ABSTRACTS BOOK

100 Anniversary

Celebrating 100 Years of the 1st EPN discovery

April 10-12, 2024
Logroño, Spain

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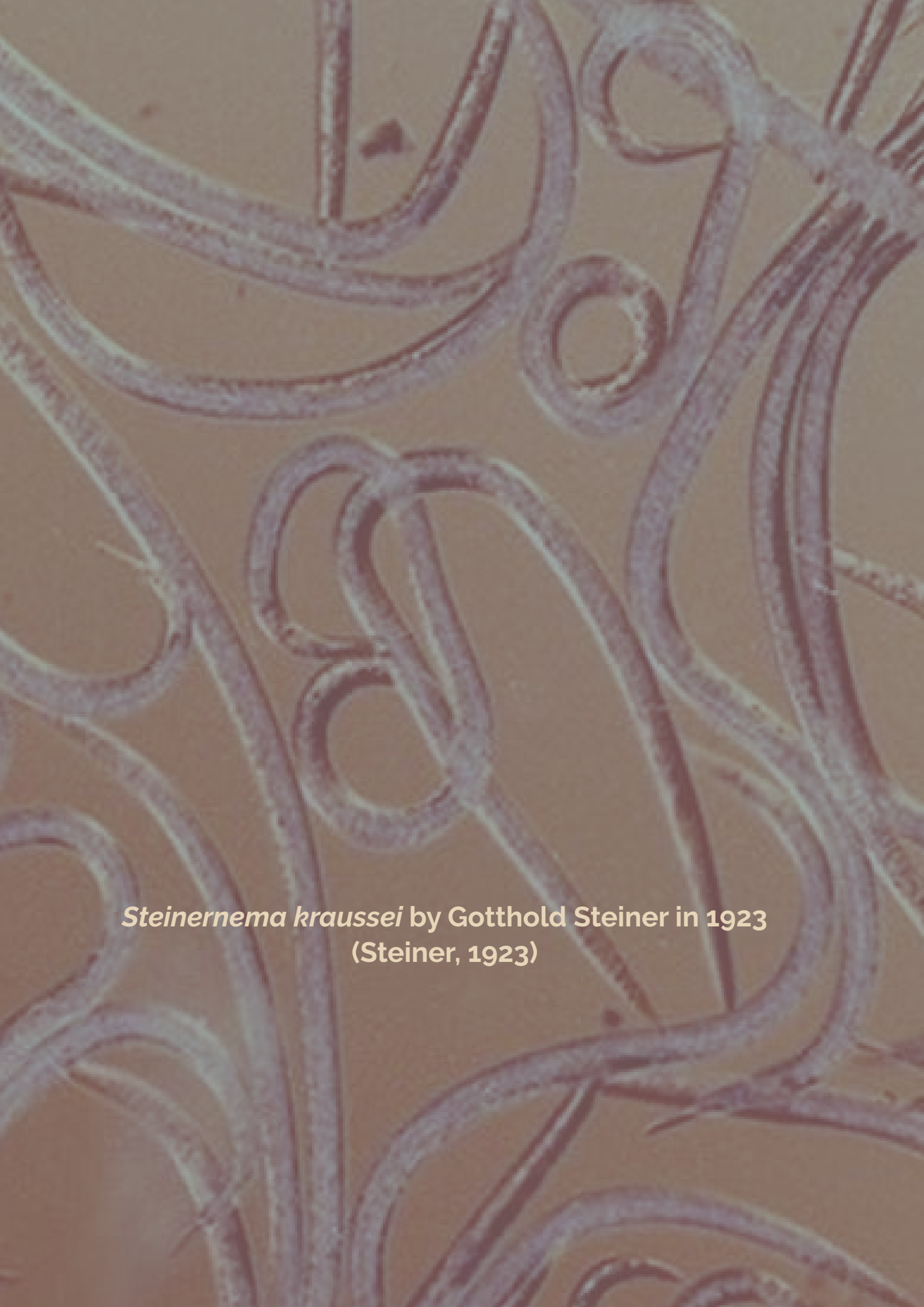


Steinernema kraussei by Gotthold Steiner in 1923
(Steiner, 1923)

Welcome

We are delighted to announce the upcoming congress commemorating the 100th anniversary of the first description of the entomopathogenic nematode Steinernema kraussei by Gotthold Steiner in 1923 (Steiner, 1923). This event will be a unique opportunity to celebrate a century of scientific achievements in the field of nematology and entomology. Join us in celebrating the centenary of the discovery of Steinernema kraussei and contribute to the advancement of knowledge and solutions for sustainable agriculture and pest management.

The congress will be held in Logroño, Spain from Wednesday 10th April, 2024 until Friday 12th of April, 2024. Organized by Raquel Campos-Herrera, this event will take place right before the 2024 meeting of the European Society of Nematologists (www.esn-online.org) in Cordoba, providing participants with an opportunity to attend both events and maximize their knowledge and networking experiences.

We will bring together renowned experts, researchers, and students from around the world to discuss the latest findings, innovations, and challenges related to entomopathogenic nematodes. Symposia will be organized to include competent experts in the field to summarize existing knowledge and provide valuable insights and perspectives on the history, evolution, and future of nematode research. Poster presentations, workshops and networking opportunities will allow participants to engage with their peers and learn from the best in the field. We look forward to welcoming you to the congress and experiencing an unforgettable celebration of a century of nematology and entomology.

Raquel Campos-Herrera

Reference: Steiner, G. (1923) Aplectana kraussei n.sp., eine in der Blattwespe Lyda sp. parasitierende Nematodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden. Zentralblatt für Bakteriologie, Parasitenkunde, Infektions¬krankheiten und Hygiene, Zweite Abteilung 59, 14-18.

International Organizing Committee

Helge Bode (Max Planck Institute for Terrestrial Microbiology, Germany)

Raquel Campos-Herrera (ICVV, Spain)

Larry W. Duncan (University of Florida, USA)

Ralf U. Ehlers (e-nema GmbH, Germany)

Fernando García del Pino (Universidad Autonoma de Barcelona, Spain)

Sophie Gaudriault (INRAE-Montpellier, France)

Christine Griffin (National University of Ireland, Maynooth, Ireland)

Ivan Hiltpold (Agroscope, Switzerland)

Xingyue Li (Sichuan Academy of Agri. Sci., Chin)

Ricardo Machado (University of Neuchâtel, Switzerland)

Tshima Ramakuwela (University of Pretoria, South Africa)

Ernesto San Blas (Universidad de O'Higgins, ICA3, Chile)

David Shapiro-Ilan (USDA-ARS, USA)

Nelson Simões, (Universidade dos Açores, Portugal)

Patricia Stock (California State University Chico, USA)

Symposium presentations are 20 minutes long
(15 min talk + 5 min questions)
Flash presentations are 5 minutes long (no questions)

Local Organizing Committee

Rubén Blanco-Pérez (ICVV, Logroño, Spain)

Raquel Campos-Herrera (ICVV, Logroño, Spain)

Fernando García del Pino (Universidad Autonoma de Barcelona, Spain)

Anna Garriga (Universidad Autonoma de Barcelona, Spain)

María del Mar González-Trujillo (ICVV, Logroño, Spain)

Ivan Julià Brunat (Universidad Autonoma de Barcelona, Spain)

Vicente S. Marco Mancebón (Universidad de La Rioja)

Ana Morton (Universidad Autonoma de Barcelona, Spain)

José Luis Ramos Sáez de Ojer (Goverment of La Rioja)

Ignacio Vicente-Díez (ICVV, Logroño, Spain)

Speakers at Plenary Sessions

Raymond Akhurst,
Retired; formerly CSIRO Division of Entomology, Canberra ACT (Australia)

Noël Boemare,
Retired; formerly DGIMI, University of Montpellier, INRAE, Montpellier (France)

Randy Gaugler,
Distinguished Professor Emeritus, Rutgers University, Albert Einstein Professor, Chinese Academy of Sciences (USA)

Ramon Georgis,
Vice President BRANDT INTERNATIONAL LLC, Tampa, Florida (USA)

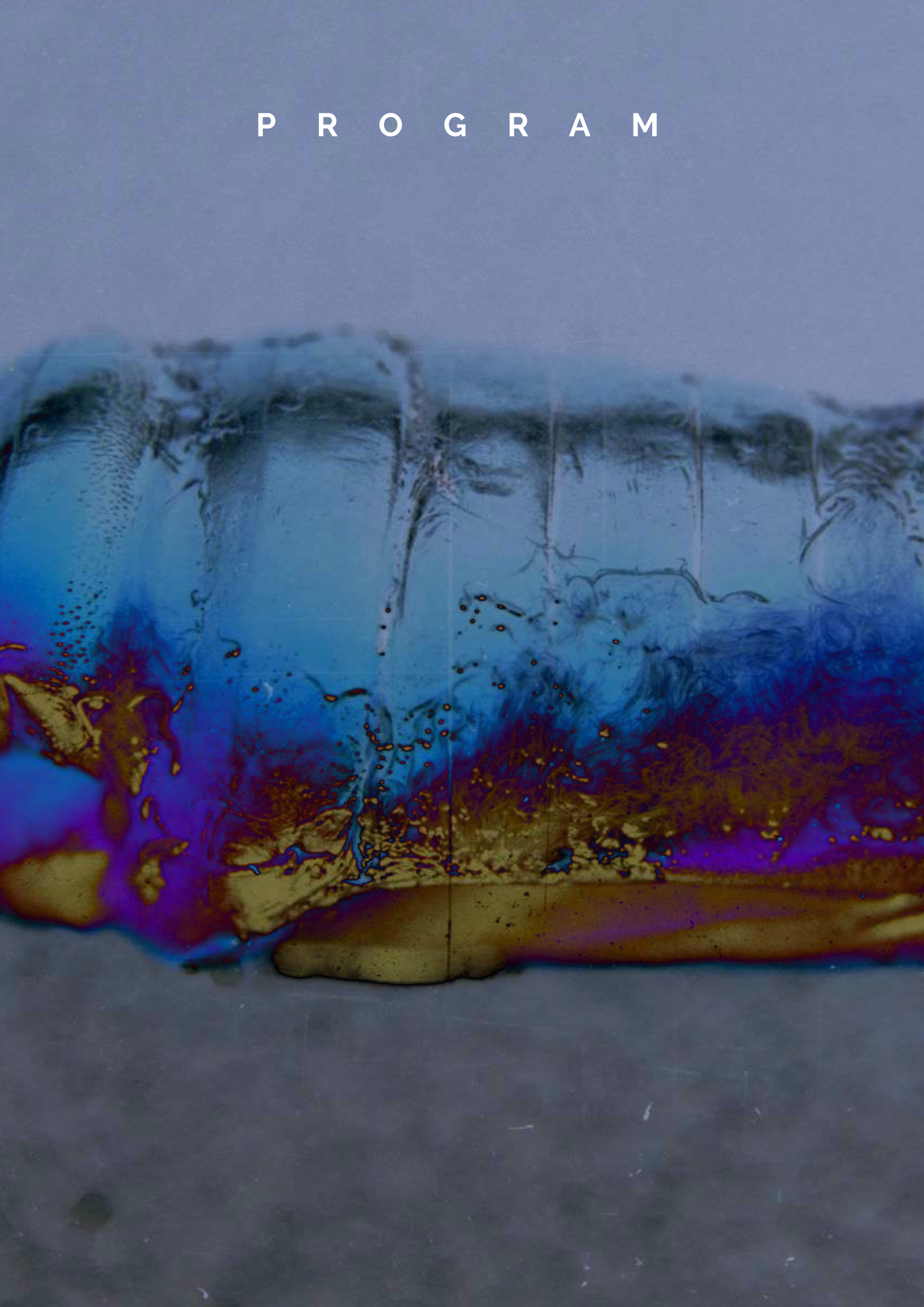
George O. Poinar Jr.,
Department of Integrative Biology, Oregon State University; Professor Emeritus University of California, Berkeley (USA) (online presentation)

Symposium presentations are 20 minutes long
(15 min talk + 5 min questions)
Flash presentations are 5 minutes long (no questions)

Topic for Discussion: Past, Present And Future

1. Systematics, diversity & biogeography
2. Bacteria biology, symbiosis, and application
3. Behavioral Ecology (dispersal, chemical signaling)
4. Population ecology
5. Survival, Virulence and immunity
6. Mass production, include safety & regulation
7. Commercial use & future targets (including inundative and conservation approaches)
8. Application technology
9. EPN omics OR Genome and evolution
10. New Frontiers in EPN (selected from abstract submission)

P R O G R A M



Tuesday, 9th April

3 LOCATIONS

University of La Rioja, Salón de actos Complejo Científico-Tecnológico

9:30 - 13:30 **Specialization course** (In Spanish)
Nematodos en los viñedos: problemática, desafíos y bioindicadores salud del suelo

ICVV (Institute of Grapevine and Wine Sciences)

16:00-18.00 **Visit ICVV and Institutional Winery**
Includes transportation by bus.

Meeting point: Fuente Murrieta Square (Logroño city centre)

Espacio Lagares (Logroño city center)

19.30-21:30 **Welcome reception and cocktail**

Wednesday, 10th April

RIOJAFORUM CONFERENCE CENTRE

8.30	REGISTRATION
9.30-10.00	CONFERENCE OPENING
9.50-10.00	***Steinernema kraussei*, Steiner 1923: The story of two scientists and the first description of an entomopathogenic nematode.** Ralf-Udo Ehlers
10.00-10.40	**Legend speaker: Ramon Georgis.** Entomopathogenic nematodes: a commercial journey
	Chair: Ed Lewis
10.40-11.10	Coffee break
11.10-12.10	**SYMPOSIUM 1** Systematics, Diversity & Biogeography
	Chairs: Ricardo Machado & Antoinette Malan

African frontier: Tapping into entomopathogenic nematodes and symbiotic bacteria. Antoinette Malan; Abate Birhan

Systematics and phylogeny of the entomopathogenic nematodes: the past, the present and the future. Vladimír Pluža; Hana Toušková; Emilie Ferreira; Martina Žurovcová

Taxonomy and systematics of the entomopathogenic bacterial genera *Photorhabdus* and *Xenorhabdus*. Ricardo Machado

12.15-12.45	FLASH PRESENTATIONS
	Chair: Ivan Hiltpold

Bioluminescence protects *Heterorhabditis*-infected cadavers from nocturnal scavengers. Maria D. Cassells; Sophie Labaude; Christine T. Griffin

***Xenorhabdus nematophila* and *Photorhabdus laumondii* (Morganellaceae) secondary metabolites for the management of *Plasmopora viticola* (Peronosporales: Peronosporaceae) on grapevine leaves**. Ignacio Vicente-Díez; Jorge Dueñas-Hernani; Raquel Campos-Herrera; Alícia Pou

Exploring the potential of symbiotic bacteria as plant-growth promoters: a comprehensive evaluation on grape and wheat seeds. Carlos Castañeda-Alvarez; Simona Prodan; Ximena Antiguay; Ernesto San-Blas; Alan Zamorano

Metabarcoding survey supports specificity of EPN-*Paenibacillus* sp. association and identifies potential bacterial antagonists of *Diaprepes* root weevil in a Florida citrus orchard. Alexandros Dritsoulas; Homan Regmi; Kamali Shokoofeh; Lukasz Stelinski; Lauren Diepenbrock; Larry Duncan.

Genomics of in vitro dauer juvenile recovery of *Heterorhabditis bacteriophora* in monoxenic liquid culture with *Photorhabdus laumondii*.
Zhen Wang; Ralf-Udo Ehlers; Carlos Molina.

12.50-13.30	**Poster session 1**
13.30-15.00	Lunch

Symposium presentations are 20 minutes long
(15 min talk + 5 min questions)
Flash presentations are 5 minutes long (no questions)

15.00-15.40	**Legend speaker: George Poinar (Virtual).** Legacy of Entomopathogenic Nematology: The early years 1932-1986
	Chair: Larry W. Duncan
15.40-16.40	**SYMPOSIUM 2** Bacteria biology, symbiosis, and application
	Chairs: Patricia Stock & Selcuk Hazir

Natural products from *Xenorhabdus* and *Photorhabdus* bacteria: Past, present and future. Bode Helge

To have or not to have a symbiont: Is that the dilemma of the *Steinernema-Xenorhabdus* partnership? S. Patricia Stock

***Xenorhabdus* and *Photorhabdus* bacteria: New sources of bioactive compounds**. Selcuk Hazir

16.40-17.10	Coffee break
17.10-18.10	**SYMPOSIUM 3** Behavioral Ecology
	Chairs: Ivan Hiltpold & Christine Griffin

Finding (and keeping) a good resource. Christine Griffin

Group dynamics of entomopathogenic nematodes: Leaders lead and followers follow. Ed Lewis; Glen Stevens; David Shapiro-Ilan

Smelling in the dark and finding hosts: entomopathogenic nematodes use environmental odors to efficiently forage. Ivan Hiltpold

18.10-19.30	**Discussion session 1.** From academia to industry
	Chairs: Roxina Soler, Roxina Soler, Diana Londono, Bart Vandenbossche, Ramon Georgis, Ralf-Udo Ehlers, David Shapiro-Ilan, & Antoinette Malan
19.30-20.30	Welcome cocktail

Thursday, 11th April

RIOJAFORUM CONFERENCE CENTRE

8.30	REGISTRATION
9.00-9.40	**Legend speaker: Raymond Akhurst.** Entomopathogenic nematode/bacterial symbioses – an historical perspective
	Chair: Patricia Stock
9.40-10.40	**SYMPOSIUM 4** Population ecology
	Chairs: Raquel Campos-Herrera & Monique Rivera

Enhancing the role of entomopathogenic nematodes in agricultural soil: advances in quantification and interaction analysis for effective biological control. Raquel Campos-Herrera; Rubén Blanco-Pérez

Deciphering tritrophic connections between insect herbivores, entomopathogenic nematodes, and their insect hosts. Monique Rivera

How to make your own IJs traceable with the use of quantum carbon dots? Ernesto San-Blas; María José Cornejo; Patricia Morales-Montero; Gabriela Lankin; Macarena Olivares; Sebastian Faundez; Carlos Castañeda-Álvarez

10.40-11.10	Coffee break
11.10-12.10	**SYMPOSIUM 5** Survival, Virulence and immunity
	Chairs: Itamar Glazer & Ioannis Eleftherianos

Disclosing virulence factors of *Steinernema carpocapsae*. Nelson Simões

Survival of entomopathogenic nematodes: Summarizing three decades of research with view to the future. Itamar Glazer

Using the *Drosophila* model to understand insect anti-nematode immunity. Ioannis Eleftherianos

12.10-13.20	**Discussion session 2**. Global platform for advancing on EPN.
	Chairs: Ralf Ehlers & Xingyue Li
13.20.13.30	GROUP PHOTO
13.30-15.00	Lunch

Symposium presentations are 20 minutes long
(15 min talk + 5 min questions)
Flash presentations are 5 minutes long (no questions)

15.00-15.40 **Legend speaker: Noël Boemare.**
Bacterial Symbionts of Entomopathogenic Nematodes

Chair: Sophie Gaudriault

15.40-16.40 **SYMPOSIUM 6**
Mass production, include safety & regulation

Chairs: Ralf Ehlers & Luis Leite

EPN contribution to transformation of agricultural practice: Production and safety. Ehlers Ralf-Udo

EPN production in bioreactors: some engineering aspects that would be considered. Norberto Chavarría-Hernández; Carlos-Inocencio Cortés-Martínez; Marco-Antonio Islas-López; Ma.-del-Rocío López-Cuellar; Adriana-Inés Rodríguez-Hernández

History of EPN production in Brazil with emphasis on a new biphase process – liquid/solid – for the control of *Sphenophorus levis*. Luís G. Leite; Julie G. Chacon-Orozco; David I. Shapiro-Ilan; Fernando B. Baldo

16.40-17.10 Coffee break

17.10-18.10 **SYMPOSIUM 7**
Commercial use & Future targets

Chairs: Tshima Ramakuwela & Stefan Toepfer

Applications of entomopathogenic nematodes for the control of below- and above-ground maize pests. Stefan Toepfer; Xun Yan; Bart Vandenbossche; Patrick Fallet; Ted Turlings; Sergio Rasmann; Bancy W. Waweru; Joelle Kajuga; Rui Tang

Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses – the Italian experience. Eustachio Tarasco; Elena Fanelli; Alberto Troccoli; Giulia Torrini; Alessio Vovlas; Giovanna Curto; Leonardo Marianelli; Mirella Clausi; Diego Leone; Giancarlo Rappazzo; Francesca De Luca

Entomopathogenic nematodes take root in South Africa. Tshimangadzo Ramakuwela

18.15-18.45
FLASH PRESENTATIONS

Chair: Xingyue Li

Soil, water, nematodes. Ralf-Udo Ehlers

Unveiling the complex ecological relationship between entomopathogenic nematodes and earthworms. Maryam Chelkha; Kyle Wickings; Raquel Campos-Herrera

Volatile organic compounds of the black truffle: attraction or repulsion to EPNs? Implications for truffle beetle biocontrol. Ivan Julià; Ivan Hiltpold; Ana Morton; Fernando Garcia-del-Pino

Identification of natural products regulating the symbiosis between entomopathogenic nematodes and their bacterial symbionts. Daniela Vidaurre Barahona; Edna Bode; Li Su; Helge B. Bode

Influence of natural products from entomopathogenic bacteria on nematode recovery. Fatemeh Sayedain; Coralie Pavesi; Peter Grün; Helge B. Bode

18.50-19.30 **Poster session 2**

20.00 **Pincho's night at Laurel street (tapas+drinks)**

Friday, 12th April

RIOJAFORUM CONFERENCE CENTRE

9.00-9.40	**Legend speaker: Randy Gaugler.** EPN Archeology: My Journey in the Golden Age
	Chair: David Shapiro-Ilan
9.40-10.40	**SYMPOSIUM 8** Application technology
	Chairs: David Shapiro-Ilan & Mayra Rodríguez

