IOBC - WPRS Meeting of the Working Group



Integrated Protection in Viticulture

3 – 5 October 2023 LOGROÑO - LA RIOJA - SPAIN **ABSTRACTS BOOK**



Instituto de Ciencias de la Vid y del Vino



Presentation

Plant protection in viticulture is matter of discussion due to the undesirable effects of pesticide use on human health and the environment. EU has launched various measures to reduce negative impacts of intensive management practices and to promote the transition towards more ecologically-based pest management methods. In addition, climate change poses particular regional challenges in the different wine-growing countries. While heat, drought and fire are pushing viticulture to its limits here, other areas are struggling at times with continuous rain and fungal diseases. The particular challenge here is to develop plant protection and cultivation systems that are as sustainable and effective as possible for each region. Research in this field is producing excellent results but their implementation in practice is still challenging. For our working group, which represents the wine-growing regions of the Western Palearctic, it is particularly important to understand scientifically the regional bottlenecks of sustainability and to communicate towards policy-makers that these may vary greatly from region to region. We are pleased to welcome you to the Conference of the IOBC/WPRS Working Group "Integrated Protection in Viticulture" that will take place at Logroño, Spain. This international conference (3 - 5 October 2023) aims to bring together scientists, researchers, students and consultants working on biological control and integrated protection in viticulture. This conference offers opportunities to share ideas and to discuss current research findings. Promising areas for future research will be identified. The Symposium will be organised under the auspices of the International Organisation for Biological and Integrated Control (IOBC) by the Instituto de Ciencias de la Vid y del Vino (ICVV).

We look forward to seeing you in Logroño, Christoph Hoffmann (convenor) David Gramaje (local organizer)



Proposed topics

1.

Advances in knowledge and new solutions against diseases

- a) Pathogens plant interactions
- b) Resistant varieties: strengths and weakness
- c) Inducing resistance through chemical or agro-technical means
- d) Pathogens arthropods relationships

2. New strategies in arthropod pest control

- a) Conservation biological control in viticulture
- b) Bio-ecology and management of invasive pests in viticulture
- c) Disruption in communication and pest control

3.

Sustainable management of soil and weeds

- a) Alternatives to herbicides
- b) Soil management strategies: implications for pest control

4.

IPM implementation and tools for sustainable viticulture

- a) New technologies and products aimed at reducing pesticide use
- b) Organic viticulture: opportunities and limitations

Scientific Committee

Josep Armengol (Universitat Politècnica de València, Spain)

Raquel Campos Herrera (ICVV, spain)

Ales Eichmeier (Mendel University in Brno, Czech Republic)

César Gemeno (University of Lleida, Spain)

Elisa González-Domínguez (Horta SRL, Italy)

David Gramaje (ICVV, Spain)

Saioa Legarrea Imizcoz (University of La Rioja, Spain)

Vicente Marco Mancebón (University of La Rioja, Spain)

Ignacio Pérez Moreno (University of La Rioja, Spain)

Gianfranco Romanazzi (Marche Polytechnic University, Italy)

José Ramón Úrbez-Torres (Agriculture and Agri-Food Canada)

Organizing Committee

Raquel Campos Herrera (ICVV)

Rebeca Bujanda Muñoz (ICVV)

David Gramaje (ICVV)

Saioa Legarrea Imizcoz (University of La Rioja)

Beatriz López Manzanares (ICVV)

Vicente Marco Mancebón (University of La Rioja)

Ignacio Pérez Moreno (University of La Rioja)

José Luis Ramos Sáez de Ojer (Gobierno de La Rioja)

María Elena Martínez Villar (University of La Rioja)

Opening lecture: Chloé Delmas (INRAE Bordeaux)

Grapevine vulnerability to drought and pathogens in the context of climate change

José Ramón Úrbez-Torres (Agriculture and Agri-Food Canada)

Grapevine virus diseases: why should we care? A multidisciplinary effort to mitigate their impact in British Columbia, Canada

Elisa González-Domínguez (Horta SRL) **Plant disease models** and their impact in the integrated management of the vineyards Jordi Recasens (ETSEA, Universidad de Lleida)

Cover crops and organic mulches to control weed flora in vineyards

César Gemeno (ETSEA, Universidad de Lleida) Insect senses and pest management

Closing lecture: Miguel Ángel Altieri (University of California, Berkeley)

Agroecology: Designing biodiverse and resilient vineyard agroecosystems for a planet in crisis



Integrated Protection in Viticulture

Program

Program

Program

Monday, 2nd October 2023

Accommodation in the hotel

20:00 Cocktail reception

Espacio Lagares Welcome by Dr. José Miguel Martínez Zapater, Director of the Institute of Grapevine and Wine Sciences (ICVV) Welcome by Councilor

Demonstrative video of La Rioja

(Wine Region and Cultural Heritage)

Tuesday, 3rd October 2023

08:00-09:00	Registration
09:00-09:10	Welcome by University and Local Authorities (UR; ICVV; La Rioja Government)
09:10-09:25	IOBC-WPRS Presentation Christoph Hoffmann (convenor), Dominique Mazzi (liaison officer)
09:25-09:30	OIV Presentation Christoph Hoffman (Scientific secretary of the OIV Commission Viticulture)

09:30-10:30 OP Opening Conference

Grapevine vulnerability to drought and pathogens in the context of climate change.

Chloé Delmas, INRAE, France.

10:30-12:30 Session 1

Topic 1.1.

Advances in knowledge and new solutions against diseases: bacteria, phytoplasmas and virus

Chairpersons: Agnès Calonnec and René Fuchs

10:30-10:45	O 01	High-throughput monitoring as an approach for an early detection of quarantine pests in viticulture. <u>Anna</u> <u>Markheiser</u> , Sandra Biancu, Nina Minges, Christine Seinsche, Kerstin Zikeli, Michael Maixner, Wilhelm Jelkmann, Christoph Hoffmann
10:45-11:00	002	Investigations on the transmission pathway of 16SrV-C phytoplasma from <i>Clematis vitalba</i> to <i>Vitis vinifera</i> by <i>Phlogotettix cyclops</i> . <i>Gudrun Strauss</i> , <i>Helga Reisenzein</i>
11:00-11:15	O03	Climate change impacts on mealybugs vectoring grapevine leafroll disease. <u>Maria Schulze-Sylvester</u> , Annette Reineke

11:30-12:00 Coffee break

12:00-12:30 KN1 Keynote lecture: Grapevine virus diseases: why should we care? a multidisciplinary effort to mitigate their impact in British Columbia, Canada. José Ramón Úrbez-Torres, Sudarsana Poojari, Patricia Bowen, Carl Bogdanoff, Kevin Usher, Thomas Lowery. Agriculture and Agri-Food, Canada

12:30-13:30 Session 2

Topic 1.2.

Advances in knowledge and new solutions against diseases: mildews

Chairpersons: Laura Mugnai and Josep Armengol

12:30-12:45 004 Copper alone is not enough. <u>René Fuchs</u>, Stefan Schumacher, Mario Steinger, Gottfried Bleyer

- 12:45-13:00 **O05** The current state of fungus-resistant grape varieties in Switzerland. <u>Kathleen Mackie-Haas</u>, Marie Blackford, Jean-Laurent Spring, Olivier Felix
- 13:00-13:15
 O6
 The potential of lecithins as a plant protection product in grapevine, the case of Plasmopara viticola. Diego

 Llamazares
 De Miguel, Ana María Diez Navajas
- 13:15-13:30 **O07 Predict downy mildew infection in partially resistant grapevines.** <u>Irene Salotti</u>, Federica Bove, Othmane Taibi, Vittorio Rossi

13:30-15:30 Lunch

15:30-17:00 Session 3

Topic 1.2.

Advances in knowledge and new solutions against diseases: mildews

Topic 1.3.

Advances in knowledge and new solutions against diseases: fungal trunk diseases

Chairpersons: Chloé Delmas and José Ramón Úrbez-Torres

15:30-16:00 KN2 Keynote lecture: Plant disease models and their impact in the integrated management of the vineyards. Elisa González-Domínguez, Horta srl, Italy

16:00-16:15	O08 Chitosan application to induced resistance for an innovative and sustainable management of grapev	/ine
	downy mildew. Gianfranco Romanazzi, Simone Piancatelli, Marwa Moumni	
16.15-16.30	009 A dPCR protocol for the quantification of <i>Ervsinbe necator</i> chasmothecia in granevine bark samples	

<u>Othmane Taibi</u>, Maela León, Federica Bove, Vittorio Rossi, Josep Armengol, Elisa González-Domínguez, Mónica Berbegal

- 16:30-16:45 **O10** *In vitro* biofilm formation of the Esca pathogen fungus Phaeomoniella chlamydospora. <u>Zoltán Karácsony</u>, Nikolett Molnár, Dóra Szabó, Kálmán Zoltán Váczy
- 16:45-17:00 **O11 Control of Botryosphaeria dieback and black-foot pathogens in grapevine propagation material using** *Bacillus subtilis* **PTA-271 and Trichoderma atroviride SC1.** <u>Catarina Leal</u>, David Gramaje, Florence Fontaine, Nicolas Richet, Patricia Trotel-Aziz, Josep Armengol

17:00-17:15 Break

17:15-18:00 Session 4

Topic 1.3.

Advances in knowledge and new solutions against diseases: fungal trunk diseases

Chairpersons: Cecilia Rego and Akif Eskalen

- 17:15-17:30 **O12** Field control of Botryosphaeria dieback of grapevines by using locally sourced *Trichoderma* species. <u>José</u> <u>Ramón Úrbez-Torres</u>, Jinxz Pollard-Flamand, Julie Boulé
- 17:30-17:45 **O13** Antifungal effect of Cu based nanocomposites against *Pleurostoma richardsiae*, *Dactylonectria torresensis* and *Fusarium oxysporum*. Marie Zahrádková, Kateřina Štůsková, Jan Wohlmuth, Milan Špetík, Lucie Frejlichová, Dana Homolová, Zuzana Bytešníková, Tomáš Kiss, Jakub Pečenka, Lukáš Richtera, David Gramaje, <u>Aleš Eichmeier</u>
- 17:45-18:00 **O14** Mechanically pruning vs hand-pruning: the effect on incidence and severity of grapevine trunk disease pathogens. *Minette Havenga, Matthew Webber, Lizel Mostert, Francois Halleen*

18:00-20:00 **Poster viewing session and cocktail**

Wednesday, 4th October 2023

08:30-10:00 Session 5

Topic 1.4.

Advances in knowledge and new solutions against diseases: Botrytis and black rot

Topic 1.5.

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

Chairpersons: Ales Eichmeier and Catarina Leal

- 08:30-08:45 **O15** Specific effects of copper on the mycobiome of grapevine leaf surfaces. *Falk Behrens, Yannick Ditton, Michael Fischer*
- 08:45-09:00 **O16** Combining indicators for a better understanding and management of black rot risk? <u>Agnès Calonnec</u>, Ghislain Delestre, Philippe Cartolaro

09:00-09:15	017	Etiology and epidemiology of sou	ır rot in Ontario, Canada.	. Cristina Huber,	, Debra Inglis,	Wend	y McFadden-Smith
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- 09:15-09:30 **O18** New insights into sour rot: a complex interaction between the microbial community, vinegar flies and weather. Ignacio Romero Lozano, Sébastien Hévin, Valérie Hofstetter, <u>Patrik Kehrli</u>
- 09:30-09:45 **O19 Pruning wound protection products induce alterations in the wood mycobiome profile of grapevines.** <u>Giovanni Del Frari</u>, Marie Rønne Aggerbeck, Alex Gobbi, Chiara Ingrà, Lorenzo Volpi, Teresa Nascimento, Alessandra Ferrandino, Lars Hestbjerg Hansen, Ricardo Boavida Ferreira
- 09:45-10:00 **O20** Promising future for *Botrytis cinerea* (Helotiales: Sclerotiniaceae) management using strategies based on *Xenorhabdus* and *Photorhabdus* (Morganellaceae) in vineyards. *Ignacio Vicente-Díez*, *Elizabeth Carpentero*, *Xoaquín Moreira*, *Victoria Pastor*, *Mar Vilanova*, *Alicia Pou*, *Raquel Campos-Herrera*

10:00-10:30 Coffee break

10:30-12:00 Session 6

Topic 2.

New strategies in arthropod pest control

Chairpersons: Saioa Legarrea and Carlo Duso

10:30-10:45	021	Habitat management as an integrative tool for the control of grapevines Flavescence dorée. Alan Oggier, Mauro Jermini, Marco Conedera, Christophe Debonneville, Olivier Schumpp, <u>Attilio Rizzoli</u>
10:45-11:00	022	Trapping Scaphoideus titanus (Hemiptera: Cicadellidae) in Italian vineyards: where, when and why? Bruno Bagnoli, Giovanni Benelli, Piero Braccini, Vieri Caciagli, Renato Ricciardi, Patrizia Sacchetti, <u>Andrea Lucchi</u>
11:00-11:15	023	Studies on the Scaphoideus titanus phenology in a diversified region: implications for management strategies. Gianluca Spessotto, <u>Stefan Cristian-Prazaru</u> , Francesco Pavan, Carlo Duso
11:15-11:30	024	Kaolin against Scaphoideus titanus as an alternative to natural pyrethrins in Swiss vineyards? <u>Christian</u> Linder, Michel Jeanrenaud, Patrik Kehrli
11:30-11:45	O25	Spatial and temporal distribution of leafhoppers and their parasitoids in vineyards located in north-eastern Italy. <u>Stefan Cristian Prazaru</u> , Elena Merlin, Francesca Carriero, Isabel Martinez Sanudo, Alberto Pozzebon, Francesco Pavan, Carlo Duso
11:45- 12:00	026	Sugar feeders as a tool for ant-mediated plant defense against leafcutter ants in grapevine. <u>Maria Schulze-Sylvester</u> , Francisco Sylvester, Victor Manuel Torres, José Antonio Corronca

12:00-12:15 Break

12:15-13:45 Session 7

Topic 2.

New strategies in arthropod pest control

Chairpersons: Raquel Campos-Herrera and Nicola Mori

12:15-12:45	KN3	Keynote lecture: Insect senses and pest management. César Gemeno, University of Lleida, Spain
12:45-13:00	027	Waves and tortricids: Ultrasounds, vision and pest control. <u>Alejandro Martín-Gabarrella</u> , Luis Elvira-Segura, Gregor Belušič, César Gemeno
13:00-13:15	028	Grapevine yeast attracts grapevine moth. Carles Amat, Ramon Gonzalez, Pilar Morales, César Gemeno
13:15-13:30	O29	A double dispenser for mating disruption of two grape pests: Lobesia botrana and Cryptoblabes gnidiella. <u>Renato Ricciardi</u> , Giovanni Benelli, Francesca Cosci, Andrea Iodice, Francesco Savino, Edith Ladurner, Andrea Lucchi
13:30-13:45	O 30	Oviposition by three tortricid moths on filter paper treated with different doses of gustatory stimuli. Carles

13:30-13:45 **O30 Oviposition by three tortricid moths on filter paper treated with different doses of gustatory stimuli.** Carles Amat, Margherita Marmugi, Giovanni Benelli, Andrea Lucchi, Rajendra Prasad, Renato Ricciardi, <u>César Gemeno</u> 13:45-15:45 Lunch

16:00-20:00 Congress Tour

Vivanco Winery and Museum of Wine Culture Departure from Riojaforum

Thursday, 5th October 2023

08:30-10:45 Session 8

Topic 3.

Sustainable management of soil and weeds

Topic 4.

IPM implementation and tools for sustainable viticulture

Chairpersons: Claudio Ioriatti and Andrea Lucchi

08:30-09:00	KN4	Keynote lecture: Cover crops and organic mulches for weed management in Mediterranean vineyards. <i>Jordi</i> Recasens, University of Lleida, Spain
09:00-09:15	O31	How organic mulches affect spontaneous weed vegetation and soil properties. <u>Andreu Mairata</u> , David Labarga, Miguel Puelles, Joaquín Huete, Javier Portu, Luis Rivacoba, Alicia Pou
09:15-09:30	032	Weed management by cover crops in organic Mediterranean vineyards. <u>Diego Barranco-Elena</u> , Jordi Recasens, Àurea Guiu, Bàrbara Baraibar
09:30-09:45	O33	Managing viticultural ecosystems using functional biodiversity indicators and agroecology approaches for sustainable production and consumer engagement: the case of SOGRAPE in Portugal and Spain. <u>António</u> <u>Graça</u> , Natacha Fontes, José Manso, João Vasconcelos Porto, Mafalda Guedes, Isabel Morais, María Barúa, Alberto Saldón, Luisa Freire
09:45-10:00	034	Phytoseiid mites in European wine-growing regions were influenced at the field and landscape scale. Stefan Möth, Sylvie Richart-Cervera, Maria Comsa, Rafael Alcalá Herrera, <u>Christoph Hoffmann</u> , Sebastian Kolb, Daniela Popescu, Jo Marie Reiff, Adrien Rusch, Pauline Tolle, Andreas Walzer, Silvia Winter
10:00-10:15	O35	Entomopathogenic nematodes for controlling <i>Lobesia botrana</i> in vineyards: fine-tuning of application, target area, and timing. <u>Raquel Campos-Herrera</u> , María del Mar González-Trujillo, Ignacio Vicente-Díez, Elizabeth Carpentero, Miguel Puelles, Elisabet Vaquero, Rasa Cepulyte
10:15-10:30	O 36	Entomopathogenic fungi for the control of grapevine sap-sucking insects. <u>Riccardo Barbera</u> , Nicola Bodino, Daniele Vitti, Domenico Bosco, Paola Dolci
10:30-10:45	O 37	Enhanced biocontrol of vine mealybug, <i>Planococcus ficus</i> , using hydrogel-based control of sugar-feeding ants. <i>David Haviland</i> , Stephanie Rill, Chelsea Gordon, Nathan Mercer, Kent Daane

10:45-11:30 Coffee break and

Poster viewing session

11:30-13:30 Session 9

Topic 4.

IPM implementation and tools for sustainable viticulture

Chairpersons: Tirtza Zahavi and Christoph Hoffmann

11:30-11:45	O38	Deciphering the effects of agronomical practices on Aspergillus incidence and carpospheres microbial communities of grapevine. <u>Stefanos Testempasis</u> , Christina Papazlatani, Serafeim Theocharis, Panagiotis Karas, Stefanos Koundouras, Dimitrios Karpouzas, George Karaoglanidis
11:45-12:00	O39	Impact of weather data sources on infection risk provided by a decision support system and implications in disease management. <u>Sara Elisabetta Legler</u> , Elisa Gonzalez-Domínguez, Vittorio Rossi
12:00-12:15	O 40	Parametrization and validation of a Physiologically Based Demographic Model (PBDM) for Scaphoideus titanus Ball (Fam: Cicadellidae). Marta Corbetta, Sara Elisabetta Legler, Vittorio Rossi
12:15-12:30	041	Field evaluation of endophytic and rhizospheric bacteria against grapevine trunk diseases. <i>Marcelo</i> Bustamante, Karina Elfar, <u>Akif Eskalen</u>
12:30-12:45	042	Adaptation of the VitiMeteo forecasting system to PIWIs. <u>Stefan Schumacher</u> , Caroline Mertes, Thomas Kaltenbach, Nour Sawas, Barbara Augenstein, Ronald Krause, Gottfried Bleyer, René Fuchs
12:45-13:00	043	Hybrid grapevine resistance to downy and powdery mildew in Israel. Tamir Zonenberg, Tirtza Zahavi, Ofir Degani, Meir Shlisel, <u>Mery Dafny-Yelin</u>

13:00-15:00 Lunch

15:00-16:00 Session 10

Topic 4.

