

# Annals of the 2<sup>nd</sup> Symposium on the Biology of Galls

February 25th - 27th 2021

















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#### PREFACE

The 2<sup>nd</sup> Symposium on The Biology of Galls (2<sup>nd</sup> SBG) occurred from February 25<sup>th</sup> to 27<sup>th</sup>, 2021, gathering researchers of different expertise. This book reunites the abstracts of this event, with an inherent diversity of approaches, which are uniquely brought by the study on the biology of galls. Besides the abstracts of the lectures and videoposters presented in the 2<sup>nd</sup> SBG, this book also counts with an honorable article written by Dr. Jane E. Kraus, Renê G.S. Carneiro, and Rosy M.S. Isaias, a brief history of studies on the Biology of Galls worldwide and in Brazil.

Perspectives on the organization of the 2<sup>nd</sup> SBG was born during the first edition of the event, in Ouro Preto, Minas Gerais, Brazil, in November 2018. The idea of the Symposium on the Biology of Galls was to reunite researchers, professors, and students from distinct universities and institutions to discuss the advances in the field of Cecidology in distinct fields of research. The presential event occurred in Ouro Preto allowed the discussion of interdisciplinary topics, an immersive experience of field works, and the establishment of research partnerships among Brazilian and foreigner groups.

Engaging a similar event, with rich discussions among students involved in the studies on gall biology, the organizers proposed the 2<sup>nd</sup> Symposium on The Biology of Galls at Ilha Grande, Rio de Janeiro state, Brazil. The organizing committee had the mission to maintain the quality, interdisciplinarity and integration offered by the first edition of the event. Unfortunately, the COVID-19 pandemic occurred, and our plans had to be changed.

With the financial support by the FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – Ref. 210.357/2020), the organizing committee decided to adapt the event to an online version, required by the financial agency due to the pandemic restrictions. The mission of the 2nd Symposium on The Biology of Galls in an online version was to offer our students and interested researchers a comprehensive view of the most current topics on

the Biology of Galls, and to narrow geographic gaps between the researchers of distinct regions and expertise. The event promoted and celebrated the integrative facet of Cecidology among students and researchers, setting new ideas and questions for their current study systems by rich, evidence-based, and interdisciplinary discussions.

The 2<sup>nd</sup> SBG reunited people from around the world, with 191 subscribers in the event website (https://www.even3.com.br/2ndbiologyofgalls), 16 lecturers, and 15 videoposter presenters. Even though the subscription in the event website was free of charge, the event was broadcasted in YouTube for any interested person. The *lives* reached 600 views during the transmission in the first day (February 25<sup>th</sup>, 2021), with a peak of 81 simultaneous views; 348 views in the second day (Feb 26<sup>th</sup>), with a peak of 67 simultaneous views; and 307 views in the third day (Feb 27<sup>th</sup>), with peaks of 51 simultaneous views. The videos of the event are yet available in the channel of the Neotropical Gall Group (https://www.youtube.com/channel/UCQ5MoGbXiYZyRwkTHZ2ZhoQ).

The participation of the public was successful, as can be attested by the interactions among the audience in the YouTube chat, the lecturers and the videoposter presenters with questions, comments, and suggestions about their contributions. We invite you to watch the videos and remember the best moments of the 2<sup>nd</sup> Symposium on the Biology of Galls, and also to read the abstracts of the most current topics on the Cecidology around the world. As a continuous effort to maintain the spirit of the SBG alive, we invite all gall researchers and enthusiasts around the world to contact us, and to contribute for the next events, when we hope to aggregate further perspectives on the fascinating universe of Cecidology.

The organizing committee

March 2021

#### THE 2ND SYMPOSIUM ON THE BIOLOGY OF GALLS

The Organizing Committee

- Dr. Bruno Garcia Ferreira Universidade Federal do Rio de Janeiro (President of the 2<sup>nd</sup> SBG)
- Dr. Rosy Mary dos Santos Isaias Universidade Federal de Minas Gerais (President of the Scientific Commission of the 2<sup>nd</sup> SBG)
- Dr. Renê Gonçalves da Silva Carneiro Universidade Federal de Goiás (Vice-president of the Scientific Commission of the 2<sup>nd</sup> SBG)
- Dr. Valéria Cid Maia Museu Nacional / Universidade Federal do Rio de Janeiro
- Dr. Denis Coelho de Oliveira Universidade Federal de Uberlândia
- Dr. Marcelo Guerra Santos Universidade do Estado do Rio de Janeiro

#### Institucional support

- Universidade Federal do Rio de Janeiro (UFRJ)
  - o Instituto de Biologia
  - Botany Graduate Program at Museu Nacional
  - Zoology Graduate Program at Museu Nacional
- Universidade Federal de Goiás (UFG)
  - o Instituto de Ciências Biológicas
- Universidade Federal de Minas Gerais
  - Plant Biology Graduate Program
- Universidade Federal de Uberlândia
  - Ecology Graduate Program

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#### Organization of the online transmission and mediation of the event

Chapters (Iniciação Botânica):

• Ana Maria Abreu Santos

- Gabriel Barros da Silva
- João Victor Cerqueira Nunes
- Sabrina Vasconcelos Caram
- Wallyson Herbet da Silva

#### Artwork conception and execution

• Marcelo Tomé Kubo

#### Lecturers of the 2<sup>nd</sup> Symposium on The Biology of Galls

- Dr. Beryn Otienko Research scientist/Entomologist and Assistant regional Director at KEFRI – Kenya Forest Research Institute, Kenya.
- Dr. Daniel Burckhardt Volunteer Entomologist at the Naturhistorisches museum Basel, Switzerland.
- Dr. Jack Schltuz Senior Executive Director of Research Development -University of Toledo, Toledo, USA.
- Dr. Kevin Floate Agriculture and Agri-Food Canada, Alberta, Canada
- Dr. Christian Villagra Researcher in Entomology, Ecology and Evolutionary Biology - Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile.
- Dr. Adriano Cavalleri Adjunct Professor of the Departamento de Zoologia, FURG (Fundação Universidade Federal do Rio Grande), Brazil.
- Dr. Bruno Garcia Ferreira Adjunct Professor of the Departamento de Botânica do Instituto de Biologia, Universidade Federal do Rio de Janeiro, Brazil.
- Dr. Dalva Queiroz Researcher of the EMBRAPA Florestas
- Dr. Denis Coelho de Oliveira Associate Professor of the Instituto de Biologia, Universidade Federal de Uberlândia, Brazil. Researcher of the Research National Concil (CNPq, Brazil).

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- Dr. Gilson Rudinei Pires Moreira Full Professor of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Brazil. Researcher of the Research National Concil (CNPq, Brazil).
- Dr. Jarcilene Silva Almeida-Cortez Full Professor of the Departamento de Botânica, Universidade Federal Federal de Pernambuco, Brazil. Researcher of the Research National Concil (CNPq, Brazil).
- Dr. Marcelo Guerra Santos Associate Professor of the Departamento de Ciências, Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro, Brazil. Researcher of the Research National Concil (CNPq, Brazil).
- Dr. Renê Gomçalves da Silva Carneiro Adjunct Professor of the Departamento de Botânica, Universidade Federal de Goiás, Brazil.
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- Dr.Valéria Cid Maia Full Professor of the Departamento de Zoologia do Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.
   Researcher of the Research National Concil (CNPq, Brazil).

#### Presentations of the 2<sup>nd</sup> Symposium on The Biology of Galls

#### Day 1 - February 25th, 2021

 Opening Lecture: Gall-inducing Psylloidea (Hemiptera) in the light of recent phylogenetic and taxonomic studies (Dr. Daniel Burckhardt
 Entomologist, expert in Psyllidae biology and taxonomy, co-evolution of Psylloidea and their host plants, among other research topics)

#### • Session 1: Gall inducers

- Psyllid gallers (Hemiptera: Psylloidea) as plant pests (Dr. Dalva Luiz de Queiroz, EMBRAPA Florestas, Brazil, and Dr. Daniel Burckhardt, Natural History Museum - Basel, Switzerland)
- Cecidomyiidae: State of the Art in Brazil (Dr. Valeria Cid Maia (Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil)
- Thysanoptera and their galls (Dr. Adriano Cavalleri, Universidade Federal do Rio Grande, Brazil)
- Galls induced by cecidosid moths in the Neotropics (Dr. Gilson Rudinei Pires Moreira (Universidade Federal do Rio Grande do Sul, Brazil)

#### • Videoposters: Taxonomy and diversity of gall inducers

- Insect galls on Asteraceae in Brazil: richness and geographic distribution (Ismael Cividini Flor, Alene Ramos Rodrigues, Sharlene Ascendino Horacio da Silva, Barbara Proença do Nascimento, Valéria Cid Maia).
- Spatial and temporal distribution of galling species in *Copaifera langsdorffii* Desf.: a super-host plant (Stella Sposito, Maria Virginia Urso-Guimarães, Fernando Rodrigues da Silva)
- Checklist of the gall midges (Diptera, Cecidomyiidae) in the state of Bahia (Northeastern Brazil) (Bruno Gomes da Silva, Valéria Cid Maia)
- A new species of *Brethesiamyia* Maia (Diptera: Cecidomyiidae) from Colombia with description of immature forms (Esau Adenawer Ospina-Peñuela, Francisco Serna, Carlos José Einicker Lamas, Maria Virginia Urso-Guimarães)
- New State Record of Two Gall Midge Species (Diptera, Cecidomyiidae) In Brazil (Barbara Proença do Nascimento, Valéria Cid Maia)

#### Day 2 – February 26th, 2021

#### • Session 2 - Gall ecology and interactions

- Broad host acceptance for oviposition does not go well with *Leptocybe invasa* (Dr. Beryn Otieno, Kenya Forest Research Institute, Kenya)
- The curious case of *Nothotrioza* spp. associated to Psidium spp. In Brazil (Dr. Renê Gonçalves da Silva Carneiro, Universidade Federal de Goiás, Brazil)
- Semiochemical interactions between plants, cecidogenic insects and their associates (Dr. Cristian Villagra, Universidad Metropolitana de Ciencias de la Educación, Chile)

#### • Session 3: Galls: From ecological to molecular complexities

- A possible case of gall wasp speciation induced by endosymbiotic bacteria (Dr. Kevin Floate, Agriculture and Agri-Food Canada, Canada)
- Building networks out of galls: complex tritrophic interactions (Dr. Milton de Souza Mendonça Júnior, Universidade Federal do Rio Grande do Sul, Brazil)
- What transcriptomics can and cannot tell us about gall development (Dr. Jack Schultz, University of Toledo, USA)

#### • Videoposters: Gall distribution and cool science

- Latitudinal gradients in cynipid species diversity provides open niche opportunities for poleward range expansions (Dylan Jones, Kirsten Prior)
- Phenological synchronism strategies and abundance of five *Lopesia* galls (Cecidomyiidae) with the super-host *Mimosa* gemmulata (Fabaceae) (Elaine Cotrim Costa, Vitor Martini, Aline

Silva de Souza, José Pires Lemos Filho, Denis Coelho de Oliveira, Rosy Mary S. Isaias)

- My Gall, My Life a comic book (Pedro Henrique Pereira Gonçalves, Uiara Costa Rezende, Denis Coelho de Oliveira)
- The curious case of ants that live in galls: creativity toward scientific communication (Nina de Castro Jorge, Rosy Mary S. Isaias)
- Galls from inside out: didactic game (Rosy Mary S. Isaias, Elaine Cotrim Costa)

#### Day 3 – February 27th, 2021

#### • Videoposters: Gall structure and functioning

- Cell wall traits of galls in Caatinga environment (Ravena Malheiros Nogueira, Elaine Cotrim Costa, Rosy Mary S. Isaias)
- Woolly apple aphid stem galls (Mariana De Sousa Costa Freitas, Ravena Malheiros Nogueira, Edgard Augusto de Toledo Picoli, Rosy Mary S. Isaias)
- Nematode-induced galls show host-specific signatures in their structure (Igor Abba Arriola, Rosy Mary S. Isaias)
- Anatomical and histochemical implications of sexually dimorphic galls induced by *Eriogallococcus isaias* (Eriococcidae) on *Pseudobombax grandiflorum* (Malvaceae) (Ana Flávia De Melo Silva, Thiago Alves Magalhães, Denis Coelho de Oliveira, Rosy Mary S. Isaias)
- Could a sexual dimorphism of the gall inducing insect *Lopesia* mataybae (Cecidomyiidae) determine different morphotypes of galls in Matayba Guianensis (Sapindaceae)? (Pedro Henrique Pereira Gonçalves, Uiara Costa Rezende, Denis Coelho de Oliveira)
- Xylem differentiation controled by Cecidomyiidae on *Inga ingoides* (Fabaceae) (Gracielle Pereira Pimenta Bragança, Mariana De Sousa Costa Freitas, Rosy Mary S. Isaias)

#### • Session 4 - Multidisciplinary approaches in the study of galls

- Deciphering new gall models: our story with *Ditylenchus* (Dr. Bruno Garcia Ferreira, Universidade Federal do Rio de Janeiro, Brazil)
- Diversity of galls: dry Forest x rain Forest (Dr. Jarcilene Almeida Cortez, Universidade Federal de Pernambuco, Brazil)
- Galls as fascinating models in biological studies (Dr. Denis Coelho de Oliveira, Universidade Federal de Uberlândia, Brazil)

#### • Final lecture:

 Another brick in gall walls (Dr. Rosy M. S. Isaias, Universidade Federal de Minas Gerais, Brazil)



### SUMMARY



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#### ABSTRACTS - LECTURES



SPECIAL ARTICLE

### HISTORICAL REVIEW AND ACADEMIC INTERACTIONS RELATED TO PROGRESSES OF BRAZILIAN CECIDOLOGY

Jane Elizabeth Kraus (kraus@usp.br) Rosy Mary dos Santos Isaias (rosy@icb.ufmg.br) Renê Gonçalves da Silva Carneiro (renecarneiro@ufg.br)

Cecidology is the study of galls and comprise a multidisciplinary approach. The word cecidology is originated from the greek words *kēkis* (singular), *kēkidos* (plural), diminutive *kēkidion*, in new latin, the words are *cecidium* (singular) and *cecidia* (plural). The firt cecidologist to use the latin word *galla* (singular), *gallae* (plural) was the roman GAIUS PLINIUS SECUNDUS, Plinius the Elder (23–79 AD), who designated as *galla* the structure induced by a wasp (Cynipidae) in oak leaves (Quercus sp., Fagaceae), in his book *Naturalis Historia XXIV* (Meyer & Maresquele 1983, Fernandes & Martins 1985). Galls are structures formed by a host plant in response to the activity of different organisms, such as bacteria, fungi, nematodes, mites, and in most cases insects. These organisms cause deviations in the typical plant development patterns toward the formation of new plant structures. Galls may have bizarre shapes, different colors, and importance for medicine, food, ornaments, and commercial usages (Fagan 1918, Mani 1964, Berlin & Prance 1978, Fernandes & Martins 1985, Meyer 1987).

Galls have been known in China, India and Europe since ancient times, with the first mentions about galls going back to the Greek HIPPOCRATIS (460-

375 BC), the Father of Medicine, whose legacy are the manuscripts known as *Corpus Hippocratum*, and THEOPHRATUS (371–287 BC), the Father of Botany, with the books *Historia Plantarum* and *De Causis Plantarum* (Fagan 1918, Fernandes & Martins 1985). These historical books cited above constitute the most important contribution to the science from antiquity until the renaissance period (14th-16th century).

