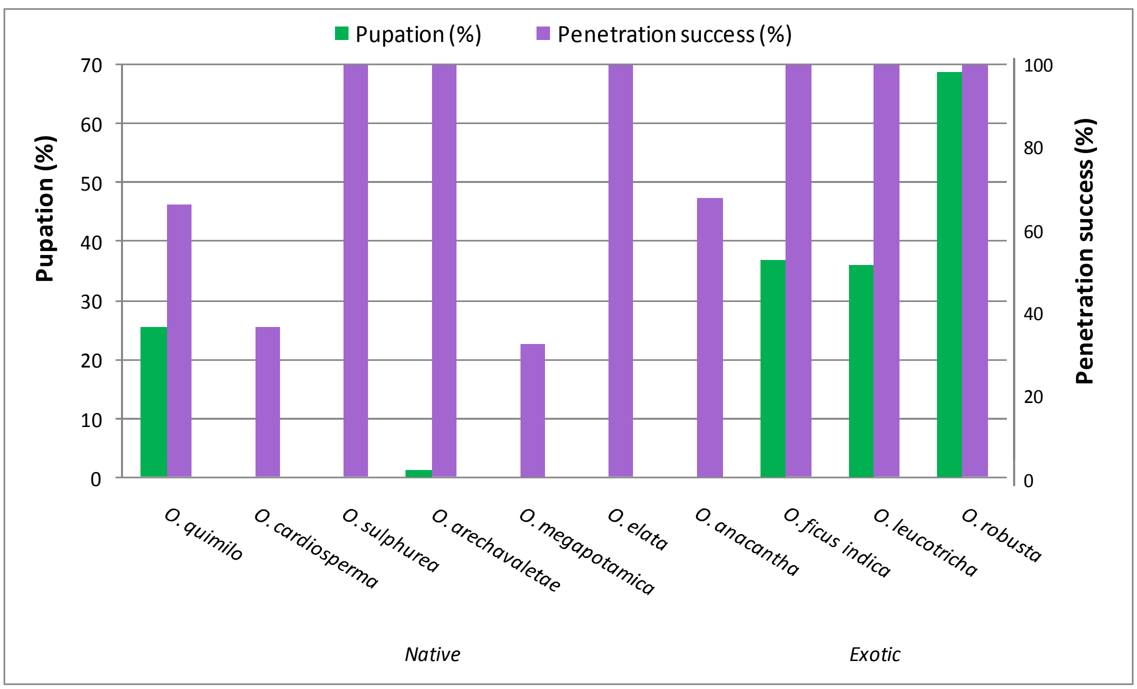


Fig. 5. Non-choice experiment: percentage of initial eggs that emerged, penetrated, fed and reached the pupal stage on *Opuntia* spp.

# LITTLE FIRE ANT

by

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## Introduction

The Little Fire Ant (LFA), *Wasmannia auropunctata*, is native to South and Central America and has spread fairly recently around the Pacific through infested nursery stock. It is now a serious pest in Hawaii where infestations are disrupting agricultural practices. Initially their stings burn intensely and cause severe itching for two or more weeks. LFA damage also pets and livestock and multiple stings to the eyes can cause blindness in animals. Efforts to suppress the pest are intensive and involve consistent applications of toxic baits. In October 2008, SABCL started a program to find natural enemies for their potential use in Hawaii.

## Field Surveys in Argentina

Three field surveys were conducted in northern Argentina to search for LFA and natural enemies. Because LFA occurs in regions independently of the type of land use (Wetterer and Porter 2003, Tenant 2004, Delabie et al. 2007), we focused in natural forests: 1) Yungas forest (little modified low mountain forest) in northwestern Argentina, 2) Paranaense forest (Atlantic semi-evergreen moist forest) in northeastern Argentina, mostly in Misiones province, and 3) Chaco/Espinal forest (mostly gallery forest, a disturbed natural forest along the rivers (e.g. Paraná, Uruguay, Paraguay, and Negro Rivers) (Cabrera and Willink 1981; Fig. 1).