IPM implementation and tools for sustainable viticulture

Chairpersons: Sara Elisabetta Legler and Gianfranco Romanazzi

- 15:00-15:15 **O44** Impact of sustainable farming interventions in the vineyard and its surrounding area on the dynamics of pest and natural enemies' populations in Rioja Alavesa, Spain. <u>Bárbara Sebastián</u>, Luis G. Santesteban, Nazareth Torres, Ignacio Arzoz, Maite Loidi, Luis Rubén Román-Fernández, Ainara Crespo-Susperregui, Gonzalo Sainz de Samaniego, Julián Palacios
- 15:15-15:30 **O45** Are Farm to fork strategy goals reasonable and achievable? State of the art of Península de Setubals winegrowers. <u>Miguel Cachão</u>, Ana Chambel, Sérgio Pinto
- 15:30-15:45 **O46** Long-term historical characterization of French vineyard exposure to pests and diseases: case study of Bordeaux and Champagne regions. <u>Marc Fermaud</u>, Anne Merot, Lionel Delbac, Leslie Daraignes, Marianne Fraysse, Nathalie Smits
- 15:45-16:00 **O47** Effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture. *Marvin Kaczmarek, Martin H. Entling, Christoph Hoffmann*

16:00-17:00 cc Closing Conference

(online participation):

Agroecology: Designing biodiverse and resilient vineyard agroecosystems for a planet in crisis. *Miguel A. Altieri, University of California, Berkeley, USA* Christoph Hoffmann, Dominique Mazzi

20:00

Winery free visit and gala dinner Bodegas Franco Española



Abstracts

Invited Speakers & Oral Presentations



Integrated Protection in Viticulture

Opening Conference OP

Grapevine vulnerability to drought and pathogens in the context of climate change

Chloé DELMAS¹

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Abstract

Climate change, particularly the increased frequency and severity of drought, has a significant impact on plant physiology, pathogen aggressiveness, and their interactions. Consequently, plant mortality and yield losses are escalating worldwide in both natural and agroecosystems, and are challenging integrated protection of crops. However, our understanding of the underlying causes of yield losses is limited due to the complex interplay between abiotic and biotic factors. This is particularly evident in viticulture, where grapevines have been experiencing declining yields and increased mortality over the past two decades. I will present an overview of our research on grapevine response to drought, fungal diseases and their interactions. We integrated ecophysiology and plant pathology to provide novel insights into the role of vine physiology, microbial communities, and their interaction with climate, as key drivers of viticulture sustainability.

Key words : climate, drought, fungal diseases, grapevine, xylem hydraulics

Session 1 **001**

High-throughput monitoring as an approach for an early detection of quarantine pests in viticulture

Anna Markheiser¹, Sandra Biancu ¹, Nina Minges¹, Christine Seinsche², Kerstin Zikeli², Michael Maixner¹, Wilhelm Jelkmann², Christoph Hoffmann¹

¹Julius Kühn-Institut (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Geilweilerhof, 76833 Siebeldingen, Germany ²Julius Kühn-Institut (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Schwabenheimer Str. 101, 69221 Dossenheim, Germany E-mail: <u>anna.markheiser@julius-kuehn.de</u>

Abstract

Global trade and tourism, in addition to climate change, increase the risk of introduction and establishment of new pests in permanent crops. Within the European territory, the bacterium *Xylella fastidiosa* (Xf) and the grapevine flavescence dorée phytoplasma (GFDp) are currently of greatest concern in viticulture. Classified as Union quarantine pests (QPs), they cause considerable economic damage and may result in a negative impact on the natural landscape. In addition, the Union regulated non-quarantine pest (RNQP) '*Candidatus* Phytoplasma solani', the causal agent of Bois noir (BN), impairs the phytosanitary quality of planting material. Due to risk posed QP and RNQP, it is crucial to detect infestations at an early stage in order to prevent further spread of these pathogens. Monitoring measures for their occurrence are therefore essential, but rely currently on time-consuming visual inspections for disease symptoms and/or the monitoring of vectors using various trapping methods in the field. As Xf, GFDp and BN are transmitted by plant sap-feeding Auchenorrhyncha, the goal of this project is to develop an efficient monitoring strategy using a high-throughput sequencing (HTS) methodology based on insect mass catches, which simultaneously detects vectors and associated QPs or RNQPs. However, compared to other arthropod groups, only few molecular reference sequences are available for the identification of Auchenorrhyncha.

Hence, we produced insect mass catches in vineyards by sweep netting and a vacuum device. The pros/ cons of these trapping methods will be highlighted. Following a taxonomic analysis of the Auchenorrhyncha species in these mass catches, individual DNA barcode sequences for both confirmed and potential vectors of the pests were elaborated. For this purpose, DNA of single insects was extracted and the Folmer region of the mitochondrial cytochrome C oxidase subunit I (COI) amplified by polymerase chain reaction (PCR) and sequenced. DNA barcodes for more than 70 species could be generated, belonging to the Cicadellidae (subfamilies: Deltocephalinae, Cicadellinae, Agallinae and Megophtalminae), Aphrophoridae, Cercopidae, Dictyopharidae and Cixiidae family.

To establish a reliable HTS methodology for the identification of Auchenorrhyncha species within an arthropod mass catch, identified Auchenorrhycha were remixed with the remaining arthropods and used for COI amplicon sequencing. Auchenorrhyncha species identification results using different databases (German Barcode of Life (GBOL), Barcode of Life Data System (BOLD) and COins) were verified by comparison to the species list from the previous taxonomic analysis. The COI barcode sequences generated previously were implemented into the data analysis workflow. The results achieved so far will be presented and discussed, as they are a promising approach for an early, efficient and labor saving detection of pests in the field and the maintenance of plant health.

Key words: vectors, high throughput sequencing, grapevine, phytoplasmas, identification

Session 1 Advances in knowledge and new solutions against diseases: bacteria, phytoplasmas and virus

Investigations on the transmission pathway of 16SrV-C phytoplasma from *Clematis vitalba* to *Vitis vinifera* by *Phlogotettix cyclops*

Gudrun Strauss¹& Helga Reisenzein¹,

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Abstract

Flavescence dorée (FD) is a yellows disease of grapevine associated with phytoplasmas belonging to the 16SrV taxonomic group (subgroups C and D). FD affects the vitality and yield of infected grapevines as well as the quality of the wine. The main leafhopper vector of flavescence dorée phytoplasma (FDp) from grapevine to grapevine is Scaphoideus titanus (Ball, 1932) (Cicadellidae, Deltocephalinae). However, other host plants, especially Alnus sp. and Clematis vitalba L., and some other leafhopper species are involved in the epidemiology of FD. Previous investigations on potential vectors in Austria revealed that the leafhopper species Phlogotettix cyclops (Mulsant & Rey, 1855) frequently occurred on C. vitalba nearby to vineyards and also on cultivated grapevines in different viticulture areas (Strauss & Reisenzein, 2018). A high percentage (46%) of captured P. cyclops on C. vitalba harboured the 16SrV-C phytoplasma, displaying a high genetic similarity on different gene loci with the FD-C strains from C. vitalba and infected grapevines (Reisenzein & Strauss, 2019). These results led to the hypothesis that P. cyclops may transfer FDp from clematis to grapevine, which can then lead to sporadic FD outbreaks in areas where S. titanus also occurs. Within the EUPHRESCO research project FLAVID, several acquisition and transmission trials with FDp, P. cyclops and S. titanus were carried out. Studies were conducted to evaluate, if S. titanus could acquire FDp from test plants previously infested by P. cyclops and then infect healthy test plants by this means. The overall aim of these studies was to better assess the risk of P. cyclops as a vector of FDp from 16SrV-C phytoplasma infected C. vitalba to grapevine.

In 2021, a series of acquisition and transmission trials were conducted under controlled conditions in an insect-proof quarantine greenhouse at AGES, Vienna, Austria. *C. vitalba* infected with 16SrV-C phytoplasma was used as the source of FDp infection in all experiments, onto which field collected *P. cyclops* nymphs were placed in small groups for FDp acquisition. *P. cyclops* was then placed on either healthy *Vicia faba* L. or *Vitis vinifera* L. for phytoplasma inoculation. After completion of the inoculation access period, *P. cyclops* was removed and *S. titanus* adults were placed on the test plants.

In trial 1 it was investigated, whether FDp previously transmitted by *P. cyclops* to *V. faba* subsequently could be acquired by *S. titanus* and be transmitted to non-infected *V. faba*. In trials 2 and 3 was tested, whether FDp previously transmitted by *P. cyclops* to potted *V. vinifera* (cv. CH) or *V. vinifera* seedlings could subsequently be acquired by *S. titanus*.

The experimental design and results of the acquisition and transmission trials is presented. The role of *P. cyclops* as a vector in the epidemiology of FD is discussed based on these findings and results from previous studies.

Key words: insect vector, epidemiology, grapevine, phytoplasma, transmission pathway

Climate change impacts on mealybugs vectoring grapevine leafroll disease

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 $\mathbf{003}$

Abstract

Climate change impacts crop plants, plant pathogens, and their insect vectors and hence adds abiotic stress to the triangle of plant-virus-vector interactions. Grapevine is among the most widely grown fruit crops worldwide and grapevine leafroll disease (GLRD) is probably the most widespread viral infection in vineyards. It is transmitted through infected propagation material and insect vectors, such as the vine mealybug Planococcus ficus (Signoret). Knowledge on climate change impacts on mealybugs in general or as virus vectors, in particular, is scarce. Similarly, we lack information on grapevine plant defence against pathogens under climate change conditions. Plant-virus-vector interactions are usually very species-specific hence conclusions cannot be derived from other pathosystems. It is therefore unclear how climate change influences the plant-virus-vector interactions of GLRD. Here, we aim to fill this gap by evaluating in a first step the life-history parameters of the insect vector, Planococcus ficus, under elevated CO₂ (eCO₂) in greenhouse and field experiments (Schulze-Sylvester & Reineke, 2019). The greenhouse experiments with an eCO₂ showed a significant increase in survival rates, a strong trend towards declining body size, and an increasing fecundity of female mealybugs, while fertility and development time did not change. However, a clear understanding of future GLRD disease progression under climate change scenarios will only emerge from studies that realistically evaluate the effects of combined climate variables on biotic interactions (Hoffmann et al. 2022). Current experiments investigate the impacts of elevated temperature (eT) and elevated CO2 (eCO2) on P. ficus and its ability to vector GLRD. At the same time, we also focus on amino acids as well as defence-related plant parameters (gene expression and phenols). Experiments are carried out in plant growth chambers. Preliminary results indicate differences in disease onset and (possibly) transmission. The obtained data will serve to close basic knowledge gaps on individual species, their interactions, and the disease they cause in current and future climate scenarios. Enhancing the knowledge of the impacts of climate change on economically important plant diseases and their insect vectors is crucial to strengthen the adaptive capacity of crop production and ensure sustainable viticulture.

Key words: elevated CO₂, elevated temperature, grapevine, GLRaV, plant-vector-virus interactions.

Session 1 Advances in knowledge and new solutions against diseases: bacteria, phytoplasmas and virus

Session 1 KN1

Grapevine virus diseases: why should we care?, a multidisciplinary effort to mitigate their impact in British Columbia, Canada

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Abstract

Grapevines (Vitis vinifera L.) host the largest number of viruses than any other agriculture plant. Among them, Grapevine leafroll disease (GLD), caused by several Grapevine leafroll-associated viruses (GLRaVs) and Grapevine red blotch disease (GRBD) cause by Grapevine red bloch virus (GRBV) are of significance importance. In North America, GLD and GRBD cause unsustanable economic losses ranging from USD\$2,665 to USD\$250,000 per hectare. Thought still relatively young and emerging, the Canadian grape and wine industry contributes over CAD\$9 billion per year to the national economy. However, industry growth relies entirely on the import of nursery material, which is one of the primary sources for the introduction of grapevine virus diseases. In the early 2010s, the British Columbia (BC) grape and wine industry started experiencing yield losses and delay in ripening, which was associated with the introduction and spread of virus diseases. The main objective of this talk is to present a multidisciplinary approach taken by the Summerland Research and Development Centre grape and wine team to first demistify the status of virus diseases affecting BC vineyards, to understand their impact and to develop and implement effective field mitigation strategies. Large scale field surveys were conducted across BC grape-growing regions between 2014 and 2018, which confirmed the presence and rapid spread of GLD. These field surveys also confirmed the introduction of GRBD in BC. Studies have been conducted to identify the vectors associated with these virus diseases in BC. This talk will present the impacts that both GLD and GRBD have on plant health and fruit and wine quality, which clearly highlights the urgent need to manage these diseases under the short growing season condictions of BC. Efforts on control of GLD and GRBD have been implementing by area-wide 'roguing', which has shown to be effective in mitigating the spread of these diseases. Results from these studies have significantly contributed to the origin of the Canadian Grapevine Certification Network (CGCN), a non-for-profit organitazion funded in 2017 and formed by all grapegrowers associations in Canada. The main goals of this organization are i) to create a clean plant network for domestically certified virus-free grapevines in Canada, ii) promote, coordinate and direct finnancing towards research on a national scale for the benefit of the Canadian grape and wine industry, and iii) to advance Canada'a grape and wine industry by ensuring a sustainable domestic supply of certified propagative grapevine material.

Key words: clean plant certification program, Grapevine leafroll disease, Grapevine red blotch disease, mealybug, sustainable control, virus diseases, *Vitis vinifera*.



Advances in knowledge and new solutionsagainst diseases: mildews

Copper alone is not enough

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Abstract

Plasmopara viticola, the causal agent of downy mildew on grapevines, is one of the most devastating pathogens in viticulture worldwide. Under optimal climatic conditions, such as those predominant mainly in Central Europe, *P. viticola* can cause total crop loss. In many southern European countries, downy mildew is a minor economic problem due to the rather hot and dry weather conditions prevailing there during the growing season. However, in the context of global warming, conditions could change, making epidemics caused by this pathogen more likely in these countries as well. In organic viticulture, downy mildew is mainly controlled with copper-containing fungicides. These agents are relatively easily washed off, which is particularly problematic because years with severe downy mildew epidemics are usually characterized by prolonged rainfalls. As a result, vineyards are often poorly accessible, making reapplication difficult. Since copper fungicides are contact fungicides that do not protect newly grown leaf surfaces, this results in unprotected plants during periods of high infection pressure. In addition, efficacy has been shown to be significantly lower on clusters than on leaves.

In the search for suitable alternatives or possible supplements to existing copper products, appropriate field trials have been carried out as part of several research projects. To this end, various basic substances, but also sulphur and potassium phosphonate, have been tested for their efficacy against downy mildew in recent years. As the results showed, the high efficacy of potassium phosphonate was repeatedly confirmed in the field. Potassium phosphonate was listed as a plant strengthening agent in Germany until 2013. Since October 2013, however, the substance has been approved as a plant protection product and is no longer permitted in organic viticulture. In addition to potassium phosphonate, sulphur, which is normally used against powdery mildew, is also effective against *P. viticola*. In contrast, other agents, including the basic and low-risk substances, do not show sufficient efficacy against downy mildew. None of the currently approved agents, except for copper and sulphur, have been shown to be sufficiently effective against *P. viticola* at moderate to high infection pressure in the field. To ensure that organic viticulture is possible in the future without economic losses in regions or years with high infection pressure, it would be useful to approve potassium phosphonate. This would provide organic viticulture with a further tool in its control strategy against downy mildew.

Session 2 Advances in knowledge and new solutions against diseases: mildews

Session 2 **005**

The current state of fungus-resistant grape varieties in Switzerland

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Abstract

Agroscope has been breeding interspecific fungus-resistant grape (FRG) varieties since 1996. The first trials resulted in two successfully marketed grape varieties: Divico and Divona. Increased mildew infections have recently been observed in older European FRG varieties, such as Regent, however, which is why multi-gene FRG varieties are now being bred. Agroscope has been working with INRAE in Colmar, France to breed this second generation of FRG varieties so that long-term protection can be provided, ideally without the use of fungicides. We also conduct rigorous grape variety testing for both the first and second breeding programs, alongside other interspecific varieties from around Europe. Testing includes assessing agronomical, as well as enological characteristics. Additionally, winegrower and consumer acceptance projects have been conducted to better understand the current state of FRG varieties in Switzerland. Generally, winegrowers are motivated by fewer fungicide applications and therefore reduced management costs and environmental consequences, while winemakers are producing both single variety and cuvee wines for the market. Consumer awareness of these wines has slowly started to increase and there has been a steady increase in vineyard area of FRG varieties in Switzerland over the last decade. However, as of 2021 FRG varieties remain only 2.8% of the total vineyard area in Switzerland. As of 2023, in an attempt to increase the vineyard area more quickly and to drastically reduce the number and amount of high-risk fungicides being used in viticulture, the Swiss Federal Office of Agriculture has begun subsidizing the planting of FRG varieties. The Swiss government has created a robust and progressive program, which can be used as an example for supporting sustainable winegrowing methods, which have positive impacts on the environment.

Keywords: fungal resistant grape, viticulture, consumer, winemaking

Advances in knowledge and new solutions against diseases: mildews

Session 2 **006**

The potential of lecithins as a plant protection product in grapevine, the case of *Plasmopara viticola*

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Abstract

Basic substances (BSs) are a type of active substances, which are not predominantly used as plant protection products but which may be valuable for it. They are regulated by the European Union and represent a safe alternative to conventional fungicides and a good candidate for their substitution. In the specific case of Plasmopara viticola, several BSs are approved to control this pathogen, but their molecular mechanism and mode of action remain unclear in grapevine. One of these BSs is soy lecithin, which is a common supplement in the food industry. In this study, the molecular mechanism of two lecithin-based commercial products in grapevine was analysed, focusing on their direct toxicity against the pathogen and on their defence stimulation capacity, both at the genetic and metabolic level. Moreover, their efficacy as a plant protection product against P. viticola was evaluated in greenhouse, using a whole-plant infection assay. According to our results, both products demonstrated a toxic effect against the pathogen and an ability to induce several defence-related genes which comprised mainly PR proteins and other genes involved in secondary metabolism, as oxylipin and phenylpropanoid biosynthesis. At the metabolic level, the products increased the production of some metabolites involved in P. viticola infection, as several stilbenes and the defence hormone salicylic acid. Other metabolites related to oxylipin and jasmonic acid biosynthesis were also affected. Finally, we saw that plants treated with the products significantly decreased P. viticola infection severity in the whole-plant infection assay. This study represents the first report on the molecular mechanism of lecithins in grapevine, and explores and supports their use against P. viticola.

Key words: grapevine, downy mildew, Plasmopara viticola, basic substances, lecithins.