After the Renaissance, the English scientist ROBERT HOOK (1635-1703) made gall observations on a microscope and described them in his book *Micrographia* (1665). He conjectured that the leaf tissues grew surrounding the insect larvae in apple-oak galls. However, the first scientific investigation of galls is credited to the Italian physician and biologist MARCELLO MALPIGHI (1628-1694), who published his observations *De Gallis*, in 1679, in the book *Anatome Plantarum II* (Mani 1992). According to MALPIGHI, "the gall formation arose from an emanation of the animal, which stimulates the behavior of the cells of the affected plant" (Krikorian 1984). Based on its accurate observations, MALPIGHI has proposed an explanation to the process of gall development, and he "made" science on this issue. In a scientific work, the most important part is the analysis and the interpretation of the results toward a valuable discussion; the discussion is the soul of the research.

However, it was only from the beginning of the 20th century that Cecidology established itself as an important area of scientific knowledge. ALESSANDRO TROTTER (1874-1967), an Italian botanist and entomologist, is responsible for the great merit of establishing Cecidology as a field of biological research. He started publishing the specialized journal *Marcellia*, a name given in honor to MARCELLO MALPIGHI, in 1902. TROTTER described about 742 galls, many caused by new species of Cecidomyiidae and Cynipidae. During the same period, in 1900, the German botanist ERNEST KÜSTER (1874-1953) defended his thesis entitled *Beiträge zur Anatomie der Gallen* (Contributions to the Anatomy of Galls). KÜSTER is the author of several books on plant anatomy, including *Die Gallen der Pflanzen: ein Lehrbuch fur Botaniker und Entomologen* (The Plant Galls: A Textbook for Botanists and Entomologists), published in 1911 (reprinted in 2010). He wrote another important book, *Anatomie der Gallen*  (Anatomy of Galls) published in 1930. KÜSTER was the editor of the *Zeitschrift für Wissenschaftliche Mikroskopie* (Journal for Scientific Microscopy) for almost 50 years.

Advances in the study of galls are conspicuous from the middle of the 20th century, when the Indians have special merits. The main reference is the illustrious, zoologist-entomologist, MAHADEVA SUBRA (MANYA) MANI (1908-2003), author of many books, highlighting the Ecology of Plant Galls, a *Magnum Opus*, the basis of the studies of galls, first published in 1964. Due to its importance, this book was reprinted in 2013. Another relevant of MANI's books was The Plant Galls of India, whose first edition is from 1973 and the second from 2000. MANI obtained the Master degree awarded by the University of Madras (Madras) and the Doctor degree by the Agra University (Agra), now called Dr Bhimrao Ambedkar University. He worked at the St. John's College (Dr Bhimrao Ambedkar University), and later at the Presidency College in Madras, both in India. An important point to emphasize on the reprinting of these famous and basic books is that they are important not only for his historical value, but because they provide important basic information, and help us avoiding to "reinvent the wheel".

The 1970 to 1990s were also important for the expansion of several areas of Cecidology, whether under the entomological, ecological, anatomical, chemical, and other scientific areas. Only some researchers will be mentioned to exemplify the range of scientists, which is much larger. In the 1970s, the evolutionary ecology of galls flourished under the guidance of PETER WILFRED PRICE, English, who completed the master's degree in Canada and the doctorate in the USA, at The Cornell University, Ithaca (USA). In 1980, PRICE went to Northern Arizona University, Flagstaff (USA), where he stayed up to his retiring in 2002. His teaching concentrated on insect ecology, entomology, and biological evolution. PRICE has numerous important publications: PRICE (1988), FERNANDES and PRICE (1988), PRICE et al. (1986, 1987, 1998) etcetera. His book entitled Insect Ecology (1975, 1984, 1997), now in the third edition, is a *Magnum Opus*. It is the world's foremost reference to the never-ending and crucial interactions of the insects, the richest taxon of organisms on the Earth,

with plants. He also published other books: Evolutionary Biology of Parasites (1980), Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions (Price et al. 1991), Biological Evolution (1995), among others.

Following a temporal line, TARACAD NARAYANAN ANANTHAKRISHNAN (1925-2015) was another important Indian entomologist and chemical ecologist of galling insects, especially in the order Thysanoptera. He completed Master and Doctorate degrees, both in the University of Madras. He founded the Entomology Research Institute at Loyola College, campus Chennai, India. He published several books, including Biology of Insect Galls (1985), Chemical Ecology in Thrips-Host Plant Interactions (1993) in collaboration with R. GOPICHANDRA; Biocommunication in Insects (1998) with A. SEN, etcetera. He had a great influence of Professor Mani, during his the time at the Presidency College.

Another contemporary cecidologist is ANATANARAYANAN RAMAN, an Indian biology educator and researcher. His achievements include research in Cecidology and tertiary education. Raman got his Master of Science by the Presidency College, and his Doctorate of Philosophy by the Loyola University at the University of Madras (1981). He is also a Doctor in Science by the University of Madras (2004). RAMAN published several books, many as co-author. Thrips and Gall Dynamics (1989) and Chemical Ecology of Phytophagous Insects (1993) with ANANTHAKRISHNAN (mentioned above), and currently works at the School of Charles Sturt University, Orange, Australia.

Following a temporal line, it is worth mentioning the publications of RAYMOND J. GAGNÉ, American specialist in Cecidomyiidae (Diptera), a family of flies known as gall midges. He works at the Systematic Entomology Laboratory, Agricultural Research Service of the Smithsonian Institute, Washington, D.C., USA. His books The Plant-Feeding Gall Midges of North America (1989), and The Gall Midges of the Neotropical Region (1994) are compendia of the current knowledge on the biology, recognition, and literature of plant-associated cecidomyiids of North America and Neotropical region.

Under the Plant Anatomy (histology) focus, the book *Anatomie des galles* (Anatomy of Galls), published in 1983, authored by JEAN MEYER and HENRI JEAN MARESQUELLE (1898-1977), is also a *Magnum Opus*. It includes a detailed study of the anatomy and development of plant galls. The numerous examples show how variable gall shapes are, and how the plant tissues undergo changes during gall development. Although with extensive production, the examples, for the most part, do not represent models for the neotropical region. The authors worked at the *Laboratoire de Cecidologie of the Université de Strasbourg* (Laboratory of Cecidology of Strasbourg University), Strasbourg (France). Several important researchers were part of this laboratory group: ODETTE ROHFRITSCH, ROBERTE BRONNER, FRANÇOISE DREGERJAUFFRET and EVELYNE WESTPHAL. MEYER is also the author of the Plant Galls and Gall Inducers, published in 1987.

In the 1990s, JOSEPH D. SHORTHOUSE from Laurentian University (Sudbury, Canada) and ODETTE ROHFRITSCH (mentioned above) published the book Biology of Insect-Induced Galls (1992), also a particularly important reference. This book provides comprehensive coverage of gall biology and its complex etiology. Several researchers wrote specific topics in plant-insect relationships; some collaborators belonged to the *Laboratoire de Cecidologie of the Université de Strasbourg*. For most of his research career, SHORTHOUSE has concentrated on the ecology, distribution and development of plant galls induced by cynipid wasps of the genus *Diplolepis* found on wild and introduced roses, genus *Rosa*.

At the beginning of the 21st century, the literature on galls flourished again. The researches range from studies on classical biology, anatomy of plant galls to immunocytochemistry, systematics of galling organisms to molecular phylogeny, populations, ecological and evolutionary gall theories, and genetics to molecular biology. Some books are basic and put on greater emphasis on the biology, behavior, and evolution of the gall-inducing arthropod and of associated organisms. For examples: Biology, Ecology, and Evolution of Gall-Inducing Arthropods (Raman et al. 2005); Biological Control of Tropical Weeds Using Arthropods (Rangaswamy Muniappan et al. 2009); Insects and Plant Defense

Dynamics (Ananthakrishnan 2001); Arthropods of Canadian Grasslands: Ecology and Interactions in Grasslands Habits (Shorthouse and Floate 2010). The book Insect Ecology: Behavior, Populations and Communities (Price et al. 2011) gives a logical and cohesive introduction to insect ecology concepts with a broad range of examples and practical applications. The Biology of Gall-Inducing Arthropods (Csóka et al. 2012) is the result of an International Symposium (1997, Hungary), and explores many facets of the ever intriguing and enigmatic relationships between plants and their gall-forming herbivores. The important Catalog of the Cecidomyiidae (Diptera) of the World by R.J. GAGNÉ and MATHIAS JASCHHOF (2017), fourth edition, registers about 6,203 known species of Cecidomyiidae, either living or fossil.

Knowing that Brazil possesses the richest flora of the world with about 40,000 species (Forzza et al. 2012) and the number of galling species is estimated about 132.000 species on the planet (Espírito-Santo & Fernandes 2007), attention should be paid to the galls that represent the interaction with these organisms. The Neotropical region, including Brazil, is known to have a great richness and diversity of species. The Neotropical region is defined as southern North America, Central America, Carribean Islands and South America (Udvardy 1975). Each ecosystem has specific characteristics that makes it unique. Hence, it is necessary to know the environmental characteristics and species in the diverse ecosystems in order to understand and preserve natural biodiversity.

The first studies on Brazilian galls were made by the Portuguese Jesuit priest JOAQUIM DA SILVA TAVARES (1866-1931), an entomologist, who surveyed the galls of the Atlantic Forest, where he was exiled from 1910 to 1913. In his studies, however, he mostly came to the level of plant families, due to the lack of specialists in the country at that time. TAVARES sent material to the American entomologist EPHRAIM PORTER FELT, and the French botanist CLODOMIR ANTONY VINCENT HOUARD (both mentioned below). Part of TAVARES collection is currently in Germany, at the *Staatliches Museum für Naturkunde Stuttgart* (State Museum of Natural History Stuttgart), according to GAGNÉ (1994). TAVARES was one of the founders of the Portuguese-Brazilian

journal *Brotéria - Revista de Sciencias Naturaes* (1902-2002), together with Cândido Azevedo Mendes (1874-1943) and Carlos Zimmermann (1871-1950), a qualified journal among Portuguese scientific publications during the past century. Most of TAVARES researches was published in *Brotéria* (TAVARES 1906, 1909, 1915, 1916a, b, 1917a, b, 1918, 1920b, 1921, 1922, 1925), and just one paper was published in *Marcellia* (1920a).

EWALD HEINRICH RÜBSAAMEN (1857-1919), German entomologist, also studied some species of Brazilian insect galls (RÜBSAAMEN 1907, 1908). As above-mentioned, FELT and HOUARD received samples of Brazilian insect galls. FELT (1868-1943), American entomologist, studied at Cornell University, USA, Ithaca, and worked mostly with Nematocera, particularly Cecidomyiidae. He wrote the book Plant Galls and Gall Makers (1940) and described about 1,000 species of gall midges. His collection is at the National Museum of Natural History, Washington D.C., USA. HOUARD (1873-1943), French botanist, worked at the Université de Strasbourg and is the author of several works with galls, notably the book Les Zoocécidies des Plantes de l'Amérique du Sud et de l'Amérique Central, 1933 (Plant Zoocecidia of South and Central America). His extensive collection of galls is preserved in the Muséum National d'Histoire Naturelle (National Museum of Natural History), in Paris (France).

In the 30s, 20th century, in Rio de Janeiro, the botanist, pathologist, FERNANDO ROMANO MILANEZ (1905-1987) carried out the first anatomical study on galls. He described a gall on guava trees (*Psidium guajava* L., Myrtaceae), published in the first issue of *Rodriguésia, Revista do Jardim Botânico do Rio de Janeiro*, in 1935. Subsequently, two unpublished doctoral theses are mentioned in the literature: the first one authored by J.A. GUIMARÃES (1957) that described in few details some leaf galls on *Copaifera langsdorffii* Desf. (Leguminosae) and *Anemopaegna mirandum* (Bignoniaceae), now *Anemopaegna arvense* 

(Vell.) Stellfeld ex de Souza, in Rio de Janeiro; the second one authored by J.P. ALVARENGA (1959) that described a stem gall on *Vernonia polyanthes* (Sprend.) Less. (Asteraceae), from Minas Gerais. Later, in the 1980s PAULO

OCCHIONI (1979, 1981) made a descriptive survey of 110 types of galls. The galls were collected in different vegetation types, distributed in 34 families of phanerogamous plants and one in a pteridophyte. However, there is no identification of the inducers and the plants are in most cases identified only to the family level.

It is in the mid-1980s that Brazilian cecidology gains a new stimulus, highlighting the ecological works developed at the Universidade Federal de Minas Gerais (Federal University of Minas Gerais), Belo Horizonte, by the mentor GERALDO WILSON FERNANDES. He did his master's and doctorate (1987, 1992, respectively) at Northern Arizona University, Flagstaff, under the guidance of PETER W. PRICE, and later post-doctorate at Stanford University, Palo Alto (both in USA). Its numerous scientific publications (460 or more) are of indisputable importance. However, it is relevant to mention the publication on the journal Ciência Hoje (1985), in which the range of gall studies is shown in a didactic way for people and students interested in the theme. The book chapter Insetos Galhadores (Galling Insects) is also very didactic (FERNANDES and CARNEIRO 2009). FERNANDES published several books with emphasis on the entitled Neotropical Insect Galls (2014), edited in collaboration with JEAN CARLOS SANTOS. The chapters were written by several researchers and reported the reaction of plant cells to the galling insects, their natural enemies, diversity and ecological evolution on these special taxa on the Neotropics. A Handbook of Sampling Methods (2021), again in collaboration with SANTOS, aims to be a reference for zoologists, entomologists, ecologists, cecidologists, botanists, students, researchers, and for those interested in arthropod science and biodiversity. It is worth mentioning that the group of researchers led by FERNANDES is the largest in the country in the area of gall ecology and evolutionary biology. The approach taken in different Brazilian biomes has brought up the knowledge on part of our biodiversity (e.g. Fernandes et al. 1988; Lara & Fernandes 1996; Price et al. 1998; Maia & Fernandes 2004). An overview of the inventories of gall-inducing insects in Brazil was made by ARAUJO et al. (2019).

It is also in the mid-1980's, that gall studies began under the anatomical approach in Brazil. The first doctoral thesis focusing on morphological, histological and ontogenetical aspects of galls was carried out by LÉA DE JESUS NEVES (1987), under the guidance of BERTA LANGE DE MORRETES, at the *Universidade de São Paulo* (São Paulo University), São Paulo. Two types of galls were studied in *Ficus tomentella* Miq. (Moraceae), one induced by Diptera and the other by Hymenoptera. However, MORRETES did not pursued this research line.