Advances in application and formulation of entomopathogenic nematodes. David Shapiro-Ilan

Considerations for the delivery of EPN in orchards. Larry Duncan

Entomopathogenic nematodes as tool for pest management in developing country: Cuba, study case. Mayra G. Rodríguez Hernández; Roberto Enrique Regalado; Jorge Hernández Núñeze; Martha R. Hernández Zaldívar; Mérida Rodríguez Regal; Yordano J. Alambares Carrió; Nilda Perez-Consuegra

10.40-11.10	Coffee break
11.10-12.10	**SYMPOSIUM 9** Genome and Evolution
	Chairs: Sophie Gaudriault & Ralf Sommer

Entomopathogenic nematode genomes. Adler Dillman; Anil Baniya; Erich Schwarz

***Pristionchus pacificus* – developmental plasticity of feeding structures and the many features of entomophilic nematodes**. Ralf J. Sommer; Hanh Witte; Waltraud Röseler

The endosymbiont and the second bacterial circle of entomopathogenic nematodes: from monoxenic paradigm to pathobiome. Ogier Jean-Claude; Pagès Sylvie; Frayssinet Marie; Akhurst Raymond; Boemare Noël; Gaudriault Sophie

12.15-12.45	FLASH PRESENTATIONS
	Chair: Fernando García del Pino

Symposium presentations are 20 minutes long
(15 min talk + 5 min questions)
Flash presentations are 5 minutes long (no questions)

The potential of ShK domains of *Steinernema carpocapsae* as bioinsecticide. Duarte Toubarro; Jorge Frias; Tiago Paiva; Nelson Simoes

Entomopathogenic nematodes to control wireworms: efficacy screening, and impact of morphometry and symbiotic bacteria. Andrea Chacon; Fanny Ruhland; Salimata Drabo; Thibaut Smeets; Brice Checconi; Raquel Campos-Herrera; François Verheggen

Injection of entomopathogenic nematodes in tropical fruit trees for xylophagous pest management. Roxana Myers; Cathy Mello; Tracie Matsumoto

Bacterial bioluminescence is an important regulator of multitrophic interactions in soil ecosystems. Ricardo Machado

Sky is not the limit: successful foliar application of *Steinernema* spp. entomopathogenic nematodes to control Lepidopteran caterpillars. Kay Moisan; Olga Kostenko; Magda Galeano; Roxina Soler; Jose E. Belda; Sjoerd van der Ent; Ivan Hiltpold

12.45-13.30	**Poster session 3**
13.30-15.00	Lunch
15.00-16.40	**SYMPOSIUM 10** New Frontiers in EPN
	Chairs:Helge Bode & Ernesto San Blas

A versatile toolkit for genetic studies in *Heterorhabditis bacteriophora*: opening doors for future EPN research. Christopher Ogaya; Zhen Wang; Giulia Godina; Ralf Ehlers; Carlos Molina

Combining multiple baiting cycles with digital droplet PCR optimizes description of the distribution of entomopathogenic nematodes in French maize fields. Elisabeth Depuydt; Jean-Claude Ogier; Nusrat Ali; Cécile Villenave; Anne Jimenez; Patrice Mahieu; Brendan Vouadec; Eric Nguema-Ona; Sophie Gaudriault

Controlling the fall armyworm in Africa with entomopathogenic nematodes. Patrick Fallet; Didace Bazagwira; Carlos Bustos-Segura; Joelle Kajuga; Stefan Toepfer; Ted C,. J. Turlings.

Entomopathogenic nematodes avoid scent of predatory mites. Shokoofeh Kamali; David Olabiyi; Homan Regmi, Lukasz Stelinski; Lauren Diepenbrock; Larry Duncan

Exosomes are virulence factors in *Steinernema carpocapsae*. Duarte Toubarro; Jorge Frias; António Marcila; Nelson Simões

16.40-17.10	Coffee break
17.10-18.30	**Discussion session 3.** Breaking the Frontiers in EPN research
	Chairs: R. Campos-Herrera & S. Patricia Stock
18.30-18.45	**CONFERENCE CLOSURE**
20.30	**Winery free visit and welcome wine.** Bodegas Franco-Españolas
21.00-00.30	**Gala dinner and drinks.** Bodegas Franco-Españolas

Posters' list

	TITLE	AUTHORS	PRESENTER INSTITUTION
01	Contributions to the study of entomopathogenic nematodes against pests of agricultural and health importance in Argentina	Daiana Eliceche; Soledad Guevara; Matias Rusconi; Matias Rosales; Augusto Salas; Agustin Balsalobre; Dario Balcazar; Marina Ibañez; Laura Morote; Graciela Minardi; Gerardo Marti; Diego Sauka; Fernanda Achinelly	CEPAVE
02	Enhancing Thrips Control in Pepper: A Synergistic EPN-Kairomone Approach	Gorkem Ates; Ozgur Ates; Tufan Can Ulu; Alper Susurluk	Bioglobal A.S
03	Does the EPN associated bacteria composition fluctuate after successive parasitic cycles in a Lepidopteran host?	Roux Chloé; Ogier Jean-Claude; Bedhomme Stéphanie; Brillard Julien	INRAE-Univ. Montpellier
04	*Metarhizium brunneum* (Petch.) vectoring by entomopathogenic nematodes in the context of compatibility to control soil-dwelling stages of *Spodoptera littoralis*	Yousef Meelad; Brillard Julien	INRAE-Univ. Montpellier
05	Assessing the performance of various spray nozzles in the application of entomopathogenic nematodes	Busra Sadic Ulu; Tufan Can Ulu	Bilecik Seyh Edebali University
06	Bioluminescence protects *Heterorhabditis*-infected cadavers from nocturnal scavengers	Maria D. Cassells; Sophie Labaude; Christine T. Griffin	Maynooth University
07	Entomopathogenic nematodes to control wireworms: efficacy screening, and impact of morphometry and symbiotic bacteria.	Andrea Chacon; Fanny Ruhland; Salimata Drabo; Thibaut Smeets; Brice Checconi; Raquel Campos Herrera; François Verheggen	University of Liege, Gembloux Agro-Bio Tech
08	Bioactivity of *Xenorhabdus szentirmaii* metabolites against the ant fungus *Leucocoprinus gongylophorus*	Julie G. Chacon-Orozco; Luis G. Leite; Ana Eugenia C. Campos	Instituto Biologico
09	Unveiling the complex ecological relationship between entomopathogenic nematodes and earthworms	Maryam Chelkha; Kyle Wickings; Raquel Campos-Herrera	Cornell AgriTech
10	Selection of a South African *Heterorhabditis bacteriophora* isolate for in vitro liquid mass production for the biocontrol of *Thaumatotibia leucotreta*	Nicholle Justine Claasen; Murray David Dunn; Antoinette Paula Malan	Stellenbosch University
11	Metabarcoding survey supports specificity of EPN-*Paenibacillus* sp. association and identifies potential bacterial antagonists of *Diaprepes* root weevil in a Florida citrus orchard	Alexandros Dritsoulas; Homan Regmi; Kamali Shokoofeh; Lukasz Stelinski; Lauren Diepenbrock; Larry Duncan	Agricultural University Of Athens

12	A novel biphasic process – liquid to solid – to produce *Steinernema rarum*, and its implementation to control *Sphenophorus levis* in Brazil	Luis G. Leite; Julie G. Chacon-Orozco; David I. Shapiro-Ilan; Fernando B. Baldo	Instituto Biológico
13	Impact of the secondary metabolites synthesized by *Xenorhabdus bovienii* on the activity of beneficial soil organisms: viability and virulence of entomopathogenic nematodes	Maria del Mar González Trujillo; Juan Artal; Ignacio Vicente-Díez; Sergio Álvarez-Ortega; Jorge Dueñas Hernani; Raquel Campos-Herrera	ICVV
14	Volatile organic compounds of the black truffle: attraction or repulsion to EPNs? Implications for truffle beetle biocontrol	Ivan Julià; Ivan Hiltpold; Ana Morton; Fernando Garcia-del-Pino	Universitat Autònoma de Barcelona
15	Are EPNs compatible with essential oils? A novel approach for the integrated pest management of the truffle beetle	Ivan Julià; Marina Seco de Herrera; Ana Morton; Daniel Tapia; Juliana Navarro-Rocha; Fernando Garcia-del-Pino	Universitat Autònoma de Barcelona
16	Evaluating the insecticidal potency of Entomopathogenic nematodes, bacterial symbionts and their products on tomato pests and natural enemies	Ariadni Papafoti; Nathalie Kamou; Vasileia Chatzaki; Apostolos Kapranas	Aristotle University Of Thessaloniki
17	Omics data provide more evidence on interactions among nematode-plant-insect	Javad Karimi; Shokoofeh Kamali; Sepideh Ghaffari; Lukasz Stelinski	Ferdowsi University of Mashhad
18	Regulation of natural product biosynthesis in *Xenorhabdus* and *Photorhabdus* by an ancient metabolite	Agnieszka Nurek; Paushali Chaudhury; Helge Bode	Max Planck for Terrestrial Microbiology
19	Genomics and Chromosome Structure of an Entomopathogenic Nematode	Vera Ogi; Dorothy Maushe; Stefan Grob; Matthias Erb; Christian Parisod; Christelle AM Robert	University of Bern
20	The OptiNEPs project: deciphering the biotic and abiotic factors influencing the isolation of native entomopathogenic nematodes in French agricultural soils	Sire Zoé; Mnasri Refka; Cabre Lisa; Chabert André; Emonet Emeric; Gaudriault Sophie; Lecerf Elodie; Le-Cointe Ronan; Nguema-Ona Eric; Pagès Sylvie; Poggi Sylvain; Siegwart Myriam; Villenave Cécile; Ogier Jean-Claude	INRAE
21	Collection Of Entomopathogenic Nematodes, Biological Resources For Use As Bio-Control Agents	Pagès Sylvie; Kamel Yascim; Antoine-Lorquin Aymeric; Ogier Jean-Claude; Gaudriault Sophie; Givaudan Alain	INRAE
22	Fine scale deposition of EPN by micro-sprinklers	Homan Regmi; Gabriel Martinez; Larry W. Duncan	CREC, University of Florida
23	Efficacy of entomopathogenic nematodes on pupae of *Eucalyptus* snout beetle, *Gonipterus* sp. n. 2	Innocent Rakubu; Agil Katumanyane; Brett Hurley	University of Pretoria, Forestry and Agricultural Biotechnology Institute (FABI)
24	Host-finding strategies of five South African entomopathogenic nematodes species	Innocent Rakubu; Agil Katumanyane; Brett Hurley	University of Pretoria, Forestry and Agricultural Biotechnology Institute (FABI)

25	Influence of natural products from entomopathogenic bacteria on nematode recovery	Fatemeh Sayedain; Coralie Pavesi; Peter Grün; Helge B. Bode	Max-Planck-Institute for Terrestrial Microbiology
26	Infection variations of Azorean *Heterorhabditis bacteriophora* strains against *Popillia japonica* from laboratory to field experiences.	Hugo Monteiro; Rubén Beltri; Angel Ros; Nelson Simões; Anna Garriga	Universidade dos Açores
27	The type strains of entomopathogenic nematode-symbiotic bacterium species, *Xenorhabdus szentirmaii* (EMC), and *X. budapestensis* (EMA): a chronicle of a twenty-year-long story	Andràs Fodor; Michael G. Klein; Maxime Gualtieri; Matthias Zeller; Eustachio Tarasco; Jànos Kiss; Katalin Lengyel; Virginia Pett; LeRoy Haynes; Andrea M. Fodor; David Chitwood; Tibor Vellai	University of Bari "Aldo Moro"
28	*Xenorhabdus* antimicrobial products: Genetic regulation of biosynthesis and perspectives of application	Zsófia Boros; János Kiss; Ferenc Olasz; Bálint Csikós; Nóra Förhécz; Anna Sebestyén; János Ujszegi; Attila Hettyey; László Makrai; Eustachio Tarasco; Tibor Vellai; András Fodor	University of Bari "Aldo Moro"
29	Non-target safety of entomotoxic protease inhibitors and lectins from higher fungi for entomopathogenic nematodes	Toepfer Stefan; Sabotic Jerica	CABI
30	Apple codling moth control with EPN: climatic parameters for optimal timing of EPN application	Bart Vandenbossche; Mike Barg; Verena Dörfler; Hartmut Kaiser; Nikolina Grabovac; Thorsten Rocksch; Ralf-Udo Ehlers	e-nema GmbH
31	Identification of natural products regulating the symbiosis between entomopathogenic nematodes and their bacterial symbionts	Daniela Vidaurre Barahona; Edna Bode; Li Su; Helge B. Bode	Max Planck Institute for Terrestrial Microbiology
32	Genomics of in vitro dauer juvenile recovery of *Heterorhabditis bacteriophora* in monoxenic liquid culture with *Photorhabdus laumondii*	Zhen Wang; Ralf-Udo Ehlers; Carlos Molina	Kiel University
33	Red imported fire ants committing suicide by taking poison under the stress of entomopathogenic nematodes	Sheng-Yen Wu; Huatao Tang; Youming Hou	Fujian Agriculture and Forestry University

Hang up posters:
Wednesday, 10th April from 08:30

Take down posters:
Friday, 12th April until 19:00

Main lobby floor -2

Venue

CONFERENCE VENUE
Riojaforum Conference Centre
Calle San Millán, 23 - 25, 26004 Logroño, La Rioja

SESSIONS ROOM: Sala Polivalente 1 + 2
POSTER EXHIBITION: Main lobby floor -2

Social Program

Tuesday, 9th April | 19:30

Welcome reception and cocktail
Espacio Lagares
Address: Ruavieja, 18-20, 26001 Logroño, La Rioja

Wednesday, 10th April | 19:30-20:30

Welcome cocktail
Riojaforum Conference Centre
Address: Calle San Millán, 23 - 25,
26004 Logroño, La Rioja

Thursday, 11th April | 20:00

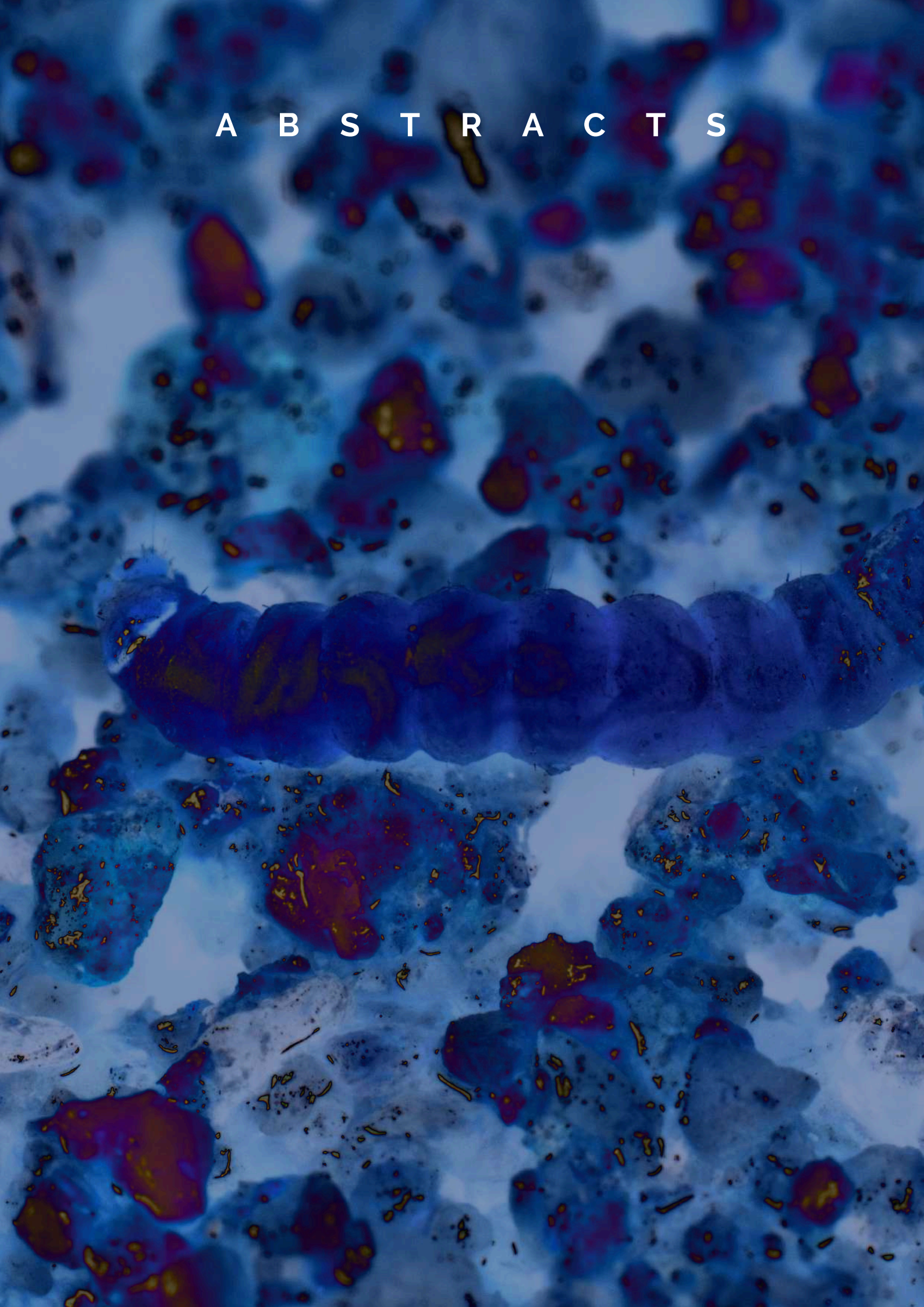
Pincho's night at Laurel street (tapas+drinks)
Meeting point: Plaza del Mercado

Friday, 12th April | 20:30-00:30

Winery free visit and welcome wine
Bodegas Franco-Españolas

Gala dinner and drinks
Bodegas Franco-Españolas
Address: Cabo Noval, 2, 26009 Logroño, La Rioja

A B S T R A C T S



Steinernema kraussei, Steiner 1923: The story of two scientists and the first description of an entomopathogenic nematode

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A B S T R A C T

Anton Krausse was born on December 29, 1878 in Heldrungen, Thuringia, Germany. He studied natural sciences in Berlin and Jena and received his doctorate in Jena in 1905 with a thesis on the antenna of ants. From 1906 to 1914 Anton Krausse lived in seclusion on the island of Sardinia, Italy and made a living collecting and selling insects and other animals to museums around the world. He made valuable contributions to the island's wildlife and described several new species. With the start of the First World War, Krausse was forced to leave all his belongings behind and return to Germany. He was offered a position as a research assistant at the Forestry University in Eberswalde, Germany. From October 1 to 5, 1917, and again from May 27 to June 10, 1918, Krausse made excursions to a forest in the Egge Mountains near Neuenheerse, near Paderborn, Westphalia, where he discovered a massive outbreak of the spruce webworm *Cephalcia abietis* L. (Hymenoptera: Pamphiliidae) on *Picea abies* with up to 600 nymphs per m^2. He took samples and identified several wasps that parasitized this pest (Krausse 1917). This insect is a common natural host for *Steinernema kraussei*. Krausse found a nymph infected with nematodes and sent it to Gotthold Steiner.

Gotthold Steiner, born April 8, 1886 in Signau, Switzerland, was a renowned nematologist. He received his higher education at the universities of Bern and Zurich and received his doctorate in 1910. Steiner took part in the German South Polar Expedition with the sailing ship "Gauss" from 1901-1903 and summarized his research in two publications on marine nematodes (Steiner 1931a,b). In 1922 he moved to N.A. Cobb to the U.S. Department of Nematology Beltsville. After Cobb's death in 1932, Steiner succeeded him as chief nematologist and head of the department. Dr. Steiner wrote 191 scientific papers, most of which dealt with free-living nematodes, including marine, freshwater and terrestrial forms, as well as the nematode parasites of plants and invertebrates. Steiner investigated the material received from Krausse and discovered the new species, which he named *Aplectana kraussei* (Rhaditida: Oxyuridae) in honor of Krausse (Steiner 1923). The publication is written in German. Travassos recognized that the classification in the genus *Aplectana* Railliet & Henry 1916 was a misjudgment and established the genus *Steineria* Travassos in 1927 (Travassos 1927a). This genus had already been assigned to a group of marine nematodes (Steineria Micoletzky, 1922), so Travassos quickly renamed the genus *Steinernema* Travassos 1927 (Travassos 1927b).

Keywords: history, discovery *S. kraussei*.

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Chairs: Ed Lewis

Entomopathogenic nematodes: a commercial journey

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A B S T R A C T

My journey with Entomopathogenic Nematodes (EPNs) started in 1976; it opens with opportunities for me to research, develop and commercialize EPN – based products in various countries across a broad range of markets. Although attractive biological control products, their commercialization has been limited to certain markets and target insects. How the obstacles to commercialization of EPN products, and how some of them can be overcome, will be outlined. The experience I obtained with EPNs (1976-1997) was the corner stone in my carrier to assume various managerial positions with various companies (1998-2024), with focus on business management, global development, and commercialization of biologicals, nutrients, and biostimulants. The new plant health approaches that combined effective and environmentally safe biological control products in pest management with micronutrients, biostimulants, and bio-fertilizers programs should be evaluated for expanding the strengthening the commercialization of EPNs.