Session 2 **007**

Predict downy mildew infection in partially resistant grapevines

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Abstract

The effect of wetness duration on the infection by *Plasmopara viticola* was studied in monocycle experiments conducted under controlled temperature conditions in 15 *Vitis* accessions, most of them carrying one or more genes of resistance to *P. viticola* or showing moderate susceptibility to downy mildew. Mathematical equations relating *P. viticola* infection severity to hydrothermal time (a physiological time accounting for the combined effect of temperature and wetness duration) were developed and used to develop variety-specific critical thresholds for predicting infection. These thresholds were applied in a simulation study for scheduling fungicide applications under three contrasting climates (mild and moist, warm and dry, and warm and moist). Applying the variety-specific thresholds led to a 48% average reduction in fungicide applications, and no fungicides were needed on highly resistant varieties. These results confirm that resistance to downy mildew can contribute to vineyard sustainability and propose new knowledge to facilitate decision-making to protect partially resistant varieties.

Key words: Plasmopara viticola, genetic resistance, Rpv, Vitis vinifera, disease management.

Session 3 KN2

Advances in knowledge and new solutions against diseases: mildews

Advances in knowledge and new solutions against diseases: fungal trunk diseases

Plant disease models and their impact in the integrated management of the vineyards

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Abstract

In the last decades the concepts and principles of integrated pest management (IPM) have been actively promoted in crop management by several policies and extension strategies. IPM relies on an integrated knowledge of the dynamic processes characterizing the agricultural ecosystems and rationalizes the interventions reducing the pesticide applications to moments in which they are justified. Despite the environmental, social, and economic benefits of IPM, its practical implementation has been perceived by the farmers as complex and time consuming compared to calendar-based application of pesticides, and this perception reduces the adoption of IPM in viticulture. Plant disease models, as part of decision tools, help viticulturists in taking informed decisions in the vineyard.

A high number of epidemiological models have been built to predict the development of the main diseases affecting grapevines, with focus on downy and powdery mildews, but also on Botrytis bunch rot and black-rot; Phomopsis leaf and cane spot disease, secondary bunch rots, and trunk diseases have been also addressed in recent years.

In this talk, key aspects are addressed related to the development of epidemiological models for grapevine fungal diseases. The modelling approach (data-based vs process-based) depends on the data and knowledge available, the level of complexity to be represented, and the type of output required. Process-based models are developed based on the scientific knowledge of the pathosystems, and knowledge gaps on the life cycle of pathogens are addressed through specific experiments. Validation of model output to test accuracy and robustness in representing the biological processes and the benefits of model use in scheduling fungicide sprays are essential aspects to be investigated and shown to farmers to promote model's adoption. Finally, decision tools, such as decision support systems, should include disease models as part of a multimodelling approach, together with plant growth models, fungicide models and biocontrol models.

Key words: integrated pest management, plant disease models, decision support systems.

Session 3 **008**

Session 3 Advances in knowledge and new solutions against diseases: mildews

Advances in knowledge and new solutions against diseases: fungal trunk diseases

Chitosan application to induced resistance for an innovative and sustainable management of grapevine downy mildew

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Abstract

Downy mildew is one of the most important grapevine diseases, caused by the Oomycete Plasmopara viticola. The management of the disease in organic agriculture can require up to 15 copper applications per year. However, copper accumulated in the soil, is phytotoxic and is toxic for organisms living in the soil, thei its use has been restricted in European Union to maximum 28 kg in 7 years. Therefore, testing of alternatives with equal effectiveness is desirable. Among those, the natual biopolymer chitosan, known to induce resistance toward a list of pathogens, proved to be effective toward downy mildew in plot experiments. The aim of our trials was to extend chitosan applications in large scale experiments in different years, cultivars and environmental conditions. Trials were carried out in 3 commercial organic vineyards, of Marche Region, Central-Eastern Italy, along 3 seasons (since 2019 to 2021). Treatments were applied on cv Verdicchio of wineries Moncaro (in Castelplanio, AN) and Belisario (in Matelica, MC), and on 'Montepulciano' of winery Moncaro, in Angeli di Varano (AN). Chitosan was applied at 0.5% along the season, in the second half of the season following copper, or combined at half rate (0.25%) with half rate of copper. Untreated and copper treated plots were used as a control. Downy mildew infections were recorded along the season, and disease incidence, severity and McKinney index were calculated. The weather conditions were variables among the years and the vineyards. Chitosan treatments reduced McKinney Index of downy mildew, compared to untreated control, on bunches of cv. Verdicchio by 41.69%, 85.48%, and 43.99%, and on cv. Montepulciano by 17.14%, 68.87%, 45.21%, respectively, in 2019, 2020 and 2021. Similar results were obtained on leaves. In 2021, combination of chitosan with reduced copper rates protected leaves and bunches from downy mildew at the same level of copper. This investigation demonstrates that chitosan can be introduced in downy mildew management strategies and can integrate or even replace copper with similar degree of protection and with lower amounts of residues on the grapes.

Key words: chitosan, copper, downy mildew, grapevine, Plasmopara viticola, Vitis vinifera.

Session 3 **OO9**

Advances in knowledge and new solutions against diseases: mildews

Advances in knowledge and new solutions against diseases: fungal trunk diseases

A qPCR protocol for the quantification of *Erysiphe necator* chasmothecia in grapevine bark samples

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Abstract

Powdery mildew is the most important disease in Spanish vineyards because environmental conditions are frequently suitable for its development. Erysiphe necator mainly overwinters through chasmothecia that have been developed at the end of the previous summer on affected leaves and clusters, and remain within the bark of the trunk and main cordons. Ascospores from chasmothecia initiate the disease epidemics, which are then continued by asexual conidia, so the higher the number of chasmothecia, the higher the disease pressure. The role of ascosporic infections has been underestimated in the past, and no control measures have been applied to reduce it. In this work, we set up a qPCR protocol to detect and quantify chasmothecia of E. necator in grapevine bark samples using previously described specific primers. The standard curve was constructed using 10-fold dilution series from a 10 ng/µL stock solution of a chemically synthesized fragment of the 18S ribosomal RNA gene that included the annealing sites for the specific primers. In a first experiment, we collected and guantified different numbers of chasmothecia under a stereoscope. These numbers were linearly correlated with the fungal DNA quantified by qPCR (P<0.001 and R²=0.935). In a second experiment, chasmothecia were extracted from naturally infested bark samples using an ad hoc protocol; the bark samples being collected from different grapevine cultivars varying in the level of powdery mildew susceptibility. The results validate the qPCR protocol for the quantification of chasmothecia in grapevine bark samples, thus improving the time-consuming visual counts.

Keywords: real-time PCR, powdery mildew, epidemiology, inoculum monitoring.

Session 3 O10

Session 3 Advances in knowledge and new solutions against diseases: mildews

Advances in knowledge and new solutions against diseases: fungal trunk diseases

In vitro biofilm formation of the Esca pathogen fungus *Phaeomoniella chlamydospora*

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Abstract

While grapevine trunk diseases (GTDs) -including Esca- are a substantial threat to viticulture and therefore attract significant attention from researchers, the pathogenesis of these syndromes is still unclear, with special regard to their latent nature. Several hypotheses were made to explain the occasional shift of the fungal causal agents of GTDs from endophytic growth to pathogenic behavior, focusing on the possible triggering effect of climatic conditions, host stress, or microbial interactions. Another explanation can be the cell density-dependent changes in fungal physiology, regulated by the so-called Quorum Sensing mechanism. In the present study, we would like to show our results suggesting the biofilm formation of the Esca pioneer pathogen *Paheomoniella chlamydospora* (Pch), the inoculum-size-dependent nature of the process, and its possible role in pathogenesis.

Three Pch isolates were used in our experiments. The possible biofilm formation of the isolates was tested on a rich medium (5 m/v% sucrose, 1 m/v% yeast extract, 2 m/v% agar) amended with the polysaccharide indicator congo red (80 mg/100 ml). All three tested strains showed the accumulation of the dye at the late stages of colony development, followed by the abundant production of extracellular polysaccharides, indicated by the formation of a black halo around the colonies. Parallel microscopic observations of young colonies showed the development of dense separate patches over the layer of mycelia, which later fused into a mature biofilm-like structure. The establishment of mature biofilms coincided with the previously mentioned production of extracellular polysaccharides. The three examined Pch strains differed in the dynamics of biofilm formation, as well as in the apical dominance of hyphal growth, with a negative correlation between these two traits. The process also depended on the inoculum density and was affected by externally added fungal Quorum Sensing molecules, tyrosol and farnesol. Microscopic detection of congo red fluorescence in the cross sections of the mature colonies revealed that most of their polysaccharide content is concentrated into a central layer of swollen cells surrounded by actively growing mycelia at the margins. Disruption of this layer led to the release of swollen mycelial cells, conidiogenous cells, and spores that entered the microcycle conidiation. These cells seemed to be glued together by an extracellular matrix, further reinforcing the hypothesis of biofilm formation. Similar structures and cells were detected on autoclaved grapevine canes inoculated with Pch and incubated on water-agar. This latter result suggests that nutrients released from decomposing grapevine wood are sufficient for the biofilm formation in Pch.

The above results suggest that Pch can form biofilms *in vitro* and possibly *in planta*. This may lead to the enhanced protection of Pch cells from antifungals produced by the host. The production of extracellular polysaccharides was previously suggested to take part in the pathogenesis of Esca and this process also seems to be linked to Pch biofilm development. The cell density dependence of Pch biofilm formation may contribute to the latent nature of Esca disease.

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Key words: Esca, Quorum Sensing, pathogenesis, grapevine, polysaccharides

Session 3 O11

Advances in knowledge and new solutions against diseases: mildews

Advances in knowledge and new solutions against diseases: fungal trunk diseases

Control of Botryosphaeria dieback and black-foot pathogens in grapevine propagation material using *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1

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Abstract

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Grapevine trunk diseases (GTDs) are a complex group of diseases that lead to major economic losses in all wine-producing countries. The investigation of biocontrol agents (BCAs) capable of forestalling or at least minimizing the development of GTDs has, recently become a priority. Nursery experiments were set up to: (i) assess the biocontrol effect of *Trichoderma atroviride* (Ta) SC1 and *Bacillus subtilis* (Bs) PTA-271, alone and in simultaneous application, against Botryosphaeria dieback (BOT)- and black-foot (BF)- associated pathogens during the grapevine propagation process, and (ii) evaluate the success of the BCA inoculation during the grapevine propagation process, using quantitative reverse transcription polymerase chain reaction (qPCR) techniques. The results demonstrated a significant reduction in the percentage of potentially infected plants and the percentage of fungal isolation from wood fragments of BOT and BF pathogens in nursery material treated with Ta SC1 and Bs PTA-271, respectively. In one of the experiments, simultaneous treatments with Bs PTA-271 and Ta SC1 caused a reduction in percentages of potentially infected plants and fungal isolation, from wood fragments containing BOT and BF pathogens. These biological treatments may be relevant components of an integrated approach, using complementary management strategies to limit infection by GTD pathogens, but further research is still needed to elucidate the effectiveness of Bs PTA-271 and the benefits of simultaneous application with Ta SC1 for the control of GTD pathogens in nurseries.

Key words: Botryosphaeria dieback; black foot; biocontrol agents; grapevine trunk diseases; grapevine propagation process.

Session 4 **O12**

Field control of Botryosphaeria dieback of grapevines by using locally sourced *Trichoderma* species

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Abstract

Botryosphaeria dieback (BD), caused by several species in the family Botryosphaeriaceae, occurs wherever grapevines are grown and is one of the main biotic factors reducing yields and limiting vineyards' lifespan. Botryosphaeriaceae spp. infect grapevines through pruning wounds and thus, pruning wound protection is currently the most effective management strategy. Currently, no control products are registered in Canada against BD. Furthermore, a demand for alternatives to chemical products and more sustainable control methods to manage grapevine diseases has significantly increased in the last years in British Columbia (BC). The main objectives of this research were to identified local biological control agents in the Trichoderma genus and evaluate their potential biocontrol activity against the BD fungi Diplodia seriata and Neofusicoccum parvum. A total of 29 Trichoderma isolates were obtained from vineyards in BC. Morphological and molecular analyses of the ITS1-5.8S-ITS2 gene and TEF-1α partial gene identified seven species, including T. asperelloides, T. atroviride, T. harzianum, T. koningii, T. tomentosum, and two novel species, T. canadense and T. viticola. In vitro confrontation assays showed several isolates to inhibit pathogen mycelial growth by up to 75%. In planta detached cane assays under controlled greenhouse conditions identified T. asperelloides, T. atroviride and T. canadense isolates from BC to provide 70% to 100% pruning wound protection against D. seriata and N. parvum for up to 21 days after treatment. Field trials conducted in 'Merlot' vines in 2019 and 2020 showed mixed-species inoculum of T. asperelloides, T. atroviride and T. canadense to provide high biocontrol activity against BD fungi for up to 60 days after treatment. Field results also showed Trichoderma spp. from BC to provide similar or better pruning wound protection when compared against commercial chemical and biocontrol products. This study provides for the first time data towards the development and registration of the first control products against BD in Canada.

Key words: Biocontrol, Botryosphaeria dieback, grapevine trunk diseases, pruning wounds, *Trichoderma*, *Vitis vinifera*.

Session 4 O13

Antifungal effect of Cu based nanocomposites against *Pleurostoma richardsiae*, *Dactylonectria torresensis* and *Fusarium oxysporum*

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Abstract: Grapevine trunk pathogens cause serious damage to grapevines and have significant economic impacts. Six batches of nanocomposites containing graphene oxide and three batches based on CuO were tested in vitro against the tree selected fungal isolates (Pleurostoma richardsiae MEND-F-035, Dactylonectria torresensis MEND-F-0360 and Fusarium oxysporum MEND-F-0174) isolated from the grapevine wood showing Petri or Black foot disease symptoms in the Czech Republic. The highest inhibitory effect of the nanocomposites (NCPs) was recorded in case of CuO group (CuO-L-3, CuO-L-7, CuO-L-9), suppressing the growth of all the three fungi. The NCP CuO-L-9 was the most effective NCPs (P<0.05), however the obtained values were very similar among CuO NCPs. Different results were obtained from in planta experiment where the grapevines cv. Sauvignon grafted on SO4 were inoculated by the fungi and hydrated with all the CuO based NCPs solutions. Based on statistics of wood lesion lengths, CuO-L-3 was effective against P. richardsiae and D. torresensis but not against F. oxysporum. On the contrary, CuO-L-7 and CuO-L-9 were effective only against F. oxysporum. Length of the whole plant and root length were also measured during the in planta experiment. CuO-L-3 and CuO-L-9 positively influenced the plant height in case of P. richardsiae inoculated grapevines and the root length was positively influenced by all the three NCPs in case of F. oxysporum inoculated grapevines. The Koch's postulates were completely fulfilled through the reisolation of the fungi from inoculated grapevines.

Key words: nanocomposites, CuO, grapevine, trunk pathogens, treatment

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Session 4 Advances in knowledge and new solutions against diseases:

Session 4 014

Mechanically pruning vs hand-pruning: the effect on incidence and severity of grapevine trunk disease pathogens

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Abstract

Grapevine trunk diseases (GTDs) cause severe dieback and economic losses to grapevines in all major grape-growing regions in the world. Dieback of infected grapevines results in stunted growth, leaf necrosis and, in severe cases, death. Mechanical pruning (MP) uses machinery to prune vines and has proved benefits of reduced labor costs, increased production and acceptable wine quality. However, the effect of MP on GTDs is unknown. The study, therefore, aimed to elucidate the effect of MP on the incidence and severity of GTDs compared to conventional hand pruning (HP). This was achieved by sampling an MP and HP vineyard in five economically important wine regions (Robertson (2019), Aan de Doorns (2020), Goudini (2020) Vredendal (2021) and Upington (2022)) in South Africa. A total of 70 vines per site were sampled. From each vine, a pruning stub with at least 4-5-year-old growth was removed from MP blocks and a distal spur with 10cm of the cordon for HP blocks. Isolations were made from all visible symptoms in the internal discoloration. Isolates were identified up to species-level using morphological and molecular techniques. In all seasons, Botryosphaeriaceae, Togniniaceae, Phaeomoniellales, Diapothales, Diatrypaceae and Basidiomycete species were identified from both pruning types. However, HP vineyards had significantly higher incidence and severity of several severe dieback and canker pathogens compared to the MP vineyards in all wine regions. Wood rot symptoms were abundant in hand-pruned samples. This is because hand pruning results in larger and more wounds whereas in mechanically pruned vines longer canes with smaller wounds appear giving the vine more opportunity to compensate when infection occurs and shoot die. Mechanical pruning is a good option to use as part of an integrated management strategy to prevent inoculum build-up and reduce wound infections increasing the productive lifespan of vineyards in several wine regions in South Africa.

Key words: Cultural practices, mechanical pruning, hand-pruning, dieback, wood-rot pathogens

Session 5 **O15**

Advances in knowledge and new solutions against diseases: Botrytis and black rot

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

Specific effects of copper on the mycobiome of grapevine leaf surfaces

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Abstract

In organic viticulture, copper is used to control downy mildew (Plasmopara viticola) and is currently the only approved plant protection product, which is effective in situations with high disease pressure. In addition to the control of downy mildew and other secondary grapevine diseases, "non-target" effects of copper on many other plant-associated fungi can be expected due to its non-specific mode of action. Therefore, the joint project VITIFIT, which is investigating ways to secure grapevine health while reducing the amount of copper used in organic viticulture in Germany, includes a focus on exploring effects of copper on the grapevine phyllosphere mycobiome. DNA-metabarcoding was applied to evaluate effects on the fungal diversity as well as on the relative abundance of individual species. Over a period of three years, the effects of copper on the mycobiome composition of grapevine leaf surfaces were investigated in field trials each located in one of five wine-growing regions of Germany (Baden, Rheingau, Palatinate, Rhinehesse and Franconia). Within these three years, at each location samples were collected for at least two years and control leaves treated with just sulphur were compared to leaves treated with sulphur and copper, the latter at a maximum amount of 3 kg*ha⁻¹*a⁻¹. Furthermore, at the Palatinate site it was tested if a reduced copper application rate can also reduce copper induced mycobiome changes. Thereby, general effects of copper on the composition of the mycobiome and specific "non-target" effects e.g. on potential antagonists were identified. The mycobiome composition was highly effected by the respective year and location. The copper treatment had a minor, yet significant effect. On the OTU level, several yeast like basidiomycota are negatively affected in their relative abundance. Some of these include genera like Filobasidium and Sporobolomyces, which are associated with antagonistic activity. In addition, several of these yeasts were affected in a dose dependent manner implying a potentially improved health promoting leaf surface mycobiome by reduced copper application rates. The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture.

Key words: copper, organic viticulture, mycobiome, phyllosphere, antagonists

Session 5 **O16**

Session 5 Advances in knowledge and new solutions against diseases: Botrytis and black rot

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

Combining indicators for a better understanding and management of black rot risk?

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Abstract

We are setting up reflections and experiments to build an epidemiological monitoring of black rot risk adapted to the agro-ecological conducts and to the evolution of the agronomic context of the culture of the vine (reduction of available fungicides, resistant varieties, grassing, productive practices, abandoned plots...). Existing risk models for black rot indicate a risk depending on climatic conditions and potentially on the phenology of the plant. However, no model takes into account the presence and quantity of primary inoculum in the plot or in the surroundings to moderate or not this risk. We are setting up projects and experiments to see if new indicators such as the maturation of the primary inoculum, the spore trap and plant sentinel systems can improve our understanding of the epidemics and refine the existing models and the management of the disease. We will present the first results of these studies.