In that same period, mid-1980s, JANE ELIZABETH KRAUS and MARGARIDA VENTURELLI (1942-1989) resumed anatomical studies on galls. KRAUS obtained her master's and doctoral degrees in Botany at the Universidade de São Paulo (1981, 1986, respectively), and initially worked with plant tissue culture in this institution. As VENTURELLI was specialist in Struthanthus vulgaris L. (Loranthaceae), a hemi-parasitic plant that hosts the galls studied on anatomical basis for the first time (Arduin et al. 1989). From the 90s onwards, KRAUS defined anatomical studies on galls as a research line, with focus on galls in the Neotropical region. She retired in 2002, but continued to contribute as she published some papers, and book chapters, with emphasis on the chapter entitled Galhas: morfogênese, relações ecológicas e importância econômica (Galls: Morphogenesis, Ecological Relationships and Economic Importance) in Interações Ecológicas & Biodiversidade (Ecological Interactions & Biodiversity) edited by TISSOT-SQUALLI (2009), a basic introduction for the study of morphogenesis in galls. Two other book chapters were published by KRAUS as a co-author with ISAIAS et al. (2014a, 2017). The most commented paper shows the distribution of metabolites on galled and non-galled leaf tissues of Tibouchina pulchra (Cham.) Cogn. (Melastomataceae) (Motta et al. 2005). However, the root galls of Cattleya guttata Lindt, Orchidaceae (Kraus & Makoto 1999; Kraus et al. 2004) are an interesting model because galls in aerial root are uncommon.

In 2000, also at the *Universidade Federal de Minas Gerais*, a group is formed for morphological, anatomical, histochemical, physiological and chemical studies on galls, having as mentor ROSY MARY DOS SANTOS ISAIAS, who became an exponent in the area. She did her master's degree at the Museu Nacional / Universidade Federal do Rio de Janeiro (National Museum of Federal University of Rio de Janeiro), under the guidance of LEA J. NEVES and her doctorate (1998), at the Universidade de São Paulo, advised by JANE E. KRAUS. ISAIAS has numerous published papers (more than 120), highlighting the Illustrated and Annotated Checklist of Brazilian Gall Morphotypes (Isaias et al. 2013), published in the journal Neotropical Entomology as her most cited paper. It is also worth mentioning her collaboration with international research groups by invitation (Martin J. Steinbauer, of the La Trobe University, Melbourne, Australia), that recognized her research (Isaias et al. 2018). She is also the co-author of several book chapters with emphasis on those dealing with gall morphotypes and the developmental anatomy of galls in the Neotropics (Isaias et al. 2014a, b) published in the book Neotropical Insect Galls (Fernandes & Santos 2014). ISAIAS has also contributed to the book coordinated by MELO JÚNIOR and BOEGER (2019), entitled Patrimônio Natural, Cultura and Biodiversidade da Restinga do Parque Estadual Acaraí (Natural Heritage, Culture and Biodiversity of Coastal Thicket of Acaraí State Park), located in Santa Catarina, Brazil (Isaias et al. 2017). ISAIAS is also the co-author of a guide to Brazilian insect galls - Guia de Galhas de Insetos de Caetité, Bahia, Brasil (Guide to Insect Galls from Caetité, State of Bahia, Brazil) published recently (Silva et al. 2020).

Concomitantly, at the beginning of the 21st century, the studies on galling insects, with emphasis on Cecidomyiidae (Diptera), were established by VALÉRIA CID MAIA. Her first contact with galls was during the under-graduation training under the guidance of RICARDO FERREIRA MONTEIRO, at *the Universidade Federal do Rio de Janeiro*, Rio de Janeiro. She obtained her master's and doctorate degrees (1994, 1999, respectively) in Zoology, at the same university, under the guidance of MÁRCIA SOUTO COURI. During her doctoral studies, she maintained contact and training with RAYMOND J. GAGNÉ, mentioned above, specialist in Cecidomyiidae. Later, she completed a post-doctorate at the *Universidade Federal de Minas Gerais*. MAIA develops research in taxonomy and ecology of galling insects (Cecidomyiidae, Diptera), with about 150 publications. She works at the *Museu Nacional / Universidade Federal do* 

*Rio de Janeiro*. Among numerous works, it is worth highlighting those that report Restinga galls (Maia 2001a, b) and the survey of galling insects carried out in the *Parque Nacional de Itatiaia* (Itatiaia National Park, State of Rio de Janeiro), where she observed a great diversity of gall morphotypes (Maia & Mascarenhas 2017). Maia has also contributed with chapters in the books *Biodiversidad de Artrópodos Argentinos* (Maia 2014) and Neotropical Insect Galls (Fernandes & Santos 2014).

It is important to mention the paper "30 years of research on insect galls in Brazil; a scientometric review" (Araújo 2018), a revision about gall scientific production. This revision shows that the most common topic studied was ecology, followed by inventory and taxonomy, which together represent 82,41%, and anatomy, revision, agriculture and genetic represent the remaining 17,59%. Unfortunately, most anatomical (histological) works have not been accounted for, giving a false idea of the research in this area. ARAÚJO also forgot that the studies on galls began, and they will be continued with botanists! In order to maintain and preserve natural environments, basic studies on the biology of the species under the focus of different areas of the biological knowledge are extremely important and constitute the basis for scientific information.

This brief historical tour of Cecidology demonstrates that there is an intricate network of relationships, which enables the advance of the knowledge on galls. Entomological studies are developed mainly at the *Museu Nacional / Universidade Federal do Rio de Janeiro*. It is also worth emphasizing that, currently, the largest and most important center of gall studies in Brazil is at the *Universidade Federal de Minas Gerais*. Morphological, Anatomical, Histochemical and Ecological Studies are the main research lines. As a training center of excellence, the disciples have been creating new groups in other universities, expanding the knowledge network.

As biologists, our fascination with galls as models of study comes from the interactions of different biological areas of the knowledge. To finish, we would like to tell the students that in the scientific studies it is necessary to interact with diverse specialists. This is enriching both from the scientific and the human point of views.

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#### LECTURE - BIOLOGY OF GALLS

#### A POSSIBLE CASE OF GALL WASP SPECIATION INDUCED BY ENDOSYMBIOTIC BACTERIA

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Aulacidea pilosellae (Hymenoptera: Cynipidae) is a European species of gallforming wasp that is being studied as a potential biocontrol agent for release in North America against invasive species of hawkweed (Pilosella spp.). Some field populations of this wasp occur only on P. officinarum, whereas other cooccurring populations develop on several Pilosella spp. excluding P. officinarum. Molecular analyses (COI gene) indicate that these two hostaffiliated types are not cryptic species, but rather are biotypes; i.e., Biotype 1 = ex. Pilosella spp., Biotype 2 = ex. P. officinarum. Our subsequent laboratory studies show that Biotype 1 is univoltine (obligate diapause) and bisexual (males and females). In contrast, Biotype 2 is multivoltine (facultative diapause) and parthenogenetic (no males). Biotype 1 females produce about 30% more eggs than do females of Biotype 2. Biotype 1 also has a shorter, more defined, period of adult emergence than does Biotype 2. Molecular screening identifies infections of Wolbachia bacteria in Biotype 2 individuals, but generally not in individuals. Biotype 1 These maternally-inherited bacteria induce parthenogenesis in other species of wasps. We speculate that Wolbachia has induced parthenogenesis in a subset of A. pilosellae populations leading to genetic divergence and subsequent effects on host-range, voltinism and other biological traits. If so, this study system may be an illustration of symbiontinduced incipient speciation.



#### LECTURE - BIOLOGY OF GALLS

#### ANOTHER BRICK IN GALL WALLS

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All living organisms are composed of cells, as states the cell theory. The cells are elementary units both of plant and gall bodies, like the bricks are the elementary units of the walls of human constructions. The investigation of the dynamics of cell wall components help us understand the cascade of events involved in gall development from the host plant metabolism perspective. The mystery involving this cascade of events has intrigued many cecidologists, and such investigation guides one of the lines of research pursued by the Neotropical gall group. The host plant-galling herbivore interactions have been interpreted as true efforts to confer protection and nutrition and keep the galling organism alive. Moreover, from the plant metabolism perspective, all the efforts to keep the galling organism alive demands keeping plant cells alive, as well. The attempt of using galls as micro-laboratories to investigate plant reactions at the cellular level has revealed important pieces to solve the great puzzle of the triggering and orchestration of gall development. We address plant responses involved in such processes by five lines of research on galls in the Brazilian flora: morphology, histology, cytology, histochemistry, and immunocytochemistry.
Our first premise is that the accumulation of each molecule in gall developmental sites indicates a response of plant cells toward the alien stimuli, but in an attempt to avoid imbalanced homeostasis and to keep their metabolic viability. The quite common histochemical detection of phenolics in gall induction sites, usually interpreted as a chemical defense against natural enemies, from the plant perspective may be interpreted as the reaction toward scavenging the excess of oxidants. The overgeneration of reactive oxygen species (ROS) due to the direct contact of galling organisms with plant tissues can be detected in two cell compartments: the symplast and the apoplast. The biochemical and biophysical reactions in the inner side of the plasma membrane and in cell walls lead to cell redifferentiation, and new tissue layer organization. The peculiar orchestration of the cell and tissue responses in each host plant-galling herbivore system culminates in the development of the variety of gall morphotypes. The ROS have been histochemically detected in nutritive cells, vascular bundles, and photosynthetic parenchyma, and the scavenging of ROS molecules performed by the phenolics modulates cell reprogramming. The co-occurrence of ROS, phenolics, and indol-3-acetic acid (IAA) in cells of some super-hosts of galling herbivores has been interpreted as evidence of the involvement of these compounds in the coordinated control of gall growth. Hence, the accumulation of phenolics as a metabolic strategy to scavenge de excess of ROS results in the blockage of IAA-oxidases, which results in the increase in IAA concentration in gall developmental sites. This molecular interaction favors the occurrence of the acid growth and consequently, of cell hypertrophy. The overlapping of ROS, IAA and phenolics in specific tissue compartments contributes to the generation of the specific gall morphotypes as their detection relates to specific spatial and temporal dynamics. As this premise solves the mystery of signaling toward cell hypertrophy, the missing brick regards the increment in cell division, the hyperplasia. Suck mystery has been investigated by histochemical and immunocytochemical detection both of IAA and cytokinins (CKs) and has been elegantly demonstrated in gall morphotypes associated to super-host plants. Curiously, gall tissues where IAAs and phenolics co-occur have higher degrees of cell hypertrophy, while sites CKs accumulation have smaller cells, as indicative of increased cell divisions, i.e., hyperplasia. The CKs were detected in tissue compartments distinct from those where IAA and phenolics occurred in galls in growth and development stage. The mapping of the phytohormones in spatial and temporal scales on distinct gall morphotypes associated to super-host plants corroborates our hypotheses on the roles of IAAs, phenolics and CKs, and help

setting lights on the mechanisms of gall shape determination. In addition, the histochemical localization both of IAA and CKs were confirmed with the use of polyclonal antibodies.

The variety of gall morphotypes observed in nature involve not only events of cell hypertrophy and tissue hyperplasia, but also alterations in subcellular level. The dynamics of cell wall components along gall developmental stages have been studied to understand how ordinary conditions of plant cells are changed toward new structural and functional profiles. Immunocytochemical studies have assessed cell wall components of galls induced by insects, mites, and nematodes. The main component of plant cell walls, the cellulose, and the remodeling of its microfibrils have been demonstrated by the calcofluor white fluorescence, and as expected confirmed that alterations in shapes demands adjustments in the isotropic and anisotropic patterns of cell growth. We have been testing if the predominance of isotropic elongation of cell axis may relate to the development of isodiametric shapes, such as the globoid morphotypes. In fact, the patterns of cell elongation vary across the different tissue compartments, i.e., the cell layers with specific structural and functional traits, and regarding gall developmental stages, even when similar gall morphotypes are compared. For the uncommon and bizarre shapes, the predominance of anisotropic elongation in cell axis in specific tissue layers is expected, and the spatial dynamics of isotropic and anisotropic elongations may explain each specific gall shape.

Together with the cellulose, hemicelluloses also have functional traits other than the ordinary role of anchorage of cellulose microfibrils in gall developmental sites. These cell wall components have contributed to the increase in resistance of secondary cell walls from young gall stages toward gall maturation, as is the case of the xylans labeled by the monoclonal antibody LM10 in cell walls of the mechanical gall tissues. Other two types of hemicelluloses, the xyloglucans and the heteromannans, have been labeled in nutritive cell walls, where their functional role has been accredited to the nutritional support to the galling herbivores. These hemicelluloses are additional carbohydrate reserves, that together with reducing sugars, lipids and proteins, may be assessed by the inducers according to their specific modes of feeding. Finally, the dynamics of pectins in galls induced by Diptera: Cecidomyiidae and Hemiptera: Psylloidea has indicated higher rigidity along gall maturation and higher porosity from the outer toward the inner tissue compartment. The cell wall porosity ensures a transferring pathway for the nutritional molecules through the apoplast of the outer tissue compartment cells toward the nutritive cells. Regarding the proteins, the functions performed by the arabinogalactan-proteins (AGPs) are still controversial as their labeling is not constant either in temporal or spatial scale in gall developmental sites. The AGPs are associated not only with the cell walls but also with the plasma membrane and the intercellular spaces, and their ordinary functional roles rely on the contribution to cell divisions and expansion. The AGPs may also be important in blocking processes of cell death that would interrupt gall development. The extensins, another class of proteins we have been investigating, should be labeled at the end of cell expansion as their classical role is determining cell final shape, but in our gall models of study, extensins have been labeled by LM1 in young galls, indicating that the cytological compartments respond peculiarly in specific host plant-galling herbivores systems.

In the ultrastructural perspective, plant cells of galls induced by insects, mites, and nematodes respond to the over accumulation of ROS by producing plastoglobules. The plastoglobules contain, among other proteins, tocopherol cyclase, a key enzyme on the biosynthesis of tocopherols, which protect the thylakoid membranes and the proteins related to photosynthesis from oxidative damage caused by the ROS. The plastoglobules are linked to each other and to the membranes of the thylakoids, which allow the exchange of lipophilic molecules between the plastoglobules. The lipophilic molecules serve as electron corridors, and protect the photosynthetic apparatus from damage caused by free radicals. Other cytological evidence of plant cell efforts to survive the impact of gall induction and development is the differentiation of multivesicular bodies, lamellar bodies, and lomassomes. The multivesicular bodies were observed in fast-dividing cells of lepidopteran galls, and are responsible for the rebuilding of the plasma membranes in meristem-like cells. lamellar bodies and lomassomes, together with multivesicular Additionally, bodies, were reported for nutritive cells of galls induced by different insect orders. The oxidative stress confirmed by the detection of ROS in nutritive cells may be compensated by the activity of the lomassomes, lamellar bodies, and multivesicular bodies, which help the endoplasmic reticulum in recycling the endomembrane systems.

In summary, our knowledge on plant structure, physiology and chemistry has been used to assemble the pieces of the great puzzle regarding the variety of gall developmental pathways toward the variety of morphotypes. Moreover, we try to understand plant potentialities to deal with the strong stress of the interaction with galling herbivores and keep their cells and tissues alive and metabolic viable. In fact, "all in all these are just bricks in the gall walls".

# BUILDING NETWORKS OUT OF GALLS: COMPLEX TRITROPHIC INTERACTIONS

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Ecological interaction networks are representations of communities that allow us to visualise not only richness but also interactions between species and ultimately community structure. From observing which interactions occur and which species actually interact on a network, we can discern ecological factors determining how communities are. Adopting a functional overview of interactions can also lead to much insight on potentially forbidden interactions limiting connectivity. Further, network topology can reveal about community stability and even coevolution patterns shaping the organisms involved.

Galls can represent a treasure trove for ecological network construction, a consistent, identifiable and culturable plant tissue structure within which at least three trophic levels could be found, not to mention associated mutualistic fungi and microorganisms, successors and even other interactions. Although our feeble taxonomic knowledge on gallers and wasp parasitoids can be a serious restriction in some cases, thoughtful morphospeciation and/or current genomic methods can partially solve this problem. Building networks with gall systems can thus help deepen our understanding of biological communities, their assembly and dynamics in time and space. Although gallers are quite specific,

there is still much to learn from how this specificity affects the network resulting from these interactions.