A total of 665 samples of leaf-litter and above-ground ants were obtained using sieves (5.8 ± 0.4 samples per site) and baits (13.7 ± 0.6 samples per site); 146 plots (0.25-$m^2$) were sampled: 55 in Yungas, 46 in Paranaense, and 45 in Chaco/Espinal. Plots were located >50m apart from edge forest. The leaf litter (leaf mold, rotten wood) of each plot was collected and sieved (1x1 cm) to a white plastic tray (12x45x55 cm). Ants were collected with forceps and kept in 96% alcohol for their identification. Baits (1-2g of canned tuna on 5x5 cm white paper cards) were exposed for 30-45 min to 1) promote worker recruitment, 2) locate LFA nests, and 3) study overall presence and abundance of terrestrial ants. Large polydomous colonies of LFA have not a central mound and colonies can be found under stones, in dead wood, in leaf-litter, in stems or under bark (Wetterer and Porter 2003). In each site, a 200-m transects was established. Baits were placed on the ground along transects every 10-20m. Ants were attracted to a total of 519 baits: 164 in Yungas, 172 in Chaco/Espinal, and 183 baits in Paranaense. All the ants attracted were kept in 96% alcohol for their identification by L.A.C. and Fabiana Cuezzo (Instituto y Museo Miguel Lillo, Tucumán, Argentina) under a dissecting scope. Bait sampling was extended to anthropic habitats (sugar cane and banana fields).

**Results.** The presence of the LFA was 3 times higher in the Chaco/Espinal (Fig. 2), occurring in 8 (72.7%) of the 11 sites, while only in 3 (23%) of the 13 sites in the Yungas and in 3 (21.4%) of the 14 sites in the Paranaense. The LFA was 3-5 times more abundant in the Chaco/Espinal. It was found in 25 (11.4%) of the 220 samples in the Chaco/Espinal, in 9 (4.2%) of the 216 samples in the Yungas and 5 (2.2%) of the 229 samples in the Paranaense.

In the Chaco/Espinal, the LFA was found in 8 sites in Santa Fe, Chaco, Formosa, and Corrientes provinces. It occurred between the 28º32´ and 25º07´ S, 58º10´ and 57º29´ W, and 45 and 81 m of altitude (Fig. 3). In the Yungas, LFA was found in 3 sites in Jujuy province (Calilegua and Bananal) at 424-750 m of altitude, while in the Atlantic Forest, it was found in three areas in south Misiones (San Javier, Aristóbulo del Valle, and Loreto) at 129-225 m of altitude. The LFA occurred in secondary forests with different level of alteration (and different vegetation composition) and in a banana plantation in the Yungas.

Other *Wasmannia* spp. occurred with LFA: 1) *Wasmannia* sp. 1 was found for the first time in Argentina in 2 (18.2%) sites and in 3 (1.4%) samples in the Chaco/Espinal at 75-81 m of altitude. This non-described species is similar to that reported by Alex Wild (*Wasmannia* cf. alw01) for Concepción, Paraguay; 2) *Wasmannia sulcaticeps* was found in 2 (15.4%) sites and in 5 (2.3%) samples in the Yungas at 690-750 m of altitude; it co-occurred with *W. auropunctata* only in Calilegua at 750 m of altitude; 3) *Wasmannia* sp. (putative *W.* cf. *rochai*) was found also for the first time in Argentina in 2 (14.3%) sites and in 3 (1.3%) samples in the Atlantic Forest at 192-243 m of altitude.

Paranaense Forest was richer in ant species than the Yungas and Chaco/Espinal. A total of 98 species was found in the Paranaense, 67 in Chaco/Espinal, and 55 in the Yungas (Table 1). However, the mean number (± SE) of species per sample was similar in the 3 regions: 2.3 ± 1.4 species for the Paranaense, 2.0 ± 1.1 for the Chaco/Espinal, and 1.8 ± 1.2 for the Yungas ($H_{2, 665}$ = 5.95, $P$ = 0.051) (Table 2). An inverse relationship was observed in the proportion of ant baited (above-ground foraging ants) and sieved (leaf-litter ants) between the Yungas and Paranaense (Table 2). The proportion of species collected with both methods was similar in the Chaco/Espinal. The LFA was better detected with sieves in Paranaense and Chaco/Espinal, but not in the Yungas (Fig. 4), where it was similarly captured with both methods.

The most common ants co-occurring with LFA were *Camponotus* cf. *yala*, *Hypoponera opaciceps, H. opacior* in 28% of the samples in Chaco/Espinal, *Pheidole* sp. 35 in 25% of the samples in Paranaense, and *Brachymyrmex depilis* in the Yungas.