Key words: grapevine, black-rot disease, primary inoculum, risks models.

Session 5 **O17**

Advances in knowledge and new solutions against diseases: Botrytis and black rot

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

Etiology and Epidemiology of Sour Rot in Ontario, Canada

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Abstract

Sour rot is frequently a problem in vineyards in Ontario Canada, especially when the preharvest period is warm and rainy. While the main *Vitis vinifera* varieties that are affected are thin-skinned, tight-clustered and early-maturing (Pinot noir, Pinot gris, Riesling, Chardonnay), in years when warm temperatures extend into November, sour rot can be a problem in Cabernet franc and C. sauvignon. We isolated organisms from sour rotted berries collected from 38 vineyards and separated them into morphotypes which were then identified to species. Isolates of each organism was inoculated to wounded and unwounded berries and assessed for sour rot development. *Gluconobacter oxydans* and *Hanseniaspora uvarum* were consistently the isolated from all vineyard sites and also the most pathogenic. Pathogenicity of solo and co-inoculations with *G. oxydans* and *H. uvarum* was tested at a range of temperatures (10-25°C). Infection was possible at 10°C but symptoms started earlier and were more severe at 25°C. This is consistent with field observations. Also noted in field observations was the relationship between fruit maturity and initiation of sour rot symptoms. Lab trials showed that there was an abrupt increase in sour rot development in inoculated berries between °Brix of 13 and 15. This information has been the basis of management strategies in Ontario vineyards.

Key words: sour rot, Gluconobacter oxydans, Hanseniaspora uvarum, temperature, fruit maturity

Session 5 **018**

Session 5 Advances in knowledge and new solutions against diseases: Botrytis and black rot

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

New insights into sour rot: a complex interaction between the microbial community, vinegar flies and weather

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Abstract

Sour rot, as defined by Hall et al. (2017) is "a syndrome that involves pre-harvest cluster decay accompanied by the smell of vinegar" and the disease affects grape berries once they reach ripeness. It is caused by yeasts and acetic acid bacteria whom are frequently vectored by vinegar flies and are all regulated by the actual weather conditions. It is commonly assumed that yeasts are the primary component of sour rot disease since they ferment in a first step sugars into alcohol. In a next step acetic acid bacteria transform the fermented alcohol into acetic acid and/or gluconic acid that initiate the pungent vinegar smell in sour rot diseased grapes. For getting new insights in the etiology of sour rot we studied and manipulated concurrently the microbial community, transmission patterns as well as meteorological conditions. Here we present how the microbial community evolved from fungi dominated by the order of Hypocreales on healthy berries towards fungi of the order of Saccharomycetales in sour rot diseased berries. Moreover, a co-inoculation experiment indicates that the two yeast genera Candida and Hanseniaspora contribute to the development of sour rot symptoms and acetic acid production in healthy grapes and that they might be able to provoke sour rot on their own. When studying the vectoring capacity of vinegar flies, females of Drosophila suzukii induced more severe sour rot damage than their males or D. melanogaster indicating the promotion of microbial colonisation and/or development by egg laying and larval development. However, we were also able to show that direct fruit to fruit transmission is common when there is physical contact between diseased grapes and healthy but injured berries. This mechanism is probably responsible for the propagation of sour rot within grape clusters frequently characterised by a direct aggregation of diseased berries. Finally, we were able to demonstrate in a laboratory experiment that the optimal temperature for the development of sour rot lays around 30°C. Above and below this temperature, acetic acid production was hampered and almost absent at 20°C and below. Besides a greenhouse experiment revealed that temperature and precipitation affected the number of eggs laid by present D. suzukii females in the berries of caged grapevines, which thereafter determined acetic acid concentration within grapes and consequently the severity of sour rot. These findings support the common perception that area-wide sour rot infection relies on extensive rainfall and warmer autumn temperatures, both also favouring activity and reproduction of vinegar flies. Overall, our studies provide additional insights in the etiology of sour rot and contribute to a better understanding of the complex interaction between microbial communities, sour rot transmission, assumed vectors and favourable weather conditions in vineyards.

Key words: Vitis vinifera, Drosophilidae, spotted wing drosophila, microbial diversity inoculation experiment.

Session 5 **O19**

Advances in knowledge and new solutions against diseases: Botrytis and black rot

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

Pruning wound protection products induce alterations in the wood mycobiome profile of grapevines

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Abstract

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Fungal pathogens involved in grapevine trunk diseases (GTDs) may infect grapevines throughout their lifetime, from nursery to vineyard, via open wounds in stems, canes or roots. In vineyards, pruning wound protection products (PWPPs) offer the best means to reduce the chance of infection by GTD fungi. However, past research suggests that some plant protection products are responsible for non-target alterations in the wood mycobiome profile of young vines. Similarly, PWPPs may affect non-target microorganisms that comprise the natural endophytic mycobiome residing in treated canes, disrupting microbial homeostasis and indirectly influencing grapevine health.

Using DNA metabarcoding, we characterized the endophytic mycobiome of one-year-old canes of cultivars Cabernet Sauvignon and Syrah in two vineyards, in Portugal and Italy, and assessed the impact of established and novel PWPPs on the fungal communities of treated canes.

Our results reveal a large fungal diversity (176 taxa), and we report multiple genera never detected before in grapevine wood (e.g., Symmetrospora and Akenomyces). We found differences in mycobiome beta diversity when comparing vineyards (p = 0.01) but not cultivars (p > 0.05). When examining PWPP-treated canes, we detected cultivar- and vineyard-dependent alterations in both alpha and beta diversity. In addition, numerous fungal taxa were over- or under-represented when compared to control canes. Among them, *Epicoccum* sp., a beneficial genus with biological control potential, was negatively affected by selected PWPPs.

This study demonstrates that PWPPs induce alterations in the fungal communities of grapevines, requiring an urgent evaluation of their direct and indirect effects on plants health with consideration of factors such as climatic conditions and yearly variations, in order to better advise viticulturists and policy makers.

Keywords: Vitis vinifera; microbiome; grapevine trunk diseases; Trichoderma; copper; biological control


Advances in knowledge and new solutions against diseases: Botrytis and black rot

Advances in knowledge and new solutions against diseases: mycobiome and sour rot

Promising future for *Botrytis cinerea* (Helotiales: Sclerotiniaceae) management using strategies based on *Xenorhabdus* and *Photorhabdus* (Morganellaceae) in vineyards

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Abstract

The pathogen Botrytis cinerea (Pers. Fr.) (Helotiales: Sclerotiniaceae) is a wound necrotrophic fungus that causes significant losses in grapevines worldwide. Several chemical and physical tools have been used to reduce fungal pathogen infections, but their efficiency and economic and environmental costs are under vivid debate. The use of microbial-based tools for pathogen management holds promise. In this study, we investigated whether the symbiotic bacteria of the entomopathogenic nematode, Xenorhabdus and Photorhabdus have antifungal capacity against B. cinerea and whether the effectiveness of the bacteria depend on the method of their usage. In particular, we evaluated the efficacy of the following control strategies: (i) bacterial cell-free supernatants, (ii) unfiltered ferments, (iii) crude bacteria isolates, and (iv) volatile organic compounds (VOCs). The antifungal efficacy of X. bovienii, X. nematophila, X. kozodoii and P. laumondii subsp. laumondii cell-free supernatants and the antifungal effect of X. nematophila and P. laumondii unfiltered ferments were tested in Petri dishes at 10 % of concentrations. A subsequent study evaluated the antifungal capacity of the crude isolate of X. nematophila and P. laumondii against B. cinerea compared with the fungicidal effect of the commercial Bacillus amyloliquefaciens (Serenade® ASO fungicide). Finally, two laboratory experiments investigated the effects of X. nematophila and P. laumondii VOCs on the growth and incidence of B. cinerea in Petri dishes and in harvested red grapes (treated with the bacterial VOCs simultaneously and preventively before the fungal infection). X. nematophila cell-free supernatant and unfiltered ferments inhibited 82 and 100 % of the B. cinerea mycelial growth compared to control (distilled water). Furthermore, P. laumondii-isolate controlled the mycelial growth of B. cinerea with similar efficacy to commercial B. amyloliquefaciens. We also found that VOCs emitted by X. nematophila and P. laumondii (vs control) reduced ~40 and ~60 % of B. cinerea colony growth after pathogen infection in Petri dishes, respectively. Moreover, X. nematophila and P. laumondii VOCs inhibited ~100 % of B. cinerea mycelial growth in harvested grapes when applied simultaneously with the B. cinerea infection. Finally, VOCs emitted by P. laumondii reduced approximately 20 % of B. cinerea incidence in harvested grapes. Overall, this study showed strong evidence of the potential antibotrytic use of those bacteria and helps to develop an innovative formulation of these bacterial products to develop an efficient biocontrol tool for grapevine growers.

Keywords: antifungal compounds, beneficial microorganims, bio-tools, Vitis vinifera.

Session 6 **O21**

Habitat management as an integrative tool for the control of grapevine's Flavescence dorée

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Abstract

Flavescence dorée (FD) is a detrimental grapevine's disease associated with the quarantine organism 'Candidatus Phytoplasma vitis' (FDp), which leads to important economic losses to European viticulture. FDp is acquired and transmitted by the leafhopper Scaphoideus titanus, which is responsible for the rapid spread of the disease. No curative methods are currently available and so far, control measures have been targeted at the vineyard scale, disregarding the role of landscape as a possible source of inoculum and as a suitable habitat for the vector population. In addition to abandoned vineyards and gone-wild grapevines, which may represent an important FDp and S. titanus reservoir and habitat, several alternative vectors and host plant species have been identified in the landscape as possible actors in the FD epidemics. In the specific case of Southern Switzerland, the East Palearctic leafhopper Orientus ishidae along with the plant species Alnus glutinosa have been described as functioning actors in the maintenance of FDp in the landscape. O. ishidae is a polyphagous insect and widely found on plant species such as Corylus avellana, Acer spp., Salix spp, and even on Vitis spp., on which it is also able to oviposit. The role that such alternative FDp epidemiological cycles may play is not negligible, considering that 28.1% of the vineyard perimeter directly borders the forest at less than 10 m due to the specific geomorphology and cultural practices of Southern Switzerland. This is additionally aggravated by the fact that previous works found up to 85% of O. ishidae specimens sampled from alder infected by FDp genotypes compatible with both S. titanus and grapevine and other FDp-like phytoplasmas, while 100% of the tested alders resulted infected. C. avellana, which is a typical forest edge species in Southern Switzerland and a highly suitable host of O. ishidae, may act as a bridge between alder stands and vineyards.

In the frame of this study, during winter 2021/22, a habitat management experiment was conducted in order to test the impact of selective removal of hazelnuts for the control of *O. ishidae*. Based on the monitoring activities of 2021, the trap positions representing *O. ishidae* capture hotspots were selected for the experiment. During the dormant period, all existing hazelnuts within a radius of 3 m from the trap position were coppiced and the resulting woody material was quantified (diameter and length) and removed from the experimental plots. The effect of habitat management on the *O. ishidae* population was assessed by placing the traps in the same locations from June 2022 until October 2022. In the treated sites, the *O. ishidae* mean populations showed a marked decreasing trend, which went far beyond the seasonal reduction observed on the control traps installed in untreated sites, confirming the positive impact of the selective removal of hazelnuts. Habitat management may thus serve as an integrative tool to lower the risk of FDp flow between the forest and the cultivated compartments by lowering the population of the alternative vector *O. ishidae*.

Key words: alternative vectors, phytoplasma, Orientus ishidae, Scaphoideus titanus, Switzerland



Trapping Scaphoideus titanus (Hemiptera: Cicadellidae) in Italian vineyards: where, when and why?

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Abstract

The Nearctic leafhopper Scaphoideus titanus Ball (Hemiptera: Cicadellidae: Deltocephalinae) is currently the main vector of the grapevine flavescence dorée (FD) phytoplasmas in Europe. Its bio-ecology, studied since the 60s, has recently been the subject of new findings, which are useful for understanding the role of this leafhopper in the FD epidemic diffusion. However, the contraction in the availability of plant protection products, deriving from a European legislation aiming at sustainability objectives, has made the efficacy assessment of S. titanus management tools urgent. In this scenario, we conducted field research in 2021 and 2022 in a vineyard located near Florence (Tuscany, Central Italy). To evaluate the efficacy leafhopper management approaches carried out against nymphs and adults, we monitored the adult catches by yellow sticky traps in July and August, over the control plots. In 2021, the experimental design included one (flupyradifurone) or two (flupyradifurone and acetamiprid) insecticide treatments carried out in June against S. titanus nymphs. In 2022, after two treatments done in June against the nymphs with the same active ingredients, a further treatment was done in the first week of August against the adults with sulfoxaflor. In the two study years, no significant differences were observed taking into consideration the number of adults catches, though a positive gradient in terms of adult preference was observed for the traps located at the edges of the plots and for the south side of each trap. Most of the S. titanus captured in the traps until the first week of August were males. The significant reduction in the average number of adults caught per trap that was observed in 2022 in the entire plot after the treatment with sulfoxaflor leads to at least three questions which deserve consideration in further research: (i) can the yellow sticky traps mask the actual efficacy of an insecticide treatment? (ii) May they result in a sort of mass trapping against adults present within the range of influence of the device? (iii) May the mobility of the adult population allow a redistribution of individuals within the vineyard, among parcels differently treated?

Key words: Deltocephalinae; flavescence dorée; leafhopper; insect mobility; Integrated Pest Management

Session 6 O23

Studies on the *Scaphoideus titanus* phenology in a diversified region: implications for management strategies

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Abstract

Scaphoideus titanus is a leafhopper native to North America. It was accidentally introduced to Europe and first discovered in France in the 1960s. This leafhopper is the primary vector of a phytoplasma (FDp) associated to the Flavescence dorée (FD) disease which is a significant threat to viticulture in Europe. The chemical control of *S. titanus* is required to prevent FD spread and this measure is mandatory in a number of European countries. In Italy, insecticide use and timing is regulated at regional level. Typically, the appearance of the fourth nymph instar of *S. titanus* is considered for the first mandatory control application. However, the variability in *S. titanus* phenology in a large area (e.g., a region) could make difficult to synchronize chemical control measures. The phenology of *S. titanus* was investigated in different viticultural areas of the Veneto region (North-eastern Italy). We selected a number of sampling areas spanning from sea-level (0 m asl) to mountain regions (561 m asl). In each sampling area, we conducted weekly samplings. Our findings revealed a 15-day difference in the appearance of the fourth instar nymphs between areas located near the Adriatic Sea and the mountain regions. These results suggest the need to improve insecticide application recommendations for regions with significant environmental variations.

Key words: Flavescence dorée, Scaphoideus titanus, pest phenology, pest control.



Kaolin against *Scaphoideus titanus* as an alternative to natural pyrethrins in Swiss vineyards?

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Abstract

In Switzerland, natural pyrethrins are currently the only registered insecticide to control the leafhopper *Scaphoideus titanus*, the main vector of grapevine Flavescence dorée, in commercial vineyards. In order to find alternative products against this insect, we tested the effectiveness of kaolin, a white inert aluminosilicate mineral, in 11 independent field trials over four consecutive years from 2018 to 2021. The use of kaolin at rates between 20 and 40 kg/ha applied two to three times at the beginning of hatching resulted in an average reduction in leafhopper densities of 36.8% with efficacy values ranging from 0 to 88.9% for the 18 different interventions. Overall, the efficacy of the different kaolin dosages and application strategies did not equal those commonly recorded for natural pyrethrins, which showed a mean efficacy of 74.8% in the 6 independent field trials and single reduction values ranging from 41.3 to 97.4%. In view of the highly variable efficacy levels of kaolin observed in our assays, we conclude that the use of kaolin to control *S. titanus* in Swiss vineyards does not provide an efficient alternative to natural pyrethrins in compulsory control areas.

Key words: *Vitis vinifera*, Cicadellidae, grapevine yellows, elm yellows group (16SrV), transmission, IPM, alternative control

Session 6 O25

Spatial and temporal distribution of leafhoppers and their egg parasitoids in vineyards located in north-eastern Italy

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Abstract

The presence of semi-natural areas contiguous to vineyards can influence the distribution of some pests and their natural enemies inside cultivated plots. We investigated the effect of woody vegetation on the spatial and temporal distribution of three leafhopper species (i.e., *Empoasca vitis, Zygina rhamni* and *Erasmoneura vulnerata*) and their potential egg parasitoids. Observations were carried out in vineyards located in Northeastern Italy and data were analyzed using SADIE. Results showed that the colonization of vineyards by *E. vulnerata* adults and egg parasitoids (*Anagrus atomus*) was clearly favored by woody vegetations, and their distributions were often associated. No clear effects resulted for the remaining leafhopper species. We exposed sentinel eggs of *E. vulnerata* and *Z. rhamni* to parasitism by *A. atomus* and both leafhopper eggs were parasitized. These results can suggest habitat management practices to improve the sustainability of viticultural agro-ecosystems.

Key words: grape leafhoppers, egg parasitoids, conservation biological control.



Sugar feeders as a tool for ant-mediated plant defense against leafcutter ants in grapevine

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Abstract

In Argentina, leafcutter ants such as Amoimyrmex bruchi are a major herbivore pest in vineyards, but biological control against these ants has not been successful so far. The Argentine ant Linepithema humile (Mayr) is a common invader of vineyards worldwide and has a strong preference for carbohydrates. In search for new biological control methods against A. bruchi, we tested if sugar supplements can trigger L. humile ants to act as plant bodyguards. In a first 10-week field experiment in Cafayate, Argentina, we planted five 2-year old vines (cv. Torrontés) close to each of 18 pre-existing leafcutter ant colonies and eight control spots. Then, we attracted L. humile ants to a group of vines using sugar feeders, while empty feeders were used for a second group. Feeders (750 mL bottles) contained 25% sucrose solution and were placed at ground level. Control plants were deployed using sticky, non-toxic paints to exclude all ants. Treatments were distributed over 2 similar sites using a randomized stratified design. In a second short field experiment, we evaluated the impact of L. humile on A. bruchi foraging behaviour using the same field site. We found that the leafcutter ant A. bruchi caused >90% leaf area reductions in vines without L. humile, while plants with L. humile were rarely attacked. The presence of L. humile decreased A. bruchi's foraging activity and the amount of material transported by it. The development of biological control strategies that include and promote the ecological services of ants as natural enemies of herbivorous pests could become a useful tool in sustainable viticulture and horticulture.

Keywords: Amoimyrmex bruchi, Linepithema humile, sugar feeders, Biological control, Grapevine

Session 7 KN3

Insect senses and pest management

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Abstract

Insects sense their environment with the five standard sensory modalities: vision, hearing, olfaction, gustation and touch. Two of them, vision and olfaction, are widely used in pest management to monitor and control pest population occurrence and abundance, respectively. Light traps and mating disruption are representative examples of this. The other three sensory modalities have received relatively less attention, but new research is shedding light on ways to employ them in pest management (e.g., biotremulation, push and pull). Insect sensation of less familiar stimuli such as CO₂, magnetic fields and IR, or even common ones such as humidity, temperature and pressure, is relatively unexplored. In the current scenario of insecticide restrictions and climate change, a better understanding of the ways that insects sense and respond to their environment could generate innovative pest monitoring and control tools. In this talk I will explore some examples with a focus on grapevine pests.