To illustrate the usefulness of gall systems to understand networks, we explored three examples differing in scope and method. One deals with a single host plant and a range of gallers and their parasitoids, in which we looked for the effects of gall structure on the network through gall-parasitoid functional coupling. Another one compares oligophagous and monophagous galler species in two phylogenetically close host plants, to check whether plant identity can change galler-parasitoid network structure and how this could impact coevolution after recent plant cladogenesis. Finally, we considered all galls and all parasitoids found in areas under restoration compared to native vegetation to assess the effect of restoring vegetation to higher-order trophic levels. All work was done in the subtropical forests of the northern part of southern Brazilian State of Rio Grande do Sul. Our general hypothesis is that galler-parasitoid networks will be more strongly connected, less modular, etc, in systems in which fewer plants are sampled, and the reverse for cases of many plants considered together.

In the first case (Luz, Goetz & Mendonça 2020, Ecol. Entomol.) we used a single host plant, Guapira opposita (Nyctaginaceae), and six Cecidomyiidae species from subtropical forests of Porto Alegre. From 1762 galls sampled, 741 parasitoids emerged (in 45 species, 60 unique interactions). The resulting bipartite network showed intermediate specialisation levels (H2' = 0.55) and low connectance (21%). We tested for ecological processes that could predict network structure, such as neutral ones (abundance), spatial, temporal or morphological coupling factors. Gall-parasitoid morphological coupling (simply comparing gall thickness to parasitoid ovipositor length for each species) explained network structure best, and thus forbidden interactions seem to determine interactions in this system. Thinner galls had higher parasitism rates and more attacking parasitoid species, implying an important adaptive role for gall shape/size in natural enemy interactions. There were two network modules (Q = 0.20), one with a single galler (the largest, thickest and hardest one among those sampled), and all its specific parasitoids, those with comparatively longer ovipositors.

Second, structure and topology of a bipartite interaction network of galling insects and associated parasitoids was studied for two closely related and recently diverged host plants - Mikania laevigata and M. glomerata (Asteraceae)(Prauchner & Mendonça, submitted). We tested for the effect of

three clustering factors: gall structure, galler phylogenetic proximity and host plant identity, on network structure, especially modularity. We sampled 4629 galls of three monophagous and four oligophagous galling species; 664 parasitoids were obtained, belonging to 37 morphospecies. Connectance was relatively higher (27.4%). All networks formed given the clustering factors were significantly modular, although not highly so (Q = 0.4). Parasitoid specificity was again intermediate but the higher modularity contrasts with what is known for other galler-parasitoid systems. We explored the delayed host-tracking hypothesis as a possible model to explain the incipient speciation expected of gallers from diverging hosts, but warning that top-down effects (from parasitoids), in a tritrophic view, need to be taken into account.

In our last study case, areas under active restoration were compared to reference areas for tritrophic networks including host plants, gallers and parasitoids (Goetz et al. 2018, Iheringia; Goetz, Luz & Mendonça, in prep.). We totaled 4132 sampled galls, belonging to 89 morphotypes on 46 host plant species; however, only from 26 gall morphotypes we obtained parasitoids. Parasitoids comprised 1175 individual wasps distributed in 62 morphospecies, with final identifications still underway. Also because of this, we here present only preliminary analyses of galler-parasitoid networks. Connectance was very low (6.3%), and specialisation also very high (H2' = 0.77). When comparing environments, even still without resorting to statistics, neither connectance (C), specialisation (H<sup>2'</sup>), number of modules or robustness to extinctions seem to be different. Thus, we conclude that the restoration attempt was successful in terms of this specific fauna.

These low levels of connectance are not unheard of in terms of other trophic (antagonistic) webs, but it is noteworthy in comparison with the higher connectances for the above single-host and double-host networks. Future analyses of topology, such as modularity, might yet reveal further discrepancies between multi-host vs. single-host situations. (Supporting information: CNPq, CAPES).

### CECIDOMYIIDAE (DIPTERA): STATE OF ART IN BRAZIL

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The family Cecidomyiidae (Diptera, Insecta) is very diverse, with more than 6,500 described species, commonly known as "gall midges", since most species are gall-inducers. Only 500 species occur in the Neotropical region, which represents less than 10% of the global diversity, and 93% of them are known only from this region.

Brazil comprehends about 50% of the Neotropical gall midge richness, being the most studied fauna, with a few fungivorous, inquilinous, predaceous and free-living species, and more than 200 gall-inducers. The gall-inducing species are associated with about 50 plant families, Fabaceae, Asteraceae and Myrtaceae comprising 37% of them. These families are among the most speciose in Brazil, adding evidences too favor the plant size hypothesis.

Concerning plant origin, 152 species of gall midges occur on native plants, five on exotic and one on a naturalized species. The native hosts comprise 50 endemic species to Brazil, 29, 4, and 1 of them being endemic to the Atlantic forest, Cerrado, and Amazon. This is a very relevant information due to the species-specificity of the gall-inducers. Based on their specificity, cecidomyiids exclusively on endemic hosts can be considered co-endemic. These data are valuable and should be used in the establishment of environmental preservation areas and conservation programs. Most inducers (n=181) are known from the Atlantic forest, 33 from Cerrado, 18 from Amazon, 11 from Pampa, three from Caatinga, and two from Pantanal. However, Brazilian inventories indicate that Amazon comprehends the greatest diversity of gall-inducing species, but none of them has been identified. As Amazon is the richest Brazilian phytogeography domain in number of plant species, we could expect that it comprises the greatest gall midge richness.

The Southeast region concentrates most records, especially the Rio de Janeiro state, with 115 species. These data reflect the greatest amount of taxonomic studies in Atlantic forest areas and in the state of Rio de Janeiro. Furthermore, about 70% of the gall-inducers are known only from the type-locality. Analyzing the geographic distribution of host plants in Brazil, we can realize that all of them have a wider distribution than that of their inducers. This scenario reflects the lack of collections. In fact, in Brazil there are many states with few or no records of cecidomyiids. However, the current state of art can be improved through collections in non-surveyed or little surveyed localities, as well as through galled exsiccates examination, since gall-inducing species can be identified based on gall morphology and host plant identification. It is interesting to add that exsiccates examination can be performed by herbaria visitation or virtually, using digital platforms.

In all Brazilian inventories, most gall-inducing cecidomyiids are identified in family, due to the high number of undescribed species and low number of taxonomists. Immature phases (larva and pupa) and adults of both sexes (male and female) are necessary to identify gall midge species. It happens because, in the past, many species were described based on a single stage of the life cycle, resulting in species known only from larva, others known only from male and so on. These incomplete descriptions result in species morphologically not comparable. Nowadays, most cecidologists agree to describe species only when larva, pupa, male and, female are available, what involves adults rearing. Nevertheless, rearing is not an easy task due to high rates of gall-inducer mortality, which can be caused by natural enemies, fungal growth, dryness of the host plant, hypersensitivity, and unknown life cycles, especially the period of adults emergence. In fact, there are few studies on gall midges life cycle in Brazil.

Phylogenetic studies are also very rare. There are only three publications with Brazilian species: Möhn 1961, Maia & Barbosa 2018, and Garcia et al. 2020. The first performed a study on Asphondyliini, without using a cladistic methodology, while the others tested the monophyly of two genera, Stephomyia

Tavares, 1016 and Bruggmanniella Tavares, 1909, respectively, using a cladistics methology.

Despite the ecological importance and diversity of habits and species, Cecidomyiidae are still little studied. Morphological, taxonomic, phylogenetic, biological and geographic distribution studies as well as a new generation of taxonomists are essential to improve the current knowledge (Supporting information: CNPq, Proc. 301481/2017-2).

#### DECIPHERING NEW GALL MODELS: OUR STORY WITH DITYLENCHUS

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Gall models are used by plant anatomists to understand how the galling organisms distinctly manipulate the host plant developmental potentialities. Variations in gall development occurs depending on the taxon of the galling organism, the feeding habit and the stimuli promoted on host plant tissues for gall induction, as well as the host plant developmental constraints. The changes in the host plant development, promoted by a galling organism, are based on the potentialities of the host plant genome. The galls are then important models to understand how the plant cell differentiation and metabolism may be manipulated, and in which conditions. The galls usually have a definite growth, demonstrating conserved developmental steps, metabolic processes, and tissue layers, generated by specific stimuli of a galling species on a determined tissue of a host plant species.

The study of distinct gall systems in the Neotropics, including galls induced by lepidopterans, dipterans, hemipterans, and mites in Brazilian host plants, has been revealing novel patterns in plant developmental changes. The study of an unknown gall system always has some challenges, but always bring interesting scientific questions to the debate. Engaged in this purpose, we decided to study some verrucous galls induced on Miconia spp. (Melastomataceae) on the Brazilian Cerrado. The first challenge step, the identification of the galling species, was overlapped by Oliveira and colleagues (Nematology, 15:179-196,

2013), which described the species as Ditylenchus gallaeformans and attested the galling potential of this species on several Miconia spp. This peculiarity of associating to several host plant species is a unique feature of these nematode galls when compared to insect-induced galls. Our story with the Ditylenchus galls began with the interest in the comprehension of some distinctions on these bizarre galls, but several others were detected while we tried to decipher the D. gallaeformans-Miconia spp system.

Our main goal, at the beginning of this project, was to understand how a same nematode species may manipulate distinct host plants, and if the galls would have distinct features linked to host plant constraints. Our first works demonstrated the capability of D. gallaeformans to induce galls in distinct plant aerial organs, with peculiarities according to the host plant species. Anatomically, these galls may be divided into an Outer Tissue Compartment (OC), with a common storage parenchyma with hypertrophied cells, and an Inner Tissue Compartment (IC), with a typical nutritive tissue (TNT), a division usually applied to insect-induced galls (Botanical Review, 85:78-106, 2019). The OC of the nematode galls are covered by uniseriate epidermis, with indumentum also varying according to the host plant species (Botany, 95:173-184, 2017). Novelties in gall anatomy were described, as the TNT with promeristematic feature in these galls. The cells of the TNT originate new emergences inside the gall chamber, and then these emergences mature into a new gall region divided into OC and IC. Therefore, the galls have a constant increasing inner surface and, consequently, the gall size and complexity increases. Such meristematic activity in the IC causes the peculiar indeterminate growth in these galls, which may be maintained for several generations of nematodes (Flora, 227:36-45, 2017). In general, the insectinduced gall systems have determinate growth, with four well-defined phases: induction, growth and development, maturation, and senescence.

Several questions arose from these novelties. Additional works mixing developmental anatomy, histochemistry, immunocytochemistry and physiology have been conducted and then we are revealing, step by step, the functioning of this incredible gall system. The OC parenchyma, with hypertrophied cells, accumulated auxins, besides starch and phenolics (Plant Biology, 21:1052-1062, 2019). The cells of the OC were ultrastructurally studied, and variations of cell wall pectins and hemicelluloses varied depending on the host plant species (Protoplasma, 257:229–244, 2020).

The IC cells of D. gallaeformans galls accumulate cytokinins, which were linked to the hyperplasia and maintenance of promeristematic feature in the TNT with indeterminate growth. Additionally, cytokinins were related to the activity of invertases in the nutritive cells. The histochemical analyses have shown that starch is broken by phosphorylase activity in the OC-IC transition, and then the invertases break the sucrose into glucose and fructose, which were densely detected in the TNT of these galls. Then, we linked the ultrastructural features with some metabolic responses, showing the uncommon functioning of these nematode-induced galls.

Additional works have shown distinct physiological responses in the gall physiological responses. For example, the accumulation of carotenoids and phenolics may be preferred, depending on the host plant species, demonstrating distinct antioxidant responses by the assessment of the chlorophyll fluorescence and phospholipid peroxidation rates (PLoS One, 13:e0205364, 2018). Miconia spp. also demonstrate different responses of heavy metal accumulation, showing an important role of galls as bioaccumulators, depending on the host plant species adaptations (Plant Physiology and Biochemistry, 154:360-368, 2020).

The study of different gall models usually brings several novel knowledge and questions, and open new perspectives. D. gallaeformans galls are important models to study distinct host plant responses and potentialities using the same galling organism as a natural manipulator. The anatomical and physiological responses of each host plant reveal distinct evolutionary histories, and then each host plant differently respond to the galling stimuli. (Supporting information: FAPEMIG, CNPq).

### **DIVERSITY OF GALLS: DRY FOREST X RAIN FOREST**

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The estimation of gall-inducing insects range from 21,000 to 211,000 species. Therefore the studies of gall-inducing insects and their host plants are fewer in dry forest compared to rain forest. Tropical dry forests represents almost 42 % of the tropical forests distributed in Africa, Asia, Oceania, Mesoamerica and South America. Caatinga is the largest areas of continuous seasonally dry forests that is located in northeast Brazil. Despite its ecological importance, there are few studies on galling insects and the association with their host-plants. A scientometry review for the period of 30 years recorded 182 papers, which dealt with insect galls in Brazil. The review highlight the Southeast region to concentrated the published papers, probably due to the highest number of research group studing biology galls.

The northeast brazilian region is cover by Atlantic forest (rain forest) and Caatinga (Tropical Dry Forest). Although the Caatinga occupies more than 50% of this region of Brazil, it still less studied when compared to the Atlantic forest. Thus the question is the Caatinga presents low gall richness or is less study in comparison to other Brazilian ecosystems? The first paper investigated the fauna of gall-inducing insects in plants from environments with different intensities of anthropic action in the Caatinga region of Xingó hydreletic. The researche recorded 25 morphotypes of galls in 18 host species of eight plant families (Anacardiaceae, Asteraceae, Bignoniaceae, Boraginaceae, Euphorbiaceae, Fabaceae, Laminaceae and Verbenaceae). The family

Fabaceae bears the largest number of morph-species of gall, with six morphotypes and Cenostigma pyramidale (Tul.) E. Gagnon & G. P. Lewis (Caesalpinia pyramidalis Tul) presents four morphotypes. In another study of the richness of gall-inducing insects in the tropical dry forest. The authors recorded 64 different types of galls of 48 species of host plants belonging to 17 families and 31 genera. in the vegetation of caatinga of Pernambuco. The host plant families that had a greater number of species of galls were: Fabaceae (23.44%), Euphorbiaceae (14.06%), Boraginaceae (6.25%), Malpighiaceae (6.25%) and Myrtaceae (6.25%). Few years late in Bahia State, one study was conducted in areas of Caatinga with different degrees of anthropogenic impact. They described twenty gall morphotypes on nine plant host belonging to eight families: Annonaceae. Bignoniaceae, Capparaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Rubiaceae and Sapindaceae. A most complete inventory of the diversity of galls was conducted in areas of Caatinga of Bahia state. This study recorded 91 different types of gall of 67 plant species of 54 genera belonging to 19 families (Anacardiaceae, Bignoniaceae, Boraginaceae, Cannabaceae, Capparaceae, Celastraceae, Combretaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Malpighiaceae, Malvaceae, Myrtaceae, Rubiaceae. Rutaceae, Sapindaceae, Rhamnaceae, Solanaceae and Verbenaceae). The family Fabaceae (39,6%) bears the largest number of galls morphotypes followed by Bignoniaceae (19,8%).