African frontier: Tapping into entomopathogenic nematodes and symbiotic bacteria

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ABSTRACT

Entomopathogenic nematodes (EPNs) and their associated bacteria have been extensively studied globally, particularly in Europe and America. In contrast, relatively few surveys have been conducted on the African continent to identify native EPN species, and to assess their occurrence and distribution. EPNs have gained considerable attention as a biocontrol agent over the past two decades, leading to multiple surveys being conducted, not only in South Africa, but also in nine other African countries. Such surveys have led to the description of multiple new EPN species from 10 different African countries, with all, apart from for those conducted in Morocco and Nigeria, describing new species. Substantial progress has been achieved by both South Africa and Egypt in uncovering and characterising new EPNs, in exploring their associated symbiotic bacteria, and in conducting pathogenicity assessments against pest insects. In South Africa alone, 16 new EPN species have been described and tested for their biocontrol potential. However, numerous other African nations are still in the initial phases of research, or have not yet commenced with such investigations. Most research conducted so far in Africa has focused on laboratory bioassays, whereas the ecological research undertaken into the use of local nematodes in large-scale field trials remains scant. Whereas the commercial *in vitro* mass-culture of EPN has been highly successful in Europe and North America, such success has been obtained only in the case of a few selected well-studied nematode species, with none of the native African species described being used. This study provides information on EPNs and their associated symbiotic bacteria from the African continent, with a focus on research being undertaken in South Africa.

Keywords: Africa, *Heterorhabditis, Photorhabdus,* South Africa, *Steinernema, Xenorhabdus*.

Systematics and phylogeny of the entomopathogenic nematodes: the past, the present and the future

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A B S T R A C T

The advancement of molecular methods in the last decades enabled a revolution in systematics of entomopathogenic nematodes, with the number of recognized EPN species growing tremendously from 22 in 1995 to 115 in the beginning of 2024. Molecular systematics of EPNs has primarily relied on sequences of the ITS and D2D3 regions of the rDNA. However, recent studies have shown that these markers lack the variability needed to distinguish closely related species. Therefore, transitioning to multilocus molecular characterization will be necessary for future EPN systematics and species descriptions. In recent years, several such markers have been tested, yielding variable results. Among the molecular markers used for phylogenetic reconstructions, the ITS region of the rDNA proved to be the most powerful tool, enabling a division of both families into well-supported main clades. However, the relationships within clades, and in the case of steinernematids, also among clades, are not well resolved, and there is a need for additional genetic markers that would enable to trace the relationships of the closely related EPN species.

Keywords: molecular systematics, species delimitation, phylogeny, coevolution

Taxonomy and systematics of the entomopathogenic bacterial genera Photorhabdus and Xenorhabdus

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A B S T R A C T

Entomopathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus* establish obligate symbiotic relationships with nematodes of the genera *Heterorhabditis* and *Steinernema*, respectively. The nematodes infest soil-dwelling small arthropods, including insects. Right upon infestation, the nematodes release their symbiotic bacterial partners, that produce a plethora of pathogenic factors that kill the infected organism. The diversity of both nematodes and bacteria is enormous. The genus *Photorhabdus* contains 30 taxa (23 species, 6 of which are divided into different subspecies), the genus *Xenorhabdus* contains 32 taxa (31 species, 1 of which is divided into two subspecies), the genus *Heterorhabdtiis* contains 22 species, and the genus *Steinernema* more than 100 species. During my talk, I will focus on different aspects of the taxonomy and systematic of the bacteria. In this context, I will give an overview on the major events in the taxonomic history of the genera *Photorhabdus* and *Xenorhabdus*, including the experimental tools that have been used to classify the different species of these two genera.

Keywords: Morphology and biochemical tests; DNA-DNA hybridization; 16S rRNA, house-keeping, and whole genome sequences; sequence identity scores.

Bioluminescence protects *Heterorhabditis*-infected cadavers from nocturnal scavengers.

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A B S T R A C T

Photorhabdus spp., the symbionts of Heterorhabditis nematodes, are the only known terrestrial bioluminescent bacteria. The ecological benefits of this bioluminescence, for both the bacteria and its symbiotic nematode, have been debated but not supported by experimental evidence. We show for the first time that the bioluminescence produced by Photorhabdus deters nocturnal scavengers, protecting the host insect cadaver and thus the nematode/ bacterial populations inside.

In both field and laboratory experiments, fewer Heterorhabditis downesi-infected cadavers than uninfected cadavers were fed on by scavengers, but only under dark conditions where the bioluminescence of Photorhabdus temperata was visible. We show that slugs (including Lehmannia valentiana) are an important component of the nocturnal scavenger community and that L. valentiana is innately deterred from feeding on uninfected insect cadavers that are in proximity to light that simulates the bioluminescence of Photorhabdus. We propose that bioluminescence works together with widely demonstrated chemical deterrents, as part of a multi-modal defence, to deter scavengers such as slugs from feeding on the host cadaver.

Key words: *Photorhabdus temperata*, aposematism, symbiotic bacteria

Chair: Fernando García del Pino

Xenorhabdus nematophila and *Photorhabdus laumondii* (Morganellaceae) secondary metabolites for the management of *Plasmopora viticola* (Peronosporales: Peronosporaceae) on grapevine leaves

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A B S T R A C T

The entomopathogenic nematode symbiotic bacteria *Xenorhabdus* and *Photorhabdus* (Morganellaceae) have proven their potential for vineyard-associated pest and disease control. Its efficacy has recently been proven against *Lobesia botrana* (Lepidoptera: Tortricidae), the European grapevine moth, and the fungal pathogen *Botrytis cinerea* (Helotiales: Sclerotiniaceae). We hypothesized that the use of the secondary metabolites produced by *Xenorhabdus* and *Photorhabdus* are able to control another vineyard-associated disease, such as the downy mildew produced by the biotrophic oomycete *Plasmopara viticola* (Peronosporales: Peronosporaceae). In this study, we investigated for the first time the use of *Xenorhabdus* and *Photorhabdus* unfiltered ferments for the control of *P. viticola.* We used Tryptone Soya Broth (TSB) unfiltered ferments produced by *X. nematophila* and *P. laumondii* after three days of fermentation on 1 cm2 vine leaf discs. We dip the leaf discs in the bacterial ferments and then let them dry for 1 hour. After applying a 107 suspension of *P. viticola* oospores, we kept them in the conditions to favour the sporulation of the disease. We quantified the sporulation using a manual image analysis protocol employing ImageJ software. Our results showed that *P. viticola* sporulation in TSB and distilled water controls measured 7.78 µm and 20.2 µm, respectively. When treated with bacterial unfiltered ferments, the sporulation reduced significantly to 0.0333 µm for *X. nematophila* and 0.167 µm for *P. laumondii* on grapevine leaf discs. Furthermore, a statistically lower incidence of downy mildew attack was observed on leaves treated with bacterial ferments compared to the control leaf discs ($P < 0.001$). These findings suggest that these bacteria can serve as a source of toxins that can be used to control critical diseases in vineyards, reducing the use of chemical pesticides and copper. Further studies on the multi-pest-multi-disease system are required to determine the feasibility of this approach.

Keywords: Biological control – biofungicide – Unfiltered ferments - EPN symbiotic bacteria

Exploring the potential of symbiotic bacteria as plant-growth promoters: a comprehensive evaluation on grape and wheat seeds

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A B S T R A C T

Symbiotic bacteria (SB) derived from entomopathogenic nematodes present a promising avenue for addressing contemporary agricultural challenges arising from pest damage. Recent studies have intriguingly proposed that these SB may establish positive interactions with plants, thereby enhancing growth. This research systematically assessed the impact of SB on seeds and various plant growth parameters, employing two distinct materials: grapes and wheat. To conduct this study, suspensions of three SB strains (*Photorhabdus antumapuensis* UCH-936, *Photorhabdus* sp. UCH-926, and *Xenorhabdus lircayensis* VLS) were prepared in Phosphate-buffered saline (PBS), after being cultivated in Luria Bertani medium. Seeds of grape (previously stratified, var. Chardonnay) and wheat were subjected to thorough disinfestation, washing, and immersion overnight in a suspension of each SB at a concentration of 1x106 colony-forming units (CFU) mL-1. These pretreated seeds were then planted in 500-mL pots (for grapes) or seedbed trays (for wheat), reinoculated with the same SB suspension, and allowed to grow until reaching the two or three-leaf stage. Upon reaching this growth stage, plants were carefully uprooted, and various parameters, including aerial and root height, germination rate, and vigor, were evaluated. As controls, PBS and a suspension of plant growth-promoting rhizobacteria were employed. The results demonstrated that all SB significantly improved the germination ratio of grape seeds compared to untreated seeds ($p<0.05$), exhibiting increased aerial and root volume, alongside enhanced germination vigor. In the case of wheat, exclusively *Photorhabdus* sp. UCH-926 recorded the highest values for plant parameters ($p<0.05$). Collectively, these findings underscore the potential of SB bacteria as effective promoters of plant growth. However, further studies are imperative to elucidate the specific nature of the interaction between plants and SB, including the identification of associated compounds. This research contributes valuable insights to the development of sustainable agricultural practices by harnessing the beneficial attributes of symbiotic bacteria.

Keywords: Microbial interactions, Rhizobacteria, entomopathogenic nematodes, beneficial bacteria.

Chair: Fernando García del Pino

Metabarcoding survey supports specificity of EPN-Paenibacillus sp. association and identifies potential bacterial antagonists of Diaprepes root weevil in a Florida citrus orchard

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A B S T R A C T

Diaprepes root weevil (DRW, *Diaprepes abbreviatus*) is a major economic pest of citrus trees in Florida and the Caribbean basin. Biological control by native entomopathogenic nematodes (EPN) has been proposed as a driver of DRW abundance across Florida's ecoregions. Weevils also typically occupy specific locations within orchards for unknown reasons. To identify potential causes of local patterns of weevil abundance and tree condition, we measured relationships between DRW and edaphic properties (biotic and abiotic) within a central Florida orchard. Adult DRW were trapped and monitored weekly for two years in 94 plots arranged in a grid pattern within a 2.5 ha area. One year after monitoring began, soil in each plot was sampled and DNA extracted from organisms recovered by sieving-sucrose centrifugation. Soil subsamples were processed for physicochemical properties and DNA was subjected to metabarcoding (Illumina NovaSeq) for three gene regions (ITS2 rDNA, 16S rDNA, and COI mtDNA). Species-specific qPCR primer-probe sets were also used to measure *Steinernema diaprepesi* and *Heterorhabditis indica.* Here we focus on a restricted set of 124 amplicon sequence variants (ASV, comprising 55 identified *Paenibacillus* species) because of the known entomopathogens in this group and the two species reported to be ectoparasites of EPNs. Soil pH was strongly associated with *Paenibacillus* ASVs ($P<0.001$). Fourteen bacterial ASVs were dissociated with DRW ($P<0.05$), whereas none were positively associated with the weevil according to Spatial Analysis by Distance Indices (SADIE). Several *Paenibacillus* species, elevation, coarse sand particles, and combined ASVs of all identified nematophagous fungi (but no EPN) were significant variables explaining 36% of DRW and tree condition variability in a redundancy analysis. Of 123 ASVs, only *Paenibacillus* sp. JF317562, an ectoparasite of *S. diaprepesi*, was highly correlated ($r=0.82$, $P<0.0001$) to that nematode measured by barcode. The ectoparasite was unrelated ($r=0.12$, NS) to *S. diaprepesi* measured by qPCR.

Genomics of *in vitro* dauer juvenile recovery of *Heterorhabditis bacteriophora* in monoxenic liquid culture with *Photorhabdus laumondii*

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A B S T R A C T

The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* is a biological control agent against insect pests. The dauer juvenile (DJ) carries cells of *Photorhabdus* bacteria, invades the host, and delivers bacterial cells into the insect haemolymph. The events chain in which the DJ perceives haemolymph signals and exits the arrested stage to reach sexual maturity is called DJ recovery. In monoxenic liquid cultures, DJs depend on unknown bacterial food signals to trigger the recovery. A rapid, synchronized, and high DJ recovery is a key factor for commercial production of EPN, and its further understanding is crucial to improve the mass production of EPN.
We have developed a DJ recovery predictor bioassay based on *Photorhabdus* supernatant to evaluate the phenotypic variability in *H. bacteriophora* wild type (WT) and EMS mutant lines. More than 150 single nucleotide polymorphisms (SNPs) were characterized within more than 160 mutant lines via high throughput genotyping, and four SNPs resulted robustly associated with the DJ recovery. Thereafter, we carried out a detailed geno- and phenotypic characterization of 14 *Photorhabdus* strains and evaluated their influence on the DJ recovery in a set of *H. bacteriophora* materials. It was evidenced that the bacterial component plays a subordinate role, whereas the nematode genetic pre-disposition is a main factor in the regulation of the DJ recovery in this species. Furthermore, we conducted RNA-seq along early DJ recovery stages (0.5 – 6 h) in two mutant lines with contrasting phenotype. We determined that *H. bacteriophora* DJs discriminate at early stages the source of the recovery signal (bacteria or haemolymph). More than 14,000 gene models were analysed in connection with functional databases and homologies with *Caenorhabditis elegans*. As outcome, nine gene models are postulated as potential targets for future approaches.

Keywords: *Heterorhabditis bacteriophora,* EMS-mutagenesis, DJ recovery, *Photorhabdus* bacterial supernatant, SNPs, RNA-seq.

Chair: Larry W. Duncan

Legacy of Entomopathogenic Nematology: The Early Years 1932-1986

George O Poinar

A B S T R A C T

Those of us that worked with the so-called EPNS (Entomoparasitic nematodes) have to search hard to find any records of that term today in Google. Yet in "The Early Years", it was Gothold Steiner working with sawfly larvae in Germany in 1923 and Japanese beetles in New Jersey in 1929 that set the wheels of *Neoaplectana, Steinernema* and *Heterorhabditis* in motion. Taking a walk back in time shows the numerous individuals and their breakthrough methods of investigating the biology, manipulation and utilization of these nematodes. In this worldwide tour we see ourselves working on long-forgotten, curious projects, a few of which are still in use today. So sit back and enjoy the trip.

Natural products from *Xenorhabdus* and *Photorhabdus* bacteria: Past, present and future

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A B S T R A C T

Bacteria of the genera *Xenorhabdus* and *Photorhabdus* live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively. Since their first description as producers of natural products 30 years ago, they have been developed into a productive model system, which allows to study the biosynthesis, structure and function of various natural products. Their ability to produce up to 25 different natural product classes is outstanding among all Enterobacteriales. Already early on, several of these natural products showed biological activity, most likely originating from important functions during the live cycle of entomopathogenic nematodes (EPNs) and their bacterial symbionts. Tools have been developed to identify these compounds and study their function in high-throughput, allowing the identification of widespread natural products with conserved functions in the EPN live cycle, as well as the identification of rare compounds with highly specific functions.

In my talk, I will summarize the history of natural product research from *Xenorhabdus* and *Photorhabdus* and will highlight recent findings from our lab probably explaining the success of EPNs as well as synthetic biology approaches allowing the generation of non-natural derivatives with improved biological activity.

Keywords: natural product function, biosynthesis pathways, ecology.

To have or not to have a symbiont: Is that the dilemma of the *Steinernema-Xenorhabdus* partnership?

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A B S T R A C T

The Steinernema-Xenorhabdus-insect partnership tripartite symbiosis is extremely diverse and represents a model system in ecology and evolution to investigate beneficial and antagonistic interactions between invertebrates and microbes. The spectrum of dependence in this symbiotic partnership is variable, ranging from tight, obligate relationship to a facultative one that impacts the fitness of the symbiotic pair. A body of evidence suggests that the reproductive fitness of the nematode-bacterium partnership is tightly associated. Furthermore, maintenance of their virulence is also critical to the conversion of the insect host as a suitable environment where this partnership can be perpetuated. Indeed, studies have shown that *Xenorhabdus* symbionts play an important role not only on the survival of *Steinernema* nematodes in the soil environment, but also on their development and reproduction in the insect host. Disruption of the symbiotic partnership can have detrimental effects on the fitness of both partners. In this presentation, I will summarize the symbiotic relationship between *Steinernema* and *Xenorhabdus*, focusing on the contributions of bacterial symbionts to nematode fitness and in their ability to successfully access and utilize an insect host.

Keywords: *Steinernema*, *Xenorhabd*us, symbiosis, fitness, reproduction, virulence

Xenorhabdus and *Photorhabdus* bacteria: New sources of bioactive compounds

Selcuk Hazir

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A B S T R A C T

Entomopathogenic bacteria in the genera Xenorhabdus and Photorhabdus are mutualistically associated with entomopathogenic nematodes Steinernema and Heterorhabditis, respectively.

These bacterial species produce different types of soluble or volatile secondary metabolites when vectored into insect hemocoel by nematodes to protect against microbial competitors. Most of these compounds are non-ribosomal derived peptides, polyketides, and/or hybrid natural products and are known to have antibacterial, antifungal, antiprotozoal, insecticidal, acaricidal, ovicidal, larvicidal, anticancer and scavenger deterrent activities. Various secondary metabolites have been identified from Photorhabdus spp. including anthraquinone pigments, rhabduscin, -lactam carbapenem, darobactin, transcinnamic acid, trans-stilbenes, benzaldehyde, phototemtide, mevalagmapeptides, isopropylstilbenes, photorin etc. and nematophin, xenorhabdin, xenortide, xenocoumacin, xenotetrapeptide, benzylidenacetone, rhabduscin, rhabdopeptide, fabclavine, ambactin, cabanillasin, indole, szentiamide, PAX peptides etc. have been isolated from Xenorhabdus spp. Among the identified compounds fabclavines are the most polyfunctional molecules with antibacterial, antifungal, antiprotozoal, ovicidal and larvicidal activities. Others such as Trans cinnamic acid, stilbene derivatives, benzaldehyde and cabanillasin also exhibit antifungal activities against plant and human pathogenic fungi. Xenocoumacine, xenorhabdin and PAX peptides possess antiprotozoal activities. Xenocoumacin showed acaricidal activity against important phytophagous mite species. Such natural products are believed to be an emerging source of novel biopesticides and pharmaceuticals. They can serve as lead compounds for the design and synthesis of new alternatives to replace current more toxic chemicals.

Finding (and keeping) a good resource

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A B S T R A C T

Entomopathogenic nematodes (EPN) such as *Steinernema* spp and *Heterorhabditis* spp include a free-living infective juvenile (IJ) stage in the lifecycle. The IJ seeks out and infects insects in soil and other cryptic places. Once inside, the IJ releases symbiotic bacteria (*Xenorhabd*us and *Photorhabdus*) that kill and digest the insect, providing a nutritive environment for development and reproduction. Choosing a host that will allow a high reproductive potential, and defending it against competing organisms, are key fitness components of the nematode-bacterial complex, shaped by the ecological conditions prevailing over the evolutionary history of the EPN. This talk will review various aspects of host selection and defence in relation to nematode fitness.

Keywords: host choice, competition, scavenger defence

Group dynamics of entomopathogenic nematodes: Leaders lead and followers follow

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A B S T R A C T

Animals form groups to accomplish actions that singletons cannot. Entomopathogenic nematodes (EPNs) are generally found in groups in the field, whether in natural populations or soon after application for pest management. EPN infective stage juveniles (IJs) do little more than find a host, but only in rare circumstances can an individual establish a successful infection. Thus, traveling, and then infecting, in groups makes evolutionary sense. We have documented several instances of group behaviors by EPNs and have shown that chemical-based communication is the glue that holds groups together. First, IJs group together in the absence of environmental cues. EPNs exhibit trail-following behaviors that are species-specific, and individual IJs are more likely to be followed after they have had contact with hosts. IJs also are attracted to remote conspecific groups of IJs, and groups that have been exposed to hosts are more attractive than unexposed groups. Finally, EPN IJs infect in groups. A host recently infected by conspecific IJs is more attractive than an uninfected nearby host, which results in some hosts being infected while others are not. Modelling group infection dynamics of EPNs suggests that infections are initiated by a small number of IJs in a group that are "risk-prone" and then followed by other IJs once the risks associated with infecting a healthy host are mitigated. All of these aspects of group behaviors require a degree of communication among EPN IJs, so like pods of whales, packs of wolves and murders of crows, EPN group dynamics provide an adaptive advantage via communication.

Keywords: group behaviour, trail following, chemical ecology, infection behaviour

Smelling in the dark and finding hosts: entomopathogenic nematodes use environmental odors to efficiently forage

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A B S T R A C T

Within the structural and ecological complexity of the soil matrix, entomopathogenic nematodes (EPNs) must identify hosts and orientate towards them in order to achieve their life cycle. In order to do so, EPN infective juveniles (IJs)rely of various physical and chemical cues. Physical cues encompass magnetic fields or vibrations whereas volatile organic compounds (VOCs) have been proposed as effective cues signaling the presence of an insect host and its quality. Indeed, VOCs isolated from insects triggered positive (attraction) or negative (repulsion) chemotaxis to cruiser IJs. Similarly, jumping was induced by insect isolated VOCs in nictating ambusher IJs. In addition to cues directly emitted by their hosts, EPNs respond to VOCs emitted by insect damage plant roots. These cues can diffuse over large distances (on the scale of EPNs) and are hypothesized to be alarm signals emitted by plants to attract natural enemies of insect herbivores and indirectly defend their root systems. Understanding and characterizing these interactions allowed to manipulate agroecological systems to enhanced pest management. Past discoveries and further perspectives will be discussed.

Keywords: chemical ecology, host finding, behavioral ecology.

Chair: Patricia Stock

Entomopathogenic nematode/bacterial symbioses – an historical perspective

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A B S T R A C T

Research on entomopathogenic nematodes (EPNs) at CSIRO commenced in the mid-1970s. After initial trials demonstrated their potential for use in biological control of insect pests, our research was focussed on developing mass production capability. An early breakthrough was our simple method for recovering EPNs that enabled us to quickly establish a collection of multiple EPN species and strains. This collection allowed the testing of the generality of the features of these EPN/bacterial associations and gave insights into natural levels of infection. Examination of the role of the bacteria in the insect/nematode/bacteria interaction provided data across multiple species and strains for issues such as symbiont specificity, its importance *in vivo* and *in vitro*, its retention in IJs, toxicity and antimicrobial activity. It also revealed that each species of the bacterial symbionts occurs in two markedly different forms, of which phase one is apparently better suited to the EPNs needs and is preferentially taken up by the IJs. This discovery opened the way to a mass production system based on otherwise low nutrient media. Studies using early molecular biological techniques showed that the DNA of the two phases was essentially identical and not driven by any known phase shift mechanisms, indicating phase variation was due to a translational or post-translational mechanism. It is evident that the existence of phase variation in all species of two genera of symbionts associated with two families of EPNs implies that it has fundamental importance in the interactions between insects, nematodes and bacteria.