Entire colonies of the LFA (Fig. 5) were collected in Puerto Ocampo, Santa Fe, in the edge between the forest and grasslands near the Paraná River. Nests were found by following foraging trails of workers (Fig. 6). These colonies showed multiple queens (up to 12 queens) and a few broods in small chambers 2 cm deep (Figs. 7 and 8). Colonies adopted queens from other colonies distant ≥ 100 m. The lack of aggression by the receptor colony suggests unicoloniality as in its introduced range (Wetterer and Porter 2003).

**<u>Screening for natural enemies</u>**

Once nests were found, LFA were placed in plastic trays (12x45x55 cm) to visually screen for arthropod natural enemies. Occasionally, workers were exposed in plastic trays (7x24x37 cm) for 30 min for the potential presence of phorid flies. The walls of the trays were coated with talc or Fluon to prevent workers from escaping. LFA trails were also checked for phorids attacks. After exposure, the workers were placed in buckets with soil, transported to the lab, separated from soil by flotation and placed in trays for a more exhaustive examination for arthropods. Sub-samples of workers were checked for the presence of diseases by grinding them individually in 1-2 ml of water with a tissue grinder. Then, a drop of the aqueous extract was examined under a phase contrast microscope (400x) for the presence of diseases.

**Results.** So far, arthropod natural enemies or pathogens were not found.

**Selected study sites**

Three sites in Chaco/Espinal with high abundance of LFA were selected for a more intense search of natural enemies (Fig. 1): 1) Chaco National Park, 2) Puerto Ocampo, and 3) Isla del Cerrito. The sites are located 150 km apart in the humid Chaco. Climate is subtropical (seasonal) with a dry season in winter (June-September) and rainfall predominantly in summer (December-March). Mean annual rainfall is 1,034-1,127 mm (De Fina 1992). Puerto Ocampo and Isla del Cerrito, on the floodplain of the Paraná River, become extensively flooded during the wet season. Mean temperature is 15.5ºC in July and 27.5ºC in January.

**Field Surveys in Dominica**

On May 11-28, 2009, an intensive survey for *Wasmannia* and the hymenopteran parasitoids *Orasema costaricensis* or *O. minutissima* was conducted in the island. Collecting sites were located in the Archbold Biological Station, Cabrits National Park, Sulphur Springs, Central Forest Reserve, Northern Forest Reserve, Syndicate Trail, Trafalgar Falls and Middleham Falls. At each site, 20 baits (5x5 cm paper cards with 1-2g of peanut butter) were located in a 200 m transect and checked for 30 min and then after 2-3 hs. The presence and abundance of ant species were recorded. Samples of the species trapped were preserved in 96% ethanol for identification.

**Results.** *Wasmannia auropunctata* was trapped in all the sampled sites and baits were discovered within the first 10 min. The highest abundance per trap was over 300 individuals. Further information on bait discovery and colonization is being processed. The LFA mounds were found in the litter, inside old trunks, and up to 750 m over the sea level. Colonies were small with few broods (Fig. 9).

**Future plans**

-Intensify the search of natural enemies in selected sites.
-Conduct lab attraction tests with LFA and other ants using LFA pheromones provided by Dr. Matthew Siderhurst (Eastern Mennonite University).
-Use specific pheromones to improve the search of LFA nests and their natural enemies.
-If natural enemies are found, study the distribution, abundance, specificity, and impact.
-Begin ecological studies in selected sites in the three regions previously surveyed.

**Field Trips**

1) Oct 24-30, 2008. To northwestern Argentina, to survey LFA and natural enemies (Calcaterra, S. Cabrera).
2) Dec 9-16, 2008. To northeastern Argentina, to continue surveys (Calcaterra, Ramírez).
3) Jan 26-30, 2009. To central northern Argentina, to continue surveys (Calcaterra, Ramírez).

**Relevant Accomplishments**

-*Wasmannia* cf. *rochai* and *Wasmannia* sp. 1 were recorded for the first time in Argentina.
-One of the most exhaustive ant inventory was built for a single survey-stratum (185 spp).

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**Fig. 1**. Natural forest regions surveyed within Argentina and sites with the highest LFA abundance.