Key words: IPM, sensory biology.



Waves and tortricids: Ultrasounds, vision and pest control

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Abstract

Sex-pheromone-based mating disruption and monitoring are major elements in the control of tortricid moths. However, this control could be enhanced by taking advantage of other sensory modalities, such as acoustics and vision. To this end, we are trying to understand visual and acoustic communication of the three major pests of grapes, peached, and apples worldwide: Lobesia botrana, Grapholita molesta and Cydia pomonella, respectively. The three species produce lowintensity ultrasound clicks in the 20 kHz-50 kHz range coincident with wingbeat frequency. Preliminary tests indicate that males don't respond to biologically relevant ultrasound sources such as typical FM foraging bat cries around 50 kHz – 80 kHz range. The three species have trichromatic retinnae endowed with three photoreceptor classes peaking at 355 nm (ultraviolet), 440 nm (blue) and 525 nm (green). The three species also show similar temporal (flicker fusion threshold) resolution at around 120 Hz. So, although each species has a different diel activity period, they do not differ in the measured visual parameters.

Key words: Tortricidae, Communication, Ultrasound, Vision, Diel Activity.



Grapevine yeast attracts grapevine moth

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Abstract

Monitoring pest populations is a key aspect of Integrated Pest Management (IPM). However, in vineyards under mating disruption against *Lobesia botrana* male response to the female sex pheromone is compromised. Therefore, new attractive volatile compounds are wanted in order to monitor *L. botrana* males and females. We tested if the volatiles released by the prominent grapevine yeast, *Hanseniaspora uvarum*, attract *L. botrana* adults in a wind tunnel assay. Two sticky trap liners were placed side by side in the upwind end of the tunnel each with a different Petri dish containing either *H. uvarum* growing on solid, half strength, MS300 (synthetic grape must) medium or uninoculated medium. Unmated males, unmated females and mated females were released on different days in groups of 50-150 individuals and left for 24h. The captures where video-recorded. Significant differences in the flight activity were detected between sexes. About 65% of the males but 6-16% of the females were captured in the traps. *H. uvarum* traps captured significantly more adult males, unmated females and mated females than the control traps. Males showed a peak of activity during the first part of the scotophase (corresponding with the female calling period) and a second peak of activity at the start of the photophase. Females presented a single peak of the activity at the start of the photophase. Females presented a single peak of the activity at the start of the photophase.

Key words: insect, Vitis, microbial, attractant, IPM, Hanseniaspora



A double dispenser for mating disruption of two grape pests: *Lobesia botrana* and *Cryptoblabes gnidiella*

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Abstract

The European grapevine moth (EGVM), Lobesia botrana (Lepidoptera: Tortricidae) and the honeydew moth (HM), Cryptoblabes gnidiella (Lepidoptera: Pyralidae) are two important pests of grapevine in Europe and South America. In the last 20 years, pheromone-based mating disruption (MD) has been successfully employed to manage EGVM populations, allowing a reduction in the use of chemical insecticides in the vineyards, thus safeguarding human health and the environment. On the other hand, no well-established strategy is available for HM management. In the last few years, a biodegradable double capillary dispenser (Isonet® LCG BIOX235), which simultaneously releases the synthetic pheromones of EGVM and HM, was evaluated for MD purposes. This experimental device was tested in two study sites (i.e., Apulia and Tuscany) at three different application densities (i.e., 300, 400 and 500 dispensers/ha). The trials were conducted monitoring EGVM and HM adult and larval population in MD vineyards, in insecticide treated vineyards (grower standards) and untreated vineyards. For both species, simultaneous MD led to a significant reduction of male catches, as well as to a reduction in the number of infested clusters, if compared to the untreated control. As a general trend, no significant differences were found between the three MD dispenser densities tested in our study. Overall, our results showed that the use of Isonet® LCG BIOX235 against EGVM and HM can contribute to the sustainable management of both pests, albeit with more difficulty against HM when highly abundant populations are present.

Key words: Honeydew moth, Pyralidae, Phycitinae, Integrated Pest Management, pheromone.

Session 7 030

Oviposition by three tortricid moths on filter paper treated with different doses of gustatory stimuli

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Abstract

Naturally occurring behaviorally modifying compounds, such as oviposition stimulants and deterrents, play a role in Integrated Pest Management programs (IPM). The effect of several gustatory stimuli on the number of eggs laid by *Cydia pomonella* (CM), *Grapholita molesta* (OFM) and *Lobesia botrana* (EGVM) was tested under laboratory conditions. Five mated females were released during the photophase in 4.5-L plastic arenas containing 4 filter paper pieces loaded with three doses (0.1 mM to 1 mM or 10⁻³ to 10⁻¹) of a given tastant plus solvent control (water or ethanol), and the number of eggs laid on each filter paper was recorded 24 h later. Sugars (fructose and sucrose), salts (KCI and NaCI) and neem oil were tested on all three species, whereas tannic acid, juglone, grape seed oil and oleanolic acid were tested on EGVM alone. Neem oil was highly deterrent to all three species, although the lowest dose in all three moth species, and salts stimulating CM at the mid dose. Tannic acid, juglone, and grape seed oil strongly reduced EGVM oviposition. Oleanolic acid had no significant effect, although the trend was to increase oviposition in the EGVM. Our study pointed out stimulatory and deterrent oviposition effects triggered by several chemical compounds under laboratory conditions. Field tests are needed to determine their potential use in IPM programs.

Key words: Tortricidae, oviposition, gustation, repellent, attractant.

Session 8 KN4

Cover crops and organic mulches to control weed flora in vineyards

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Abstract

Weed control in vineyards is usually carried out by tillage or by herbicide applications. However, there is great interest in looking for alternatives to both methods since tillage increases the risk of soil erosion and the loss of organic matter, while the use of herbicides affects the soil microbiota and increases the risk of selecting for herbicide resistant biotypes. Given this situation, the possibility of implementing cover crops or organic mulches opens up a promising scenario in the control of weeds, apart from providing other benefits to the vineyard.

The use of cover crops is useful in the containment of some perennial weeds such *Cynodon dactylon*. The shading caused by the cover during the weed's emergence period limits its subsequent summer development. Also, the type of cover crop used and the method of finishing it can have a significant impact on weed control. In this sense, it is more effective to finish the cover with a roller crimper when the cover reaches peak biomass, than with a shredder when the cover is ending its cycle. The mulch created over the soil with the roller crimper constitutes an effective obstacle to light, significantly hindering the emergence and development of *C. dactylon*.

At the same time, under the line of the vineyard it is difficult to control certain species with herbicides or by in row tillage. The application of organic mulches based on chopped pine wood or almond shell is a useful tool to control species like *Conyza bonariensis*, a fast-growing weed capable of developing resistant biotypes to different herbicides, including glyphosate. These organic mulches, in turn, create more favorable edaphic conditions for the vineyard which are reflected in an increase in yield and vigor.

Session 8 **031**

How organic mulches affect spontaneous weed vegetation and soil properties

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Abstract

Spontaneous weeds are one of the main problems for farmers in semi-arid wine regions due to the excessive competition they pose to the crop and the complicated management that this entails. Traditionally, herbicide application and tillage management have been commonly used for weed suppression despite their negative environmental impacts. Currently, there is a need for environmentally sustainable viticulture practices. This three-year study describes the effects of different soil management practices in the vine row on spontaneous weed abundance and species community, as well as the impact on physical and chemical soil properties and water retention capacity. Specifically, three soil managements with organic mulches (grape pruning debris (GPD), straw (SM), and spent mushroom compost (SMC)) and two conventional methods (under-row tillage (TILL) and herbicide (HERB)) were analysed. SM and GPD mulches delimited weed growth under 30% of weed soil coverage. In addition, they reduced the proportion of harmful weed species, increased plant diversity and ecological richness, reduced crop competence and decreased the inputs of herbicide or tillage maintenance and, consequently, improved soil quality and integrity. Among conventional practices, TILL treatment was highly sensitive to the timing of agricultural work and environmental conditions, exhibiting significant year-to-year variability. Organic mulch, particularly SMC, increased the soil's water retention capacity and the content of essential elements and organic matter. Furthermore, SMC mulch presents a weak physical barrier than SM and GPD, allowing bigger spontaneous germination in the former resulting in weeds soil coverage of more than 85%. In conclusion, organic mulches improved soil physicochemical properties and water retention capacity. Besides, SM and GPD mulches controlled the excessive growth of spontaneous weeds.

Key words: grapevine, plant suppression, circular economy, viticulture, ecosystem services.



Weed Management by Cover Crops in Organic Mediterranean Vineyards

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Abstract

Cover crops are a sustainable agricultural practice that can provide multiple ecosystem services such as reducing erosion, improving water infiltration, managing weeds, and retaining nitrogen. The appropriate species, termination method, and timing for termination are key challenges to successfully implement cover crops. The objective of this study is to determine which cover crop species and termination methods are most suitable for Mediterranean vineyards (Vitis vinifera) and their potential to control weeds. To achieve the proposed objectives, two experiments are being conducted in organic vineyards located in Raimat (Lleida, Spain). The first study has got eight treatments (four cover crop species with two roller-crimper passes dates) and a no cover crop control, distributed following a completely randomized block design with three repetitions. The species planted are black oat (Avena strigosa), barley (Hordeum vulgare), ryegrass (Lolium multiflorum), and triticale (*×Triticosecale*), that were sown at the end of October of 2022. In spring, all covers will be crimped with a roller on two different dates. Another experimental design will test the effect of cover crop termination method (roller-crimper or shredder) of two cover crop species (Triticale and Phacelia tanacetifolia) on weed emergence and cover persistence during the summer. The first results, obtained up to April 2023 indicate that all cover crops except ryegrass were providing around 75% of soil cover already in February 2023 and close to 85% in April, while ryegrass percent cover was only 35% in April. Weed cover was the lowest in barley and triticale cover crops (around 10% in April) while in the ryegrass, weed cover reached 30%, similar to the control. The expected results are that cover crops will control weeds during the summer, and crimped covers will have greater persistence on the soil than covers terminated with a shredder. These studies will contribute to the development of sustainable agricultural practices in vineyards and improve our understanding of cover crop effectiveness to manage weeds in the Mediterranean region.

Key words: weed suppression, ground cover, roller crimper, shredder, grapes.

Session 8 033

Managing viticultural ecosystems using functional biodiversity indicators and agroecology approaches for sustainable production and consumer engagement – the case of SOGRAPE in Portugal and Spain

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Abstract

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The 2022 Kunming-Montreal agreement for the United Nations Convention for Biological Diversity (CBD) saw unprecedented support and participation from global businesses that pushed governments for higher ambition in setting the Global Biodiversity Framework post-2020 (GBF). Sogrape actively participated in this landmark drive for a nature-positive world joining more than 700 global businesses from all sectors. This commitment stemmed from our pioneering adoption of IOBC-based integrated production for all Sogrape vineyards in Portugal for more than 20 years, dedicated sustainable viticulture work across five countries and the stern realization that natural resources underpinning Sogrape's wine business are threatened by the lack of efficient public governance for protection of ecosystems in wine regions. Because of this joint crosssectoral advocacy, the new GBF incorporates ambitious goals and targets aiming to make the world naturepositive by 2030. This is now the time for deploying in the field the knowledge and action required to achieve those goals. Owning 1150 hectares of vineyards in Iberia, Sogrape launched a wide project for nature-positive winegrowing integrated in its «Seed the Future» global sustainability program. Surveys were conducted to combine data on soil moisture, pedology, remote sensing, biodiversity inventories and ecosystem diagnostics and create action plans for ecosystem management and agroecology aiming to materialize a land-sharing strategy for wine production. This strategy ambitions to develop actionable indicators for vineyard ecosystem management linked to indicators of economic performance to achieve true-value reporting of natural capital management in the company's reports. A whole-of-value-chain vision meant to engage markets and citizenconsumers will be applied through brand building and wine-tourism to lead the grape and wine food system towards the UN SDG 12: Responsible Consumption and Production. In this work, we present data and action plans obtained from surveys in Portugal and the field-deployed actions in Portugal and Spain to bring the values of vineyard biodiversity and ecosystem-management into wine brand equity and engagement with consumers and citizens.

Key words: functional biodiversity, ecosystem services, agroecology, resilience, sustainability



Phytoseiid mites in European winegrowing regions were influenced at the field and landscape scale

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Abstract

Viticultural practices at the field scale and landscape composition at the landscape scale are important factors for biodiversity and ecosystem services in vineyards. Phytoseiid mites are important natural enemies against phytophagous mites on vines in this context. In this study, we investigated in 156 vineyards, in five European wine-growing regions the effect of farming type, inter-row vegetation management and landscape composition on phytoseiid mites. Our results demonstrated that phytoseiid mite species diversity was mainly dominated by one or two species across the investigated vineyards. Depending on the wine-growing regions, phytoseiid mite densities were higher in integrated and conventional farming compared to organic farming. Moreover, phytoseiid mite densities increased through spontaneous vegetation cover in the vineyard interrow compared to bare soil or seeded cover crops. Interestingly, phytoseiid mite populations increased when the proportion of vineyards increased in the landscape. Effects of the farming type could be linked to the adverse effect of a high pesticide use, especially of active ingredients which are harmful for phytoseiid mites such as sulphur in organic vineyards. The beneficial effect of spontaneous vegetation cover may be related to a more suitable supply of the additional food resource pollen, which is depending on plant species richness in the vinevard inter-row. Our research demonstrated, that phytoseiid mites as natural enemies in European vineyards benefited from a reduced use of pesticides and vegetation cover in the inter-row. Furthermore, an important factor which should be considered, is the proportion of viticultural area at the landscape scale for stable phytoseiid mite population.

Key words: predatory mites, biological control, vineyard, inter-row management, landscape composition



Entomopathogenic nematodes for controlling *Lobesia botrana* in vineyards: fine-tuning of application, target area, and timing

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Abstract

Lobesia botrana (Lepidoptera: Tortricidae), also known as the European grapevine moth, is one of the major harmful pests detected in worldwide vineyards. The current control methods have limitations, and expanding the available biological control agents can provide new strategies in both IPM and organic viticulture. Entomopathogenic nematodes (EPN) are well-known biological control agents for numerous soil pests. Thanks to the current application systems, such as the use of adjuvants, their implementation against aerial pests is now possible. Previous laboratory experiments probed the control capability of the EPNs Steinernema feltiae and S. carpocapsae against L. botrana at various larval stages and pupae. We hypostatized that by selecting the best combination of EPN-adjuvant, target area in the grapevine and the timing (season/temperatures), we can enhance EPN efficacy as a biocontrol agent against L. botrana. The aim of this study was to screen for the best EPN-adjuvant mix and to determine the best ecological scenarios for their use against L. botrana. We investigated (i) survival, viability, and adherence on leaves of EPNadjuvant mix, (ii) protection capability (mortality of L. botrana and reduction of its damage) in leaves, grapes, and trunk bark, and (iii) EPN activity against L. botrana at low temperatures as a proxy of the overwintering period. The screening of five adjuvants (Multi-Us, Maximix, Dash HC, Nu-Film-17 and Adrex) and a subsequent combination (Multi-Us+Maximix), showed generally high compatibility with all the adjuvants, resulting Maximix as the best candidate for the EPNs S. feltiae and S. carpocapsae. Compared with the noapplication treatment, Maximix combined with EPN increased their killing against L. botrana L3 on grapes and leaves with significant reduction of the damage. The study of the pupae in the trunk bark system at 22°C, 14°C, and 10°C showed that mortality caused by EPNs decreased with temperature, from ~60% at 22°C to values ~20% at 10°C, and the use of Maximix in this scenario did not improve their efficacy. Overall, the results showed that the combination with Maximix can enhance the survival, infectivity, and adherence of the EPN S. feltiae and S. carpocapsae. In addition, these species combined with Maximix reduced the damage caused by L. botrana L3 in leaves, and grapes. However, this combination did not improve their efficacy against pupae. Finally, we suggest their application at sunset and late spring/ealy autunm (March/September) to target mid-temperatures (22°C-15°C). Further validation in field conditions is necessary for their full implementation.

Keywords: adjuvants, grapevine, Steinernema, overwintering.



Entomopathogenic Fungi for the control of grapevine sap-sucking insects

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Abstract

Several sap-sucking Hemipteran can cause severe damages in vineyards, both directly, by feeding in the grapevine phloem, and indirectly, by transmitting phytoplasmas and viruses.

Despite defense strategies are currently based on chemical treatments, in the last years, the control of insect pests has been increasingly oriented towards green biological approaches. In this context, this research aims to study an alternative control strategy, consisting on the use of entomopathogenic fungi (EPF).

In spring 2022, EPF strains were isolated from soil samples collected in vineyards, fields and woods of Piedmont region (northwest Italy) by using two different approaches: plating on selective medium and Galleria bait method. Finally, 314 strains were isolated and submitted to both morphological and molecular identification by ITS-PCR.

The most abundant species found were *Metarhizium anisopliae* (38%), *Purpureocillium lilacinum* (24%), *Metarhizium robertsii* (17%), *Penicillium citrinum* (7%) and *Clonostachys rosea* (4%).

A preliminary screening was performed to test the pathogenic activity of the 314 fungal strains on the model leafhopper *Euscelidius variegatus*. The trials were carried out by "conidic shower" method consisting in reverting a mycelium plate on top of a jar where insects are located, showering the targets with conidia for 8h; after the shower the insects are transferred in a microcosm and monitored for the next week, during which mortality is assessed.

The preliminary tests are currently in progress and will allow the selection of promising EPF candidates to be used against three target pests: *Scaphoideus titanus*, *Empoasca vitis* and *Planococcus ficus*.

In addition, during summer 2022, 61 EPF strains from the collection were tested for their capacity in the endophytic colonization of grapevine, as a possible strategy to augment their persistence in field compared to direct propagule spraying. In the future, further endophytic trials will be performed with the most promising fungal strains selected by direct pathogenicity tests on the target insects.

Keywords: entomopathogenic fungi, grapevine, conidic shower, Hemiptera, endophytization



Enhanced biocontrol of vine mealybug, *Planococcus ficus*, using hydrogel-based control of sugarfeeding ants

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Abstract

Vine mealybug, Planococcus ficus, is the most significant pest of grape production in California vineyards. Effective management requires a combination of chemical and biological controls, often augmented by mating disruption. Over the past few years, reductions in pesticides that can be used in vineyards have made it more difficult to control mealybugs, and have made it nearly impossible to control sugar-feeding ants. These ants protect vine mealybug from predators and parasitoids that would otherwise provide conservation biological control. Our research focused on ant management through an experimental method to apply liquidbased baits to the vineyard as a solid. This was accomplished by hydrating polyacrylamide crystals with water containing 25% sucrose (to simulate honeydew) and small amounts of a toxicant, and then applying baits with a traditional bait or fertilizer spreader at rates ranging from approximately 20 to 40 liters per hectare. Screening various toxicants identified thiamethoxam (conventional) and spinosad (organic) as two of the most effective toxicants when applied at rates of active ingredient per acre that are approximately 1-2% of the amount of active ingredient that can be applied to the foliage or through the drip system according to current labels. During the most recent studies in 2023, a series of two applications at approximately 3-4 week intervals in the spring can provide good to excellent control for a period of 2-3 months. Parallel studies conducted in wine grapes and citrus have shown similar efficacy on other sugar-feeding ant species, including Argentine ant, *Linepethima humile*. Data on the impacts of ant removal on parasitism rates of vine mealybug, and on subsequent fruit infestation levels at harvest, are in the process of being collected and will be available for release by the fall of 2023..