Chairs: Raquel Campos-Herrera & Monique Rivera

Enhancing the role of entomopathogenic nematodes in agricultural soil: advances in quantification and interaction analysis for effective biological control

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A B S T R A C T

As effective biological control agents, entomopathogenic nematodes (EPNs) are crucial in agricultural soil ecosystems. To optimize their efficacy, it is imperative to understand their natural occurrence in soil, necessitating improved quantification methods. EPN assessment has evolved from identifying epizootic events to systematic quantification techniques. The insect bait technique, introduced in 1975, marked a significant advancement, allowing exploration despite the complexity of identifying EPNs amidst diverse nematode communities due to their soil-living infective juveniles (IJs) stage. By the late '90s, a formula utilizing insect bioassays enabled the estimation of IJ abundance. Molecular tools, introduced in 2007, overcame limitations, enabling more frequent detection of EPN sympatric distributions. Recent technologies like High Throughput Sequencing (HTS) and Digital qPCR enhance precise species identification in soil samples. Integration with other tools reveals insights into EPN interactions with competitors and natural enemies, crucial for understanding factors influencing their distribution and pest control efficacy. This presentation focuses on the conservation biological control approach, presenting regional and field studies elucidating EPN occurrence patterns across various habitats, crop types, and management practices. Key findings modulating EPN distribution in agricultural soils and implications for nematode applications are discussed. The exploration extends to EPN interactions with other soil organisms, including entomopathogenic and nematophagous fungi, impacting competition for hosts in diverse manners (synergistic, additive, or antagonistic). The role of EPNs as scavengers in the presence of opportunistic organisms, such as free-living nematodes or saprophytic fungi, is also examined. Overall, understanding these interactions is pivotal in shaping the role of EPNs as biological control agents in soil ecosystems, offering insights into environmental and biotic factors influencing their effectiveness.

Keywords: population density, soil food web, biotic interactions, population dynamics.

Deciphering tritrophic connections between insect herbivores, entomopathogenic nematodes, and their insect hosts

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A B S T R A C T

Entomopathogenic nematodes (EPNs) are soil-dwelling obligate parasites of insects. In the darkness of the soil, EPNs use a multitude of non-visual signals to find their soil-dwelling insect hosts. Over the last decade, research has explored the cues used by EPNs to detect insect hosts. The primary focus, however, has been on plant-derived cues elicited from insect root feeding. However, there are additional dynamics at play which influence the effectiveness of plant-derived cues. In this talk, I will discuss a suite of cues utilized by EPN including information cues from plant defence reactions. Overall, the interactions provide information about how we could potentially enhance the use of EPNs in agroecosystems and potential routes to improvement will be discussed.

Keywords: tritrophic interactions, plant defense response, plant domestication

How to make your own IJs traceable with the use of quantum carbon dots?

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ABSTRACT

Carbon quantum dots (c-dots) are nanostructures capable of fluorescing in various colors (depending on their design) when excited by a specific energy source (UV light in this case). Two c-dots were used for experimentation: one green, synthetized from urea and citric acid, and another blue, from ethylene-diamine and citric acid. Infective juveniles (IJs) exposed to both c-dots showed no significant differences in mortality due to toxicity, nor sublethal effects in terms of pathogenicity, penetration, and host-killing capacity. Fluorescence acquisition in exposed IJs revealed a gradual increase, peaking between six and seven days of exposure, with fluorescence persisting indefinitely (four months). Additionally, experiments were conducted by placing IJs form two different populations of *Steinernema feltiae* marked with green and blue c-dots in vertical olfactometers (15 cm) with *Galleria mellonella* larvae in one end. After four days, the IJs were extracted, indicating that those from the green-marked population significantly moved further in the olfactometer, consistent with control treatments (same populations placed individually in olfactometers and unmarked). Utilizing c-dots enables the accurate tracking of nematodes with distinct origins, cohorts, or species in the same experiments because different colors are only need. This significant advancement offers a cost-effective and straightforward method for conducting ecology, biology, and behavioral studies, providing valuable insights into the intricate dynamics of these organisms.

Keywords: Population dynamics, behavior, *Steinernema feltiae*, nanotechnology.

Disclosing virulence factors of *Steinernema carpocapsae*

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A B S T R A C T

The interaction of *Steinernema carpocapsae* and insect host is complex and involves a wide range of issues at the cellular, molecular, and organismic levels. It is obviously the excreted secreted products (ESP) play a foremost role particularly in virulence and modulation of host defenses. The identification and characterization of ESP has been achieved by omics approaches, producing a large set of genes and proteins. Part of these proteins are released by a classical secretory pathway, whereas others are released in exosomal vesicles. Proteases are the most abundant proteins in ESP. The purification of some of these proteases and the production of others in a recombinant form, allowed its characterization and the identification of targets in the insect host thus understanding the role they may play in the pathogenic process. Moreover, it was evidenced that these molecules have domains other than the catalytic with functional roles in the interaction with insect cells and functional proteins. Among such, ShK domains, that are cysteine riche domains typically blockers of voltage gate channels. These domains are suggested to cause paralysis in infected hosts, but can be also associated to many other disfunction, namely immune defenses. The knowledge of these virulence factors allows the establishment of a framework that must drive the search on these nematodes for the next decades. The selection in the nature of the most active and resistant strain to a specific insect pest, the improvement of beneficial traits by the modulation of specific genes /pathways and the use of identified virulence factors as new biopesticides with improved lethality and specificity, will be discussed.

Key words: Virulence factors, proteases, ESP, nematode improvement, biopesticides.

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Chairs: Itamar Glazer & Ioannis Eleftherianos

Survival of entomopathogenic nematodes: Summarizing three decades of research with view to the future

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A B S T R A C T

As entomopathogenic nematodes (EPNs) are used as biological control agents, their survival and persistence is crucial to ensure success in application against insect pests. EPNs survival is dependent on abiotic and biotic factors in the environment. Abiotic stress environments such as desiccation, temperature, and ultraviolet radiation (UV) severely impact their performance on field. Hence, a comprehensive understanding of the underlying survival mechanisms enabling protection and tolerance is imperative to realistically enhance their performance on field. Thus, identifying key players among each stress could invariably contribute towards developing more robust, reliable solutions towards application strategies including genetic tools and formulation technologies. The current knowledge and future prospects will be presented.

Key words: Abiotic stress, survival, mechanisms, efficacy

Using the *Drosophila* model to understand insect anti-nematode immunity

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A B S T R A C T

The fruit fly *Drosophila melanogaster* is extensively used as a model species in biomedical research. It is also widely studied for its innate immune system to expand our understanding of immune host defenses against numerous pathogens. More precisely, studies using both natural and nonnatural *Drosophila* pathogens have provided a better perspective of pathogen infection strategies and immunity processes than any other model organism. The entomopathogenic nematodes *Heterorhabditis* and *Steinernema* form mutualistic complexes with Gram-negative bacteria. These insect parasites have emerged as excellent research tools for studying nematode pathogenicity and elucidating the features that allow them to persist and multiply within the insect host. Recent work has demonstrated the power of using the *Drosophila* infection model to identify novel parasitic nematode infection factors and elucidate the genetic and functional bases of host antinematode defense. Here, I will describe the interaction of *Drosophila* with recently characterized entomopathogenic nematode secreted virulence factors that each contributes to infection through modulation of host responses. A better understanding of the molecular mechanisms of nematode infection and host anti-nematode processes will lead to the development of novel means for parasitic nematode control.

Keywords: entomopathogenic nematode, innate immunity, infection, pathogenicity.

Chair: Sophie Gaudriault

Bacterial Symbionts of Entomopathogenic Nematodes

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A B S T R A C T

Around 1974, I started bacteriological studies on the microbiota of EPNs, following the request of my colleague nematologist C. Laumond (INRA Antibes France). I contacted people concerned by this subject that were at this stage the pioneers of the subject, the Prof. Poinar (University of Berkeley, USA), Dr Lysenko bacteriologist of the Czech Invertebrate Pathology laboratory of Prof. Weiser, and the Australian team working on EPNs in CSIRO in Hobart, Drs Akhurst and Bedding. It was the time that the concept of the symbiotic association with a specific bacterium discovered by Prof Poinar named *Xenorhabdus*, was confirmed and extended to several species of *Steinernema* by Akhurst (PhD thesis,1982) and, later, species of *Heterorhabditis*. However, the bacterial species isolated from EPNs were not the same every time. Lysenko found species of *Pseudomonas,* and from my side, though I found *Xenorhabdus* every time, I also randomly found Pseudomonadaceae and Enterobacteriaceae (PhD thesis,1983). In that time, the most urgent topic was to develop the taxonomy of the *Xenorhabdus* in terms of phenotypic and genotypic properties. I engaged in a cooperation with Dr Akhurst to study the specificity of these symbiotic associations in order to define new species of *Xenorhabdus* for the symbionts of different species of *Steinernema* (Akhurst and Boemare, *Bergey's Manual* Vol. 2, 2005) and to define a new genus, *Photorhabdus*, removed from *Xenorhabdus*, for all the symbiont species associated with *Heterorhabditis (*Boemare and Akhurst, *ibid.* 2005). The main topics of this talk will develop the results of this very fruitful cooperation.

Keywords: Bacterial symbionts, *Xenorhabdus*, *Photorhabdus*, *Enterobacteriaceae*, *Pseudomonadaceae*, Taxonomy

EPN contribution to transformation of agricultural practice: Production and safety

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A B S T R A C T

Agricultural practice is at a crossroad. Insecticides loose efficacy, new products have limited target spectrums and toxic insecticides are banned. Markets call for alternatives. EPN can fill the gap. Liquid culture of EPN has much advanced during the last two decades. At the three major players production sites (BASF, e-nema and Koppert), the total capacity has reached 1 million L, a volume that yields between 50 and 100 tones wet weight of nematode DJs for treatment of 40-200×103 ha, at 5 or 1 billion DJs ha-1 application density. This capacity can be run approximately 20 times per year and provide EPN for maximum 2 million ha. Although standard process hardware is used and much experience exists on production of microorganisms, culturing of EPN is not yet a standard operation. Major challenge is the maintenance of monoxenic conditions as the process lasts appr. 14 days. With increasing scale, the loss can easily surpass 10 k€ only for the medium. For monitoring for contaminants qPCR technology was introduced. Genetic stability is frequently assessed and quality monitored continuously throughout up- and downstream processing, storage and formulation. Monitoring quality does not end at the doorstep of the factory. The huge potential of molecular marker assisted breeding for improvement of the reproduction potential, stress tolerance and shelf life is so far only applicable for heterorhabditid nematodes. EPN are exceptionally safe biocontrol agents. Application technology is the key to reduction of application density and together with reduction of production costs EPN are competitive with synthetic insecticides. Together with reduction A comprehensive summary on safety aspects has been published (Ehlers 2003) and can be made available on request. It is often postulated that native strains are better adapted to site specific climatic conditions and therefore achieve higher efficiency. However, convincing evidence is missing. Consequences of xenophobic regulation will be discussed.

Keywords: Liquid culture, volume, downstream processing, production costs, paradigm shift

EPN production in bioreactors: some engineering aspects that would be considered

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A B S T R A C T

The application of infective juvenile stages (IJ) of entomopathogenic nematodes (EPN) has been convenient for the biological control of pest insects in certain agro-systems. Then, the mass production of IJs is required, making the submerged monoxenic culture (SMC) an appropriate option for EPN production. This bioprocess must have pure cultures of the EPN and its symbiotic bacterium. Furthermore, the SMC still presents critical challenges from the point of view of bioprocess engineering since the yields and productivities of IJs can vary greatly. An orthodox approach to setup and control this process involves the dimensional analysis, using dimensionless numbers such as Reynolds (Re) to evaluate the prevailing flow conditions, as well as Sherwood number (Sh), to evaluate the oxygenation conditions in culture, and in different configurations of bioreactor (i.e., Erlenmeyer flask, mechanically stirred reactor, or airlift reactor and bubble columns). For this purpose, we must know the properties of the fermentation broth, such as viscosity and density, as well as the respiratory rate of the bacteria and NEPs, besides other essential factors like composition of the culture media and specific "details" of the specimens in turn (i.e., size, density, specific growth rates, among others). These factors illustrate how vital the fermentation bioprocesses tools are in the framework of biological control using NEPs.

Keywords: Basic Bioengineering, Mixing, Oxygen demand.

History of EPN production in Brazil with emphasis on a new biphase process – liquid/solid – for the control of *Sphenophorus levis*

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A B S T R A C T

Entomopathogenic nematodes (EPNs) have been mass produced in vitro in monoxenic systems using two different approaches: the sponge (polyurethane) process soaked with symbiotic bacterial culture, and by liquid fermentation conducted in submerged bacterial culture. Another approach to produce EPNs is the biphasic process, starting with the liquid culture and ending with solid culture. In Brazil, EPNs have been developed as biopesticide since the year 2000, but only recently a commercial production was established when the synthetic polyurethane sponge commonly used for solid production was replaced by phenolic foam usually used for rooting seedlings. Advantages of using phenolic foam to grow EPNs include 1) eliminating the need to extract/harvest and formulate the nematodes (because the foam serves as support for the nematode growth as well as the final formulation), 2) enabling storage of the nematodes with high stability on the shelf without contaminants, 3) facilitating extraction of nematodes by crushing the sponge after the end of the production process, which allows direct application of the nematodes together with the foam debris/particles, just after its extraction, without clogging the nozzles of the application system. In Brazil, *Steinernema rarum* has been assessed against sugarcane pests, mainly against the sugarcane billbug *Sphenophorus levis* (Coleoptera: Curculionidae) that infests more than 1,000,000 ha of cane and caused up to 74%, control at rates of 1–3 × 108 IJs/ha. Thus, following biphasic production, *S. rarum* has already been used in Brazil to control *S. levis,* on sugarcane fields just after crop harvesting at rates of 1 x 108 IJs/ha. The phenolic sponge biphasic system is a highly efficient approach for producing entomopathogenic nematodes, and is advantageous relative to other systems because the sponge acts as a medium for both production and storage; moreover; the sponge-nematode mixture can be directly applied to the field without any extraction or harvest steps.

Keywords: entomopathogenic nematode, liquid culture, solid culture, sugarcane billbug.

Applications of entomopathogenic nematodes for the control of below- and above-ground maize pests

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A B S T R A C T

Maize (*Zea mays*, corn) is crucial for food security. Numerous coleopteran and lepidopteran pests, as well as some minor other pests, infest maize in most agricultural regions. This leads to an influx of insecticides into this agro-ecosystem. Nevertheless, biological control agents, such as entomopathogenic nematodes have so far only been used to a limited extent against these insect pests. This is probably because maize is generally not a cash crop, despite its importance. Nevertheless, some nematode products have been registered for use against maize pests in several countries. We have reviewed field studies and registered products for the use of nematodes against the most important soil and above-ground insect pests in maize. It appeared that soil and above-ground nematode application techniques for field crops have largely improved. In addition, recent bans of several soil insecticides and insecticidal seed coatings have increased the demand for alternative solutions and could lead to wider use of entomopathogenic nematodes in maize. As part of a three-year Kenya-Switzerland-Rwanda SOR4D project (2024-2026), we will focus in particular on research and development for biological control of the larvae of the fall armyworm, *Spodoptera frugiperda*.

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Keywords: corn, field crop, beneficial entomoparasitic nematodes, *Spodoptera frugiperda*

Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses – the Italian experience

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A B S T R A C T

The term "microbial control" has been used to describe the use of microbial pathogens (bacteria, viruses, or fungi) or entomopathogenic nematodes (EPNs) to control various insect pest populations. EPNs are widely recognized as valuable assets in soil ecosystems due to their role as effective biological control agents (BCAs). They are among the best biocontrol agents, and major developments in their use have occurred in recent decades, with many surveys having been conducted all over the world to identify EPNs that may have potential in the management of insect pests. For nematodes, the term "entomopathogenic" means "causing disease to insects" and is mainly used in reference to the bacterial symbionts of *Steinernema* and *Heterorhabditis* (*Xenorhabdus* and *Photorhabdus*, respectively), which cause EPN infectivity. A compendium of our multiannual experiences on EPN surveys and on their collection, identification, characterization, and use in agro-forestry ecosystems is presented here to testify and demonstrate once again that biological control with EPNs is possible and offers many advantages over chemicals, such as end user safety, minimal damage to natural enemies, and lack of environmental pollution, which are essential conditions for an advanced IPM strategy.

Keywords: Steinernematidae, Heterorhabditidae, microbial control, survey, EPN native strains

Entomopathogenic nematodes take root in South Africa

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A B S T R A C T

Traditionally, the entomopathogenic nematode (EPN) bacterial complex is applied for biological control of soil-borne insect pests. A number of EPN products are commercially available internationally (mainly, in Europe and USA) contributing to the biocontrol agents' market. Similarly, they hold great potential for biological control of insect pests in South Africa. However, these products are under development, hence there are currently no products based on indigenous EPN in South Africa. The current EPN commercialization status and research contributing towards commercialization of EPNs in South Africa will be highlighted. To date, several steps in the development of EPNs have been achieved: i) Surveys to collect indigenous EPNs in South Africa; ii) identification and description of several new species; iii) development of mass production media; iv) Formulation; v) conducted efficacy bioassays and trials for control of key agricultural pests. Furthermore, key challenges, considerations and future direction, in South African context will be presented.

Chair: Xingyue Li

Soil, water, nematodes

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A B S T R A C T

Movement of nematodes in the soil, including entomopathogenic nematodes (EPNs) (Steinernematidae, Heterorhabditidae) depends on their biology, mobility, tolerance to environmental factors (soil moisture, temperature), the edaphic parameters and the soil water dynamics. All textbooks tell us that nematodes need a water film to move in the soil. The poster indicates that this was a wrong interpretation of results obtained with an in vitro experimental setup conducted in the 1960s. The free-living stage of EPNs is the 3rd juvenile stage, the so-called dauer juvenile (DJ). Due to its diameter of 25-43 µm (depending on the species), it can only move through coarse soil pores (defined at > 10 µm diameter). Considering that nematode can only move when these pores are lined with water, movement would be impossible once these pores are dry. Since the coarse pores are empty at a water potential (pF) > 2.5, infestations of insects by EPN would be impossible. However, results of field trials show that control was obtained at lower values. The poster explains why the assumptions based on previous experiments were misinterpreted. But how much water is necessary to successfully establish EPN in the soil?

Keywords: dauer juvenile, movement, water potential

Chair: Xingyue Li

Unveiling the complex ecological relationship between entomopathogenic nematodes and earthworms

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A B S T R A C T

The complex and intricate associations between earthworms and entomopathogenic nematodes (EPNs) have far-reaching implications for soil ecosystems, plant health, and agricultural pest control. As the most dominant soil biomass, earthworms influence EPNs through habitat modification, predation, phoresy, and the effects of their cutaneous excreta. These factors shape the behavior and reproductive patterns of EPNs, essential agents for controlling pests that feed on plant roots in agricultural contexts.

While earthworms generally enhance the biological control potential of EPNs against root-feeding pests, the specific impacts on nematode virulence and reproductive capability vary based on the EPN and earthworm species and their excretions. Besides the direct effect of their activity, recent laboratory mesocosm experiments have highlighted that earthworms can also modify chemical communication between maize roots and EPNs. However, discrepancies exist, particularly in the effects of earthworm mucus on EPN behavior. Understanding these interactions is crucial for sustainable agricultural practices and developing effective pest and disease control strategies. Further comprehensive research under natural conditions will be essential to unravel and bridge the existing knowledge gaps. This review will explore the factors that shape the intricate relationship between earthworms and entomopathogenic nematodes. It will provide a foundation for future studies to expand our understanding of multitrophic interactions in soil ecosystems, ultimately paving the way for targeted and sustainable agricultural practices.

Keywords: Earthworm, entomopathogenic nematode, biological control, phoresy, interaction.

Chair: Xingyue Li

Volatile organic compounds of the black truffle: attraction or repulsion to EPNs? Implications for truffle beetle biocontrol

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A B S T R A C T

The European truffle beetle, Leiodes cinnamomeus, is the most important pest in black truffle (Tuber melanosporum) plantations. Entomopathogenic nematodes (EPNs) are a promising biological control agent against L. cinnamomeus. EPNs may employ multiple sensory cues for their host-seeking behaviors, such as volatile organic compounds (VOCs) and CO2 gradients. We report for the first time the ability of truffle fruitbodies to attract EPNs, identifying some VOCs that appear to play a key role in this attraction. We conducted olfactometer assays to investigate the attraction behavior of Steinernema feltiae and Steinernema carpocapsae towards both T. melanosporum fruitbodies and larvae of L. cinnamomeus. Subsequently, a chemotaxis assay using agar plates was performed to determine which of the 14 main VOCs emitted by the fruitbodies elicited an attraction behavior to S. feltiae at two different concentrations. Both EPN species were attracted to mature fruitbodies of T. melanosporum, which may enhance the likelihood of encountering L. cinnamomeus during field applications. L. cinnamomeus larvae in the presence of truffles did not significantly affect the behavior of EPNs 24 hours after application, underscoring the importance of the chemical compounds emitted by truffles themselves. Chemotaxis assays showed that four long-chain alcohol compounds emitted by T. melanosporum fruitbodies attracted S. feltiae, especially at low concentration, providing a first hint in the chemical ecology of a still-eluded ecological system of great economical value. Further studies should be conducted to gain a better understanding of the tritrophic interactions between T. melanosporum, EPNs, and L. cinnamomeus, as this knowledge may have practical implications for the efficacy of EPNs in the biological control of this pest.