Therefore, there are more studies conducted in rain topical forest: Amazonia forest and Atlantic forest. For example, an inventory of gall inducing insects and their host plants in a urban fragment of Atlantic forest of Pernambuco (387,4 ha) recorded thirty and two morphotypes of galls had been associated with 13 families of host plants: Burseraceae (24,1%), Lecythidaceae (20,4%), Annonaceae and Melastomataceae with 11,1% each. A most wide study of the Atlantic forest of Pernambuco recorded one hundred thirty-six different morphotypes of insect galls on 79 host plant species of 53 genera belonging to 35 plant families. The study reported the host plant families with the largest number of galling species were: Lecythidaceae (6.6%), Myrtaceae (6.6%), Nyctaginaceae (5.2%), Burseraceae (5.9%), Clusiaceae (5.2%), Fabaceae (5.12%), Lauraceae (5.2%), Melastomataceae (5.2%), Polygonaceae (5.2%), Rubiaceae (4.4%). To other studies conducted at a 3500-ha remnant of in the Atlantic Forest (Alagoas) was recorded 50 gall morphospecies on 37 host plant species, the most important plant families were Melastomataceae (32%) and

Miconia prasina (Melastomataceae) was the host plant that had the highest number of individuals with galls (21).

The number of studies conducted in Caatinga are extremely limited. One explanation could be that in general the most of studies were done close to the research center most of far from the Caatinga areas. Fortunaly, nowadays new researches groups studing the biology of galls inducers and host plants are forming in university and research center located close to Caatinga. (Supporting information: FACEPE, CNPq, CAPES).

# GALL-INDUCING PSYLLOIDEA (HEMIPTERA) IN THE LIGHT OF RECENT PHYLOGENETIC AND TAXONOMIC STUDIES

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Psyllids are small hemipterous insects with slightly over 4000 described and at least as many undescribed species worldwide. Adults are always winged and range from 1–10 mm in body length. They have a simple life cycle with sexual reproduction passing through five immature instars. They are generally phloem feeders displaying several adaptations to overcome this unbalanced diet: mid gut with filter system, bacteriome with endosymbionts, secretion of honeydew and wax. Many psyllids induce galls which is a way to improve the nutritional quality of their host. Psyllids have generally narrow host ranges at species and often at higher taxonomic rank.

Immature psyllids are either free-living or develop in open or closed galls. Some species as the South African Ctenarytaina melanota Burckhardt are associated with witches' broom possibly induced by Phytoplasma they transmit. The earliest mention of psyllid galls and of psyllids in the literature is by Bauhin (1620), professor of Anatomy the University of Basel, Switzerland, describing the galls of Livia junci (Schrank). Psyllid galls display a wide range of shapes usually on leaves but also on stems, flowers and roots. They are generally induced by the first instar but exceptionally also by oviposition and the egg.

Psyllid hosts belong mostly to the eudicots, to a lesser content to magnoliids and only a few to monocots and conifers. Fabaceae, Myrtaceae and Asteraceae are the most important host families in terms of associated psyllid genera, followed by the much less species-rich Sapindales. Hosts of this order are colonised by four subfamilies of three families of Psylloidea and represent perhaps the primitive host association. Gall inducing species are present in some subfamilies and lack in others. In some subfamilies both types can be found. Limataphalara Hodkinson exemplifies a genus with monophagous psyllid species inducing galls on different Nectandra species (Lauraceae) producing a pattern of vicariance. Quite the opposite is found in Copaifera langsdorffii (Fabaceae) which hosts 13 species of the genera Colophorina Capener, Jataiba Burckhardt & Queiroz and Mitrapsylla Crawford. The species of the first genus are gall-inducer, those of the other two are not. Often four or more psyllid species are found on the same tree, hence qualifying as superhost.

There is a host of literature on psyllid galls from various authors published in the late 19th and early 20th centuries. Most of these publications are descriptive and often lack good identifications of the plants and/or the psyllids. Many of these records are dubious and difficult to elucidate. Examples are the genera Neotrioza Kieffer, Neotrioza tavaresi Crawford [=Nothotrioza tavaresi] and Ozotrioza Kieffer. Apart from some unresolved old names and records, the major challenge for a better understanding of gall diversity among Psylloidea is to describe the large number of new species and genera represented in our collections. For many psyllid species the host is still unknown or needs confirmation and little or nothing is know about gall induction. This requires also targeted field work.

#### GALLS AS FASCINATING MODELS IN BIOLOGICAL STUDIES

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Galls are fascinating models for biological studies, which have attracted the attention of several researchers around the world. These researchers use different techniques to answer several questions about the biology of galls, an amazing Interdisciplinary approach. Galls are products of host plant cells and tissues, which are changed by a phenotype manipulator - the galling organism generating a high diversity of shapes in extremely specific interactions. As an example of a plant hosting a wide range of gall diversity relies the superhost Copaifera langsdorffi, which is associated to different gall morphotypes induced by different species of cecidomyiid. This superhost shows a classical phenological behavior of species from the Brazilian Cerrado, with leaf falling in the dry season and leaf sprouting starting by the beginning of the wet season. These remarkable seasonality demands that the galling insects synchronize their life cycles with the plant phenological cycle culminating with predominant univoltinism. Another interesting systems involve the galls induced by Eriogalococcus Isaias (Eriococcidae) on Pseudobombax grandiflora and Bystracoccus mataybae (Eriococcidae) on Matayba guianensis. P. grandiflora lose all its leaves during the dry season and the galling insect shelters into the bark depressions of the stem to overwinter. When the new leaves sprout, the galling insects move and induce their leaf galls. With a similar behavior, B. mataybae move to the stem at the beginning of the dry season and induce

rudimentary stem galls before overwinter. Both species of galling Eriococcidae have univoltine life cycles, however, the univoltinism is not a rule in the Neotropics. For instance, Mimosa gemmulata hosts different gall morphotypes induced by different species of cecidomyiids, most of them with multivoltine life cycles. So, efficient strategies to explore the host plant resources depends, firstly, on the availability of reactive plant sites and of synchronization of host plant and galling herbivore life cycles. The early events of insect-plant recognition are associated to salivary compounds of the galling herbivore, followed by the production of reactive oxygen species (ROS) in host plant tissues. The ROS seems to act as signaling molecules leading to biochemical alterations, triggering plant responses and gall phenotype formation. The excess of ROS demands the scavenging mediated by phenolic compounds, with phytohormones, especially which interact auxins, as already histochemically and immuocytochemically mapped in gall tissue sites. The ROS-phenolics-phytohormones interaction is associated to cell division, growth and elongation, essential steps for gall development. The rate of cell divisions, gall tissue compartmentalization and functional complexity may depend on how long the galling herbivore feeds inside the gall and on the differentiation of new meristematic sites in galls, as reported in the horn-shaped gall on C. langsdorffi. The neoformed tissues in galls seems to be associated to differential cell wall composition on the gall tissue compartments, especially regarding pectins and hemicelulloses. Hemicelluloses, for example, is a carbohydrate that can be an additional support to the galling diet, when detected in the nutritive tissue. In the root-knot nematode galls, the changes in the cell wall composition can determine the infection and the diet of inducing nematodes, especially by the impact on the differentiation of the giant cells, the sites of the root-knot nematode feeding. Another cell wall components, the lignins are the most affected by oxidative stress, as their biosynthesis depends on the generation of ROS, especially the hydroxyl radical (?OH). Such phenolic derivatives are detected in the mechanical zone of some galls, forming a sheath of sclerified cells, which can act out as stress dissipator molecules, which are in fact a primary cell response to the high oxidative stress induced by the galling herbivores. In addition, changes in cell wall may indicate not only the levels of oxidative stress, but the cell wall lignification can work out as a mechanism of stress dissipation and help to maintain the redox homeostasis in the cells of gall developmental sites. Other phenolic derivatives important for the functionality of galls are the anthocyanins, strongly associated to color variation. The biosynthesis of anthocyanins can be induced by high light exposure, on the

abaxial and adaxial leaf surfaces, as reported in galls induced bv Cecidomyiidae (Diptera) on Qualea parviflora (Vochysiaceae) submitted to different light condition. The globoid galls induced by Palaeomystella oligophaga (Lepidoptera) on Macairea radula are large and have a huge range of coloration, from light green to red. The red coloration in M. radula galls seems to be related to the concentration of water-soluble polysaccharides as carbohydrate concentration can stimulate anthocyanin biosynthesis (fabricational noise hypothesis) leading to the red color. As related here, galls represent one of the most fascinating biological systems in nature, for as models of study, we can navigate through a wide diversity of biological areas, which together may solve mysteries on plant developmental processes.

### GALLS INDUCED BY CECIDOSID MOTHS IN THE NEOTROPICS

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Cecidosidae (Lepidoptera) is a small group (ca. 21 species worldwide) of monotrysian micromoths that have a Gondwanic distribution, occurring in Southern South America, Africa and New Zealand. Most of them induce either caulinar or leaf galls in Anacardiaceae, with the exception for the monospecific Neozeland Xanadoses Hoare & Dugdale whose larvae are bark-miners. The majority of gall-inducer species are associated with Schinus Linnaeus and Searsia F. A. Barkley, in South America and Africa, respectively. Twelve cecidosid species are recognized for Africa, which belong to a single genus (Scyrotis Meyrick). They reach the greatest diversity in South America, where six genera (eigth recognized species) occur: two species in Di¬cranoses Kieffer & Jörgensen and two in Oliera Brèthes, and a total of four in the monotypic genera Cecidoses Curtis, Euce-cidoses Brèthes, Cecidonius Moreira & Gonçalves and Andescecidium Moreira & Vargas. Several species await for formal description, coming particularly from the mountain regions of coastal Brazil and northern Argentina. Thus, diversity is expected to be greater for the lineage in the region, their scarcity in collections resulting from low sampling activity.

South American and African lineages form a monophyletic group nested within Adeloidea moths, and that started diversification in the former region circa 120 Mya. Galls of South American species are either spherical or cylindrical in shape, and are species-specific regarding their hosts. They supposedly have

recent and rapid co-speciated with their host species (ex. Eucecidoses minutanus Brèthes), which remains to be better explored.

With the exception of Oliera species, whose galls develop fully under bark, they grow externally at least during later ontogeny. Apparently all have one-year lifecycle, adults flying either in later Spring (Dicranoses, Oliera and Eucecidoses species) or Fall (Cecidoses). In some species (ex., Cecidonius pampeanus and Andescecidium parrai) galls are dehiscent, the last larval instar pupating in the soil within the galls. In C. pampeanus, larvae overwinter in a diapause condition, adult flying in early Spring.

Anatomy and histology of such galls have been studied in detail for a few species (ex. Cecidoses eremita Curtis). Apparently, all develop a specialized nutritional tissue, from which larvae feed upon. They are covered externally by a hard cortex and in most cases have an operculum through which the adult emerges. From an ecological perspective, they call someone attention by the great number of associated fauna, such as parasitoids, predators, cecidophages, inquilines and successors, which in most cases are not known yet from a taxonomic perspective. These, among other factors such a high host-specificity, localized distribution and apparently low dispersion, make the cecidosid moths promissory candidates to be used in studies from an evolutionary ecology perspective. (Supporting agencies: FAPERGS, CNPq, CAPES).

### **PSYLLID GALLERS (HEMIPTERA: PSYLLOIDEA) AS PLANT PESTS**

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Psyllids constitute one of the four superfamilies of Sternorrhyncha and can become pests in agriculture and forestry, as well as on ornamental plants. The damage inflicted to the plants is manyfold. The feeding weakens the host or damages leaves and flowers. The secreted honeydew burns the leaves or provides a substrate for sooty mould. Toxin in the saliva may damage some organs. Economically by far the most devastating is the transmission of bacterial deseases as those transmitted by the citrus, pear, apple or tomatopotato psyllids. The induction of galls reduces the photosynthesis of the affected leaves and thereby reduced the plant vigour.

Economically relevant species are Triozoida limbata Enderlein (Triozidae) on guava. The pest probably originates from Central America and is now widespread in tropical America. The immature induce leaf roll galls. A recent study, modelling the potential distribution using climatic data, predicts good conditions for the pest in India, one of the most important guava producers worldwide. Gyropsylla speggazziniana (Lizer) (Aphalaridae) is a severe pest on erva-mate in Argentina and southern Brazil. The immatures induce blister galls affecting the entire leaf. Severe infestation can kill young plants. In northern India, Nepal and Bangladesh, the mango-shoot psyllid, Apsylla cistella (Buckton) (Aphalaridae) is a severe pest on mango. The reproductive and vegetative buds develop into galls, reducing or preventing fruit setting on the

affected plants. An example of psyllids damaging ornamental plants is Trioza tabebuiae Burckhardt & Queiroz (Triozidae) developing on several species of trumpet tree (Handroanthus, Bignoniaceae) in Brazil. The psyllids induce irregular leaf rolls. Heavily infested young plants can die.

Most of the psyllids inducing galls on crop plants are of local or temporal significance only, and the damage is difficult to assess. A very recent example is Crucianus latipennis Burckhardt & Lauterer (Aphalaridae) described from Malaysia. Nothing was known about this species until last year when it was discovered in French Guiana on Spondias dulcis (Anacardiaceae). The species induces leaf roll galls and is associated with witches' broom. The psyllid was probably only recently introduced into the New World along with the host. The damage inflicted by the psyllid appears massive and the species seems to be spreading rapidly.



## SEMIOCHEMICAL INTERACTIONS BETWEEN PLANTS, CECIDOGENIC INSECTS AND THEIR ASSOCIATES

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Changes in plant secondary metabolism after gall induction by insects are one of the frontiers of knowledge for plant-insect interactions. There is a developing corpus of evidence either suggesting these metabolic alterations are the semiochemical result of plant defense responses towards cecidogenesis as well as indications that gall insects and/or their microbiological associates trigger these changes. Regarding changes in volatile organic compounds (VOCs) these alternatives have been barely explored. In this presentation I review current evidences regarding changes in VOCs related to gall induction and describe new evidence of such changes found in the association between the central Chile xeric shrub Haplopappus foliosus DC. (Asteraceae) and its gall midge: Haplopappusmyiia gregaria Maia & Villagra 2017 (Diptera: Cecidomyiidae). This cecidogenic insect triggers the development of complex fruit-shaped spherical galls on the apical meristems of its hosts. We fieldcollected vegetative VOCs released from healthy apical branches of H. foliosus versus H. gregaria's induced galls in this plant using dynamic headspace technique and identified compounds by gas chromatography coupled to mass spectrometry. We found proportional changes in semiochemicals released when comparing gall vs. healthy apex VOCs. Haplopappus foliosus' healthy branches volatile mixture was mainly constituted by monoterpene hydrocarbons, such as p-cymene, a semiochemical recognized as an herbivore deterrent. In addition we found oxygenated monoterpenes and sesquiterpenes. Meanwhile, when parasitized by gall midges, this plant modifies VOC emission increasing limonene, a VOC related to host's indirect induced defense through the attraction of gall midge's parasitoids. In galls we also detected a reduction in the release several monoterpene hydrocarbons with negative effects against insect and microorganisms.