Key words: conservation biocontrol, ants, sugar, honeydew, hydrogel



Deciphering the effects of agronomical practices on Aspergillus incidence and carposphere's microbial communities of grapevine

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Abstract

Aspergillus bunch rot is considered one of the most important diseases of grapevines resulting in severe yield losses and, major qualitative deterioration of grape products due to the production of mycotoxins. We investigated, in a two-year field study, the impact of agronomic practices like defoliation to enhance grape microclimate (DF), pruning method to reduce grape bunch density (LBD), and irrigation cut-off (NIR), at three developmental stages of grapevine (Pea size berry, Veraison, and Harvest), on (i) grape composition (Titratable acidity; TA, pH, and Total soluble solids; TSS), (ii) on the frequency of occurrence of Aspergillus on grape berries and (iii) on the overall composition of grape carposphere microbiome. The density of Aspergillus on grape berries was significantly reduced by the applied management practices (DF, LBD, NIR). Amplicon sequencing analysis showed that both the phenological stage and the agronomic practices employed (particularly NIR and DF), imposed significant changes in the α -diversity and β -diversity of the grape carposphere bacterial and fungal communities. In the NIR, LBD, and DF treatments, which supported lower Aspergillus populations, network analysis revealed negative co-occurrence patterns between Aspergillus and several bacterial genera (Streptococcus, Rhodococcus, Melitangium) known for their antifungal properties, and suggesting potential natural attenuation mechanisms for the control of Aspergillus.

Keywords: Bunch rot disease, microbiome, viticultural practices, amplicon sequencing analysis, bunch density, leaf removal, irrigation, disease management.



Impact of weather data sources on infection risk provided by a decision support system and implications in disease management

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Abstract

Decision Support systems (DSSs) for sustainable crop management are web-based systems that collect real-time information about different crop components (air, soil, plants, pests, and diseases) and analyse these data by using advanced modelling techniques. Models implemented into a DSS can simulate plant growth, risk about different diseases and pests, abiotic stresses, and the protection level provided by the application of plant protection products. All these models need input data related to site-specific crop characteristics and to environmental conditions (air temperature and humidity, rainfall, leaf wetness, etc). In recent years, technologies to collect environmental data have improved substantially, and DSSs need to be flexible in receiving and using these data. The impact of different environmental data sources/technologies on models' output, and on the consequent management actions, has been scarcely investigated. In this work, we compared outputs of epidemiological models for grapevine downy and powdery mildews and consequent disease management by using weather data measured by physical weather stations and weather data estimated by climate models. Comparisons were performed in 50 vineyards from different viticultural areas of Italy and Spain in the period from 2018 to 2022. Preliminary results confirm the potential of using estimated weather data with mean of 80% of coincidence between models' outputs from estimated and measured weather data.

Key words: disease management, grapevine, decision support system, meteorological climate models.

Session 9 **040**

Parametrization and validation of a Physiologically Based Demographic Model (PBDM) for *Scaphoideus titanus* Ball (Fam: Cicadellidae)

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Abstract

The sap-sucking leafhopper Scaphoideus titanus Ball (Hempitera: Cicadellidae) plays a major role in spreading flavescence dorée (FD) phytoplasma from affected grapevines to healthy ones. Its high capacity to acquire and transfer FD, especially at the adult stage, is mainly due to the vector dependence on Vitis spp. to complete the biological cycle. As a Neartic pest accidentally introduced, S. titanus have not afford any natural limitations factors unless its own thermal limits and its strict ampelophagy; therefore its diffusion has increased over years and is still increasing. The FD management relies on vector control and eradication of infected plants. Due to the decreasing availability of pesticides and the major use of biological products that need a more precise timing of application, a model could be helpful for guiding monitoring and control activities of S. titanus. A Physiologically Based Demographic Model (PBDM) developed by Gilioli et al. (2016) for arthropod populations featured by discontinuous stages, structured with continuous time- and agestructure within a stage, and calibrated for the moth Lobesia botrana, was adapted and calibrated for S. titanus. Adaptation involved the model structure, which is based on the insect life cycle from overwintering eggs to adult stage, showing one generation per year. Calibration involved the development of temperaturedependent rate functions specific for each insect developmental stage, based on literature data. A dataset of independent data of population dynamics collected in different vineyards of Piedmont (Italy) from 2013 to 2022 was used for model validation. The model provides realistic dynamics of the S. titanus dynamics, and is currently available within the Decision Support System (DSS) vite.net® - a real-time web platform used on a large scale in different European countries, to support decision making for Integrated Pest Management.

Key words: Population dynamics, Physiologically Based Demographic Model, Integrated Pest Management, *Scaphoideus titanus*

Session 9 **041**

Field Evaluation of Endophytic and Rhizospheric Bacteria against Grapevine Trunk Diseases

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Abstract

Grapevine trunk diseases (GTD) represent a serious threat to the sustainability of viticulture worldwide and their management remains challenging. The current trend of reducing the use of chemical pesticides makes biocontrol an environmentally friendly strategy to mitigate the impact of GTDs. Three bacterial isolates obtained from woody tissues and the rhizosphere of grapevines were selected by their antifungal activity against GTD-causing pathogens *in vitro* and further evaluated as biocontrol agents (BCAs) in field conditions. The BCAs were fermented for the production of secondary metabolites in liquid media and applied adopting four approaches: (i) infiltrated in dormant propagation material before grafting in nursery settings; (ii) poured as a soil drench in the vineyard; (iii) injected in the trunk and cordon; and (iv) sprayed onto pruning wounds of mature vines. Results revealed that the isolates of *Bacillus velezensis* and *Pseudomonas chlororaphis* exerted a positive effect when infiltrated in propagation material and as soil drench treatments by reducing the lesion length caused by artificially inoculated GTD-causing pathogens. When comparing the levels of disease control, a better performance was observed against *Eutypa lata* and *Phaeoacremonium minimum* than against *Neofusicoccum parvum*.

Key words: grapevine, antifungal, trunk diseases

Session 9 **O42**

Adaptation of the VitiMeteo forecasting system to PIWIs

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Abstract

Downy mildew (*Plasmopara viticola*) is the leading disease in viticulture in years with high precipitation. Traditional grape cultivars must therefore be protected against *P. viticola* infections by fungicides over the entire season. For optimal planning of fungicide applications, forecasting and decision support systems like VitiMeteo (<u>https://www.vitimeteo.de</u>) are useful tools in integrated pest management.

The cultivation of fungus-resistant grapevine cultivars (PIWIs; German: pilzwiderstandsfähige Rebsorten) is an effective measure to reduce plant protection products in viticulture. As a result from crossings of susceptible traditional grape cultivars and resistant wild grape varieties, PIWIs combine wine quality with natural disease resistance. To prevent adapted genotypes of *P. viticola* from overcoming resistance in PIWIs, targeted fungicide applications are recommended. Ideally, on the one hand these treatments are timed for the optimal use of a PIWIs natural potential for pesticide reduction. On the other hand the treatments should provide protection during the susceptible developmental stages of the cultivar. Therefore, plant protection strategies for PIWIs were developed and integrated into the VitiMeteo forecasting system as part of the VITIFIT (https://www.vitifit.de) project.

In the course of the project, the resistance characteristics of two traditional grape cultivars and six different PIWIs were analyzed. Resistance to *P. viticola* of grape leaves and clusters was therefore determined in infection experiments. To represent the complete phenological development of the vine during the season, seven different phenological stages (BBCH 53-55: inflorescences clearly visible -inflorescences swelling, flowers closely pressed together; 57-59: inflorescences fully developed, flowers separating; 65-68: full flowering: 50 - 80 % of flower hoods fallen; 71-73: fruit set: young fruits begin to swell, remains of flowers lost - berries groat-sized; 75: berries pea-sized, bunches hang; 77-79: berries beginning to touch - majority of berries touching; 81-83: beginning of ripening: berries begin to develop variety-specific color) were selected for inoculation.

PIWIs showed only weak sporulation on leaves, but reacted with strong necrosis depending on the variety. Traditional grape cultivars showed decreasing susceptibility with increasing leaf age. On clusters, increasing resistance was observed in all grapevine cultivars as phenological development progressed, although the timing was strongly cultivar-dependent. While the traditional cultivars were resistant to *P. viticola* only in the last tested phenological stage (BBCH 81-83), PIWI cultivars showed strong resistance already at or immediately after flowering. In contrast, the early developmental stages of the clusters of all analyzed cultivars were susceptible to the pathogen under the high infection pressure applied in the experiments.

The experimentally obtained results were integrated into VitiMeteo and will be an extension of the existing model "VM Plasmopara" in the future. The practicability of the extension was validated in the field during the years 2022 and 2023 and publication on the website is planned in 2024.

Key words: Plasmopara viticola, VitiMeteo, decision support system, PIWI, fungus resistant cultivar.

Session 9 **O43**

Hybrid grapevine resistance to downy and powdery mildew in Israel

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Abstract

Vitis vinifera grapevines are susceptible to downy and powdery mildews (Plasmopara viticola and Erysiphe necator, respectively). During the growing season, 4–10 chemical treatments are typically applied to control these diseases; an alternative solution is to use resistant varieties. Some American and Asian grape species are naturally resistant to powdery mildew (PM) and downy mildew (DM). Breeding programs for PM and DM resistance have been conducted worldwide for decades. Recently, resistant hybrid cultivars have been brought to Israel from Weinbauinstitut Freiburg (WBI, Germany) breeding programs. Our research aim was to test their suitability to Israel's local climate conditions. Grapevines from the different programs were planted in four different climatic regions in the north of Israel for observation. Results: (i) In the lab and under natural weather conditions, 4 hybrid grapevine varieties were found to be significantly less sensitive to PM and DM than the control V. vinifera varieties. (ii) Monthly sprays of fungicides during the summer (to prevent the development of isolates that might overcome the vine's resistance), significantly reduced PM incidence and severity in the V. vinifera variety, and reduced PM infection in the resistant variety to zero. (iii) Metabolites profile of infected and healthy leaves of the different varieties was analyzed using LCMS-MS. It was found that the flavonoids compound: Catechin, Isorhamnetin, Kaempferol, Luteolin, Nictoflorin, Piceid, Trans-resveratrol, Trifolin - concentration was higher in Tempranillo (Vitis vinifera sensitive verity) leaves compared with the tolerant varieties Prior and Cabernet Carbon. Miquelianin flavonoid (Quercetin 3-glucuronide) was lower in Tempranillo leaves-relative to the tolerant varieties Prior and Cabernet Carbon. The current study deepened our understanding of the mechanism of PM tolerance in the two hybrid varieties.

Key words: Plasmopara viticola, Erysiphe necator, Hybrid grapevine, flavonoids

Session 10 IPM implementation and tools for sustainable viticulture)ΔΔ

Impact of sustainable farming interventions in the vineyard and its surrounding area on the dynamics of pests and natural enemies' populations in Rioja Alavesa, Spain

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Abstract

Traditional viticulture has been affected in recent decades by intensive vinevard management that has a direct effect on biodiversity loss. The simplification of landscapes indiscriminately eliminating the elements that separate plots, weeds, and native shrub flora as a reservoir of useful fauna, are some of the main causes of the problem of the current proliferation of pests in agriculture. In organic vineyard management, where more environmentally friendly products are used, pest control options are scarce, and the use of insecticides, when possible, must be done with broad-spectrum active ingredients that not only affect the pest, but also eliminate many natural enemies. For this reason, among the means of pest control, biological control is becoming more and more frequent. This involves the use of predators and parasitoids as alternative/complementary means to phytosanitary products since they are much more specific. Biological pest control can be promoted in a natural way, by introducing certain modifications in the vineyard environment that may favour the presence of native natural enemies of pests. In Rioja Alavesa, so far, the incidence of vineyard pests is not high, but one of the effects of climate change on pests includes changes in their geographical distribution, and some pests are starting to cause problems in warm years. The pests that are of greater concern are the yellow spider mite, Eotetranychus carpini Oud (Acari: Tetranychidae) and the tea green leafhopper, Empoasca vitis Gothe (Hemiptera: Cicadellidae). Phytoseiid mites and mymarids are known as natural enemies of *E.carpini* and *E.vitis* respectively.

The aim of this study was to assess the impact of the implementation of cover crops (spontaneus and flowerdriven cover), green corridors, dry stone walls with native shrubs and vineyard biodiversity hotspots on E. carpini and E.vitis and of their natural enemies in Rioja alavesa. To do so, their population dynamics were studied from May to September 2022 in four vineyards where ecological infrastructures (EI) had been implemented in 2020. E.carpini was rarely found, so the study of its population dynamics was not possible. The results obtained show that the population of phytoseiid mites is favoured by spontaneous cover crops but not affected by other EI. Flower-driven cover crop and dry-stone wall with native shrubs such as Rubus ulmifolius Schott and Rosa canina L increased the presence of mymarids, whose population dynamic was opposite to that of E.vitis.

Key words: Conservation biological control, grapevine, biodiversity, habitat management

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Are Farm to fork strategy goals reasonable and achievable? State of the art of Península de Setubal's winegrowers

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Abstract

The European Union's "farm to fork" strategy sets out several objectives to be achieved by farmers, who, among others, relate to increasing biodiversity, protecting soils and reducing the use of pesticides. At a time when the amendments to the national plans of Sustainable Use of pesticides are being discussed, it is important to understand what is the Setúbal Peninsula region status. Today, the main challenges for farmers are the impact of pesticides on public health, environmental protection, waste reduction, bees and non-target organisms protection, the removal of many active ingredients and climate change. Faced with these challenges, the use of pesticides in 235 winegrowers in the Palmela region was evaluated between 2016 and 2021. To support some of the answers, a socio-economic survey was also carried out. The data analyzed included the number of treatment, the dosages used, compliance with the pre-harvest interval, the reason why winegrowers performed phytosanitary treatment and how they chose a pesticide. For each year, it was found that, on average, farmers spray seven times, although the trend was to decrease. The most used pesticides belong to groups 3 (Triazol), M02 (Inorganic) and M04 + 4 (Ftalimidas + Phenyl Amids), according to the FRAC Codes. Regardless of the climatic conditions and the pressure of the disease in the vineyard, winegrowers sprayed every 14 days. These data were also related to climatic conditions, the existence of technical assistance and socio-economic data.

In addition to the analysis of the records and the interpretation of their relationship with the other data referred to above, residue analyses were carried out at the entrance of the grapes into the winery to assess whether the MRL was exceeded and whether were not authorised pesticides were used in the vine. This procedure was repeated in 2019, 2020 and 2021. The discussion around waste has been very intense in civil society. If, on the one hand, pesticides are indispensable to agricultural production, it is also true that their use must always take into account food security and environmental protection. It is important that the message is clear, transparent and assertive and that the consumer is also interested, critical and understandable. The second reason for the analysis of waste is related to the withdrawal of active substances and the existence of alternatives for farmers. In field trials, it has been noticed that the so-called "biological alternatives" are sufficient in situations of low/medium pressure of disease, but inefficient in medium/high pressure situations. By analyzing data from plant protection records and waste analysis, it was concluded that fear of diseases and pests and "empirical experience" sometimes go beyond knowledge and technology. In addition, the weak valorization of grapes and discouragement with the implementation of some poorly reported strategies are factors that fuel the concern about the difficulty in achieving the goals.

Keywords: Pesticides, Farm to fork strategy, Sustentability, vineyard

Session 10 **046**

Long-term historical characterization of French vineyard exposure to pests and diseases: case study of Bordeaux and Champagne regions

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Abstract

The French agricultural warning service has historically published weekly reports and annual summaries of the pressure from pests and diseases (grouped and named "pests" hereafter). The summaries are based on a large number of plots, notably vineyards, monitored in different regions with different local editions for each region. The information, issued from field observations conducted by experts, constitutes a highly valuable body of literature on the presence and overall damage of pests in vineyards. In this work, we used this literature to develop a textual analysis and build an integrative score of annual pest occurrence over a long-term period.

To transform the warning service bulletins into annual pest indicators, we proceeded as follows: (1) For every pest and every year, we extracted from the annual reports the keywords related to (a) pest occurrence within a given area and (b) associated damage. (2) We transformed these data into semi quantitative scores ranging from absence to (a) widespread pest or (b) severe damage. (3) We combined the occurrence and severity scores to obtain an annual score for each pest. After an analysis of historical surveys from 1961 to 2020 in the Bordeaux and Champagne regions, we established a long-term database of annual indicators, including various grapevine diseases (mildews, rots, trunk diseases, etc) and phytophagous or disease vector animals (moths, mites, scale insects, leafhoppers, etc.).

Using these indicators, we found that in the Bordeaux region, vineyards were exposed to a regular but rarely generalized presence of *Lobesia botrana*, which could be very severe locally, in contrast to *Eupoecialia ambiguella*. For diseases, black rot was constantly present and, after a phase of relative regression (from 1980 to 2013), has become more widespread over the last decade. Rot Brenner is completely unknown in this winegrowing region.

The Champagne vineyards were characterized by regular and widespread populations of *E. ambiguella*, sometimes with important damage but decreasing over time. *L. botrana* was present only locally but with increasing severity. For diseases, rot Brenner was historically present with local damage but is now in decline (even disappearing). On the other hand, black rot, which appeared 25 years ago, was recently more frequently observed locally and with low severity.

We reconstructed the pest occurrences that affected grapevines over time in two contrasting French winegrowing regions. This tool is very useful for characterizing the epidemiological status of various years and analysing long-term trends *versus* isolated events. This analysis helped to better characterize the conditions that prevailed in some phytosanitary situations. We can therefore better understand past pest evolutions and link them to socioeconomic contexts. This information will contribute to anticipating the necessary evolution of grape protection against quantitative and qualitative losses to adapt to global warming and regulatory and/or marketing conditions and meet changing societal demands.

Key words: grapevine, crop protection, history, pests, database

Session 10 047

Effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture

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Abstract: In the past decades, arthropod biodiversity has strongly declined in many agricultural landscapes, which is, among other reasons, attributed to intensified agriculture. Policy measures, such as the European Green Deal, include increasing the share of organic agriculture, reducing pesticide use, and increasing the proportion of semi-natural habitats (SNH) to enhance biodiversity and to counteract the strong decline in recent years. However, it is not known whether negative trends of arthropod biodiversity are also occurring in viticulture. Here, the conditions for species may have recently improved in the context of integrated plant protection with no further use of insecticides in our study region and the establishment of green cover in the inter-rows of vineyards. Besides the use of either synthetic chemicals in conventional viticulture or inorganic compounds such as copper and sulfur in organic viticulture, the frequency of pesticide sprayings can have an impact on arthropods. The cultivation of fungus-resistant grape (FRG) varieties allows more than 80 % fewer applications of pesticides compared to classical varieties and may thus be beneficial for biodiversity. Greening of inter-rows as well as SNH structures in the surrounding landscape may also promote biodiversity by providing resources for feeding and reproduction for numerous species.