Keywords: attraction behavior, Steinernema, Tuber melanosporum, VOCs, Leiodes cinnamomeus, chemical ecology.

Chair: Xingyue Li

Identification of natural products regulating the symbiosis between entomopathogenic nematodes and their bacterial symbionts

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ABSTRACT

The bacteria *Photorhabdus* and *Xenorhabdus*, members of the Enterobacteriaceae family, establish mutualistic associations with entomopathogenic nematodes (EPNs) belonging to the genera *Heterorhabditis* and *Steinernema*, respectively. The life cycle of EPNs involves a free-living stage known as infective juvenile (IJ). The primary function of the IJ is to locate and infect potential insect hosts, carrying the bacterial symbiont within its intestinal tract. Upon entering the insect through natural openings, the bacteria are released into the hemocoel, producing a diverse array of natural compounds, including toxins and enzymes. These compounds serve to digest insect tissues, providing a nutritional source that facilitates the optimal development and reproduction of the nematode. This maturation process is marked by an initial recovery phase during which IJs transition into the adult stage. Following 2-3 nematode generations and depletion of the food source, the offspring undergo a developmental shift, transforming into the next generation of IJs that retain the bacterial symbiont. Subsequently, these IJs emerge from the insect, actively seeking a new host. In the case of *P. luminescens*, signals associated with the nematode life cycle include isopropyl stilbene (IPS) and intermediates of its biosynthetic pathway. The aim of this project was to investigate the influence of natural products (NPs) synthesized by the bacterial symbiont on nematode development. Comparative experiments were conducted in *S. diaprepesi* using wild-type (WT) and mutant strains of *X. doucetiae* deficient in NP synthesis. These strains included those lacking the global post-transcriptional regulator protein Hfq, a phosphopantetheinyl transferase (PPTase), deletions and promoter exchange related to tryptophan/phenylalanine decarboxylase (DC) linked to acyl amide biosynthesis, and promoter exchange associated with gene clusters responsible for NP production. Based on the obtained results, it has been determined that amines/amides and protegomycin are essential for the proper development of EPNs. Conversely, excessive production of GameXPeptide has a detrimental effect.

Keywords: Nematode development, protegomycin, amides, amines, GameXPeptide

Chair: Xingyue Li

Influence of natural products from entomopathogenic bacteria on nematode recovery

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A B S T R A C T

Entomopathogenic nematodes (EPNs) and their bacterial symbionts play a crucial role as biocontrol agents of insect pest larvae in the soil ecosystem. Recovery and development of nematode infective juveniles (IJs) inside insect larvae are the principal stages of their life cycle and it seems that natural products produced by bacterial symbionts have an important function in this process. In this study, the role of natural products produced by *Xenorhabdus szentirmaii* and *Xenorhabdus nematophila* in completing the life cycle of *Steinernama rarum* and *S. carpocapsae* respectively has been investigated. Bacterial mutants have been created using the *easyPACID* approach (easy Promoter Activation for Compound Identification) and gene deletions. The results indicated natural products produced by non-ribosomal peptide synthetases (NRPS) or polyketide synthases (PKS) are involved in *S. rarum* recovery while natural products affected by the global regulator Hfq are responsible for *S. carpocapsae* recovery. Molecular methods and mass spectrometry-based network analysis suggested specific natural product classes responsible for the recovery of *S. rarum* infective juveniles and these results will be presented.

Keywords: Secondary metabolites, *Xenorhabdus*, *Steinernema*.

Chair: David Shapiro-Ilan

EPN Archeology:
My Journey in the Golden Age

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A B S T R A C T

A deeply personal dive through the "Golden Age" of entomopathogenic nematology. My role in events that helped facilitate EPN development including registration, the Asilomar meeting, books, companies, Cobb Foundation, and the New York Natural History Museum's Biodiversity Hall. Reference will be made to key researchers and publications impacting the era. Concludes with brief comments on my post-EPN career (Gaugler 2.0) and retirement.

Keywords: history.

Advances in application and formulation of entomopathogenic nematodes

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A B S T R A C T

Entomopathogenic nematodes (EPNs) have been proven to be powerful microbial control agents that are used to control a wide variety of economically important pests. However, mechanisms are needed to improve control under field conditions. EPNs are formulated to enhance storage capacity and ease-of-handling. The nematodes can be applied with various application equipment. Several approaches to improve biocontrol efficacy based on application and formulation technology will be described in this presentation. Novel formulations that have recently been developed include gel-based mixtures and nanoparticle-based applications. These innovations in formulation provide added protection to EPNs from environmental extremes such as UV radiation and desiccation. Mechanisms to improve application technology include the use of EPN-infected-hosts as the application vehicle, and the use of boosters such as ascaroside pheromones. EPN pheromones enhance nematode dispersal and infectivity. Thus, application of EPNs following pheromone exposure has resulted in superior biocontrol efficacy under greenhouse and field conditions. Further refinement and advances in formulation and application technology can be expected in the future.

Keywords: biocontrol, efficacy, pheromone, nanoparticle.

Chairs: David Shapiro-Ilan & Mayra Rodríguez

Considerations for the delivery of EPN in orchards

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A B S T R A C T

Orchard soil communities experience few perturbations compared to those in other agricultural or natural habitats, due to stable water relations (irrigation) and lack of tillage. Indeed, orchards would seem to provide ideal conditions for use of entomopathogenic nematodes (EPN), which can be efficiently distributed and incorporated into moist soil during routine irrigation. Orchards also tend to have relatively diverse, abundant endemic EPN communities, suggesting the capacity for persistence by augmented EPN. Here I consider some advantages and limitations of EPN delivery via existing irrigation systems and some implications for EPN conservation. An even distribution of EPN to the soil surface maximizes the probability that an adequate number of nematodes will encounter and kill a target pest. Despite some reports of large variability in the delivery of EPN via drip irrigation lines, other studies and work in Florida citrus orchards have shown drip and microsprinkler systems to distribute infective juvenile nematodes (IJ) across several tens of hectares with near perfect efficiency and consistency. By contrast, the fine scale IJ distribution by these systems is uneven – discretely spaced by 30 cm in most drip systems, while IJ deposition rates from microsprinklers can vary incrementally with distance from the emitter by as much as 20-fold. Modification of microsprinkler systems is necessary to reduce this variability and increase EPN insecticidal efficacy. Tactics to conserve native EPN services can affect biological control in habitats with abundant natives. For example, EPN augmentation can reduce subsequent pest control by endemic EPN; therefore, optimum application timing can depend on the phenology of natural biological control, not only that of the pest. Other tactics with demonstrated potential to conserve native and commercial EPN services in orchards involve engineering soil properties (e.g., texture and pH), nematicide application timing, mulching, and use of crop varieties capable of recruiting EPN in response to herbivory.

Entomopathogenic nematodes as tool for pest management in developing country: Cuba, study case

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A B S T R A C T

In Cuba, since 1988 the National Program of Biological Control has been develop; today 199 cottage laboratories (so called CREE), one pilot and four industrial plants produce several biological control agents. The entomopathogenic nematodes (EPN) were studies in Cuba from the 80s, but their mass production star during 1992-1994. Their reproduction have been develop in 14 provinces using, exclusively, *in vivo* methodology with larvae of *Galleria mellonella* as bioreactors for *Heterorhabditis indica* P2M strain and *Heterorhabditis amazonensis* HC1 strain. Near a trillion of Infective Juveniles (IJ) were produce in 2015, the maximum production in the country but, during the last years, problems with substrates for *G. mellonella* has been impact in IJ production and almost 500 000 millions were produce in 2022 and use in, at least, 30 000 hectares, mainly in small farms. Several pest, belong to Lepidoptera, Coleoptera, Thysanoptera, among other orders and plant parasitic nematodes have been management in crops such as rice, citrus, sweet potato, guava, vegetables, corn, cocoyam, coffee, ornamental, beans, chickpea, *Musa* spp., among others. The doses are variables, but the farmers use 20 million of IJ per hectare using application pump or sprinkler. The biggest difficulties with EPN in Cuba are the *in vivo* reproduction, which is very laborious and the yields that do not cover the demand and the "formulation", although some laboratories deliver the IJ in bags with sponges (5 millions of IJ per bag), most deliver IJ in aqueous solutions in different containers. Some scientific centers and productive entities are currently investigating the development of liquid *in vitro* culture and solid formulations, to achieved greater use of entomopathogenic nematodes. Another challenge is relative to increase the culture of biological control use by farmers, taking account to, each year, hundreds of "new" agriculture stakeholders are involved in food production and needs instructions.

Keywords: biological control, cottage mass rearing, small farms

Chairs: Sophie Gaudriault & Ralf Sommer

Entomopathogenic nematode genomes

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A B S T R A C T

Nematodes have been a focus of genomics since the beginning of large-scale sequencing initiatives in the early 1990s. The use of genome sequences and postgenomic tools has had a significant impact on different fields of biology. Several entomopathogenic nematode (EPN) genomes have been sequenced, with many more on the way. Genomes of EPNs are invaluable to understanding the molecular mechanisms of parasitism, symbiosis, and host-parasite interactions. We discuss the current state of EPN genomics, including an overview of the 11 currently available EPN genomes. We discuss new draft genome sequences for *Heterorhabditis indica* (N50 ~1.1 Mb; total size 68.5 Mb), *Steinernema glaseri* (N50 ~1.2 Mb; total size 85.6 Mb), *S. hermaphroditum* (N50 ~17.7 Mb; total size 91.1 Mb), and *S. scapterisci* (N50 ~4.0 Mb; total size 77.5 Mb). We discuss analyses of genome completeness, predicted gene numbers, chromosomal synteny, conservation of parasitism-associated genes, orthologous gene comparisons, and gene family expansions and contractions in EPN genomes.

Keywords: genomes, genomics, sequencing

Pristionchus pacificus – developmental plasticity of feeding structures and the many features of entomophilic nematodes

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A B S T R A C T

When studying the association of nematodes with insects, Gotthold STEINER not only described *Steinernema*, but also other nematodes now known as *Pristionchus*. *Pristionchus* ssp., as members of the family Diplogastridae, are typically found in association with scarab beetles and other insects with currently more than 50 described species. Modern research on *Pristionchus* started in 1996 with the description of *Pristionchus pacificus* Sommer, Carta, Kim and Sternberg, 1996, which has since been developed as a major model system to investigate developmental plasticity, from its genetic and molecular basis to its ecological and evolutionary significance. The international *Pristionchus* community investigates multiple aspects associated with mouth-form plasticity, from nervous system wiring, regulation of feeding structure polyphenism, predation to self-recognition, the first type of self-recognition found in nematodes. I will provide an overview on the biology of *Pristionchus* highlighting similarities and differences between the *Pristionchus* system and entomopathogenic nematodes.

The endosymbiont and the second bacterial circle of entomopathogenic nematodes: from monoxenic paradigm to pathobiome

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ABSTRACT

Single host–symbiont interactions should be reconsidered from Koch's postulates to the notion of a 'pathobiome'. We revisit here the interactions between entomopathogenic nematodes (EPNs) and their microbiota. The isolation of the bacteria from the infective juveniles (IJs) of *Steinernema* and *Heterorhabditis* species systematically led to the identification of *Xenorhabdus* and *Photorhabdus* species, respectively, supporting the concept of a symbiotic relationship. In this 'endosymbiotic bacterium-focused view', the dogma of natural monoxenicity between the nematode and the endosymbiotic bacterium has become widely accepted as a rule in the scientific community. However, recent high-throughput sequencing studies have shown that EPNs are also associated with other bacterial communities, referred to here as the second bacterial circle of EPNs. Our team profiled the microbiota of *Steinernema carpocapsae* IJs. Multigenic metabarcoding (16S and *rpoB* markers) showed that the bacterial community associated with laboratory-reared IJs consisted of the core symbiont (*Xenorhabdus nematophila*) together with a frequently associated microbiota (FAM) consisting of about a dozen of Proteobacteria (*Pseudomonas*, *Stenotrophomonas*, *Alcaligenes*, *Achromobacter*, *Pseudochrobactrum*, *Ochrobactrum*, *Brevundimonas*, *Deftia*, *etc.*). We validated the profile of the *Steinernema* FAM by culturomic approaches, isolating diverse bacterial taxa. Within the FAM, two species, *Pseudomonas protegens* and *P. chlororaphis*, displayed entomopathogenic properties suggestive of a role in *Steinernema* virulence and membership of the *Steinernema* pathobiome. We also hypothesise a potential positive contribution of the second bacterial circle to the whole infectious process and to completion of the main phases of the EPN life cycle. The microbial communities of low complexity associated with EPNs will permit future microbiota manipulation experiments to decipher overall microbiota functioning in the infectious process triggered by EPN in insects and, more generally, in EPN ecology.

Keywords: Microbiota, Metabarcoding, Pathobiome, *Xenorhabdus*, *Pseudomonas*

Chair: Fernando García del Pino

The potential of ShK domains of *Steinernema carpocapsae* as bioinsecticide

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A B S T R A C T

The excreted secreted products of the infective stage of *S. carpocapsae* contain a large sum of proteins and domains predicted with toxic activity. Among them the ShK belonging to cysteine riche domain family are largely represented. These domains are known as channel blockers with many effects including insecticidal activity. In the ESP of *S. carpocapsae* we identified 22 ShK domains, which structure was predicted by I-Tasser and Alpha-fold. Both analyses show these domains are extremely divergent despite they are highly conserved and constrained due to stability conferred by 3 S-S bridges (Cys1-Cys6, Cys2-Cys4 and Cys3-Cys5). The toxicity prediction of these domains by ClanTox identified 7 domains belonging to P3 (toxin like), 9 to P2 group (probably toxin like and the others as P1 (probably not toxic). Using rigid-docking approach against *Drosophila* K+ channels, *Shaker* channel was evidenced as the most promising target for all the ShK domains. These data support our decision in producing recombinant ShK using different vectors to perform *in vivo* assays. The best activity against larvae of *Drosophila* was achieved in ShK expressed in fusion with Dsbc, which probably is supporting the correct folding of the peptide. The application in insect by injection allows highest rate of mortality than treatments by feeding suggesting limitations in the uptake of the fused peptides. Thus, we formulate the fusion peptides in nanoparticles of chitosan. Our data showed that the treatment with these peptides formulated in chitosan has the same insecticidal activity they have by injection. This data opens new avenues in the use of encoded proteins in *S. carpocapsae* as bioinsecticides.

Key words: ShK, toxic domains, channel blockers, heterologous expression, nanoparticles.

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Chair: Fernando García del Pino

Entomopathogenic nematodes to control wireworms: efficacy screening, and impact of morphometry and symbiotic bacteria.

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A B S T R A C T

Entomopathogenic nematodes (EPNs) are increasingly recognized as a tool in controlling many crop pests, especially soil-dwelling pests, with which they have a common habitat. Several studies have been able to prove the efficiency of EPNs against different soil dwelling insects, however, their ability to infect and kill their host greatly. In particular, wireworms are highly resistant to EPN populations and require high amounts of infective juveniles (IJs) to achieve an optimal mortality rate. These polyphagous insect larvae can cause significant losses in agricultural systems. Pesticides used so far for their control have been banned, which raises concerns about a potential rise in damages caused by these larvae in the upcoming years.

In this study, we aimed to understand which factors may influence the efficacy of EPNs when used for biological control of *Agriotes* larvae. We hypothesize first that smaller nematodes are more effective in penetrating wireworms. Second that some species of symbiotic bacteria are associated with higher virulence. Thirteen nematode populations were considered. The tested EPN populations either came from populations already used in the control of soil pest insects or were isolated from agricultural sites in the Wallonia region (Belgium). Each wireworm was exposed to 250 IJs/cm^3 and monitored until week eight. Then, the morphometry (size and diameter) of IJs was evaluated. After being isolated, the symbiont bacteria of each EPN population were identified, and a metabolic characterization was performed using Analytical Profile Index (API) tests. By means to multiple comparison, we evaluated which of these factors could influence the infectivity of EPNs.

We identified three populations with potential infectivity against wireworms, all belonging to the genus *Steinernema*. Infective juveniles morphometrics emerged as an important factor involved in EPN efficacy, with smallest diameter EPNs being the most virulent. Interestingly, the species of the symbiont bacteria and their metabolic activity did not appear to impact mortality rates. We suggest considering the morphology of EPNs in the design of future applications to control wireworms' populations.

Keywords: Elateridae, Belowground interactions, Biocontrol, *Heterorhabditis*, *Xenorhabdus*, *Photorhabdus*

Chair: Fernando García del Pino

Injection of entomopathogenic nematodes in tropical fruit trees for xylophagous pest management

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A B S T R A C T

Queensland long-horned beetle (QLB) is a newly introduced invasive species in Hawaii with a broad host range. The larvae, up to 5 cm in length, cause structural damage in tropical trees by feeding and tunnelling through trunks and branches. Xylophagous pests, such as QLB, are challenging to control due to the cryptic habitats where they reside. Whereas chemical and biological insecticides are unable to directly target these pests within their galleries, entomopathogenic nematodes (EPNs) can effectively navigate the tunnels and hunt down beetle larvae. A compounded benefit is observed when subsequent generations of nematodes emerge from infected cadavers to hunt additional larvae within the tree. This discussion will focus on the treatment of tropical fruit trees by injection of a local isolate of *Heterorhabditis indica*. EPNs were injected with a syringe and needle into galleries through openings exuding frass, sawdust, or sap and through areas of spongy bark. The treated tree species included citrus, avocado, cacao, breadfruit, passionfruit, and kukui. The treatment efficacy ranged from slightly effective to highly effective depending on environmental conditions. On days with high humidity, extensive cloud cover, and high moisture levels, treatments were most effective and required no additional reapplication. We will review the best management practices for effective delivery of this technology. As this is the only existing control option for QLB and commercial EPNs are not available in the State of Hawaii, growers are adopting this approach by using EPNs reared by the local invasive species committee or rearing their own from starter cultures.

Keywords: cryptic habitats, tropical crops, xylophagous insects.

Chair: Fernando García del Pino

Bacterial bioluminescence is an important regulator of multitrophic interactions in soil ecosystems

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A B S T R A C T

Apart from its entomopathogenic abilities, an intriguing biological trait of the bacterial genus *Photorhabdus* is the production of bioluminescence. Bioluminescence is the chemical production and emission of light by living organisms. This trait has evolved multiple independent times and occurs in more than eight hundred genera across the tree of life. While bioluminescence is well-studied in aquatic ecosystems, less is known about its ecological and evolutionary significance in terrestrial ecosystems, and almost nothing is known about the role of bioluminescence in soil ecosystems. My group uses bioluminescent *Photorhabdus* bacterial symbionts as a model to understand the biological relevance of bioluminescence in the soil. *Photorhabdus* symbionts live in association with *Heterorhabditis* entomopathogenic nematodes. These nematodes penetrate soil-dwelling insects, move towards the insect hemocoel and release their *Photorhabdus* bacterial symbionts. Following the infection, *Photorhabdus* bacteria reproduce, produce toxins and immune suppressors that kill the insect prey. Nematodes then feed on bacteria-digested insect tissues, and reproduce inside the insect cadaver before emerging as infective juveniles to search for a new host. During the colonization process, *Photorhabdus* bacteria produce bioluminescence, which results in a characteristic glow of the infected cadavers. How this type of bioluminescence impacts the behaviour, performance, and physiology of other soil-dwelling organisms including entomopathogenic nematodes, plants, and predatory and scavenging insects, remains unknown. During my talk, I will present our findings in this context and will show that this unique bacterial trait is a powerful regulator of multi-trophic interactions in soil ecosystems.

Keywords: Soil ecology, *Photorhabdus* bacteria, hidden ecological regulators

Chair: Fernando García del Pino

Sky is not the limit: successful foliar application of *Steinernema* spp. entomopathogenic nematodes to control Lepidopteran caterpillars

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A B S T R A C T

Entomopathogenic nematodes (EPNs) are notorious soil-thriving organisms that use chemical cues to seek and infect soil-dwelling arthropods, yielding to various levels of control. In the last 30 years, scientists and practitioners started to explore the application of EPNs onto the foliage of crops in attempts to manage leaf-dwelling arthropods too. Because of their underground nature, and so the need of the wet, compact, dark and protected environment, it remained uncertain whether EPNs could indeed survive phyllospheric environment, and successfully control foliar insect pests. In this context, we tested the potential of *Steinernema feltiae* and *S. carpocapsae,* 2 commercially established strains, in controlling Lepidopteran pests of economic importance, e.g. *Tuta absoluta* and *Spodoptera* spp. caterpillars. We first tested the survival, host penetration time, and infectivity of the two EPN species against the Lepidopteran larvae when applied onto tomato and sweet pepper plants or lettuce, respectively, in semi-field and field trials. Additionally, we explored the behavioural response of the EPNs in response to environmental cues in the tomato phyllosphere infested with *S. exigua*. Our results show that *S. feltiae* and *S. carpocapsae* can both successfully survive and infect caterpillars, reaching similar level of control to a standard chemical pesticide in the commercial field settings. Remarkably, both EPN species survived up to four days on the phyllosphere of tomato plants, and needed only a few hours to succesfully penetrate *S. exigua* caterpillars. Moreover, *S. feltiae* showed positive chemotaxis towards volatiles from *S. exigua*-infested tomato leaf, suggesting that this EPN may actively forage toward its host, although it has never been exposed to leaf-borne volatiles during its evolution. The present study shows the very high potential of Steinernematids in managing major foliar pests in agri-ecosystems. Additionally, the positive chemotaxis of *S. feltiae* shades light on new aspects of EPN chemical and behavioural ecology.