# THE CURIOUS CASE OF NOTHOTRIOZA SPP. ASSOCIATED WITH PSIDIUM SPP. IN BRAZIL

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The study of triozid galls in Brazil goes back to the early 20th century, when Tavares reported globoid galls of an unspecified psylloid on an unidentified species of Malpighiaceae, later described by Crawford in 1925 as Neotrioza tavaresi. Crawford assigned the new species to Neotrioza with hesitation. The species was not mentioned again until 2003 when, some 80 year later, Butignol and Pedrosa-Macedo reported it as the inducer of globoid galls on Psidium cattleianum (Myrtaceae) from Paraná state, south Brazil. In 2011, globoid galls were also found on Psidium myrtoides from Minas Gerais, south-eastern Brazil, and the inducer was identified as Neotrioza cf. tavaresi by Burckhardt. To learn more about these psyllids and galls, thorough studies of available specimens were conducted. In 2013, Carneiro, Burckhardt and Isaias erected Nothotrioza as a new genus, described Nothotrioza cattleiani associated to Psidium cattelianum, Nothotrioza myrtoidis associated to Psidium myrtoides, and transferred Neotrioza tavaresi to Nothotrioza as a new combination. Research on the biology of Nothotrioza spp. led to the discovery of striking novelties,

unveiling a fruitful scenario of new hypotheses and discussions on the complex structure and metabolism of galls induced by sucking insects, which were classically believed to be simple and non-nutritive. In fact, the similar globoid galls of N. cattleiani and N. myrtoidis were found to accumulate primary and secondary metabolites, forming different gradients along gall tissues. Nutritive cells around the vascular bundles were ultrastructurally characterized for the first time, and together with immunocytochemical analyses on cell wall components, such galls were shown to be true extended phenotypes of their inducers, despite striking similarities. In 2019, two new species of Nothotrioza have been found in Brazil during field expeditions in the states of Goiás, centerwestern Brazil, and São Paulo, south-eastern Brazil, both also associated with Psidium spp. The galls of Nothotrioza sp. nov. on Psidium cf. laruotteanum were shown to retain great anatomical similarities with the previously studied galls, with peculiar characteristics due to harsh environmental conditions of the Cerrado from Goiás state. As all the Nothotrioza spp. we found are associated with Psidium spp., we suggest that the "Malpighiaceae" of Tavares may in fact be a misidentified Psidium species. Field trips to Bahia state, municipality of Camassari, in north-eastern Brazil, where Tavares' material comes from, should help elucidating the identity. An intriguing perspective on the study of Nothotrioza spp. is the wide distribution of their galls in plants occurring from the Atlantic forest at the state of Paraná toward the states of São Paulo and Bahia along the coast. In the state of Minas Gerais, galls are found in transitional Atlantic forest - Cerrado formation, and in Goiás, galls occur in Cerrado sensu strictu. As the phylogenetic relationships within the genus are yet unknown, time-based analyses should help understand the natural history of the Nothotrioza to Psidium association of spp. along the diverse phytophysiognomies of Brazil, as well as evolutionary trends concerned to gall morphology, ontogeny, and metabolism. As Nothotrioza galls on Psidium species are the best-known systems involving co-generic species in the Neotropics, from insect systematics to plant cell biology, their case is indeed curious, isn't it?

#### THYSANOPTERA AND THEIR GALLS

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Thrips, the minute insects of the order Thysanoptera, have pierce and sucking mouthparts that are used to feed on plant, animal or fungal tissues. About 400 species of thrips are known to induce leaf-galls, almost them belonging to the Phlaeothripidae family. In contrast to most insect-induced galls, those induced by thrips results from feeding, not oviposition. The galling habit has arisen on several evolutionary events, in groups of thrips that are not closely related. These structures range from simple a simple leaf-fold or leaf-roll gall, to complex structures that provide shelter to many hundreds of thrips. Most of the described gall-inducing thrips are from the Oriental and Australian region, but such behaviour is far more common in the Neotropics than the available literature suggests. In arid Australia, galls seem to represent a valuable resource for Thysanoptera, providing protection from low humidity and high insolation. In the South America, most galling thrips species were described from tropical and subtropical forests, and are especially diverse in leaves of the family Myrtaceae. Unfortunately, most of these associations have not been studied, and the thrips species involved are largely unidentified or undescribed in the scientific collections. Moreover, the internal anatomy and histochemistry of thrips-induced galls have been studied in only a few species. The purpose of this presentation is to summarise the relevant information of the taxonomical diversity and ecology of galling Thysanoptera, in order to stimulate further ecological, evolutionary and biochemical approaches.

# WHAT TRANSCRIPTOMICS CAN - AND CANNOT - TELL US ABOUT GALL DEVELOPMENT

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Cecidology has a long history of adopting new techniques and approaches to understand gall development. From gross visual examination through the use of increasingly sophisticated microscopy to histochemical techniques, cecidologists have applied current approaches to understanding the unique interactions between plant host and diverse galling species. At the present time, we see increasing use of molecular techniques, particularly transcriptomics, to investigate arthropod gall development. While these methods have been used for quite some time in plant pathology, including the study of gall-inducing microbes and nematodes, they are now being applied to plant-arthropod systems.

It is widely acknowledged that gall development arises from the galler's ability to redirect expression of the host plant's genes, altering the identities of normal plant cells and tissues and redirecting their developmental fates. If we can characterize and understand which genes experience altered gene expression and what these changes mean for development, this may open a window on the events occurring during gall development and even point at the way(s) in which galling arthropods bring them about. Transcriptomics is the study of gene expression, and the sum of all gene expression is an organism's or tissue's transcriptome. This approach can be used to compare the expression of any or all genes in different tissues, or tissues experiencing different conditions or

treatments. Changes in the transcriptome underlie the development of galls from normal tissues. This fact has led to an increasing number of published studies using transcriptomics methods to study gall development. These studies have revealed or confirmed widespread gall characteristics, including strong metabolic sinks, suppressed photosynthesis, hypoxic conditions, suppression of immune responses and appropriation of reproductive networks, among others.

In this presentation I illustrate the use of transcriptomics to examine the potential role of the phytohormone auxin in early stages of the development of a gall elicited by the insect Daktulosphaira vitifoliae (grape phylloxera) on grape leaves. The first response to phylloxera feeding on the adaxial leaf surface is expansion of a 30-cell wide circle of cells around the feeding insect with little cell division. Working through the steps that are typical of a comparative transcriptomic study, we find gene expression evidence that auxin is synthesized and expression of auxin-responsive genes is activated during this earliest phase of gall development. This is consistent with the developmental events seen in the leaf tissue. It implies that phylloxera stimulates auxin production rather than providing auxin exogenously.

There are pitfalls and limitations in the use of transcriptomics. While it is relatively straightforward to compile lists of genes of interest from the literature in this example auxin metabolism and response genes – merely finding them active in the gall tissues does not reveal their influence on outcomes. Because expression of some genes in any functional list may suppress outcomes when upregulated while others may promote outcomes when downregulated, one must understand the function of each gene to ascertain the likely consequences of the expression of the collection of genes. We also need a reference genome (or transcriptome) to be able to locate putative genes in the galler's hostplant. While over 1,000 plant genomes have been sequenced, few of these are gall hosts and even fewer include functional annotation - the function of most genes in most plant species is unknown. Taken together, finding plant genomes with which to align genes isolated from a host plant, then finding functional annotation for the differentially-expressed genes and the need to understand each gene's impact on development make transcriptomic studies of galls a lengthy and often difficult task. Even when successful, transcriptome studies are correlative. Showing that differential expression of a gene or genes impacts development requires manipulative experiments such as knockouts. This means that the host plant must be amenable to genetic transformation.

This situation is improving. It is now relatively easy to construct a de novo sequence of any plant species. Understanding gene functions is increasing rapidly. And CRISPR gene editing may allow us to test hypotheses about the impact of particular genes on gall development without transformation. At present it is uncommon for cecidologists to have these techniques in hand, so we must develop and form strong collaborations among researchers working across an array of disciplines to gain a firm understanding of gall biology.

This project was supported by the National Science Foundation, Division of Integrative Organismal Systems, grant NSF-IOS-1757358 (JCS HMA). RNAseq data that were generated for this study are available at NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under study accession GSE118569.

# WILL THE INVASIVE LEPTOCYBE INVASA INDISCRIMINATELY LAY EGGS TO ITS END?

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The eucalyptus gum chalcid, Leptocybe invasa (Hymenoptera: Eulophidae), Fisher & LaSalle (2004), is the most widespread, invasive and damaging insect pest of Eucalyptus species (Myrtaceae) around the world. Larvae of this tiny insect induce galls in actively dividing tissues, altering the patterns of resource allocation and vegetative growth. Leaves twist and curl, resulting in stunted growth and reduced productivity of infested plants. Variability in resistance to galling by L. invasa has been reported at all levels of genetic differentiation from species to genotypes. Variability in resistance of species and genotypes of species of Eucalyptus to L. invasa has been widely reported. However, there has been no record of host preference and acceptance behaviour and how their relationship could impact the sustenance of invasions. The female preference-larval performance hypothesis proposes that females identify host plant modules and locations that will best support larval survival before depositing their eggs to maintain optimal fitness for the species. This presentation, on the contrary, highlights the disparity in host acceptance for oviposition and larval survival on selected genotypes of Eucalyptus camaldulensis Dehnh.

With an aim of determining the impact of mothers' choice for oviposition on the fitness of the species, seedlings were raised from selected 29 genotypes of

Eucalyptus camaldulensis representing 7 subspecies (acuta, arida. camaldulensis, refulgens, obtusa, minima and simulata). Seedlings of the selected hosts were planted out in nurseries then later transplanted in common garden arboreta and pots in the nursery. Natural infestation was allowed in the common garden arboreta whereas potted plants randomly positioned in cage covered in cloth mesh had wasps introduced from twigs with mature galls. When the wasps had spent enough time to lay eggs, the number of ovipincture marks on twigs were counted for each genotype while other twigs were allowed time for deposited eggs to hatch and develop galls. Additionally, twigs from potted, non-infested plants were availed to individual wasps and oviposition observed under microscope. Phytochemical analysis of non-infested sample plants was carried out and profiles of the different genotypes compared. Analysis of variance was used to compare the oviposition as well as galling between different subspecies. Correlation was used to show the relationship between oviposition and galling of the different genotypes.

Our results indicate lack of variability in preference for oviposition on E. camaldulensis genotypes, even though some subspecies are not suitable for successful gall development. Although the genotypes of E. camaldulensis have shown close resemblance in terpene composition and been considered as chemotypes along the subspecies grouping, the preference for oviposition of these genotypes of was not influenced by their chemistry and showed no discrimination of any subspecies.

The oviposition intensity had no correspondence to success in gall formation while the incidence of oviposition on host genotypes had a negative, though non-significant correlation with the incidence of galling of the genotypes. Although the insect may have other Eucalyptus hosts other than E. camaldulensis, the lack of ability to discriminate cues against larval development could have adverse effects on the fitness of the insect in the invaded regions. Population of the insect in the East Africa, where invasions reduced productivity of Eucalyptus plantations have been observed to reduce and with a seasonal abundance. Moreover, several parasitoids have been reported associated with the galls of insect in the invaded regions including Africa, which could further reduce the damages associated with the pest.

#### **VIDEOPOSTER - BIOLOGY OF GALLS**

# A NEW SPECIES OF BRETHESIAMYIA MAIA (DIPTERA: CECIDOMYIIDAE) FROM COLOMBIA WITH DESCRIPTION OF IMMATURE FORMS

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Brethesiamyia colombiana Ospina & Urso-Guimarães, a new species of Cecidomyiidae (Diptera) is described from Colombia, which represents the first record of the genus for the country. We described the adult male and female, immatures pupa and the larva of the third instar, and its plant-induced gall found in leaves of Myrcia sp. (Myrtaceae). Samples of galls and host plants were collected at the foothills of the woodland connection of the Andes and the Amazon basin from Colombia. Type specimens were deposited in the taxonomic collection at the Museo Entomológico Universidad Nacional Agronomía Bogotá (UNAB), and the Diptera collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP). The genus Brethesiamyia belongs to Schizomylina, a subtribe with worldwide distribution, whose largest richness is found in the neotropics. Up to now, the only representative of this subtribe in Colombia had been Schizomyia manihoti. Brethesiamyia resembles the genus Stephomyia, these genera have neotropical distribution and the host plants belong to the family Myrtaceae. Moreover, both of them have circumfila reticulated; palpus with one segment; female flagellomeres nine to twelve
progressively shortened; mesobasal lobes present and pupal antennal horns short. However, Brethesiamyia have other distinctive characters; for instance, in Brethesiamyia the ovipositor is longer than in Stephomyia, and the cerci are separated apically, besides the pupae of Stephomyia have facial papillae while the pupae of Brethesiamyia have none. The adults and pupae from all collected specimens of the new species, Brethesiamyia colombiana, presented these distinctive characters. The adults of Brethesiamyia colombiana are characterized by having flagellomere completely reticulated in the female antennae; sternite 7 about twice longer than protrusible portion of ovipositor; cerci triangular and hypoproct weakly bilobed in males, unlike to Brethesiamyia myrciae who has circumfila uncompletely reticulated; cerci reniform and hypoproct deeply bilobed in males. The pupa of Brethesiamyia colombiana has antennal horns triangular, single pointed and about to 4 times longer than in Brethesiamyia myrciae who has antennal horn bifid. Third larval instar was described at the first time for this genus, it's characterized by one lateral papilla setose on each side of the spatula and terminal segment without papillae, unlike Stephomyia which has two lateral papillae on each side of the spatula and two pairs of setose terminal papillae. The larva of B. colombiana has spatula with two triangular and tapered apical teeth, and ventral anus rounded. Brethesiamyia colombiana induces cylindrical galls and Brethesiamyia myrciae tear-shaped galls. Both B. colombiana and B. myrciae induce leaves galls on species of Myrcia (Myrtaceae), a genus who has a wide distribution on Neotropical region, particularly in South America, which suggests that the genus Brethesiamyia could be much more diverse and exists in different types of biomes in the Neotropical region than it was previously known. (Minciencias)

# ANATOMICAL AND HISTOCHEMICAL IMPLICATIONS OF SEXUALLY DIMORPHIC GALLS INDUCED BY ERIOGALLOCOCCUS ISAIAS (ERIOCOCCIDAE) ON PSEUDOBOMBAX GRANDIFLORUM (MALVACEAE)