In this project, we investigated how organic and conventional management, reduced pesticide use through the cultivation of FRG varieties, and SNH in the surrounding landscape affect the arthropod biodiversity in vineyards. From April to September in 2020 and 2021, using e.g. Malaise traps and yellow pan traps, we assessed biodiversity in the wine-growing region Palatinate in southwest Germany in 32 vineyards in a crossed design of management (organic vs. conventional) and pesticide use (regular vs. reduced in FRG varieties). The pairs of vineyards were located in 16 landscapes, which form a gradient in the proportion of SNH within a radius of 500 m and 1,000 m of the vineyards. We measured the biomass of the captured specimens and used metabarcoding to assess the total arthropod biodiversity and further used morphological and acoustic species identification to investigate effects on wild bees (Apiformes) as well as grasshoppers and crickets (Orthoptera). Biomass was almost one-third higher in conventional vineyards compared to organic ones, while organic vineyards had almost 50 % more bees. Densities of herb-dwelling Orthoptera were 2.9 times higher in FRG varieties under organic management than in the other vineyard types. Higher proportions of SNH increased taxa richness of arthropods as well as abundance and richness of aboveground-nesting bees and further changed community composition of arthropods including bees and orthopterans. The results provide information on the importance of local management and landscape structure for the arthropod biodiversity and form as part of the "National Monitoring of Biodiversity in Agricultural Landscapes" (MonViA) the basis for a long-term monitoring in viticulture.

Key words: Community composition, DNA metabarcoding, Fungus-resistant grape variety, Organic versus conventional farming, Semi-natural habitats.



Agroecology: Designing biodiverse and resilient vineyard agroecosystems for a planet in crisis

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Abstract

Climate change, the pandemics, the input cost hikes linked to the Russia-Ukraine are bringing economic uncertainty to vineyard owners and managers forcing many to cut costs of production and adapt to extreme weather variability. These scenarios are convincing many farmers to look at agroecology (the science of applying ecological principles to the design and management of cropping systems) as a promising pathway for realizing more sustainable vineyard systems. Agroecological practices including crop diversification, intercropping, agroforestry, integrating crop and livestock, and soil enhancing management measures provide the basis to design more complex farming systems, more likely to maintain ecological services under global change pressures and at the same time have positive environmental, production (yield) and economic outcomes.

Drawing from examples of vineyards in Mediterranean regions (California, Chile and Italy), we describe strategies that break the monoculture nature of vineyards by adapting diversification strategies at the farm and landscape level. Cover cropping provides opportunities to build soil fertility and quality while providing habitat for natural enemies of insect pests. Intercropping of annual crops between vines can also be beneficial as is the integration of animal grazing in appropriate times. Farmers can also manipulate the surrounding landscapes with corridors, natural borders, shelterbelts etc. with important effects on pest control, water conservation and wildlife preservation.

Protecting the soil from erosion, drying up, and improving soil moisture levels and water circulation is also a fundamental strategy to enhance the resiliency of vineyard agroecosystems. Cover crop mulching and green manures offer great agroecological potential to conserve water, to activate soil ecology and biological species diversity with consequences in nutrient cycling and biocontrol of pathogens and thus to stabilize crop yield under moisture stress.

Key words: agroecology, resilience, biodiversity, biological control







Protection in Viticulture

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- P02 DNA metabarcoding reveals that grapevine parts harbor different, niche-specific mycobiome. Adrienn Geiger, Carla Mota Leal, Zoltán Karácsony, Richard Golen, Kálmán Zoltán Váczy, József Geml
- **P03** *In vitro* interactions between *Armillaria* sp. and *Trichoderma* sp. collected from mushroom crop residues. *David Labarga, Ignacio Vicente-Diez, Miguel Puelles, Andreu Mairata, Alicia Pou.*
- P04 Increasing microalgae biomass feedstock by valorizing wine gaseous and liquid residues. *Miguel Cachão, Ana Chambel*
- P05 Nature resistance to *P. viticola* in interspecific grapevine genotypes revealed by MASS and transcriptomic. <u>Martin Hádlík</u>, Kateřina Baránková, Viera Kovacova
- P06 New Biocontrol agents for fungi obtained from vermicompost of sewage sludge and grape bagasse. <u>Vanesa Redondo</u>, Nadia Cambra, Pablo Pérez, Manuel Aira, Jorge Domínguez
- **P07** *In vitro* evaluation of the antifungal activity of vermicompost teas against the phytopathogenic fungus Botrytis cinerea. <u>Vanesa Redondo</u>, Nadia Cambra, Pablo Pérez, Manuel Aira, Jorge Domínguez
- P08 Preliminary trial of the effect of *Trichoderma atroviride* I-1237 on the control of *Phomopsis* Cane and Leaf Spot caused by *Phomopsis viticola* (Sacc.) *Jorge Sofia*, *Raul Sofia*, *João Vila-Maior*
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Posters' Abstracts



Integrated Protection in Viticulture

Characterization of Czech PIWI cultivars using SSR markers linked to mildew resistance loci

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Abstract

A breeding program focused on resistance to fungal diseases began intensively in the Czech Republic at the end of the 1980s. Vinselekt – wine breeding station in Perná became the main workplace that focused on breeding for resistance in the late 1990s. The first registered variety in the Czech Republic was the white vine variety Malverina in 2001. In 2004, the registration of the red wine variety Laurot followed. Subsequently, the Savilon, Rinot, Vesna and Nativa varieties were registered. Currently, the registration of another 4 varieties - Runa, Marcus blanc, Pinot écru and Riesling gris - is being completed, and two more are in the registration process - Ruby Pinot and Flower Riesling. In presented research, 5th cycle of interspecies crossing is now being evaluated, which is based on the previous results of our own breeding program and includes the world's most important new interspecific hybrids. The goal is to combine many genetic sources and achieve a significant pyramiding of genes, especially genes from V.amurensis and V.rotundifolia. The mentioned varieties were tested using SSR markers that are linked to the resistance loci Rpv 1, 3, 4, 7, 10, 12, Run 1, 2 and Ren 3, 9. The analyzes confirmed the presence of loci that could be expected due to the known resistance donors used in the crossing. The most loci of resistance were confirmed in varieties Marcus blanc and Ruby Pinot. The presented varieties are characterized by good wine guality and especially the older registered varieties have already found their customers and their popularity among Czech winemakers is increasing every year.

Key words: Czech PIWI varieties, resistance loci, SSR markers, powdery mildew, downy mildew

DNA metabarcoding reveals that grapevine parts harbor different, niche-specific mycobiome

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Abstract

Grapevine is an important plant cultivated in more than 80 countries around the world. To date, it is still unclear how infectious diseases, such as grapevine trunk diseases (GTDs) modify the microbiome of grapevine. In this study, we examined the mycobiome in 200 samples from of asymptomatic and Esca type GTD symptomatic grapevines to see how health state affects grapevine mycobiome. We also tested the influence of cultivar, season, vintage and microhabitats with respect to the grapevine mycobiome. We hypothesized that fungal communities would differ in composition among microhabitats and seasons due to the contrasting abiotic conditions, while we expected little variation among cultivars and vintages. DNA metabarcoding data were generated from bark, soil and woody tissue from four different cultivars in 2020 February and August, and 2021 February and August. The most common fungal functional groups were plant pathogens, wood saprotrophs, soil saprotrophs, litter saprotrophs and mycoparasites. Plant pathogens were dominant, comprising mainly GTD-associated plant pathogens in bark and wood, and non-GTD pathogens in soil. Microhabitat turned out to be the strongest driver of grapevine mycobiome. We observed larger compositional differences of fungi among different plants parts within grapevine plants than among individual grapevines and between health states, vintages and seasons. Wood saprotrophs and mycoparasites were present mostly in bark, while soil and litter saprotrophs were most diverse and abundant in soil. Abundance and richness values differed across microhabitats but not among asymptomatic and Esca disease symptomatic grapevines. Seasonality and cultivar did not affect the mycobiome. Richness values of 2021 were lower in case of plant pathogens, wood saprotrophs and mycoparasites, which may be due to the drier vintage. Overall, our suggest that abiotic environmental factors are important shaping the fungal mycobiome of grapevine, and further studies are needed to investigate the effect of abiotic conditions on grapevineassociated fungal communities with respect to wine regions and management practices.

Key words: grapevine microbiome, Esca disease, trunk diseases, environmental filtering

In vitro interactions between *Armillaria* sp. and *Trichoderma* sp. collected from mushroom crop residues

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Asbtract

Armillaria mellea (Agaricales: Physalacriaceae) is a fungus naturally occurring in the soil, which may penetrate the grapevines' roots, causing their death in the long term. Many chemical products have been tested to manage this fungus with limited success. Nowadays, uprooting and non-prolonged cultivation is the only field-allowable and effective solution. Trichoderma is a well-known fungus used as a biocontrol agent. However, it is also known to be a contaminating fungus for the cultivation of mushrooms (Agaricus sp.). We hypothesized that T. Harzianum from contaminated mushroom cultivation is an effective biological control agent of A. mellea in vitro and could be used as a treatment in the vineyard. In this sense, a circular economic model would be favored in which, on the one hand, the waste reuse from mushroom cultivation would be encouraged, and on the other hand, an effective and sustainable solution would be proposed to combat some fungal diseases. To accomplish this, field strains of T. Harzianum and A. mellea were isolated on Malt Extract Agar (MEA). Dual confrontation tests on Potato Dextrose Agar (PDA) plates were performed to evaluate the inhibitory effect of the T. harzianum strain on A mellea. For this purpose, A. mellea was plated 14 days before T. harzianum. The two fungi were also individually cultivated as positive controls. All plates were kept at 25°C throughout the experiment. We observed that T. harzianum inhibited the in vitro growth of A. mellea. Specifically, the A. mellea growth stopped when both fungi met, while T. harzianum continued to grow above A. mellea. The results obtained support our hypothesis of the potential of T. harzianum as a biocontrol agent for A. mellea. However, it must be validated in field experiments. Future research will focus on analyzing whether mushroom crop residues infected with T. harzianum could be reused as an organic mulch treatment against A. mellea in the vineyard.

Key words: Biological control, mulch, vineyard and waste reuse.

Increasing microalgae biomass feedstock by valorizing wine gaseous and liquid residues

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Abstract

Global warming due to greenhouse gases (GHG) has become a serious worldwide concern. The new EU Green Deal aims t0 achieve GHG emissions reduction by at least 55% by 2030 and a climate neutral EU economy by 2050. The deal strongly encourages GHG reducing measures at local, national and European levels. The REDWine project will demonstrate the technical, economic and environmental feasibility of reducing by, at least, 31% of the CO2 eq. emissions produced in the winery industry value chain by utilizing biogenic fermentation CO2 for microalgae biomass production

REDWine concept will be realized through the establishment of an integrated Living Lab demonstrating the viability of the system at TRL 7. The Living Lab will be able to utilize 2 ton of fermentation off-gas/year (90% of total CO2 produced in the fermenter) and 80 m3 of liquid effluent (100% of the liquid effluent generated during fermenter washing) to produce 1 ton (dry weight) of Chlorella biomass/year. This biomass will be processed under a downstream extraction process to obtain added-value extracts and applied in food, cosmetic and agricultural end-products and to generate a new EcoWine. REDWine will focus on the recovery of off-gas from a 20.000L fermenter of red wine production existing in Adega Cooperativa de Palmela (ACP, located in Palmela, Portugal).

REDWine's microalgae were tested in 2022 and 2023 with 4 purposes in vineyard: improve flowering stages, contribute to high temperature resistance, biofungicide against downy mildew and increasing in nitrogen content in ripening to help fermentation and improve aromatic compounds. It was also used in winemaking processes as a clarificant or anti-oxidant.

So far, results were interesting on wine making process but need more trials and results to assess vineyard activity.

Keywords: CO2 sequestration, microalgae, vineyards, wine making

Nature resistance to *P. viticola* in interspecific grapevine genotypes revealed by MASS and transcriptomic

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Abstract

The fungus Erysiphe necator (Schwein.) Burrill and the oomycete Plasmopara viticola (Berk. & M.A. Curtis) Berl. & De Toni are among the most frequently occurring pathogens in the vineyards, causing significant economic losses every year. Protection is mainly limited to chemical spraying, while in rainy years the vineyard requires up to 10 chemical spraying, often based on undesirable copper. One of the eco-friendly variants of vineyard protection against pathogens appears to be the cultivation of PIWI varieties. These hybrids of the European V. vinifera L. with resistant American and Asian species such as V. amurensis Rupr., V. labrusca L. or M. rotundifolia Michx. can significantly limit or even stop the spread of the pathogen in leaf cells. The genomic regions responsible for this resistance are generally called 'resistance genes' and to date 31 Rpv loci (against downy mildew) and 14 Run/Ren loci (against powdery mildew) are known. In breeding for resistance, the MASS (Marker Assisted Seedling Selection) method is increasingly used, thanks to which promising individuals with desired R-loci combination can be selected from the progeny. These genomic regions can be relatively easily detected using SSR markers that are linked to resistant loci. In our study, we focused on the analysis of a set of 20 interspecific hybrids for the presence of 4 Rpv loci (Rpv3, Rpv4, Rpv7 and Rpv10) and 2 Ren loci (Ren3 and Ren9). 11 genotypes were Czech origin. The analysis revealed that some of them are comparable in terms of the number of resistant loci to globally known German, French or Hungarian varieties, which only highlights the quality work of Czech breeders. In the next part, the intention was to gain a deeper understanding of the molecular nature of the pathogen-resistant genotype interaction with different resistance loci combination, based on transcriptomic analysis. On the basis of the MASS described above, genotypes with a combination of resistant loci were selected for which transcriptomic analysis has not been published yet (Rpv12, Rpv12+1, Rpv12+1+3). Subsequently the differentially expressed genes in genotypes with different combinations of R-loci were identified in different time points after inoculation. From the analyses we have carried out so far, we registered more significant differences in transcriptomic activity with the 6HPI samples if compared with sampling at 24HPI. We believe that obtained results will be appreciated not only by breeders for the appropriate selection of genotypes based on their deeper knowledge, but also more generally for effective measures that would reduce the ecological burden in the protection of vineyards.

Keywords: grapevine; fungal pathogens; SSR; resistance; transcriptome

New Biocontrol agents for fungi obtained from vermicompost of sewage sludge and grape bagasse

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Abstract

Vermicompost has an enormous potential for protection of crops against pests, parasitic nematodes, and plant diseases. Vermicomposting drastically modifies bacterial and fungal composition of the initial substrates, generating new microbial communities which were not present at the beginning of the process. Over last few years, searching for new Biocontrol agents (BCAs) is one of the strategies for reducing chemical pesticides based on living microorganisms or their metabolites, and products of natural origin that control the population of plant pathogens. As a result of the conducted studies, many bacterial and fungal strains have been employed as BCAs, including Trichoderma spp., which allow their use in plant protection, biostimulation, and biofertilization. The aim of this study was, on the one hand, to study the microbial populations before and after vermicomposting of two types of wastes: sewage sludge, and grape bagasse and, on the other hand to search for new potential BCAs from vermicompost samples. Vermicompost were produced in pilot-scale vermicomposting reactors housed in the greenhouse facilities of the Animal Ecology Group (GEA) at the University of Vigo (Spain), using the earthworm species Eisenia andrei. Samples of both, two types of waste and vermicompost were processed as follow: freshly sample were added to sterile distilled water 1:100 (g/mL) and dispersed by stirring with magnetic bars for 10 min, then 5 serial dilutions were prepared. For plate count experiments, 200-µl aliquots from different dilutions were transferred to petri dishes containing the malt extract agar media supplemented with streptomycin and spread over the surface. Plates were incubated at 20°C in the dark. Colonies former unit (CFU) were counted after 72- and 96-hours incubation and after 1 week, colonies resembling Trichoderma species were transferred into potato dextrose agar plates and incubated in same conditions. As a result, vermicomposting changed the microbiota composition, decreasing CFUs and increasing fungal population, leading to a higher fungal-to-bacterial ratio, though in all cases, bacterial communities had higher incidence than fungal ones. Respecting BCAs, a total of 15 Trichoderma strains of six different morphotypes were selected. Work is still in progress to evaluate the biocontrol potential of the Trichoderma strains isolated from vermicompost.

Key words: biocontrol, Trichoderma, vermicompost.

In vitro evaluation of the antifungal activity of vermicompost teas against the phytopathogenic fungus Botrytis cinerea

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Abstract

Botrytis cinerea, the causal agent of grey mould or botrytis bunch rot in grapes is responsible for significant economic damage in vineyards worldwide. This polyphage pathogen is very resistant of many fungicides and there is global interest in finding out new alternatives to control it, such as through valorization of organic wastes. Vermicomposting is the process by which organic waste is broken down through the synergistic actions of earthworms and microbial communities, and converted into vermicompost, which is an excellent biofertilizer. Vermicompost and vermicompost teas has been shown to reduce impacts of plant pathogens, increase plant growth, and has other favorable benefits on plant performance. The aim of this study was to evaluate the effect of two vermicompost teas, derived from sewage sludge, and grape bagasse against the phytopathogenic fungus B. cinerea. Vermicompost were produced in pilot-scale vermicomposting reactors housed in the greenhouse facilities of the Animal Ecology Group (GEA) at the University of Vigo (Spain), using the earthworm species Eisenia andrei. Vermicompost teas were prepared by diluting solid vermicompost in water in a 1:12 (g/mL), then the mixtures were subjected to forced aeration for 24 hours and were allowed to settle for another 24 hours, after which the resulting liquid was filtered through a 1 mm mesh sieve, to avoid possible suspended solids. In addition, 70% methanolic extracts were prepared from the teas. Biocontrol tests against B. cinerea were performed adding different quantities of vermicompost teas and methanolic extracts to potato dextrose agar (PDA) plates. Filtrate-free PDA plates were used for the controls. An 8 mm agar plug with active growing mycelium of B. cinerea was placed in the centre of each fresh agar plate. Radial growth of pathogen isolates was measured, and percent inhibition of average radial growth was calculated. Vermicompost teas had not a biocide effect over B. cinerea whereas the methanolic extracts had. In fact, the 10% methanolic extract of grape bagasse reduced B. cinerea growth 73%. Further research is needed in other to know the chemical composition of the vermicompost teas and their mechanism of action.

Key words: Botrytis cinerea, vermicompost teas, methanolic extracts, grey mould.