Keywords: Chemotaxis; biological control; field trials; foliar application; phyllosphere; volatiles

A versatile toolkit for genetic studies in *Heterorhabditis bacteriophora:* opening doors for future EPN research

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ABSTRACT

Within the past 10 years, e-nema GmbH invested large resources to establish platforms enabling marker-assisted breeding and genome-based studies for the improvement of *Heterorhabditis bacteriophora* beneficial traits. What is the legacy of this initiative for the future? The present contribution gives an overview of the different resources that are available for further cooperations and elaborates on selected results. Concerning biologic materials, 48 WT inbred lines have been derived out of natural *H. bacteriophora* isolates collected worldwide. These lines are highly homozygous (>95%) and constitute an "eternal" resource for large comparative phenotyping studies. Up to now the lines have been phenotyped for stress-tolerance and virulence. The genome from two of these WT inbred lines has been also re-sequenced and annotated. Further on, >4.000 SNPs have been characterized in these lines, and association analyses identified markers linked to stress-tolerance and virulence. Future approaches offer the possibility to feed this system with new quantitative data and identify markers linked to any other measurable *H.bacteriophora* property. Aside, a set of >160 highly homozygous (>95%) EMS-mutant lines has been produced, and the lines were phenotyped for DJ-recovery. More than 190 SNPs have been as well identified for 96 of these lines and association analysis identified markers linked to DJ-recovery using this platform. This mutant collection is also a consolidated system for future phenotype-genotype correlation studies. Concerning *in silico* data, two large RNA-seq experiments were carried out to identify expression changes along stress- and DJ-recovery-induction for >14.000 genes. The transcript information has been annotated in the published *H.bacteriophora* genome draft as well as in the two re-sequenced genomes. This valuable data should be further mined and combined with genotypic- (SNP) and functional information. Future approaches should profit from this toolkit within the framework of collaborations and new projects in which e-nema GmbH may be involved.

Keywords: high throughput pheno- and genotyping, beneficial traits, genomics.

Combining multiple baiting cycles with digital droplet PCR optimizes description of the distribution of entomopathogenic nematodes in French maize fields

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ABSTRACT

Entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis* are soil-dwelling parasites that feed on insect larvae. They are valuable auxiliaries in the fight against insect pests of crops used in market gardening and through the addition of non-native EPNs. However, a better description of the natural distribution of EPNs in agricultural soils will be required to foster their use in crop management programs. The objective of this study was to develop an optimal methodology for obtaining an accurate picture of the presence and diversity of EPNs in the maize fields around Pau in South-West France. We combined different approaches for the detection of EPNs in 43 maize plots. We optimized a method for isolating EPNs directly from soil samples based on multiple baiting cycles with *Galleria mellonella*. With this approach, *Steinernema* and *Heterorhabditis* were isolated from 25.5% and 2.5% of the plots, respectively. We also extracted the soil nematofauna. An initial morphological identification of the EPNs present in these samples led to the detection of *Steinernema* and *Heterorhabditis* in 2.5 % and 7% of the plots, respectively. We then applied molecular detection techniques to the nematofauna samples, focusing on *Steinernema*. We detected *Steinernema* in 16.5% of plots by quantitative real-time PCR (qPCR) and in 35% of plots by digital droplet PCR (ddPCR). We propose a combination of multiple baiting cycles on soil samples with soil nematofauna extraction followed by ddPCR to optimize the detection of EPNs for the analysis of their distribution in agricultural soils.

Keywords: EPNs, France, quantitative PCR, multi-method approach

Chairs:Helge Bode & Ernesto San Blas

Controlling the fall armyworm in Africa with entomopathogenic nematodes

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A B S T R A C T

The fall armyworm (FAW), *Spodoptera frugiperda* Smith, is a voracious pest of maize originating from the Americas. Recently, it has spread across Africa and Asia where it is causing tremendous plant damage and yield losses, thereby threatening the livelihood and food security of millions. In their attempts to control FAW, farmers have drastically increased the use of insecticides. The negative impacts of these toxins on public health and the environment have prompted calls for safer and more sustainable alternatives. Herein, we developed an innocuous gel formulation made of carboxymethyl cellulose to apply entomopathogenic nematodes (EPN) directly into the whorl of maize plants, where the caterpillars preferentially feed. In laboratory assays and in field trials in Rwanda the application of a low dose of a locally isolated EPN formulated in the gel considerably reduced FAW infestation and plant damage, translating into an increased grain yield.
We now explore ways to further increase the efficacy of the formulation. In one approach, we are testing UV protectants in the formulation to enhance EPN longevity. Another approach is to supplement the formulation with odorous compounds that influence the behaviour of caterpillars and moths. Certain compounds might encourage caterpillars to feed on the EPN-gel – as an attract-and-kill strategy – while others could discourage moths from laying their eggs on the treated plants. In addition, we are evaluating different cost-effective and more practical application approaches to meet the specific needs of both small- and large-scale maize cropping systems.
A newly funded SOR4D project from the Swiss National Science Foundation will allow us to to collaborate with *icipe* (International Centre of Insect Physiology and Ecology), Dudutec Biocontrol in Kenya and CABI to conduct large scale trials to test the efficacy of our formulation under realistic conditions and to transfer the technology to farmers across Africa.

Keywords: biological control, Integrated Pest Management, sustainable agriculture, food security, invasive pest.

Entomopathogenic nematodes avoid scent of predatory mites

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A B S T R A C T

The utility of entomopathogenic nematodes (EPNs) in integrated pest management programs is limited by their poor persistence in soil. Abiotic soil conditions such as temperature, moisture, and texture modulate persistence of EPNs, and top-down regulation by predators and diseases may also drive survival rates. To better understand how EPNs cope with microarthropod predators, we conducted assays to measure chemotactic responses of the EPN, Heterorhabditis bacteriophora, to the mesostigmatid mite, Stratiolaelaps scimitus. Preliminary observations in a sand/organic material substrate showed that mixed life stages of the mites reduced the H. bacteriophora IJs recovered with Baermann funnels by more than 80% within one week. When the nematode IJs were placed in two-choice T-tube assay units filled with washed sand (>150µ), they moved toward H. bacteriophora and away from either S. scimitus, or S. scimitus combined with H. bacteriophora. Using a 'push–pull' headspace collection system combined with gas chromatography-mass spectrometry (GC-MS), two citral isomers, neral and geranial, were recovered from S. scimitus. Both compounds were repellent to H. bacteriophora in T-tube assays. Research to detect additional EPN semiochemicals that may interact with nematode response to these compounds is ongoing. Our results suggest that EPN have may have evolved the ability to detect and respond to neral and/or its isomers as a kairomone indicating danger (i.e. predation).

Exosomes are virulence factors in *Steinernema carpocapsae*.

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A B S T R A C T

The entomopathogenic nematode *Steinernema carpocapsae* is able to kill the host short after the infection. The pathogenicity of this parasite is ascribed as excretory/secretory products (ESP) released by infective juveniles (IJ). Here we report for the first time exosome-like vesicles (EVs) into ESPs of *S carpocapsae*. Ultra-thin sections of IJ revealed a huge number of EVs near the cuticle layer, at the level of pharynx and mid-gut and, released near to nematode lateral fields, along the coronal plane. NTA analysis of purified EVs reveled 2.70 e+8 EVs per ml of ESPs, corresponding to 5.4 e+3 EVs per IJ. Two groups of EVs were discriminated, one group with a mean size of 146.7 ± 6.4 nm corresponding to 85 % of total, and a second group of larger vesicles with a mean size of 245 ± 5.2 nm. Ms-Ms analyses of EVs protein cargo identified 88 proteins, including exosomal proteins signatures, like anoctamin (TMEM16) and ferlin (C2A-F) and neprilysin (CD10). Furthermore, proteins typically non-secreted like cytoskeletal proteins, heat shock proteins, membrane transporters and some already known virulence factor were identified also. The high number of proteins identified in vesicle cargo that lack the signal peptide (46%) evidenced the importance of vesicles as a process of "non-classical" secretion in *S carpocapsae*. Concerning molecular functions, EV proteins were essentially categorized into catalytic activity, binding activity, and protease inhibitors. The catalytic group is mainly composed by proteases, particularly serine proteases but also aspartic and metalloproteases. An "in vitro" assay clear shown that purified EVs were internalized by hemocytes of *G. mellonela*. Our finds reveal for the first time that exosomes are a new described mechanism by which EPNs interact with the host providing a way for delivery of molecular effectors.

Key words: Exosomes, anoctamin, ferlin, neprilysin, serine proteases.

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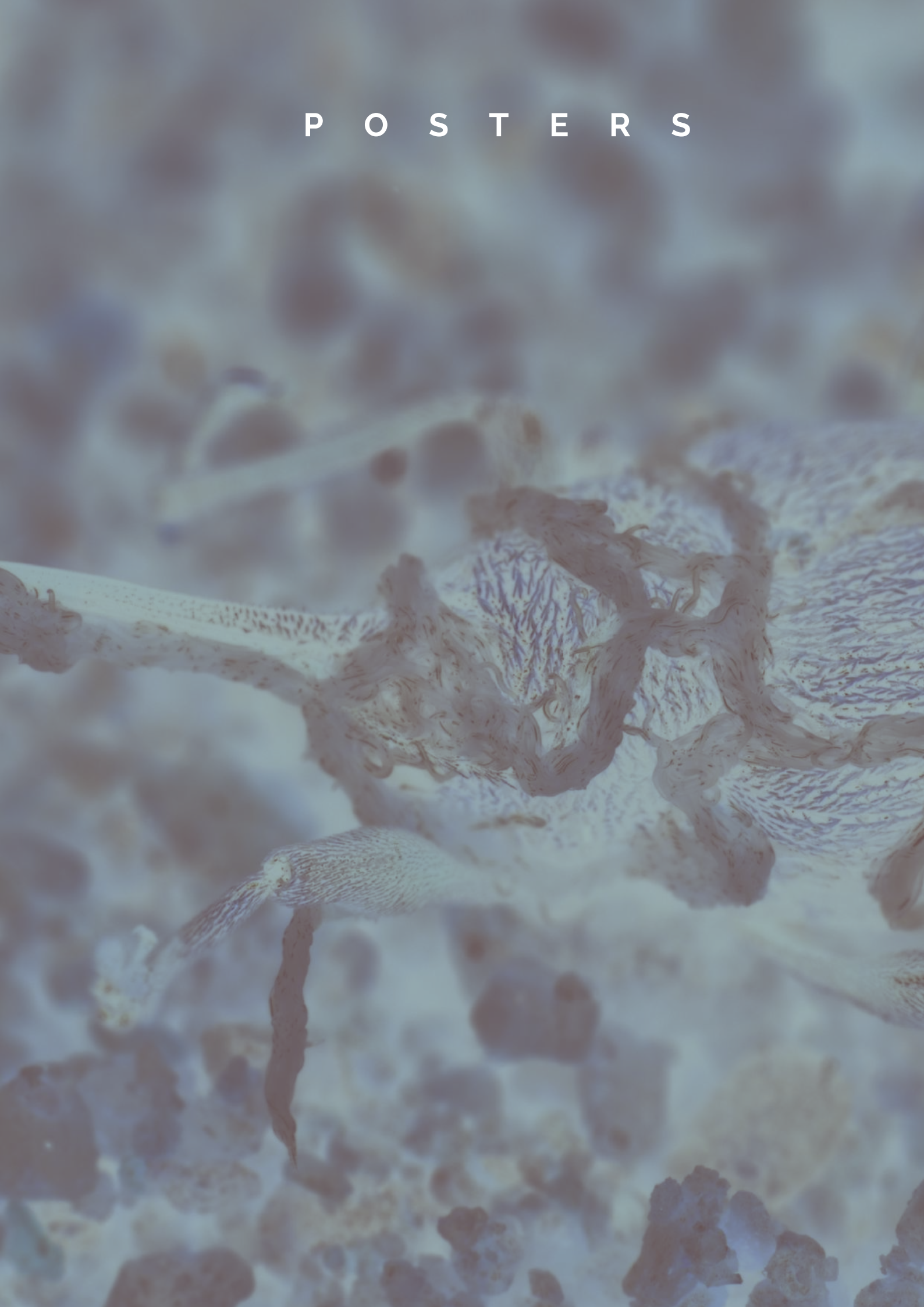
POSTERS



P.01

Contributions to the study of entomopathogenic nematodes against pests of agricultural and health importance in Argentina

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A B S T R A C T

The results of the research about entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) carried out by members of the laboratory of free-living and agroeconomic importance nematodes (CEPAVE) are summarized. The studies were mainly developed in the humid Pampa, one of the major agricultural areas of Argentina and were focus on biology and taxonomy of nematodes, factors affecting parasitism and evaluation of pathogenesis against agricultural and health insect pests at laboratory and field conditions. Mortality greater than 80% (depending on concentrations) were reached against coleoptera larvae (*Phyrdenus muriceus, Lobiopa insularis, Diloboderus abderus),* lepidoptera larvae (*Spodoptera frugiperda, Hylesia nigricans*) and orthoptera larvae (*Anurogryllus muticus, Neocurtilla claraziana*). In addition, mortality was observed in *Armadillidium vulgare* (Crustacea: Isopoda) a soybean crop pest under no-tillage systems, although at high doses. *Heterorhabditis bacteriophora* SUP isolated from this region was effective at field to control the weevil *Phyrdenus muriceus* (Curculionidae) an emergent pest of eggplant (*Solanum melongena* L.). A slight reduction of *P. muriceus* populations, was evidenced after a second application by a decrease of the foliar damage in the eggplant crops. Adults of *P. muriceus* parasitized by *H. bacteriophora* were observed in the field one month after the second application. This constituted the first report of infectivity by EPNs in these weevils. Results of the nematicide effect in the digestive tract of the bacteriophagous nematode *Panagrellus redivivus* (Rhabditidae: Panagrolaimidae) caused by the intake of *Photorhabdus laumondi laumondi* (strain LP1900) are also presented. The use of EPNs in bioinsecticide baits for the control of insect vectors of medical importance as kissing bugs is currently developed within the framework of a START-UP project and progress will be shown.

Keywords: horticultura, Buenos Aires, field application, kissing bugs, bait traps

P.02

Enhancing Thrips Control in Pepper: A Synergistic EPN-Kairomone Approach

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A B S T R A C T

Thrips (Thysanoptera: Thripidae) stands out as a highly destructive pest in agricultural production, posing a significant threat to variety of crops. Their rapid reproductive capability leads to swift resistance development against chemical pesticides, resulting in high crop losses, especially through flower damage. Due to the limitations of chemical solutions, alternative methods are essential, and biological control emerges as an important choice. Entomopathogenic nematodes (EPN) represent a noteworthy biocontrol agent in thrips management. However, it's essential for experts to conduct applications to ensure successful efficacy in the field. A different and inventive approach for thrips management includes employing semiochemicals, especially kairomones. Using the lure and kill technique, sticky traps containing kairomone effectively capture thrips, resulting in a more successful approach compared to conventional traps. However, as the efficacy of both methods varies under field conditions, we investigated whether applying them together would have a synergistic effect. This study explored the simultaneous use of *Steinernema feltiae* and a commercial thrips kairomone against western flower thrips, *Frankliniella occidentalis* on pepper plants in a greenhouse in Mersin, Turkey. According to results, combined application led to a significant reduction in the thrips population compared to individual applications. This research underscores that the collaborative use of diverse biological control agents can enhance effectiveness, demonstrating a promising approach for thrips management.

Keywords: thrips, kairomone, biological control, pepper.

P.03

Does the EPN associated bacteria composition fluctuate after successive parasitic cycles in a Lepidopteran host?

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A B S T R A C T

Entomopathogenic Nematodes (EPNs) have a great potential to control various insect crop pests, but their killing efficiency greatly depends on environmental conditions. The insect killing ability of the endosymbiotic bacterial species (i.e., *Xenorhabdus* or *Photorhabdus*) found in the EPNs' gut (*Steinernema* or *Heterorhabditis*, respectively) has been known for years. Recently, the whole bacterial microbiota associated to these EPNs has been characterized in the laboratory. Several additional bacterial species were found and may also contribute to the parasitic success of the EPNs [Ogier et al., Trends In Microbiol 2023, 10.1016/j.tim.2023.01.004]. In a context of global change, particularly at temperatures higher than those usually encountered, the future efficiency of these EPNs holobionts could be impaired [Guyer et al., Journal of chemical ecology 2021, doi:10.1007/s10886-021-01303-9]. In particular, the composition of the associated microbiota could vary and may consequently modify the parasitic success in the insect. Using a *S. carpocapsae* strain isolated in a French orchard a few years ago, we investigated this hypothesis by generating EPNs lineages obtained after serial passages *in vivo* in a lepidopteran species, *Galleria mellonella*, at different temperatures. The EPNs' bacterial microbiota composition of these lineages, identified by a metabarcoding approach, will be presented. A better understanding of the various factors involved in the parasitic success of these EPNs should help improve the biocontrol efficiency in the future.

Keywords: EPNs microbiota, temperature, insect killing efficiency

P.04

Metarhizium brunneum (Petch.) vectoring by entomopathogenic nematodes in the context of compatibility to control soil-dwelling stages of *Spodoptera littoralis*

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A B S T R A C T

Both entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) may be good agents to control edaphic stages of several insect pests. The present study seeks to assess the compatibility between the EPF *Metarhizium brunneum* strain EAMa 01/58-Su and the EPNs *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. The objective is to evaluate their effectiveness in controlling last instar larvae and pupae of a lepidopteran species, *Spodoptera littoralis*. Also, we delve into the nematovectoring of EPF spores by EPNs. For *S. littoralis* larvae control, the results showed high compatibility between *M. brunneum* and both EPN species with highest larval mortalities (90-100%) achieved in combined treatments compared to single ones (60-70%). In the case of pupae, EPNs treatments did not significantly affect *S. littoralis* adult emergence. However, EPF treatment increased the occurrence of abnormal pupae and it caused a significant reduction of adult emergence. Also, the EPNs were able to vectorize fungal spores to infect *S. littoralis* larvae and pupae. The nematovectoring occurred by both EPNs species and caused fungal infections in *S. littoralis* larvae and pupae. These results indicate that the combination of EPF with EPNs not only improve the efficiency of both control agents but also shed lights, for the first time, on the phenomenon of nematovectoring which may maximize the output IPM (integrated pest management) programs to control the edaphic stages of noctuid pests.

Keywords: entomopathogenic fungi, biocontrol agents' interactions, nematode vectorisation

P.05

Assessing the performance of various spray nozzles in the application of entomopathogenic nematodes

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A B S T R A C T

Entomopathogenic nematodes (EPNs) are microscopic organisms utilized in the biological control of agricultural pests. While EPNs can be administered through various methods, their field effectiveness exhibits variability. One of the primary reasons for this is that the application methods are not optimized for EPN use. As a result, expert applicators typically conduct field applications. With the advancement of technology, drone applications have become increasingly prevalent in agriculture. Some studies indicate the feasibility of applying EPNs via drones; however, the diverse array of spray nozzles used in drones may not all be suitable for EPN application. In this study, we investigated the effects of eight different spray nozzles on EPN discharge potential and the viability of EPNs. A battery-powered back-pack sprayer served as the reservoir and applicator, with nozzle holders connected to the backpack sprayer through a 3D printed adapter. A total of 500 thousand nematodes in one liter of water were applied in glass beakers, and the performance of the spray nozzles was assessed by calculating the number and viability of discharged nematodes. The study findings reveal that the nozzle diameter significantly influences EPN application performance, underscoring the importance of selecting the correct nozzle type during application. This study is anticipated to enhance the understanding of EPN applications with drones.

Keywords: *Heterorhabditis*, *Steinernema*, coarse, herbicide

P.06

Bioluminescence protects *Heterorhabditis*-infected cadavers from nocturnal scavengers.

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A B S T R A C T

Photorhabdus spp., the symbionts of Heterorhabditis nematodes, are the only known terrestrial bioluminescent bacteria. The ecological benefits of this bioluminescence, for both the bacteria and its symbiotic nematode, have been debated but not supported by experimental evidence. We show for the first time that the bioluminescence produced by Photorhabdus deters nocturnal scavengers, protecting the host insect cadaver and thus the nematode/ bacterial populations inside.

In both field and laboratory experiments, fewer Heterorhabditis downesi-infected cadavers than uninfected cadavers were fed on by scavengers, but only under dark conditions where the bioluminescence of Photorhabdus temperata was visible. We show that slugs (including Lehmannia valentiana) are an important component of the nocturnal scavenger community and that L. valentiana is innately deterred from feeding on uninfected insect cadavers that are in proximity to light that simulates the bioluminescence of Photorhabdus. We propose that bioluminescence works together with widely demonstrated chemical deterrents, as part of a multi-modal defence, to deter scavengers such as slugs from feeding on the host cadaver.

Key words: *Photorhabdus temperata*, aposematism, symbiotic bacteria

P.07

Entomopathogenic nematodes to control wireworms: efficacy screening, and impact of morphometry and symbiotic bacteria.

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A B S T R A C T

Entomopathogenic nematodes (EPNs) are increasingly recognized as a tool in controlling many crop pests, especially soil-dwelling pests, with which they have a common habitat. Several studies have been able to prove the efficiency of EPNs against different soil dwelling insects, however, their ability to infect and kill their host greatly. In particular, wireworms are highly resistant to EPN populations and require high amounts of infective juveniles (IJs) to achieve an optimal mortality rate. These polyphagous insect larvae can cause significant losses in agricultural systems. Pesticides used so far for their control have been banned, which raises concerns about a potential rise in damages caused by these larvae in the upcoming years.

In this study, we aimed to understand which factors may influence the efficacy of EPNs when used for biological control of *Agriotes* larvae. We hypothesize first that smaller nematodes are more effective in penetrating wireworms. Second that some species of symbiotic bacteria are associated with higher virulence. Thirteen nematode populations were considered. The tested EPN populations either came from populations already used in the control of soil pest insects or were isolated from agricultural sites in the Wallonia region (Belgium). Each wireworm was exposed to 250 IJs/cm3 and monitored until week eight. Then, the morphometry (size and diameter) of IJs was evaluated. After being isolated, the symbiont bacteria of each EPN population were identified, and a metabolic characterization was performed using Analytical Profile Index (API) tests. By means to multiple comparison, we evaluated which of these factors could influence the infectivity of EPNs.