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Galling insects, males or females of the same species, are able to manipulate the potentialities of the host plant tissues creating highly specific morphotypes for each sexes. Several genera of Eriococcidae have sexual dimorphism which is reflected in the gall phenotype. The males and females of Eriogallococcus isaias Hodgson and Magalhães (Hemiptera: Coccoidea: Eriococcidae) induce galls on a deciduous host plant, Pseudobombax grandiflorum (Cav.) A. Robyns (Malvaceae). The duration of the life cycle of the males is shorter than that of the females, however, their galls are morphologically similar. The objective of this work is to test the hypothesis that the galls may reflect the sexual dimorphism in anatomical level and that the histochemical profiles of the galls are distinct between the sexes of E. isaias on leaflets of P. grandiflorum. Samples were included in Paraplast® for structural characterization. For histochemical analyses, handmade sections from recently collected samples were immersed in specific reagents for detecting proteins (bromophenol blue), lipids (sudan III), starch (lugol) and reducing sugars (fehling). The galls are green, intralaminar, conical-shaped and have a single central larval chamber. They are covered by uniseriate epidermis and have a predominantly homogeneous cortical parenchyma, bicollateral vascular bundles are located near the nymphal chamber, which is delimited by sclerenchymatic layer. No nutritive cells differentiate. The sexual dimorphism of the E. isaias results in subtle structural differences in the mature stage, as the shape of the nymphal chamber. The galls induced by females have a bigger and rounded nymphal chamber, which assumes the females body shape whereas those induced by males have elongated chambers similar to the shape of their bodies. The cells on top of the chamber have phenolic substances with a visually higher concentration in galls induced by females when compared to the galls induced by males. The histolocalized primary metabolites are related to the metabolic maintenance of the cells during gall development. Proteins and reducing sugars were detected in the inner tissue compartment, with accumulation decreasing toward the outer layers of gall cortical parenchyma for both sexes. Lipid droplets were detected in the cells of the inner tissue compartment and in the cell walls at the top of the chamber; this detection was more intense in the galls induced by females. Starch grains were absent in galls induced by males, which do not feed during the pupa stages and thus do not appear to stimulate a strong sink of photoassimilates. However, galls induced by females store starch, which indicates a stronger sink due to the metabolic cell demand regarding the longer life cycle of the females. The deposition of suberin acts as a barrier against any biological or physical stress during the male and female galls structural development. The reducing sugars available in all phases are energy sources that are rapidly assimilated to maintain the growth and development rates of male and female gall tissues. Current data corroborate the hypotheses that the sexual dimorphism of E. isaias reflects in the structural and histochemical profiles of the galls. (CAPES, CNPQ, FAPEMIG)

## **CELL WALL TRAITS OF GALLS IN CAATINGA ENVIRONMENT**

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The Caatinga environment tends to impose constraints on the development of host plants and in their associated galls. Mimosa tenuiflora (Willd.) Poir is a common species in semi-arid environments, which hosts a bivalve-shaped leaf gall induced by Lopesia mimosae Maia, 2010. Changes in cell wall structure occur along gall development and in response to water stress. Samples of nongalled pinna-rachis and mature galls were subjected to anatomical and immunocytochemical analyses to map the traits that can be expressed in Caatinga environment. For immunohistochemistry, we used monoclonal antibodies (LM20, LM5, LM6) for the detection of pectin epitopes. The pinnarachis presents uniseriate epidermis with glandular and non-glandular trichomes, isodiametric parenchymatic cells, and bicollateral vascular bundles. The mature galls have uniseriate epidermis with glandular and non-glandular trichomes, common storage tissue, sclerenchyma layers, and collateral vascular bundles forming the gall outer compartment, while the typical nutritive tissue forms the gall inner compartment. The epitopes of methylesterified homogalacturonans were weakly marked by LM20 on the parenchyma cell walls of the pinna-rachis. In galls, they were weakly marked on the cell walls of the common storage tissue, the vascular bundles and the typical nutritive tissue. The epitopes of galactans were not labeled by LM5 in cell walls of the pinnarachis, and were weakly labeled in cell walls of the gall common storage tissue. The epitopes of arabinans were moderately labeled by LM6 in cell wall junctions of epidermis and weakly labeled in cell walls of the adaxial cortical parenchyma, and of the pinna-rachis phloem. In the galls, LM6 were weakly labeled the arabinans in the cell walls of epidermis, common storage tissue, and typical nutritive tissue. Homogalacturonans, in association with arabinans and galactans, provide greater porosity to the cell walls of the typical nutritive tissue of mature galls and are key elements for cellular hydration. The galactans and arabinans confer flexibility and hydrophilic traits to the cell walls of the common storage tissue, favoring gall tolerance to drought along maturation. The morphogenetic potentials of the host plant are modulated toward gall tissues in response to the environmental stresses, and the dynamics of pectins guarantees the hydration status of cell walls, favoring M. tenuiflora bivalve-shaped galls development in the adverse environmental conditions of the Caatinga.

(CAPES, CNPq)

# CHECKLIST OF THE GALL MIDGES (DIPTERA, CECIDOMYIIDAE) IN THE STATE OF BAHIA (NORTHEASTERN BRAZIL)

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The Cecidomyiidae family is one of the most speciose among Diptera, with about 6,500 species. Most of them are gall-inducers, because of it, they are commonly known as gall midges. In Brazil, there are about 260 species of Cecidomyiidae (Diptera); little is known about their geographic distribution. Few catalogues and/or checklists have been published in the country. Only the gall midge fauna of Rio de Janeiro, Minas Gerais and Espírito Santo states was compiled. Therefore, the species richness and composition of most Brazilian states are still unknown. The main objective of this study was to provide a checklist of the cecidomyiids in Bahia based on literature data. Bahia was chosen due to the high number of species described from this state mainly in the beginning of the 20th century. As result, 44 gall midge species (Cecidomyiidae, Diptera) of 28 genera were compiled on 17 plant families. Fabaceae stood out for hosting the greatest number of gall midge species, followed by Burseraceae, Calophyllaceae, Myrtaceae, and Nyctaginaceae. Lopesia Rübsaamen 1908, Dasineura Rondani, 1840 and Bruggmannia Tavares, 1906 were the best represented gall midge genera. Six species, Costadiplosis maricaensis Viceconte & Maia, 2001, Dasineura couepiae Maia, 2001a, D. globosa Maia, 1996, D. marginalis Maia, 2005, Lopesia andirae Garcia, Lima, Caldo & Urso-Guimarães, 2017, and L. marginalis Maia, 2001

induce galls in endemic plants. Nine host plants are useful and together host 19 gall midge species (43% of the total). Concerning the geographic distribution in Bahia, each cecidomyiid species was recorded in a single phytogeographic domain. Atlantic Forest totaled 32 species, Cerrado five and Caatinga three. As the municipalities of occurrence of Cleitodiplosis graminis (Tavares, 1916), Dasineura braziliensis (Tavares, 1922), and Haplusia plumipes Karsch 1877 were not stated, their domains could not be established. Bahia shares 19 cecidomyiid species with the state of Rio de Janeiro, 13 with Espírito Santo, and 10 with Minas Gerais, corresponding to 0.25, 0.34 and 0.24 of fauna similarity, respectively. Nineteen species are only known from Bahia, eighteen of them were described between 1877 and 1922, and have never been recollected. Most species have been recorded on the coast of Bahia and most of the state area remains uninvestigated.

# COULD A SEXUAL DIMORPHISM OF THE GALL INDUCING INSECT LOPESIA MATAYBAE (CECIDOMYIIDAE) DETERMINE DIFFERENT MORPHOTYPES OF GALLS IN MATAYBA GUIANENSIS (SAPINDACEAE)?

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Galls are new plant organs developed by chemical and/or mechanical stimulus of specialized organisms, especially insects. In general, a single species of galling insect induces only one gall morphotype on the host plant. However, insects of the same species can induce galls in distinct host plant species or in different organs of the same plant. Induction of more than one gall morphotype indirectly increases plant resource variability that galling insects can access, and this peculiarity can be related to the sexual dimorphism of the galling organism. Nevertheless, Lopesia mataybae (Diptera: Cecidomyiidae) has a clear sexual dimorphism, and this species induces two gall morphotypes on the leaflets of Matayba guianensis (Sapindaceae). These galls differ in thickness and size, allowing to address whether these differences could be related to the sexual dimorphism determining different morphotypes of galls. In the present study, the morphological, anatomical, and histochemical attributes of galls occupied by male and female insects were examined and compared. From the 124 galls of each morphotype bred in plastic pots up to the adults' emergence, 36 adult females emerged only from the globoid galls and 13 males emerged only from the cylindrical galls. Both gall morphotypes presented the galling

insect in larval or pupal stages, and the emerged insects from different galls were sexually distinct, that is, the sexes occupied different morphotypes. Galls occupied by females were significantly bigger (height average = 4,67mm, S=0,43/ width average = 4,59mm, S=0,70) than galls occupied by males (height average = 2,95 mm, S=0,46/ width average = 2,34 mm, S=0,45), and the number of cell layers and size of parenchymatic cells in female-induced galls were higher. The galls developed initially from cell hypertrophy and tissue hyperplasia of the leaflet abaxial epidermis and spongy parenchyma. Neoformed vascular bundles were noted on the base of the gall. In the point of insertion, both types of galls maintained only portions of the spongy parenchyma. The gall was covered by the epidermis that continued from the abaxial leaf epidermis. In addition, galls had projections which were composed of homogeneous parenchyma with elongated cells. There were no differences in histochemical compounds detected either in male or in female galls. Cecidomyiidae male insects usually emerges earlier from the galls and their life cycle is shorter than that of the females, thus, this difference between the life cycles of the sexes can be a possible explanation for the occurrence of sexually dimorphic galls. The gall shape determination depends on the galling insect feeding behavior, which differently stimulates the process of gall tissue development, therefore, the gall tissues differentiation and growth depends on the constant specific galling insect feeding stimulus. So, females by staying longer in galls than males, may promote more stimuli in the plant, which can result in more cell hypertrophy and hyperplasia, and consequently the development of larger and sexually dimorphic galls.

#### GALLS FROM INSIDE OUT: DIDACTIC GAME

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Didactic games are playful ways to mediate the teaching-learning process. The central theme of the game "Galls from Inside Out" is an animal-plant interaction that results in the formation of "plant tumors", the galls. The game consists of 2 boards and 48 cards (for 2 players) or 4 boards and 96 cards (for 4 players), and can be assessed in Portuguese, English and Spanish in the site of the Neotropical Gall Group (https://www.neotropicalgallgroup.com/cool-scienceeducational-games). Each player has his board representing the life cycle of an inducer and his host plant and begins the game by drawing 5 cards from the previously shuffled deck, which lies between the boards. The game aims to introduce basic concepts about the life cycle of organisms involved in the establishment and development of galls. Also, biotic and abiotic factors that may interfere with the cycle are presented. To start the game, one of the players must have the [OVIPOSITION] or [GALL INDUCTION] card and must place it in the corresponding box (= OVIPOSITION or GALL INDUCTION) on your board. If the initial 5 cards do not come to [OVIPOSITION] or [GALL INDUCTION], the player may exchange 1 to 5 cards in the deck. After starting the game with the [OVIPOSITION] card or the [GALL INDUCTION] card, to continue the game, the player chooses from the five cards in hand whether to trade 1-2 in the deck or to use one of the cards in hand. Each player put one card on the board and draw another card in the deck (always with 5 cards in

hand). The ultimate goal is to complete the life cycle stages. (Cards placed on the board need not follow the order of the life cycle). The player, at the time of his play and before the exchanging of cards, can interfere with the other players' life cycle through the cards that represent the biotic and abiotic factors. When attacked by the opposing player with one of the biotic [PREDATOR / PARASITOID] or abiotic [DROUGHT / FIRE] factors, the player who does not have the corresponding card of defense [LIGNINS or RAIN] is stopped in the cycle until he gets the corresponding card of defense. The attack and defense are worth a move, which is completed by buying 1 card on the board, recomposing the 5 cards in hand. The player / inducer who first completes his life cycle with the full board is the winner. We have the opportunity of testing this game with the visitors of the exposition "Black Professors of UFMG" in November 2019, in the Knowledge Space of the Federal University of Minas Gerais, Brasil. The game efficiency for didactic purposes may be evaluated by the degree of involvement of the players, and by their appropriation of the scientific-technical terminology illustrated in the board and cards. After playing, the students may be challenged to look for and photograph galls in nature, and also produce text reports on the new knowledge they got with the experience.

# INSECT GALLS ON ASTERACEAE IN BRAZIL: RICHNESS AND GEOGRAPHIC DISTRIBUTION

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In the last 30 years, several insect gall inventories in different Brazilian phytophysiognomies have been published. Most of them comprise galls on Asteraceae and indicate this family as one of the most important in gall richness. The main goal of this study is to present a panoramic view of insect galls on Asteraceae in Brazil. The specific objectives were to inventory the gall morphotypes on Asteraceae species in Brazil and to register the distribution of the galls in the phytogeographic domains. We used the Web of Science database to find publications about insect galls from 1988 to 2020. We restricted this study to host plants identified in species. The Botanical names were updated, and data on geographic distribution in Brazil were verified in Flora do Brasil, 2020. We found 88 insect gall papers in Brazilian areas: 24 of them without Asteraceae as hosts, 64 with morphospecies of Asteraceae and 51 with identified species. Asteraceae species appear as host of insect galls in 34 of these papers, and this family is indicated as one of the super-hosts in 21 inventories. They covered all six Brazilian phytogeographic domains, being the

Atlantic Forest and Cerrado the most studied, with 33 and 29 inventories, respectively. The surveys totaled 487 insect gall morphotypes in association with 157 species of 42 genera. Baccharis and Mikania showed the highest number of galled species, 42 and 29, and the greatest richness of galls, 218 and 76 morphotypes, respectively. Both genera are widely distributed throughout Brazil and they are the best represented in the number of species within the Asteraceae family, favoring their association with gall-inducing insects. The super-host plant species were: Baccharis concinna (n=18 morphotypes), B. dracunculifolia (n=17), B. platypoda (n=17), B. reticularia (n=17), B. retusa (n=16), B. minutiflora (n=14), Eremanthus erythropappus (n=12) and Mikania glomerata (n=10). The presence of super hosts is common in some tropical regions; hence contributing to the increase of the local and regional gall richness in the communities. The Atlantic Forest was the domain with the highest number of galls morphotypes (n=200), followed by Cerrado (n=150), Pampa (n=5), Pantanal (n=3) and Amazon rainforest (n=2). Caatinga (dry forest) presented the lowest number of galls, with only one morphotype. Most of the Brazilian insect gall inventories with Asteraceae were performed in the Atlantic Forest and Cerrado biomes, which are relevant global biodiversity hotspots, with these biomes also being considered hotspots of gall-inducing insect diversity. The highest gall richness of Atlantic Forest and Cerrado can be explained by the fact that these biomes have been more intensely surveyed and additionally they house the first research centers focusing on gall-inducing insects in Brazil. This study provides important data about the presence of galling insects in one of the richest families of Angiosperms in Brazil. Such studies are rare in Brazil and important to consolidate the current knowledge, and to show the diversity of these insects in Brazil. They also provide subsidies to other studies such as biogeography and conservation.

# LATITUDINAL GRADIENTS IN CYNIPID SPECIES DIVERSITY PROVIDES OPEN NICHE OPPORTUNITIES FOR POLEWARD RANGE EXPANSIONS

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Anthropogenic change has enabled increased re-organization of the Earth's biota. Climate change is causing species to shift their ranges poleward or to higher elevations. Species in a community will likely respond to climatic shifts differently due to variation in life history traits, with the potential of species shifting ranges asynchronistically. Species diversity generally decreases towards the poles, "Latitudinal gradient hypothesis," and species undergoing poleward range expansions will likely experience less diverse communities in their expanded range. As a result, range-expanding species could experience open niche space in lower diversity communities in their expanded range, resulting in "ecological release" (i.e., higher performance). Here, we study a recent poleward range-expansion by Neuroterus saltatorius (Hymenoptera: Cynipidae), an oak gall wasp that occurs on Quercus garryana from northern California to Washington State, United States. This species recently expanded its range to Vancouver Island, British Columbia where it is outbreaking on Q. garryana. This species co-occurs with a diverse community of cynipids throughout its range. We investigate patterns of cynipid species richness, abundance, composition of gall types, and species associations along the range of Q. garryana from northern California to Vancouver Island, British Columbia. At 18 sites, we collected and identified cynipids on 540 trees (30 per site over 3 survey periods), and measured habitat and abiotic variables. In total, we documented 25 cynipid morphotypes across our surveys. As expected, we found a negative relationship between cynipid richness and latitude. We also found a negative relationship between cynipid abundance and latitude, but only without N. saltatorius. N. saltatorius has a positive relationship with latitude, outbreaking in the expanded range. Cynipid composition also changes across latitude, with Vancouver Island, British Columbia having distinctly different community compositions when compared to the rest of the range. N. saltatorius associates (on trees) most closely with similar gall types (foliar detachable galls), and its associations are lost in northern latitudes. We pose that ecological release may be a result of changes in cynipid communities across latitude, which could ultimately lead to open niche space and/or release from direct and indirect competition. This work offers valuable insight into how latitudinal patterns in diversity may affect the dynamics of poleward range-expansions.