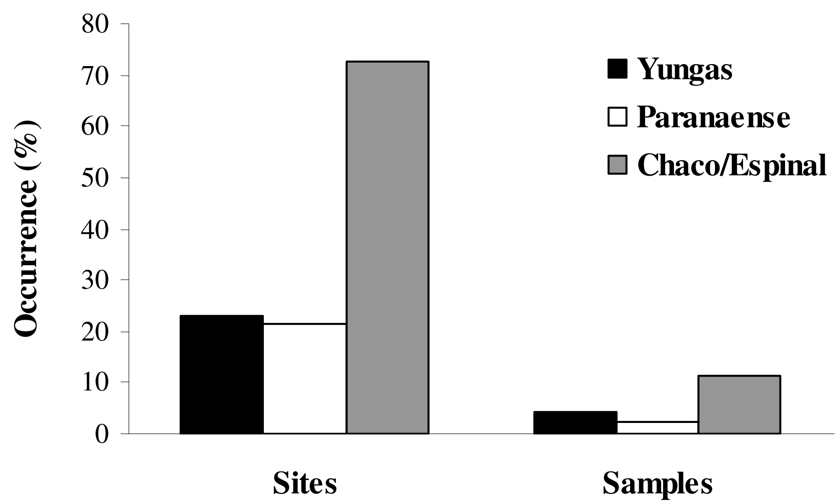


**Fig. 2**. Presence (sites) and abundance (samples) of LFA in three forests surveyed in northern Argentina.

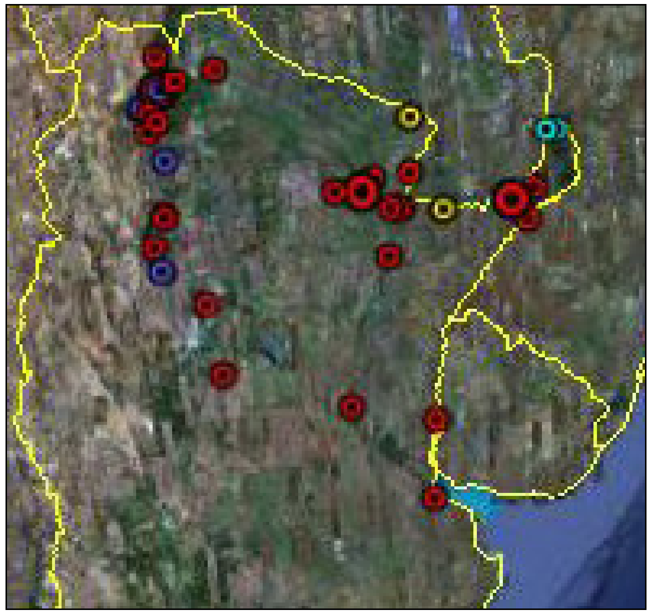

**Fig. 3**. *Wasmannia* sites found during the surveys and some previous unpublished records from Miguel Lillo Museum: *Wasmannia auropunctata* (●), *W. sulcaticeps* (●), *Wasmannia* cf. *rochai* (●), and *Wasmannia* sp. 1 (●)