We identified three populations with potential infectivity against wireworms, all belonging to the genus *Steinernema*. Infective juveniles morphometrics emerged as an important factor involved in EPN efficacy, with smallest diameter EPNs being the most virulent. Interestingly, the species of the symbiont bacteria and their metabolic activity did not appear to impact mortality rates. We suggest considering the morphology of EPNs in the design of future applications to control wireworms' populations.

Keywords: Elateridae, Belowground interactions, Biocontrol, *Heterorhabditis*, *Xenorhabdus*, *Photorhabdus*

P.08

Bioactivity of *Xenorhabdus szentirmaii* metabolites against the ant fungus *Leucocoprinus gongylophorus*

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A B S T R A C T

Leaf-cutter ants (Hymenoptera: Formicidae) are dominant herbivores on the American continent. These social insects remove the leaves of economically important plant species to maintain their colony's food reserves, the symbiotic fungus *Leucocoprinus gongylophorus* (basidiomycete). To control these ants, agrichemicals are still the main options even though there has been an increasing public concern regarding the noxious effects of chemical pesticides on the environmental and human health. The bacteria *Xenorhabdus szentirmaii* that is associated simbioticaly with the entomopathogenic nematode *Steinernema rarum* produces secondary metabolites that are antimicrobial and exhibit high toxicity against various fungal, and can be used for applications as fungicide molecules. The antifungal activities of the metabolites produced by *X. szentirmaii* PAM25 were assessed in vitro against the symbiotic fungus *L. gongylophorus* after different times of bacterial growth (6 and 9 days) and different inoculum concentration (10 and 30%). Moreover, the metabolites were also assessed in respect to their thermo-stability in autoclave. To obtain the metabolites free of the bacteria, different methods were used (filtration and autoclavation). All treatment were diluted in potato dextrose agar (PDA). To confirm the metabolites efficacy, control treatments in the media PDA (alone) and PDA+TSB (mixed) were included and assessed simultaneously for 30 days. The bacteria diluted 10% and 30% caused 25% and 51% inhibition of the symbiont fungus, respectively, when obtained from culture with nine days of bacterial growth, as well as 18% and 43% inhibition when obtained from culture with six days of bacterial growth. The metabolites showed thermo-stability when autoclavation per 20 min. The results imply that these metabolites inhibit the development of the symbiont fungus and should be incorporated into an integrated pest management strategy of leaf-cutter ants on the field.

Keywords: biological control, entomopathogenic nematodes, leaf cutter ants, secondary metabolites.

P.09

Unveiling the complex ecological relationship between entomopathogenic nematodes and earthworms

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A B S T R A C T

The complex and intricate associations between earthworms and entomopathogenic nematodes (EPNs) have far-reaching implications for soil ecosystems, plant health, and agricultural pest control. As the most dominant soil biomass, earthworms influence EPNs through habitat modification, predation, phoresy, and the effects of their cutaneous excreta. These factors shape the behavior and reproductive patterns of EPNs, essential agents for controlling pests that feed on plant roots in agricultural contexts.

While earthworms generally enhance the biological control potential of EPNs against root-feeding pests, the specific impacts on nematode virulence and reproductive capability vary based on the EPN and earthworm species and their excretions. Besides the direct effect of their activity, recent laboratory mesocosm experiments have highlighted that earthworms can also modify chemical communication between maize roots and EPNs. However, discrepancies exist, particularly in the effects of earthworm mucus on EPN behavior. Understanding these interactions is crucial for sustainable agricultural practices and developing effective pest and disease control strategies. Further comprehensive research under natural conditions will be essential to unravel and bridge the existing knowledge gaps. This review will explore the factors that shape the intricate relationship between earthworms and entomopathogenic nematodes. It will provide a foundation for future studies to expand our understanding of multitrophic interactions in soil ecosystems, ultimately paving the way for targeted and sustainable agricultural practices.

Keywords: Earthworm, entomopathogenic nematode, biological control, phoresy, interaction.

P.10

Selection of a South African *Heterorhabditis bacteriophora* isolate for *in vitro* liquid mass production for the biocontrol of *Thaumatotibia leucotreta*

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A B S T R A C T

The *in vitro* liquid mass production of entomopathogenic nematodes (EPNs) is a highly advanced biotechnological process, which is critical for EPN commercial success as a biocontrol product. EPNs have a complex relationship with their associated bacteria, which is important to the successful production of high concentrations of infective juvenile yields. *Heterorhabditis bacteriophora* is the most common EPN species found in South African soils, with previous research having shown its high pathogenicity against a variety of soilborne insects. Currently, a foreign isolate of *H. bacteriophora* is registered in South Africa as a biopesticide product for the control of false codling moth (FCM), *Thaumatotibia leucotreta*, which is a key pest of citrus. Four local isolates of *H. bacteriophora* were used in this study. Each isolate, together with their associated *Photorhabdus* symbiotic bacteria, was isolated and identified using molecular techniques. The four *H. bacteriophora* isolates were laboratory-screened to select the best candidate in terms of pathogenicity against FCM, and in terms of their ease of *in vitro* culture. The screening was conducted by doing bioassays, along with isolating the monoxenic bacteria involved. The isolate SGI_170 was chosen for having the highest pathogenicity, with strong bioluminescence. Two of the *H. bacteriophora* isolates, CRI_LC and LLM, showed slight bioluminescence, while SGI_170 and Px_SPH showed strong luminescence, indicating that it could be two different symbiotic bacterial species that are associated with the same nematode species. Molecular analysis of the 16S gene indicate three different bacterial species, of which two are the same and two possibly new. The *H. bacteriophora* isolate, SGI_170, which showed the highest pathogenicity against FCM last-instar larvae with ease of culture was chosen for the development of the *in vitro* liquid production protocol.

Keywords: Bioluminescence, pathogenicity, *Photorhabdus*, symbiotic bacteria.

P.11

Metabarcoding survey supports specificity of EPN-*Paenibacillus* sp. association and identifies potential bacterial antagonists of Diaprepes root weevil in a Florida citrus orchard

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A B S T R A C T

Diaprepes root weevil (DRW, *Diaprepes abbreviatus*) is a major economic pest of citrus trees in Florida and the Caribbean basin. Biological control by native entomopathogenic nematodes (EPN) has been proposed as a driver of DRW abundance across Florida's ecoregions. Weevils also typically occupy specific locations within orchards for unknown reasons. To identify potential causes of local patterns of weevil abundance and tree condition, we measured relationships between DRW and edaphic properties (biotic and abiotic) within a central Florida orchard. Adult DRW were trapped and monitored weekly for two years in 94 plots arranged in a grid pattern within a 2.5 ha area. One year after monitoring began, soil in each plot was sampled and DNA extracted from organisms recovered by sieving-sucrose centrifugation. Soil subsamples were processed for physicochemical properties and DNA was subjected to metabarcoding (Illumina NovaSeq) for three gene regions (ITS2 rDNA, 16S rDNA, and COI mtDNA). Species-specific qPCR primer-probe sets were also used to measure *Steinernema diaprepesi* and *Heterorhabditis indica.* Here we focus on a restricted set of 124 amplicon sequence variants (ASV, comprising 55 identified *Paenibacillus* species) because of the known entomopathogens in this group and the two species reported to be ectoparasites of EPNs. Soil pH was strongly associated with *Paenibacillus* ASVs ($P<0.001$). Fourteen bacterial ASVs were dissociated with DRW ($P<0.05$), whereas none were positively associated with the weevil according to Spatial Analysis by Distance Indices (SADIE). Several *Paenibacillus* species, elevation, coarse sand particles, and combined ASVs of all identified nematophagous fungi (but no EPN) were significant variables explaining 36% of DRW and tree condition variability in a redundancy analysis. Of 123 ASVs, only *Paenibacillus* sp. JF317562, an ectoparasite of *S. diaprepesi*, was highly correlated ($r=0.82$, $P<0.0001$) to that nematode measured by barcode. The ectoparasite was unrelated ($r=0.12$, NS) to *S. diaprepesi* measured by qPCR.

P.12

A novel biphasic process – liquid to solid – to produce *Steinernema rarum*, and its implementation to control *Sphenophorus levis* in Brazil

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A B S T R A C T

Entomopathogenic nematodes (EPNs) have been mass produced in vitro in monoxenic systems using two different approaches: the sponge (polyurethane) process soaked with symbiotic bacterial culture, and by liquid fermentation conducted in submerged bacterial culture. Another approach to produce EPNs is the biphasic process, starting with the liquid culture and ending with solid culture. In Brazil, the synthetic polyurethane sponge commonly used for solid production of EPNs was replaced by phenolic foam, which consists of a sterile substrate, made of phenolic resin, usually used as a substrate for rooting seedlings. Advantages of using phenolic foam to grow EPNs include 1) eliminating the need to extract/harvest and formulate the nematodes (because the foam serves as support for the nematode growth as well as the final formulation), 2) enabling storage of the nematodes with high stability on the shelf without contaminants, 3) facilitating extraction of nematodes by crushing the sponge after the end of the production process, which allows direct application of the nematodes together with the foam debris/particles, just after its extraction, without clogging the nozzles of the application system. In Brazil, *Steinernema rarum* has been assessed against sugarcane pests, mainly against the sugarcane billbug *Sphenophorus levis* (Coleoptera: Curculionidae) that infests more than 1,000,000 ha of cane and caused up to 74%, control at rates of 1–3 × 108 IJs/ha. Thus, following biphasic production, *S. rarum* has already been used in Brazil to control *S. levis,* on sugarcane fields just after crop harvesting at rates of 1 x 108 IJs/ha. The phenolic sponge biphasic system is a highly efficient approach for producing entomopathogenic nematodes, and is advantageous relative to other systems because the sponge acts as a medium for both production and storage; moreover; the sponge-nematode mixture can be directly applied to the field without any extraction or harvest steps.

Keywords: entomopathogenic nematode, liquid culture, solid culture, sugarcane billbug.

P.13

Impact of the secondary metabolites synthesized by *Xenorhabdus bovienii* on the activity of beneficial soil organisms: viability and virulence of entomopathogenic nematodes

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A B S T R A C T

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are effective biological control agents against various pest insects. They carry symbiotic bacteria, *Xenorhabdus* and *Photorhabdus*, respectively, which produce secondary metabolites (SMs) crucial for the nematode-bacteria complex's killing ability. While *Xenorhabdus* spp. SMs show potential for managing pests and diseases, but their impact on non-target organisms is poorly understood. This study assesses the effect of four *X. bovienii* strain SMs on beneficial nematodes *S. feltiae* 107 and *H. bacteriophora* VM-21. We hypothesize that applying SMs to EPNs would not compromise their viability and virulence. Experiments included two approaches: (i) *in-vitro* viability (4 and 24 hours) and virulence (2 and 5 days) evaluations post-SM exposure (concentrations: 10%, 40%, and 90%) and (ii) *in-vivo* virulence assessments (15 and 30 days) following SM application (10 and 20 mL, two applications) on tomato plants, with water as controls. Two independent trials were conducted with n=3-5 per treatment. *In-vitro* SM exposure had minimal impact on EPN viability (*H. bacteriophora* VM-21 <3% mortality and *S. feltiae* 107 <11%) ($P < 0.05$). However, SM1-90%, SM2-90%, and SM2-40% treatments reduced *S. feltiae* 107 virulence ($P < 0.001$). *In-vivo* exposure (15 days) to SM1 and SM3 reduced EPN virulence ($P < 0.001$), while SM2 and SM4 also affected to one of the EPN species tested. The EPN activity decreased significantly after 30 days, indicating short-term virulence reduction due to SM application, suggesting potential undesired effects on non-target organisms. These findings emphasize the importance of further studies on biopesticide impacts on non-target organisms.

Keywords: *Steinernema feltiae*, *Heterorhabditis bacteriophora*, infectivity, tomato plants, non-target effects.

P.14

Volatile organic compounds of the black truffle: attraction or repulsion to EPNs? Implications for truffle beetle biocontrol

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A B S T R A C T

The European truffle beetle, *Leiodes cinnamomeus*, is the most important pest in black truffle (*Tuber melanosporum*) plantations. Entomopathogenic nematodes (EPNs) are a promising biological control agent against *L. cinnamomeus*. EPNs may employ multiple sensory cues for their host-seeking behaviors, such as volatile organic compounds (VOCs) and CO2 gradients. We report for the first time the ability of truffle fruitbodies to attract EPNs, identifying some VOCs that appear to play a key role in this attraction. We conducted olfactometer assays to investigate the attraction behavior of *Steinernema feltiae* and *Steinernema carpocapsae* towards both *T. melanosporum* fruitbodies and larvae of *L. cinnamomeus*. Subsequently, a chemotaxis assay using agar plates was performed to determine which of the 14 main VOCs emitted by the fruitbodies elicited an attraction behavior to *S. feltiae* at two different concentrations. Both EPN species were attracted to mature fruitbodies of *T. melanosporum*, which may enhance the likelihood of encountering *L. cinnamomeus* during field applications. *L. cinnamomeus* larvae in the presence of truffles did not significantly affect the behavior of EPNs 24 hours after application, underscoring the importance of the chemical compounds emitted by truffles themselves. Chemotaxis assays showed that four long-chain alcohol compounds emitted by *T. melanosporum* fruitbodies attracted *S. feltiae*, especially at low concentration, providing a first hint in the chemical ecology of a still-eluded ecological system of great economical value. Further studies should be conducted to gain a better understanding of the tritrophic interactions between *T. melanosporum*, EPNs, and *L. cinnamomeus*, as this knowledge may have practical implications for the efficacy of EPNs in the biological control of this pest.

Keywords: attraction behavior, *Steinernema*, *Tuber melanosporum*, VOCs, *Leiodes cinnamomeus*, chemical ecology.

P.15

Are EPNs compatible with essential oils? A novel approach for the integrated pest management of the truffle beetle

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A B S T R A C T

The European truffle beetle, *Leiodes cinnamomeus*, is the most important pest in black truffle (*Tuber melanosporum*) plantations. Entomopathogenic nematodes (EPNs) are promising biological control agents against *L. cinnamomeus*. Essential oils (EOs) are also recently being investigated for the control of the adults of this pest. Therefore, both control methods could be combined in Integrated Pest Management (IPM) programs to enhance their efficacy. However, limited information exists regarding the effects of the EOs on EPNs and so their compatibility. The aims of our work were to study the effects of three previously described insecticidal and nematicidal essential oils, *Allium sativum*, *Mentha suaveolens*, and *Satureja montana,* on the survival, infectivity, reproduction, and attraction behaviour of three EPN species: *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. Therefore, we conducted four experiments under laboratory conditions: lethal effect assay, sublethal effect assay, fumigant effect assay and chemotaxis assay. *Allium* caused the highest mortality rates in all three EPN species at 24 and 72 hours after application whether through direct contact in the lethal assay (97-99%) or fumigation (40-42%), and it also reduced their infective capacity on *Galleria mellonella*. *S. montana* EO caused significantly higher mortality rates (6-8%) than control (0-3%) at 72 hours in the lethal assay. It also displayed repellent properties against *S. feltiae* and *H. bacteriohora* in the chemotaxis assay. In contrast, *M. suaveolens* EO exhibited minimal impact on the survival, infectivity and reproduction of all three EPN species. Our results suggest *M. suaveolens* oil may be the most compatible EO for use integrated with EPNs. However, further validation under field conditions and in the presence of *L. cinnamomeus* is necessary to confirm the practical applicability of these findings.

Keywords: *Allium sativum*, *Satureja montana*, *Mentha suaveolens*, *Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*

P.16

Evaluating the insecticidal potency of Entomopathogenic nematodes, bacterial symbionts and their products on tomato pests and natural enemies

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A B S T R A C T

The use of biocontrol agents, such as predators and entomopathogenic nematodes, is a promising approach for the effective control of the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). In this context, two entomopathogenic nematode species were investigated: *Steinernema carpocapsae* and *Heterorhabditits bacteriophora*, as well as their respective bacterial symbionts, *Xenorhabdus nematophila* and *Photorhabdus luminescens*, were applied as bacterial cell suspensions and as crude cell-free liquid filtrates on fresh tomato leaves inoculated with larvae of *T. absoluta*. The same treatments were also tested in the beneficial predatory mirid *Nesidiocoris tenuis*. The results of our study are discussed in the context of selecting new biocontrol tools for integrated pest management.

P.17

Omics data provide more evidence on interactions among nematode-plant-insect

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A B S T R A C T

The first part of our story which was published as Kamali et al, 2021 (DOI: 10.1111/mec.16254) showed some evidence that *Meloidogyne* nematode triggered the immune plant defense in plant roots. Also, we found that beneficial nematode modulates plant immunity against *Meloidogyne* nematodes mainly via the active expression of some enzymes. Here we have more evidence about this story. We investigated the interaction among tomato (*Solanum lycopersicum*) to two groups of nematodes: plant parasite (*Meloidogyne javanica*) and entomopathogen (*Steinernema carpocapsae*) along with a leaf-mining insect (*Tuta absoluta*). There were eight treatments on the plant. 1.*Meloidogyne* nematode, 2. *Steinernema carpocapsae* , 3. tomato leaf miner, 4. bot Meloidogyne and *Steinernema* nematodes, 5. both *Steinernema* and leaf miner , 6. *Meloidogyne* and leaf miner, 7, all three organisms, and 8. control plant. We sequenced the RNA from all treatments and analyzed those data. Here we will discuss those interactions. We will provide more robust information on how beneficial nematodes and parasitic nematodes are interacting. We will discuss the effect of the presence of a beneficial nematode alone or in the presence of a harmful nematode, as well as during the activity of an herbivorous insect on the plant. The main discussions will be about important defense pathways in plants, such as pathways related to plant hormones and plant secondary metabolites. We also provide new evidence regarding the interaction between two nematodes and the possible effect of Ascarocide from *Steinernema* on root-knot nematodes and herbivorous insects. These data could be useful to extend our knowledge about the plant rhizosphere and ultimately could be useful for better understanding the system toward efficient plant protection strategies.

Keyword: microbial control; insect pathology; transcriptomic analysis; plant defense; entomopathogenic nematode

P.18

Regulation of natural product biosynthesis in *Xenorhabdus* and *Photorhabdus* by an ancient metabolite

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A B S T R A C T

Bacteria of the genera *Xenorhabdus* and *Photorhabdus* live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively. They are well-known producers of natural products with different biological functions acting as signals, antibiotics and insecticidal compounds. While the structures and biosynthesis pathways of several of these compounds have been described in the past 30 years, almost nothing is known about the regulation of the biosynthesis pathways. Recently, the RNA chaperone Hfq together with its small regulatory sRNA ArcZ has been described as a key player for global regulation of natural product biosynthesis in both *Xenorhabdus* and *Photorhabdus*, which has also been developed into a molecular tool for studying the natural and function of novel natural products.
More recently, we have identified another global mechanism based on the well-known second messenger cAMP that is also a major regulator of natural product biosynthesis. Metabolome and proteome analysis of mutants defective in cAMP production in combination with bioinformatic analysis of promoter regions followed by biochemical assays allowed the identification of a regulatory network previously unknown to affect natural product biosynthesis in bacteria to the level observed in *Xenorhabdus* and *Photorhabdus* as we will present in our poster.

Keywords: natural product biosynthesis, regulation, second messenger.

P.19

Genomics and Chromosome Structure of an Entomopathogenic Nematode

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A B S T R A C T

Entomopathogenic nematodes (EPNs) play a significant role in agriculture for their role as natural pest control agents, targeting subterranean insects and mollusks. However, the genetic architecture of EPNs remains unknown. Here, we investigated the chromosome structure of the *Heterorhabditis* genus. We performed a *de novo* assembly and annotated the genome at a chromosome-scale in *H. bacteriophora*. The fixation Index (FST) landscape analysis between closely related species, Hi-C mapping, and the distribution of genes and transposable elements along the chromosomes indicated a monocentric chromosome structure. Imaging studies will be conducted to further confirm this hypothesis. These findings challenge the holocentric paradigm generalized from *Caenorhabditis elegans*. Collectively, these results suggest that *H. bacteriophora* may possess a monocentric chromosome structure, or at least a genomic organization, that deviates from strict holocentrism, inviting a reevaluation of nematode chromosome structure theories.

Keywords: Monocentric, Holocentric, population genetics.

P.20

The OptiNEPs project: deciphering the biotic and abiotic factors influencing the isolation of native entomopathogenic nematodes in French agricultural soils

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A B S T R A C T

Context: Entomopathogenic Nematodes (EPNs) are beneficial organisms that are naturally present in soils and in agriculture for biological control through augmentation, involving the introduction of non-native strains into soils. This approach is costly and primarily implemented in high-value crops. Furthermore, the persistence of non-native EPNs in soils is low, limiting their beneficial effects. Conservation biological control, aiming to stimulate natural ennemies to manage insect pests, offers a more cost-effective and sustainable alternative.

Objectives: The OptiNEP project (2022-2024), funded by the French Ecophyto II program, focuses on two primary objectives: i) characterizing the presence and diversity of native EPNs in soils of two economically significant French crops—potatoes and apples; ii) identifying biotic factors (the total nematofauna diversity/abundance), abiotic factors, and farming practices influencing the presence, abundance, diversity, and entomopathogenicity of native EPNs.

Methods: Native EPNs were isolated using the baiting method from soil samples collected in 43 potato fields and 47 apple orchards. Taxonomic characterization of EPN isolates was performed through ITS sequencing, and their entomopathogenicity was assessed against the larvae of two major crop pests: the codling moth (*Cydia pomonella*) and the wireworm (*Agriotes spp*). Total nematofauna was extracted using the elutriation method, with nematode families identified morphometrically.