## MY GALL, MY LIFE – A COMIC BOOK

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When walking through the Cerrado biome, it is possible to observe a series of interactions between different organisms. Among these interactions, we can highlight insect-plant interactions, which can be beneficial for both or cause damage to one of the associated organisms. In the comic book entitled "BIO HQ: Biology in comics", an interesting story is shown based on a post-graduate research project in ecology at the Federal University of Uberlândia. These comics explained how the interaction of a plant species common to the Cerrado (Macairea radula) with a lepidopteran that causes "tumors" (Palaeomystella oligophaga) and with parasitoids that feed of the lepidoptera (Calliephialtes sp.), a complex relationship called the multitrophic system. In addition to being complex, this relationship is not at all smooth: there are disputes, losses and damages.

# NEMATODE-INDUCED GALLS SHOW HOST-SPECIFIC SIGNATURES IN THEIR STRUCTURE

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Unlike the root-knot nematodes, some plant-parasitic nematodes are specialists on manipulate plant tissues and induce the neoformation of galls on aerial organs. These structures confer protection for the nematode colonies against biotic and abiotic factors, at the same time which supplies these organisms with food and water, while the feeding stimulus lasts. Ditylenchus gallaeformans induce galls on leaves and inflorescences of several Miconia species (Melastomataceae) of the Brazilian Cerrado. These galls were previously described as occurring on leaves and inflorescences of Miconia albicans and M. ibaguensis, with the outstanding feature of indeterminate growth conferred by the totipotent capacity of the nutritive cells. By extending the knowledge on the structural profile of the galls induced by D. gallaeformans on leaves of M. corallina and M. lacunosa, we compared these galls to those previously described on M. albicans and M. ibaguensis by cluster and principal component (PCA) analyses, expecting to detect similarities and divergences among the galls induced on the four plants. As expected, the galls induced on the four Miconia species share similar anatomical features, including the indeterminate growth potential. Other aspects, such as the trichomes and emergences diversity expressed in the galls were strictly related to the host plant constraints. These traits of each host plant species supported the separation between M.

lacunosa in the PCA (PC1+PC2 = 84.5% of explained variance) and the clustering of the other three Miconia species, which looks like to coincide with the phylogeny of Melastomataceae. Anatomical features of these galls such as the occurrence of cristalliferous and tanniferous idioblasts also indicate adaptations to the maintenance of the homeostasis in the gall microenvironment and the protection against stressful conditions of the Brazilian Cerrado. Besides that, the gall induction on surfaces and the release of pectinases by D. gallaeformans promote the totipotency observed on these galls. (CAPES, FAPEMIG, CNPq).

## NEW STATE RECORD OF TWO GALL MIDGE SPECIES (DIPTERA, CECIDOMYIIDAE) IN BRAZIL

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Anisodiplosis waltheriae Maia, 2005 induces galls on leaves, buds and inflorescences of Waltheria indica L. (Malvaceae). The galls are spherical to conical, covered with short light yellow to light brown trichomes and have one chamber where a single larva can be found. W. indica is a perennial herbaceous plant considered a common invader of Cerrado areas, and found in all Brazilian territory. Asphondylia sanctipetri Urso-Guimarães & Amorim, 2002 induces green and glabrous galls on leaf veins and petioles of Schefflera morototoni (Aubl.) Maguire et al. (Araliaceae) a tree commonly known as "morototo". S. morototoni occurs in all Brazilian states and it is very susceptible to the attack of fungi and insects. The objective of this study was to register the first record of A. waltheriae and A. sanctipetri in the state of Mato Grosso, Brazil. The field work was done in Parque Nacional da Chapada dos Guimarães (PNCG), in Chapada and Cuiabá municipalities, Midwest Region of Brazil, for 15 days each. Individuals of Waltheria indica and Schefflera morototoni were investigated for galls in ten different trails. This is the first record of Anisodiplosis and Asphondylia species to the state of Mato Grosso. It is also the first record of Anisodiplosis to Cerrado areas. This new record is important because the Cecidomyiidae fauna is poorly known in Brazil. Mato Grosso is the third largest Brazilian state, in its west-central area, and the only one with the

presence of three phytogeographic domains: Amazon rainforest, Cerrado and Pantanal. There are so far five species of Cecidomyiidae known to Mato Grosso: four of them were collected at PNCG: Bruggmannia chapadensis Proença and Maia, 2018; Lopesia andirae Garcia, Lima, Calado and Uso-Guimarães, 2017; Lopesia chapadensis Garcia & Urso-Guimarães, 2018 and Lopesia mataybae Garcia & Urso-Guimarães, 2018; and one of them from the campus of Universidade Federal de Mato Grosso, Schizomyia tuiuiu Urso-Guimaraes & Amorim, 2002. Therefore, this is the sixth and seventh record of a Cecidomyiidae species in Mato Grosso. A. waltheriae was previously recorded in the states of Minas Gerais and Pernambuco, in areas of Atlantic forest and Caatinga, respectively. A. sanctipetri was previously recorded in the states of São Paulo, Minas Gerais, Goiás and Pernambuco, in areas of Cerrado and Atlantic forest. Both host plant species occur in all Brazilian states, but the distribution of the gall midges is more restrict. This restriction could be explained by the scarcity of insect galls inventories in many areas of Brazil. Therefore, according to the hypothesis of specificity of gall midges, we assume that probably A. waltheriae and A. sanctipetri may occur where their host plants are distributed.

# PHENOLOGICAL SYNCHRONISM STRATEGIES AND ABUNDANCE OF FIVE LOPESIA GALLS (CECIDOMYIIDAE) WITH THE SUPER-HOST MIMOSA GEMMULATA (FABACEAE)

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Mimosa gemmulata Barneby (Fabaceae) is a super-host plant of five pinnarachis gall morphotypes induced by five Lopesia spp. (Diptera – Cecidomyiidae). We use phenological analyses of the M. gemmulata host plants, and of the gall life cycles to evaluate the phenological synchronism strategies among galling insects sharing the same microhabitat in a single super-host plant. In addition, we relate the abundance of the five Lopesia galls with the plant phenophases, as well as the temperature and the precipitation of the neotropical savanna climate. Mimosa gemmulata is a semi-deciduous shrub, with periods of leaf flushing and leaf falling related to the contrasting periods of drought and raining in the neotropical savanna climate. The generations of the five leaf gall morphospecies on M. gemmulata revealed multivoltine life cycles for the five Lopesia spp.. The success of the Lopesia spp. sharing the same microhabitat depends both on the synchrony of the induction phase with the leaf flushing of the super-host plant, and to the asynchrony among their life cycles. The asynchrony of the multivoltine life cycles of Lopesia spp. is favored by the constant leaf flushing in M. gemmulata, and favors the non-overlapping of gall induction periods. The flowering phase did not affect the life cycles of the five leaf gall morphospecies. The leaf falling is a strategy for reducing future losses to the nutrient sinks, and for redirecting the investment to fruit development. The phenological synchrony of leaf falling and fruit development relates to a decrease of the populations of the galling Lopesia, and consequently the low gall abundance. The five Lopesia galls co-occurred on M. gemmulata throughout one-year time, but the abundance of the bivalveshaped galls was higher during the rainy season and decreased during the dry season. The gall life cycle and abundance of the five Lopesia galls follow the phenology of M. gemmulata, which obeys the seasonal pattern of water availability in the neotropical savanna climate.

## SPATIAL AND TEMPORAL DISTRIBUTION OF GALLING SPECIES IN COPAIFERA LANGSDORFFII DESF.: A SUPER-HOST PLANT.

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Interactions between plants and galling insects comprise one of the most attractive topics within the studies of ecology, biogeography and evolution. Unlike the interactions between frugivorous vertebrates and plants that tend to be unskilled and mutualistic, galling insects demonstrate strong specificity with a host plant and with the host organ. An interesting pattern is the fact that some species of plants, called super-hosts, harbor many species of galling insects. However, little is known about the mechanisms influencing the coexistence of galling insects in super-host plants. In this study, we evaluated the mechanisms that allow the coexistence of multiple species of galling insects in super-host plants. For this, we tested two non-excluding hypotheses: i) Spatial partition hypothesis: galling insects are distributed in different plant organs; and ii) Temporal partition hypothesis: galling insects occupy the host plants in different months or seasons. We found that the spatial partition is an important pattern for the distribution of galling insects, but it does not occur according to the hypothesis because most of the galls occur on the leaves. The galling insects partition the space through colonization in different niches of the same superhost plant (leaves) or by the induction of galls in other individuals of C. langsdorffii Desf. The temporal partition hypothesis is consistent with the results

found, as galling insect species oviposit in different months of the year, according to the season, allowing the coexistence of different species in a single super-host plant.

## THE CURIOUS CASE OF ANTS THAT LIVE IN GALLS: CREATIVITY TOWARD SCIENTIFIC COMMUNICATION

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The scientific community has been investing time and energy on the promotion of science communication from the academy toward the elementary and high schools. Different ways for communicating science discoveries have been created, e.g., videos, social medias, podcasts, and texts with accessible language. Among these variety of tools, our choice relied on the production of a paradidactic book using part of the doctoral project of Nina de Castro Jorge entitled "Stem galls in Eremanthus erythropappus (DC.) McLeisch: anatomical changes and ecophysiological implications". The book is entitled "The curious case of ants that live in galls" (ISBN 978-65-00-13341-7) and has version in three languages: English, Portuguese and Spanish, always with the preoccupation of keeping accessible language. It is directed to 8 to 12 years-old children and it is suggested for science and literature classes. Our paradidactic text can contribute to enrich teaching interaction by presenting plant-insect interactions that are not easily visible for the common people, bringing new questions, opening the student and professor's view of science and the natural environment, creating new teaching methodologies and didactic resources toward amplifying the scientific context, and consequently motivating students. In a methodological perspective, we propose the reading of the text together with the students or the individual reading, followed by a debate guided by questions like these: (1) what are your first impressions about the book? (2) What are the highest curiosities about the story? And (3) can you retell the story or part of it? Along the debate, it will be possible to confer the scientific content appropriated by the students. Additionally, the teacher can use the story as a motivation to address the programmed content for science classes. For literature classes, it is possible to observe the students' ability to abstract from the basic concepts, to practice their abilities to the construction of narratives, intertextuality, description of characters, identification of implicit and explicit information, title-text relationship, and plot structure. We hope that the use of this type of text can put students closer to scientific research and enable more dynamic and attractive learning.

## WOOLLY APPLE APHID STEM GALLS

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The woolly apple aphid, Eriosoma lanigerum (Hemiptera: Aphididae) Hausmann, 1802, is a pest crop that attacks the root and stem of apple trees worldwide. Even though E. lanigerum is an exophytic aphid, its feeding on the phloem results in galls, affects the water conductivity of the host organs, and reduces the tree vigor. The aphid stays on stem and root barks, and despite no larval chamber is formed, there are peculiar tissue alterations. The E. lanigerum reproduces parthenogenetically and produces a wax covering that protects its body from environmental factors. The host species, Malus domestica, has worldwide economic importance, with 130 million tons produced by year, the third fruit production of the world. Thus, the woolly apple aphid represents a great economic risk for apple producers, and the study of E. lanigerum gall development on M. domestica aim to find windows of opportunity to block the infestation. Samples of non-galled and galled stems were collected in a private orchard in Ervália municipality, Minas Gerais state, Brazil, fixed in FAA, embedded in Paraplast and sectioned in a rotatory microtome. Non-galled stems have a compacted suberized periderm with lenticels, the cortical parenchyma has phloemic fibers arranged in arcs around the vascular system,

and the cambium produces a continuous cylinder of secondary phloem outward and secondary xylem inward. The stem galls are fusiform and recognized by the intumescence of the galled stem as product of intense cell division in the xylem region stimulated by the sucking activity of E. lanigerum. The cambium and the phloem occupy the periphery of the redifferentiated organ, and the cortical parenchyma stem region is thinner in sites where E. lanigerum colonies are found. The feeding activity stimulates the cambium to overproduces parenchymatic cells inward, a few but wider vessel elements, and generates cell reorientation both in radial and axial system. The main aphid feeding site is the phloem, but the major changes in the secondary xylem suggest that the aphid also feeds in water conducting cells. In summary, the observed anatomical traits suggest, so far, that the woolly apple aphid feeds not only in the phloem, but also in the xylem, corroborating previous information of literature that E. lanigerum inserts its stylet intercellularly and intracellularly, until it reaches the phloem, its feeding site, but can reach the cambium and the xylem. Next steps will look for evidencing the energetic molecules related to the nutritional requirements of the aphid, and for checking its effect over the water conductivity. (FAPEMIG, CAPES, CNPg)

## XYLEM DIFFERENTIATION CONTROLED BY CECIDOMYIIDAE ON INGA INGOIDES (FABACEAE)

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Galling herbivores influence the development and differentiation of plant tissues forming new structures, the galls with alterations in dermal, ground, and vascular system generating new tissue patterns. The new pattern of xylem differentiation is influenced by the action of phytohormones and the position of the gall in its host organ. The galls can be extralaminar when attached to the host leaf by a peduncle or can be intralaminar when they are in a morphological continuum with the host organ. The pattern of differentiation, cytometric and histometric characteristics of the xylem, as well as the influence of cytokinins and auxins during gall development were evaluated in three gall morphotypes (globoid, lenticular and fusiform) induced by Cecidomyiidae on Inga ingoides (Rich.) Willd. (Fabaceae: Caesalpinioideae). The detection of auxins is related to the differentiation of new vascular cells. The origin of the xylem cells, the intracellular variation, and the position of the gall in relation to the host plant organ are related to mechanisms that positively compensate the hydraulic conductivity in the galls. Extralaminar galls (globoid and lenticular) develop as appendages of adventitious origin, which theoretically imply in rupture with the host leaf ontogenetical pattern. Based on the fact that gall position influences xylem cell features, this rupture is associated with the origin of the vascular tissues and the action of auxins in the differentiation of narrow vessel elements. In intralaminar galls, the activity of the vascular cambium and the continuum with the host organ imply in the differentiation of wide vessel elements. The xylem morphogenesis involves the synergism between auxins and cytokinins in cells recruited to redifferentiate in vascular elements both in the extralaminar and intralaminar galls on I. ingoides. To ensure efficient hydraulic conductivity, three strategies are triggered: (1) a high rate of differentiation of parenchyma cells in extralaminar galls, (2) a high differentiation of vessel elements in the intralaminar gall, and (3) differences in the width of vessel elements in both gall morphotypes. (CAPES, CNPq, FAPEMIG)