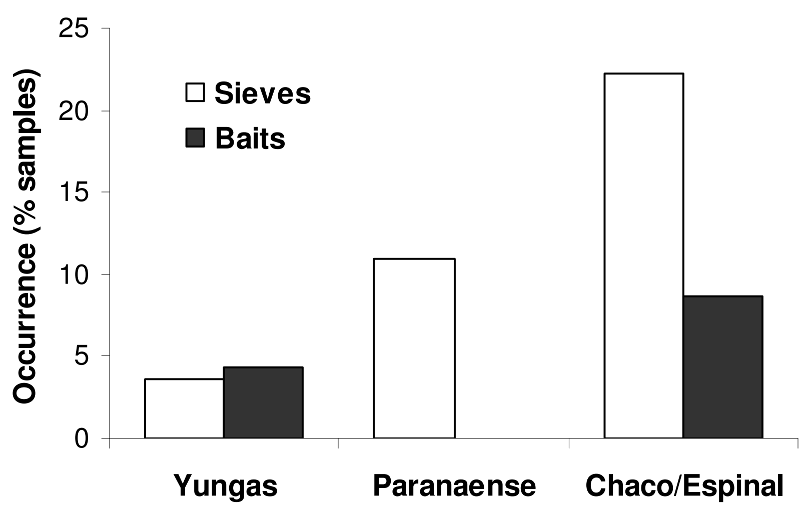


**Fig. 4**. Presence of the LFA according to the collection method in the three regions surveyed.

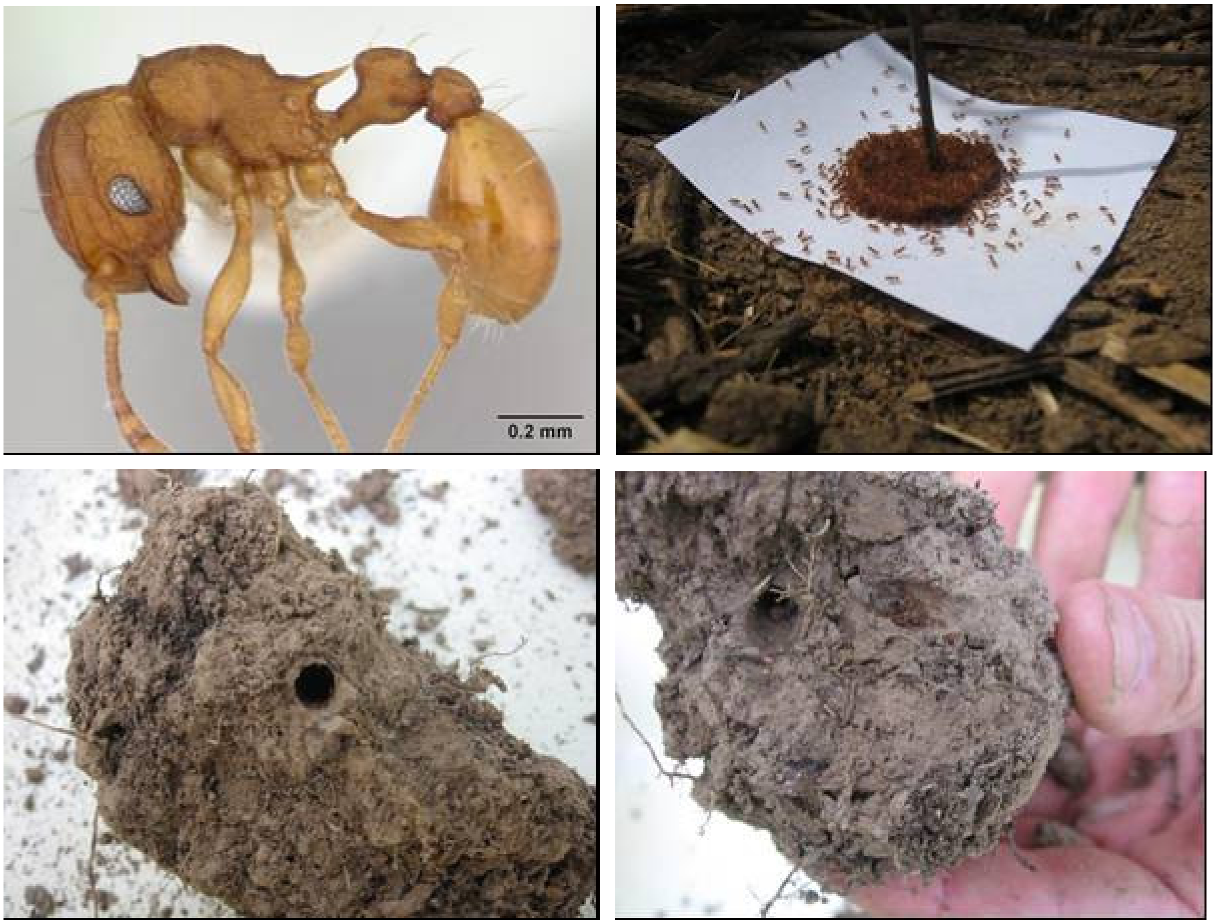


**Fig. 5-8.** LFA worker, workers recruited to tuna baits, nest entrance, and small chamber with queens, workers, and brood.