Preliminary Results: Native EPNs were found in 15 potato plots (34% positive) and 9 orchard plots (20% positive). The number of isolates was higher in apple orchards (40) than in potato fields (19). In apple orchards, three EPN species (Heterorhabditis bacteriophora, Steinernema carpocapsae, and S. feltiae) were identified, while in potato fields, a dominant EPN species (17 S. feltiae isolates) and a minor species (two S. affine isolates) were found. Additionally, several entomopathogenic-like nematodes (Oscheius spp.) were isolated. The assessment of entomopathogenic properties of the EPN isolates and statistical tests aimed at identifying factors influencing the presence and efficacy of native EPNs in agricultural soils are currently underway.

Keywords : *Entomopathogenic nematodes, conservation biological control, orchards, potatoes, biotic and abiotic factors, cultivation practices*

P.21

Collection of entomopathogenic nematodes, biological resources for use as Bio-control agents

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A B S T R A C T

Background: Entomopathogenic nematodes (EPNs) of the *Steinernema* and *Heterorhabditis* genera are commercialized since the 1970s to control crop-damaging insect pests. However, their utilization remains quite low in the bio-pesticide market. They are particularly effective against Lepidoptera and Coleoptera. These EPNs carry their bacterial symbionts *Xenorhabdus* and *Photorhabdus* (Morganellacae) in their digestive tract. Once the EPNs have penetrated insect larvae in the soil, the symbiotic bacteria multiply and kill their hosts by septicemia within 24 to 48 hours.

Since 1999, the DGIMI unit maintains a unique collection of living EPNs containing 140 strains. The collection includes 26 species of the *Steinernema* genus, totaling 100 strains (stored at 9°C), and 3 species of the *Heterorhabditis* genus, comprising 40 strains (stored at 15°C). This unique resource, rich in biological diversity, originates from soils of international provenance (Czech Republic, Lebanon, South Africa, USA, Ireland, Italy, Belgium, ...). Since 2016, the collection has been associated with the environmental pillar of the Agronomic Resource Center for Research: https://www.brc4env.fr/BRCs-and-collections/Invertebrates/Nematodes/Entomopathogenic-nematodes.

Recently, prospective soil sampling has been carried out in cultivated fields in various French regions (Pyrénées-Atlantiques, Bretagne, Normandie, Languedoc) for national research projects and private collaborations. These soil samples were used for *ex-situ* isolation of EPN. 150 additional isolates have been added to the initial collection. Each isolate is characterized in terms of taxonomy, pathology on different insects and description of the associated microbiota. Given the exponential growth of the EPN collection, we develop a database (Postgresql with phpPgAdmin web interface) to improve the internal management of the EPN collection, guaranteeing the traceability of isolates and their associated metadata.

Keywords: Entomopathogenic nematodes, *Steinernema, Heterorhabditis,* collection, data base, traceability

P.22

Fine scale deposition of EPN by micro-sprinklers

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A B S T R A C T

Orchards in Florida are typically irrigated using low-volume systems that deliver the water via drippers or micro-sprinklers. Numerous studies have shown that, when water pressure is well-regulated, these systems can deliver infective juvenile stages (IJ) of entomopathogenic nematodes (EPN) evenly to individual plants across the landscape. In drip irrigation systems IJs are usually deposited in discreet locations separated by 30 cm in plant rows, *i.e.,* no farther than 15 cm from a target insect in narrow bedded crops. In citrus orchards, micro-sprinklers are used to irrigate circular areas beneath the tree canopy with typical radii of up to 180 cm, depending on the age of the tree. The deposition rate of IJ S. riobrave measured at 30 cm increments from the micro-sprinkler emitter varied systematically. The number of IJs deposited at 90 cm was 4-fold that at 30 cm and 40-fold that at 150 cm. Ongoing trials are 1) measuring the effect of this deposition pattern on the insecticidal activity of S. riobrave against Diaprepes abbreviatus and 2) testing options such as varying water pressure, IJ delivery duration, and utilizing different emitter configurations to increase uniformity in IJ deposition and, presumably, insecticidal efficacy.

P.23

Efficacy of entomopathogenic nematodes on pupae of Eucalyptus snout beetle, *Gonipterus* sp. n. 2

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A B S T R A C T

Gonipterus sp. n. 2 causes a considerable reduction in the production of eucalypt trees in South Africa and other countries. The pest is primarily controlled with a mymarid egg parasitoid, *Anaphes nitens,* but only partial efficacy is achieved. Identifying additional biocontrol agents that target other life stages of *Gonipterus* sp. n. 2 could improve the control efficacy of this pest. Entomopathogenic nematodes (EPNs) have provided acceptable control against many important soil pests. As both the pupal stage of *Gonipterus* sp. n. 2 and EPNs coexist in the soil, it gives a window of opportunity for EPN application. In this study, we screened five South African EPN species for their virulence and lethal dosage on uncased *Gonipterus* sp. n. 2 pupae in bioassay plates. Four EPN species, namely *Steinernema jeffereyense*, *S. fabii*, *Heterorhabditis neonieputensis,* and *H. safricana* provided low mortality of less than 40% of uncased pupae, while *S. yirgalemense* provided the highest pupal mortality of 100%. Each EPN species was inoculated at 200 infective juveniles (IJs) per pupa in 50 µl of sterile water and pupal mortality was recorded two days post-inoculation. *Steinernema yirgalemense* was selected and applied at different concentrations; 0, 12, 25, 50, 100, 200, and 400 IJs/pupa to determine its lethal concentration. Based on the Probit analysis, the LC50 and LC90 of *S. yirgalemense* are 48.46 and 257.51 IJs/pupa, respectively. Our findings suggest *S. yirgalemense* has the potential as a biological control agent of *Gonipterus* sp. n. 2.

Keywords: *Steinernema, Heterorhabditis*, Bioassays, Lethal concentrations, Pupal mortality

P.24

Host-finding strategies of five South African entomopathogenic nematodes species

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A B S T R A C T

The infective juveniles (IJs) of entomopathogenic nematodes (EPNs) shows a continuum of host-finding strategies ranging from cruise foragers to ambush foragers. The knowledge of these foraging strategies has been documented by studying the movement behaviour of a selected number of popular EPN species. However, this knowledge is lacking for most newly identified EPN species/ isolates. This study aimed to investigate the host-finding strategies of five local South African EPN species, including *Heterorhabditis noenieputensis*, *H. safricana*, *Steinernema fabii*, *S. jeffreyense* and *S. yirgalemense,* by assessing their dispersal behavior. *Heterorhabditis noenieputensis*, *H. safricana*, *S. jeffreyense* and *S. yirgalemense* showed a positive response to the presence of wax moth larvae in a 9 cm diameter Petri dish, whereas *S. fabii* showed a negative response. The four EPN species that showed a positive response caused 100% mortality of wax moth larvae that were buried in sand at a depth of 10cm in plastic test tubes, whereas *S. fabii* caused the lowest mortality of 34%. The average distance traveled by all five EPN species decreased on a rough textured substrate compared with a smooth textured substrate. The results of the study suggest that *H. noenieputensis*, *H. safricana*, *S. jeffreyense* and *S. yirgalemense* use a cruiser host-finding strategy whereas *S. fabii* uses an ambusher host-finding strategy.

Keywords: *Heterorhabditis*, *Steinernema*, responsiveness, ambusher, cruiser, wax moth

P.25

Influence of natural products from entomopathogenic bacteria on nematode recovery

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A B S T R A C T

Entomopathogenic nematodes (EPNs) and their bacterial symbionts play a crucial role as biocontrol agents of insect pest larvae in the soil ecosystem. Recovery and development of nematode infective juveniles (IJs) inside insect larvae are the principal stages of their life cycle and it seems that natural products produced by bacterial symbionts have an important function in this process. In this study, the role of natural products produced by *Xenorhabdus szentirmaii* and *Xenorhabdus nematophila* in completing the life cycle of *Steinernama rarum* and *S. carpocapsae* respectively has been investigated. Bacterial mutants have been created using the *easyPACID* approach (easy Promoter Activation for Compound Identification) and gene deletions. The results indicated natural products produced by non-ribosomal peptide synthetases (NRPS) or polyketide synthases (PKS) are involved in *S. rarum* recovery while natural products affected by the global regulator Hfq are responsible for *S. carpocapsae* recovery. Molecular methods and mass spectrometry-based network analysis suggested specific natural product classes responsible for the recovery of *S. rarum* infective juveniles and these results will be presented.

Keywords: Secondary metabolites, *Xenorhabdus*, *Steinernema*.

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Infection variations of Azorean *Heterorhabditis bacteriophora* strains against *Popillia japonica* from laboratory to field experiences.

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A B S T R A C T

The Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae) is an insect pest with high impact in Europe, and it was detected in the Azorean Islands in the early 1970s. Since then, entomopathogenic nematodes (EPNs), especially *Heterorhabditis bacteriophora*, have been promising candidates for biological control, although infectivity rates proved inconsistent in field applications. Thus, there is a need to study newly isolated native strains to ensure an efficient strategy control adapted to the environmental conditions. The aim of this work was to test the virulence of six new Azorean isolates of *H. bacteriophora* and two established strains to third instar larvae of *P. japonica* in laboratory and field conditions. In laboratory conditions, all eight strains were able to infect larvae with a preliminary median lethal time (LT50) of 60h for the most virulent FA16 and Az29 to more than 86h for FAPF. The field experiment was performed in constrained PVC tubes with a mean of 5 larvae each and five strains of EPNs (Az29, Az148, SMMO, SMLV, FA16). Az148 was the most virulent strain, with 45% of infected larvae while, FA16 could barely infect (10%) in field conditions. The initial results evidence the contrasting virulence achieved between experiments and the need to test several native strains under environmental conditions.

Keywords: Biological control, native strains, field application.

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The type strains of entomopathogenic nematode-symbiotic bacterium species, *Xenorhabdus szentirmaii* (EMC), and *X. budapestensis* (EMA): a chronicle of a twenty-year-long story

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A B S T R A C T

To join the past with the future, we review two decades of international cooperation research started in Hungary, continued in "laboratories without walls" internationally on the entomopathogenic bacteria (EPB), *Xenorhabdus szentirmaii* DSM16338(T) (EMC), and *X. budapestensis* DSM16342(T) (EMA), symbiotic partners of entomopathogenic nematodes (EPN) species *Steinernema rarum* and *S. Bicornutum,* before returning to Hungary. A mystery of EPN/EPB symbioses is the cessation of bacterial propagation in the gut of the IJs. We suppose that a similar mechanism of homeoviscous membrane adaptation is responsible for that. Our EMA/EMC project includes identifications and characterizations. EMC releases a large quantity of iodinin (we identified as 1,6-dihydroxyphenazine 5,10-dioxide) in a water-soluble form into the media, which condenses to form spectacular insoluble crystals. The first functional annotation of the draft genome of EMC revealed 71 genes encoding antimicrobial synthesizer (non-ribosomal peptide synthases and polyketide synthases) enzymes with plant protection and veterinary perspectives. We turned to the antimicrobial potential of EMA and EMC since multidrug resistance (MDR) is a global challenge not only in clinical, but veterinary and agricultural aspects as well. Replacing antibiotics with antimicrobial peptides (AMPs) produced by soil-born organisms for protecting (soil-born) plants seems a preferable alternative. The natural role of AMPs produced by EPBs is to sustain optimally balanced pathobiome conditions for the EPN/EPB symbiotic complexes in polyxenic conditions (the colonized insect cadaver and soil), and they serve as abundant sources of drug candidates. When drawing attention to EMA's, and EMC's outstanding antimicrobial potential, we probably initiated and/or facilitated the fabclavine project. Fabclavines were discovered in EMA, and their biosynthetic pathway was revealed in EMC in the Bode lab, Frankfurt, Germany. The "crown" of that project, (the easyPACId technique) was first discovered in Germany, in the Bode lab. Since then, we have also reconstructed and used it, (see Boros presentation).

Keywords: non-ribosomal templated antimicrobial peptides, iodinin exocrystal,

P.28

Xenorhabdus antimicrobial products: Genetic regulation of biosynthesis and perspectives of application

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A B S T R A C T

Antimicrobial production of bacterial symbionts (EPB) of entomopathogenic nematodes (EPN) is positively controlled by the *hfq gene. Deletion of hfq* turns off the expression of numerous biosynthetic gene clusters (BCG) encoding for biosynthetic cooperating enzymes responsible for the production of antimicrobial-active end-products. If any of the ***hfq***-controlled BGC operons are reactivated in a *hfq mutant, then the strain will produce only one type of antimicrobial. The so-called "easy Promoter Activated Compound Identification" (easyPACId) approach (Bode et al., 2019) revolutionized the search for EPB-produced drug candidate molecules. We also use this remarkably* reproducible technique for drug-hunting in *Xenorhabdus szentirmaii (EMC), and X. budapestensis (EMA) discovered and first characterized in our labs. We recently published that the cell-free conditioned media (CFCM) of the wild-type strains of both species severely inhibit the chytrid fungus Batrachochytrium dendrobatidis (Bd) both in vitro and in vivo*. (Bd is the causative agent of the epidemic amphibian disease, chytridiomycosis). The EMC-CFCM proved to efficiently reduce the efficiency of artificial Bd-infection load on juvenile common toads (*Bufo bufo*) without any harmful side effects. The CFCM of each of the *hfq*-deleted (EMA, and EMC) strains performed a significantly ($P<0.005$), but not completely, lower antifungal activity than those of the respective wild-type strain. We reactivated several BGCs one by one in our Δ*hfq mutants and tested their antagonistic potentials on different Gram-positive and Gram-negative bacteria, and also eukaryotic pathogens of clinical, veterinary*, and plant pathogenic significance. We also focus on the strength and the cell-specificity of cytotoxic side effects in the organisms to be protected. The aim is to identify drug-candidate bioactive ingredients.

P.29

Non-target safety of entomotoxic protease inhibitors and lectins from higher fungi for entomopathogenic nematodes

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A B S T R A C T

Certain soil insects are becoming increasingly difficult to control due to recent bans on some insecticides. An alternative and safer approach could be the development of biopesticides based on entomotoxic defence proteins of higher fungi. Many of these proteins are protease inhibitors or lectins. Some of them have been shown to adversely affect insects and are currently being studied in more detail for their potential use as biocontrol agents. However, soil pests are also often controlled with biological control agents based on entomopathogenic nematodes. We wanted to know whether some of the promising higher fungal proteins have non-target effects on entomopathogenic nematodes. In laboratory bioassays, we tested the effects of six protease inhibitors and nine lectins from 15 different higher fungi for their potential effects on the survival and pathogenicity of entomopathogenic nematodes. Preliminary results indicate that most protease inhibitors and lectins have no effect on the tested nematodes *Heterorhabditis bacteriophora* and *Steinernema longicaudum*, probably due to the fact that their infective juveniles do not feed and therefore cannot ingest such proteins. We will conduct further studies on the safety of entomotoxic defence proteins of higher fungi to provide the basis for the potential development of new biopesticides.

This work was jointly funded by the Slovenian Research and Innovation Agency (ARIS) (J4-2543, P4-0432) and the National Research, Development and Innovation Office of Hungary (NKFIH) (134356 SNN20). CABI is supported through its core donors (https://www.cabi.org/about-cabi/who-we-work-with/key-donors/).

Keywords: side effects, biopesticides, beneficial entomoparasitic nematodes

P.30

Apple codling moth control with EPN: climatic parameters for optimal timing of EPN application

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A B S T R A C T

The product nemapom® contains entomopathogenic nematodes *Steinernema feltiae* for the biological control of overwintering larvae of the apple codling moth *Cydia pomonella*. Nematodes are sprayed in autumn or spring on the tree bark. To secure success of the application, it is recommended to "keep the bark moist for approximately 4 hours". This rather sketchy recommendation is not appropriate for modern fruit production. The aim of the NemaSens Project is to define the range of the climatic parameters (e.g. humidity, temperature, radiation, wind etc.), at which the nematodes can find and kill the host insects and under which conditions EPN ultimately die. To investigate the survival on bark, we developed a bark spraying assay and collected the nematodes 1,2 and 4 hours after application. Two and four hours after spraying, 85% and 51% of the nematodes had survived on bark, respectively. Low temperature activity of *S. feltiae* was tested in incubators at 5, 6, 7,5 and 8˚C. *S. feltiae* infected and killed *C. pomonella* larvae at temperatures as low as 5˚C, but after 14 days. Although the time to kill the larvae is long, this low temperature activity is advantageous as the larvae overwinter in the bark for several months. These data together with field data will be used to produce decision support systems for the farmer to decide when optimal conditions prevail long enough to obtain high control results.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL).

Keywords: *Cydia pomonella*, decision support systems, *Steinernema feltiae*, temperature, humidity

P.31

Identification of natural products regulating the symbiosis between entomopathogenic nematodes and their bacterial symbionts

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A B S T R A C T

The bacteria *Photorhabdus* and *Xenorhabdus*, members of the Enterobacteriaceae family, establish mutualistic associations with entomopathogenic nematodes (EPNs) belonging to the genera *Heterorhabditis* and *Steinernema*, respectively. The life cycle of EPNs involves a free-living stage known as infective juvenile (IJ). The primary function of the IJ is to locate and infect potential insect hosts, carrying the bacterial symbiont within its intestinal tract. Upon entering the insect through natural openings, the bacteria are released into the hemocoel, producing a diverse array of natural compounds, including toxins and enzymes. These compounds serve to digest insect tissues, providing a nutritional source that facilitates the optimal development and reproduction of the nematode. This maturation process is marked by an initial recovery phase during which IJs transition into the adult stage. Following 2-3 nematode generations and depletion of the food source, the offspring undergo a developmental shift, transforming into the next generation of IJs that retain the bacterial symbiont. Subsequently, these IJs emerge from the insect, actively seeking a new host. In the case of *P. luminescens*, signals associated with the nematode life cycle include isopropyl stilbene (IPS) and intermediates of its biosynthetic pathway. The aim of this project was to investigate the influence of natural products (NPs) synthesized by the bacterial symbiont on nematode development. Comparative experiments were conducted in *S. diaprepesi* using wild-type (WT) and mutant strains of *X. doucetiae* deficient in NP synthesis. These strains included those lacking the global post-transcriptional regulator protein Hfq, a phosphopantetheinyl transferase (PPTase), deletions and promoter exchange related to tryptophan/phenylalanine decarboxylase (DC) linked to acyl amide biosynthesis, and promoter exchange associated with gene clusters responsible for NP production. Based on the obtained results, it has been determined that amines/amides and protegomycin are essential for the proper development of EPNs. Conversely, excessive production of GameXPeptide has a detrimental effect.

Keywords: Nematode development, protegomycin, amides, amines, GameXPeptide

P.32

Genomics of *in vitro* dauer juvenile recovery of *Heterorhabditis bacteriophora* in monoxenic liquid culture with *Photorhabdus laumondii*

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A B S T R A C T

The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* is a biological control agent against insect pests. The dauer juvenile (DJ) carries cells of *Photorhabdus* bacteria, invades the host, and delivers bacterial cells into the insect haemolymph. The events chain in which the DJ perceives haemolymph signals and exits the arrested stage to reach sexual maturity is called DJ recovery. In monoxenic liquid cultures, DJs depend on unknown bacterial food signals to trigger the recovery. A rapid, synchronized, and high DJ recovery is a key factor for commercial production of EPN, and its further understanding is crucial to improve the mass production of EPN.
We have developed a DJ recovery predictor bioassay based on *Photorhabdus* supernatant to evaluate the phenotypic variability in *H. bacteriophora* wild type (WT) and EMS mutant lines. More than 150 single nucleotide polymorphisms (SNPs) were characterized within more than 160 mutant lines via high throughput genotyping, and four SNPs resulted robustly associated with the DJ recovery. Thereafter, we carried out a detailed geno- and phenotypic characterization of 14 *Photorhabdus* strains and evaluated their influence on the DJ recovery in a set of *H. bacteriophora* materials. It was evidenced that the bacterial component plays a subordinate role, whereas the nematode genetic pre-disposition is a main factor in the regulation of the DJ recovery in this species. Furthermore, we conducted RNA-seq along early DJ recovery stages (0.5 – 6 h) in two mutant lines with contrasting phenotype. We determined that *H. bacteriophora* DJs discriminate at early stages the source of the recovery signal (bacteria or haemolymph). More than 14,000 gene models were analysed in connection with functional databases and homologies with *Caenorhabditis elegans*. As outcome, nine gene models are postulated as potential targets for future approaches.

Keywords: *Heterorhabditis bacteriophora,* EMS-mutagenesis, DJ recovery, *Photorhabdus* bacterial supernatant, SNPs, RNA-seq.

P.33

Red imported fire ants committing suicide by taking poison under the stress of entomopathogenic nematodes

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A B S T R A C T

The red imported fire ant (RIFA, *Solenopsis invicta* Buren) poses a significant threat to human health and the agricultural economy. Current ant management relies heavily on chemical interventions, leading to environmental contamination. In contrast, entomopathogenic nematodes (EPNs) offer natural solutions with benefits like host specificity and safety. However, conventional methods using aqueous EPN suspensions can prompt ant nest relocation, reducing treatment efficacy. To address this, our study explores using EPN pre-infected insect cadavers for RIFA control. This method minimizes aqueous solutions, allowing gradual EPN release. Despite its potential, there's a lack of comprehensive evaluation of these pre-infected cadavers. We identified *Heterorhabditis bacteriophora* (56%) and *Steinernema riobrave* (49%) as most lethal to ants among species tested. Subsequent experiments showed cadavers infected with these species deterred worker ants, ensuring successful nematode emergence. Compared to traditional methods, pre-infected cadavers demonstrated enhanced pest control efficacy and increased bait consumption by worker ants. This suggests pre-infected cadavers not only heighten RIFA mortality but also boost bait intake. In conclusion, EPN pre-infected insect cadavers offer an innovative tool in combating RIFA, warranting further research and practical applications in prevention and control strategies.

Keywords: insect-parasitic nematode, nematode-infected insect cadaver, biological control, insect pest management

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Celebrating 100 Years of the 1st EPN discovery

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