**Fig. 9.** *Wasmannia auropuctata* nest in an old trunk in Dominica.

**Table 1**. List of ant species collected with baits and sieves in the three regions surveyed.

| Yungas | Chaco/Espinal | Paranaense |
|---|---|---|
| *Anochetus altisquamis* | *Anochetus sp.* | *Acanthostichus sp.* |
| *Brachymyrmex depilis* | *Apterostigma sp.* | *Acromyrmex sp.* |
| *Brachymyrmex sp. 6* | *Azteca alfari* | *Apterostigma pilosum* |
| *Camponotus blandus* | *Brachymyrmex sp. 1* | *Brachymyrmex sp. 1* |
| *Camponotus sp. 4* | *Brachymyrmex sp. 3* | *Brachymyrmex sp. 2* |
| *Camponotus cf. mus* | *Brachymyrmex sp. 5* | *Brachymyrmex sp. 3* |
| *Crematogaster sp. 1* | *Camponotus cf. yala* | *Brachymyrmex sp. 4* |
| *Crematogaster sp. 2* | *Camponotus lespesi* | *Camponotus lespesi* |
| *Crematogaster sp. 3* | *Camponotus mus* | *Camponotus ogloblini* |
| *Cyphomyrmex quebradae* | *Camponotus rufipes* | *Camponotus rufipes* |
| *Cyphomyrmex transversus* | *Camponotus sp. 2* | *Camponotus sericeiventris* |
| *Discothyrea neotropica* | *Camponotus sp. 3* | *Camponotus sp. 1* |
| *Dorymyrmex thoracicus* | *Cephalotes sp.* | *Camponotus trapezoides* |
| *Ectatomma edentatum* | *Crematogaster sp. 3* | *Carebara sp.* |
| *Gnamptogenys rastrata* | *Crematogaster sp. 5* | *Crematogaster sp. 1* |
| *Gnamptogenys triangularis* | *Crematogaster sp. 6* | *Crematogaster sp. 2* |
| *Hypoponera sp. A4* | *Cyphomyrmex rimosus* | *Crematogaster sp. 4* |
| *Hypoponera sp. 8* | *Ectatoma edentatum* | *Cyphomyrmex olitor* |
| *Hypoponera sp. 9* | *Ectatoma permagnum* | *Dinoponera australis* |
| *Myrmelachista elongata* | *Gnamptogenys rastrata* | *Ectatomma edentatum* |
| *Odontomachus chelifer* | *Hylomyrma balzani* | *Gnamptogenys rastrata* |
| *Pachycondyla striata* | *Hypoponera opaciceps* | *Gnamptogenys sp. 1* |
| *Paratrechina cf. alw03* | *Hypoponera opacior* | *Heteroponera dolo* |
| *Paratrechina pubens* | *Hypoponera sp. 3* | *Heteroponera mayri* |
| *Pheidole radoszkowskii* | *Hypoponera sp. B4* | *Heteroponera sp.* |
| *Pheidole bruchi* | *Hypoponera sp. 7* | *Hylomyrma balzani* |
| *Pheidole cf. asperithorax* | *Linepitema humile* | *Hylomyrma reitteri* |
| *Pheidole grundmanni* | *Linepithema gallardoi* | *Hypoponera sp. 1* |
| *Pheidole rosae* | *Linepithema micans* | *Hypoponera sp. 2* |
| *Pheidole scapulata* | *Mycocepurus goeldii* | *Hypoponera sp. 3* |
| *Pheidole sp. 1* | *Odontomachus cheliter* | *Hypoponera sp. 4* |
| *Pheidole sp. 2* | *Odontomachus haematodus* | *Hypoponera sp. 6* |
| *Pheidole sp. 3* | *Pachycondyla striata* | *Hypoponera sp. C8* |
| *Pheidole sp. 4* | *Paratrechina sp. 1* | *Labidus preadator* |
| *Pheidole sp. A5* | *Paratrechina sp. 2* | *Linepithema angulatum* |
| *Pheidole sp. 5* | *Paratrechina sp. 3* | *Linepithema micans* |
| *Pheidole sp. 6* | *Paratrechina sp. 4* | *Linepithema pulex* |
| *Pheidole sp. 7* | *Paratrechina sp. 5* | *Linepithema sp. 1* |
| *Pheidole sp. 8* | *Paratrechina sp. 6* | *Linepithema sp.2* |
| *Pheidole sp. 9* | *Pheidole radoszkowskii* | *Megalomyrmex drifti* |
| *Pheidole sp. 10* | *Pheidole nubila* | *Megalomyrmex sp.* |
| *Pheidole sp. 11* | *Pheidole cf. triconstricta* | *Nesomyrmex sp.* |
| *Pheidole sp. 12* | *Pheidole obscurithorax* | *Octostruma rugifera* |
| *Pheidole sp. 13* | *Pheidole sp. C* | *Odontomachus cheliter* |
| *Pheidole sp. A* | *Pheidole sp. 13* | *Odontomachus haematodus* |
| *Pheidole sp. B* | *Pheidole sp. 14* | *Odontomachus minutus* |
| *Solenopsis interrupta* | *Pheidole sp. 15* | *Oxyepoecus sp.* |
| *Solenopsis sp. 1* | *Pheidole sp. 16* | *Pachycondyla harpax* |
| *Solenopsis sp. 2* | *Pheidole sp. 17* | *Pachycondyla striata* |
| *Solenopsis sp. 3* | *Pheidole sp. 18* | *Pachycondyla sp. 1* |
| *Solenopsis sp. A (Carabella?)* | *Pheidole sp. 19* | *Paratrechina sp. A1* |
| *Strumygenys lousianae* | *Pheidole sp. D* | *Paratrechina sp. B2* |

**Table 1.** (continued)

| Yungas | Chaco/Espinal | Paranaense |
|---|---|---|
| *Wasmannia auropunctata* | *Pheidole sp. E* | *Paratrechina sp. C3* |
| *Wasmannia sulcaticeps* | *Pogonomyrmex sp. 2* | *Paratrechina sp. D4* |
| | *Pseudomyrmex sp. 1* | *Paratrechina sp. E5* |
| | *Solenopsis sp. B* | *Pheidole radoszkowskii* |
| | *Solenopsis sp. C* | *Pheidole aberrans* |
| | *Solenopsis sp. D* | *Pheidole bergi* |
| | *Solenopsis sp. E* | *Pheidole inversa* |
| | *Solenopsis sp. F* | *Pheidole obscurithorax* |
| | *Solenopsis sp. G* | *Pheidole cf. triconstricta* |
| | *Solenopsis sp. H* | *Pheidole sp. 15* |
| | *Solenopsis sp. I* | *Pheidole sp. 20* |
| | *Strumygenys elongata* | *Pheidole sp. 21* |
| | *Trachymyrmex sp.* | *Pheidole sp. 22* |
| | *Wasmannia auropunctata* | *Pheidole sp. 23* |
| | *Wasmannia sp. 1 ALW0?* | *Pheidole sp. 24* |
| | | *Pheidole sp. 25* |
| | | *Pheidole sp. 26* |
| | | *Pheidole sp. 27* |
| | | *Pheidole sp. 28* |
| | | *Pheidole sp. 29* |
| | | *Pheidole sp. 30* |
| | | *Pheidole sp. 31* |
| | | *Pheidole sp. 32* |
| | | *Pheidole sp. 33* |
| | | *Pheidole sp. 34* |
| | | *Pheidole sp. 35* |
| | | *Pheidole sp. 36* |
| | | *Pheidole sp. 37* |
| | | *Pheidole sp. 38* |
| | | *Pheidole sp. 39* |
| | | *Pogonomyrmex sp. 1* |
| | | *Pseudomyrmex sp. 2* |
| | | *Solenopsis invicta* |
| | | *Solenopsis sp. J* |
| | | *Solenopsis sp. K* |
| | | *Solenopsis sp. L* |
| | | *Solenopsis sp. O (or Carabella sp.?)* |
| | | *Solenopsis sp. P* |
| | | *Solenopsis sp. Q* |
| | | *Solenopsis sp. R* |
| | | *Solenopsis sp. S* |
| | | *Solenopsis sp. T* |
| | | *Solenopsis sp. U* |
| | | *Strumygenys louisianae* |
| | | *Wasmannia auropunctata* |
| | | *Wasmannia rochai* |

**Table 2**. Sampling effort, diversity indicators of ants, and LFA presence in the three regions surveyed in northeastern Argentina.

| Number | Yungas | Paranaense | Chaco/Espinal |
|---|---|---|---|
| Total samples collected (sites) | 219 (13) | 229 (14) | 217 (11) |
| Bait samples | 164 (13) | 183 (14) | 172 (11) |
| Sieve samples | 55 (8) | 46 (8) | 45 (9) |
| Total species found | 55 | 98 | 67 |
| from baits (% of total) | 47 (86) | 56 (57) | 45 (67) |
| from sieves (% of total) | 36 (66) | 77 (79) | 45 (67) |
| Mean (± SE) species per sample | 1.8 ± 0.08 | 2.3 ± 0.14 | 2 ± 0.1 |
| from baits | 1.3 ± 0.05 | 1.5 ± 0.06 | 1.7 ± 0.07 |
| from sieves | 3 ± 0.22 | 5.6 ± 0.35 | 3.2 ± 0.26 |
| Total LFA occurrence | | | |
| percentage of sites | 23 | 21 | 73 |
| percentage of samples (ranking) | 4.1 (15-17º) | 2.2 (28-35º) | 11.5 (3º) |

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