Preliminary trial of the effect of *Trichoderma atroviride* I-1237 on the control of Phomopsis Cane and Leaf Spot caused by *Diaporthe ampelina*

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Abstract

A field trial with artificial infection was designed and performed to verify the potential effect of the application, immediately after pruning, of the commercial product "Esquive®" containing the biological control agent *Trichoderma atroviride* strain I-1237, on later symptom expression of Phomopsis Cane and Leaf Spot (PCLS) caused by *Diaporthe ampelina* (Berk. & M.A. Curtis) R.R. Gomes, C. Glienke & Crous The trial, set up on a vineyard of cv. Touriga Nacional grafted on 1103P, located in Nelas, Viseu, Portugal, comprised four treatments: "Witness (W)", that remained in natural conditions without artificial infection and no reference product spray; "Infected witness(IW)", with artificial infection and no reference product (RP)", with artificial infection and fungicide application, and "Test product (TP)" where "Esquive®" was applied as per the label, immediately after pruning (BBCH 01), and where all replicates were subject to artificial infection. At BBCH 07 the reference product was applied just to "RP", while all the other treatments were sprayed just with water. The trial consisted of 6 replicates/treatment with three plants/replicate. Incidence and severity of PCLS were assessed on the first four internodes in all the canes of all trial plants during development stages BBCH 71 to BBCH 73.

While safeguarding that the present results are preliminary, resulting of a trial carried out in just one viticultural campaign, it was observed that all the treatments showed values of incidence and severity lower than those of "IW", with a statistically significant reduction in incidence for all the treatments when compared to "IW". Further testing in following viticultural campaigns will be necessary to gauge these preliminary results.

Key words: biocontrol, grapevine, Phomopsis Cane and Leaf Spot, field trial, artificial infection.

Survey and diversity of Grapevine Pinot gris virus in La Rioja region of Spain

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Abstract

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Grapevine Pinot gris virus (GPGV) belongs to the order *Tymovirales*, the family *Betaflexiviridae*, the class *Trivirinae* and the genus *Trichovirus*. It was initially reported in *Vitis vinifera* L. cv. Pinot gris in 2012 in the Trentino region of Italy and is closely related to Grapevine berry inner necrosis virus. GPGV is widespread in many grape growing countries worldwide. However, the evaluation of the economic impact of GPGV is challenging because many strains do not cause grapevine leaf mottling and deformation (GLMD) symptoms. To examine the prevalence of GPGV, a survey of 30 grapevines cv. Tempranillo was conducted across six vineyards in La Rioja region (northern Spain). Disease symptoms were recorded, and RNA was subsequently extracted from selected grapevines. The coding regions of the movement and coat protein as well as a region of the RNA-dependent RNA polymerase domain was sequenced in all positive isolates. A phylogenetic analysis of the GPGV isolates was performed to establish their relationship with other known GPGV isolates. Additionally, qPCR was implemented to quantify the amount of GPGV titre in leaf tissues of the selected grapevines. Out of the thirty grapevines, fourteen were asymptomatic and tested negative for GPGV. Of the remaining sixteen RNA isolates, only eleven exhibited GLMD symptoms in grapevines, despite testing positive for GPGV.

Key words: Grapevine Pinot gris virus, sequencing, grapevine, RT-PCR, RdRp, MP/CP, qPCR

REVINE Regenerative agricultural approaches to improve ecosystem services in Mediterranean vineyards

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Abstract

REVINE is a 3 year European projected funded by PRIMA programme which proposes the adoption of regenerative agriculture practices with an innovative and original perspective, in order to improve the resilience of vineyards to climate change in the Mediterranean area. The potential for innovation lies in developing and combining new approaches that make agriculture more environmentally sustainable and enable a circular economy capable of improving farmers' incomes. Primarily REVINE aims to improve soil health and biodiversity by promoting the multiplication of soil saprophytic microorganisms and the presence of useful microorganisms linked to the life cycle of the plant, such as rhizobacteria (PGPR) and fungi (PGPF) that promote plant growth which, in addition to increasing plant performance, increase tolerance to biotic and abiotic stresses.

The project has the main goals to improve the biodiversity in vineyard and the fertility and water availability of soil.

Regenerative agriculture ameliorates soil structure and microbial biodiversity that, in turn, leads to crop resilience against biotic and abiotic stressful factors. Moreover, enrichment of beneficial microbes in the rhizosphere, such as PGPR and PGPF, are known to trigger the plant immunity inducing the priming state. REVINE intends to improve the biodiversity in the vineyards by using multiple approaches, including: i) screening of tolerant grapevine genotypes; ii) consociation of the grapevine with profitable cover crops; iii) the use of cultivation practices able to enhance soil biodiversity and the beneficial rhizosphere microorganisms.

REVINE, by means of Regenerative Agriculture, intends to rebuild soil organic matter and restore degraded soil biodiversity, resulting in both carbon drawdown and water cycle improvement, by using biofertilizers and amendments (fermented manure, compost and biochar). In particular, biochar is a carbon-rich substrate that has multiple effects and can be used as soil amendment. It increases soil water-holding capacity and nutrient-availability for plants, thus positively affecting plant growth and preventing water stress. Moreover, by improving soil's physical and chemical properties, biochar modifies microbial habitats and fosters the presence of plant beneficial microbes. Biofertilizers and amendments will be produced from crop residues. In this way, REVINE intends to valorize agricultural waste and to increase farmers income, promoting the circular economy.

Keywords: Regenerative agriculture, vineyards, soil microbial biodiversity, biofertilizers.

Effect of *Trichoderma atroviride* SC1 and *Bacillus subtilis* PTA-271 on grapevine rhizosphere microbiome and plant defenses

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Abstract

The rhizosphere is where soil-borne pathogens and plant beneficial microorganisms exert influence on the growth and health of plant hosts. Soil-borne plant pathogens are a major limitation in yield production and are strongly recalcitrant to management, compared to pathogens that attack the aboveground parts of the plant. The use of microorganisms as biocontrol agents (BCAs) to manage soil-borne pathogens has been widely studied, due to their multiple assets, such as, enhancing plant defenses and antagonism. In this study, we tested the effect of two biocontrol agents, Trichoderma atroviride SC1 (Ta SC1) and Bacillus subtilis PTA-271 (Bs PTA-271), on the grapevine rhizosphere bacterial and fungal microbiome, and on plant gene expression, using high-throughput amplicon (ITS/16s) sequencing (HTAS), and quantitative real-time PCR (qPCR), respectively. Additionally, we quantified both Ta SC1 and Bs PTA-271 in rhizosphere overtime using droplet digital PCR (ddPCR). The results suggest that Bs PTA-271 established better in clay soil, where although its quantity was lower than sandy soil at 1mpi, it was preserved at 3mpi. In contrast, Ta SC1 established better in sandy soil, starting with a lower quantity at 1mpi, but increasing quantity over time. Regarding the impact of BCAs on rhizosphere microbiome, bacterial diversity was not affected by the inoculation of one or two BCAs. However, inoculations with Ta SC1 reduced fungal biodiversity on sandy soils. Bs PTA-271 presented a stronger effect than Ta SC1 on positive and negative interactions between the most prevalent bacterial and fungal taxa, although its effect varied with the type of soil. According to the selected plant defense markers, plants living in sandy soil appeared to be more impacted by BCA inoculation. Plants inoculated with Ta SC1 in sandy soil demonstrated a strong suppression of both salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) - dependent defense genes 24hpi, that converged in strongly stimulated defenses at 4dpi, with a significant overexpression of phenylalanine ammonia-lyase (PAL) and stilbene synthase (STS). On the other hand, in clay soil, BCA-treated plants showed a slight increase in the expression of plant defense genes at 24 hpi that intensifies at 4 dpi. In conclusion, the effect of Bs PTA-271 and Ta SC1 in grapevine rhizosphere appears to be soil-dependent, where a sandy soil favors the fungal BCA (Ta SC1) establishment, while a clay soil favors the bacterial BCA (Bs PTA-271) establishment.

Key words: biocontrol, droplet digital PCR, high-throughput amplicon sequencing, microbiome, rhizosphere.

Endophytic microorganisms for trunk diseases control in Chilean patrimonial vineyards

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Abstract

Grapevine Trunk Diseases (GTDs) are a major problem for Chilean patrimonial vineyards, which mainly belong to País, Moscatel, Cinsault and Carignan cultivars, reducing the productivity and longevity of the vines. Previous research reported the ability of apple bacterial endophytes to revert trunk diseases symptoms and demonstrated that they can successfully antagonize fungal pathogens growth and also induce defense related genes on apple plants. Thus, the objectives were to determine the endophytes antagonistic activity against trunk fungal pathogens of patrimonial vineyards, their ability to colonize grapevine plants cv. País and to induce plant natural defense responses. Endophytes (n=2) were confronted to virulent isolates of the main pathogens affecting patrimonial vineyards (n=8), such as Diplodia, Neofusicoccum, Arambarria and Seimatosporium, in dual cultures. The bacterial isolates were able to inhibit mycelial growth by up to 80%, compared to the control. Moreover, experiments were performed to evaluate the inhibitory activity of their volatile and diffusible metabolites against the pathogens, finding that volatile metabolites were able to inhibit fugal pathogens growth up to 85%, and diffusible metabolites up to 35%. To evaluate the ability of the endophytes to colonize grapevines as endophytes and to study the defense responses, bacterial suspensions were inoculated on 1 to 1.5 cm diameter x 30 cm length canes cv. País. After 24, 48 and 72 h incubation on flowing water, wood samples were collected from 1, 15 and 30 cm from the inoculation point, superficially disinfected and plated on nutrient agar. DNA was extracted from the colonies and amplified by BOX-PCR. The banding patters on agarose gels produced by inoculated and reisolated bacteria were compared, indicating that both endophytes were able to colonize the canes before 72 h. Furthermore, sawdust was collected from the inoculated canes, RNA was isolated, and candidate genes were analyzed by quantitative RT-PCR (qPCR). Results showed the overexpression of defense-related genes like NPR1, PR2, PAL and SOD. These studies revealed the promising role of bacterial endophytic isolates as highly promising biological control agents against GTDs on patrimonial vineyards.

Key words: biological control, endophytes, patrimonial vineyards, grapevine trunk diseases.

Implementation of qPCR for the assessment of Grapevine trunk diseases in Chilean patrimonial vineyards

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Abstract

Grapevine trunk diseases (GTDs) are a major threat for wine industry in Chile. Meanwhile in commercial cultivars like Cabernet Sauvignon, Sauvignon blanc, Merlot and Chardonnay, Grapevine Trunk Diseases (GTDs) are well studied, in Patrimonial Vineyards, mostly from País, Moscatel, Cinsault and Carignan cultivars, there is lack of information regarding these diseases. The most frequent and virulent pathogens are Neofusicoccum parvum, Diplodia seriata, D. mutila, Seimatosporium vitifusiforme and Arambarria destruens. There are not eradicative methods yet available for their control, hence, the early diagnosis is essential in order to manage the infection in the vineyard on a suitable way. The traditional isolation methods are sometimes inaccurate and slow. In contrast, well-developed quantitative real-time PCR (qPCR) have overcome the deficiencies. Thus, the objective of this work was to develop a rapid quantitative method for the assessment of GTDs in Chilean patrimonial vineyards. A survey was conducted from 2019 to 2022 on patrimonial vineyards, in the south of Chile. DNA was isolated from branches showing dieback symptoms and internal discoloration. The quantitative real-time PCR (qPCR) systems were developed and specific primer pairs for N. parvum, D. seriata, D. mutila, S. vitifusiforme and A. destruens were used to identify and quantify the infection levels of these pathogens. Despite the fact that various fungal species were present in the samples, the qPCR method was efficient to identify the pathogens of interest, showing a good correlation between pathogens and the amount of discolored wood. This correlation was confirmed by both re-isolation and biomass measurement through qPCR. It also showed a detection efficiency, with limits of 180 and 60 fg of gDNA. Our study showed discrimination even among genetically closely-related species, with a high sensitivity and a reliable quantification, becoming an efficient alternative to detect these deleterious pathogens.

Key words: qPCR, grapevine, GTDs diagnosis.

The roles of *terroir* and vintage in the composition of the grapevine pathobiome

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Abstract

In viticulture and oenology, the terroir concept is widely used to explain differences among wines. The concept itself partly is based on spatial differences in edaphic and mesoclimatic factors. These environmental differences likely affect plant-associated microbes also, with implications for plant health. In this study, we compared the compositional dynamics of plant pathogenic fungi in 144 samples collected three different microhabitats: soil, woody tissue, and bark of grapevine cv. Furmint, a white variety that is endemic to the Carpathian basin and is the main cultivar in the Tokaj wine region. We collected samples in late winter and summer of 2020 and 2021 in three different terroirs in the Tokaj wine region. We hypothesized that fungal communities would differ in composition among microhabitats and among seasons due to the contrasting abiotic conditions, while we expected little variation among vintages. Sequence data of the ITS2 region of the ribosomal DNA repeat were generated by Illumina NovaSeg. Of the 123 plant pathogenic genera found, Diplodia, Phaeomoniella, and Fusarium showed the highest richness in bark, wood, and soil, respectively. Both richness and abundance differed significantly among microhabitats, with plant pathogenic fungi known to cause grapevine trunk diseases (GTDs) showing highest richness and abundance in wood and bark samples, and non-GTD pathogens dominating soil. We found significant compositional differences among terroirs, season, and vintage, with terroir explaining 14.5-24.7%, season 1.8-2.98%, and vintage 3.7-6.4% of the variance in community composition. Some of the observed differences likely are caused by environmental filtering both at microhabitat and terroir levels, while weather and fungicide applications may explain the observed temporal dynamics of fungi.

Key words: grapevine microbiome, Esca disease, trunk diseases, environmental filtering

In vitro and nursery evaluation of electrolyzed water to control fungal grapevine trunk pathogens

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Abstract

Electrolyzed water has emerged in recent years as a sustainable alternative for disinfection because it is generated from water, salt, and electricity through electrolysis. The electrochemical oxidation of chloride generates Cl₂ at the anode that dismutes generating HCl and HClO with a strongly acidic pH. In this study, the effect of in vitro treatments with three different electrolyzed water (EW) based products on the mycelial growth of grapevine fungal trunk (GTD) pathogens was evaluated. Eight GTD species were studied: Botryosphaeria dothidea (Bd), Cadophora luteo-olivacea (Clo), Dactylonectria torresensis (Dt), Eutypa lata (EI), Ilyonectria liriodendri (II), Lasiodiplodia theobromae (It), Neofusicoccum parvum (Np), Phaeoacremonium minimum (Pm) and Phaeomoniella chlamydospora (Pch). Agar discs with mycelium of each of these fungi were treated by immersion in each of the products. The treatments lasted 30 s, and 1-, 5-, 15- and 30-min. Mycelium survival (%) of each fungal species at the different products-time of treatment combinations was determined. The effect on conidia germination was also evaluated for Cadophora luteoolivacea (Clo), Dt, II, Pm and Pch. Conidial suspensions of each fungus adjusted to 20 x 10⁶ conidia/ml were mixed with 950 µl of different EW products for 0, 15, 30, 60 or 300 s. Exposure was stopped by adding 9 ml of neutralizing buffer at pH 7.2. Drops (20 µl) of the spore suspension were plated on water agar and incubated at 25°C for 24 h. After incubation, the drops were observed for conidia germination quantification. All experiments were repeated once. The effect of the different products on the mycelial growth and conidial germination was variable according to the products, treatment times and fungal species. In general, the longest treatments were more effective to reduce mycelial growth and conidia germination. A nursery experiment was carried out in a nursery located in Valencia province (eastern Spain) in 2020. For the treatments, 10% freshly produced EW (pH 5,3, Chlorine concentration 500 ppm, Oxidation reduction potential 970 mv) was used. Cuttings of 110 R rootstock subsequently grafted with Macabeo cultivar were treated with EW at three stages during the grapevine propagating process: i) a 24-h soak in EW prior to grafting; ii) the application EW by watering the sawdust at stratification; and iii) a 1-h soak of the basal parts of the plants in EW before planting in the rooting field. The untreated control involved treatments with water at each of the three stages. For the EW treatment and the control, there were four replicates of 100 plants, which were managed separately. Grafted plants were planted in a nursery rooting field in May 2020, and were arranged in a randomized complete block design. Plants were uprooted in October 2020. Thirty plants per treatment and replicate were selected randomly and taken to the laboratory for fungal isolation and identification. Results on EW-treated plants showed a reduction in the mean percentage of plants infected by GTD pathogens associated with Petri and Black-foot diseases, which were approximately halved compared to untreated plants. These results showed the potential of EW treatments for their use in grapevine nurseries.

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Key words: Black-foot disease, Botryosphaeria dieback, grapevine nursery, Petri disease

Innovative biopesticides to control grapevine fungal pathogens

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Abstract

The essential oil components (EOCs) are widely used as natural bioactive molecules of plant origin with antimicrobial activity. However, their high volatility, high reactivity and low water solubility limit their applications. One way to overcome these drawbacks is to develop controlled release systems of these natural antimicrobials against fungal plant pathogens. In this sense, the development, synthesis, and characterization of EOCs encapsulated in mesoporous silica microparticles capable of releasing the natural bioactive molecules in a controlled manner, can significantly increase their antimicrobial activity compared to free bioactive molecules. A key innovative aspect of this project is the development of functionalized materials with molecular gates consisting of mesoporous silica materials loaded with natural bioactive molecules and functionalized with saccharide derivatives or sugars. This will reduce the high volatility of natural bioactive molecules and increase their fungicidal activity through preferential delivery of molecules by opening the biomolecular gates in the presence of amylases excreted by fungi. In this study, the effect of in vitro treatments with new formulations of different EOCs on the mycelial growth and conidial germination of representative grapevine fungal pathogens: Botrytis cinerea (causal agent of grey mold disease), Dactylonectria torresensis and Ilyonectria liriodendri (causal agents of black-foot disease), and Phaeoacremonium minimum and Phaeomoniella chlamydospora (causal agents of Petri disease), was evaluated. The goal is to obtain new innovative biopesticides using natural resources in the context of sustainable agriculture.

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Key words: Biopesticides, black-foot disease, essential oil components, grey mold disease, Petri disease

Grapevine dieback caused by Botryosphaeriaceae species Paraconiothyrium brasiliense, Seimatosporium vitis and Truncatella angustata in Piedmont: characterization and pathogenicity

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Abstract

In recent years, reports of Grapevine Trunk Diseases (GTD) caused by fungi increased worldwide. GTDs cause major economic losses due to reduced yields and costs of agronomic practices, especially in Mediterranean countries. Typical internal symptoms of the different diseases in GTDs include wood necroses, discolouration and black streaking whereas external symptoms include typical striped leaves due to interveinal necrosis and discolouration, stunted growth, dieback of shoots and leaf wilting which over the years or even in the same season lead to the death of affected vines. During 2021-2022, a survey was conducted in Piedmont to investigate the species diversity and distribution of GTD pathogens. A total of 12 cultivars were sampled, including national ('Albarossa', 'Barbera', 'Cortese', 'Erbaluce', 'Grignolino', 'Moscato', 'Nebbiolo', 'Rossese Bianco', 'Timorasso') and international cultivar ('Cabernet Sauvignon', 'Merlot', 'Sauvignon Blanc') from five different vineyards located in Cuneo province. In the sampled vineyards, more than 30% of the plants showed Botryosphaeria-dieback related symptoms and about 5% of plants were affected by general decline with severe dieback and death. Morphological and multi-locus phylogenetic analyses led to the identification of different fungal species. Botryosphaeria dothidea, Diplodia seriata, Diplodia mutila and Neofusicoccum parvum, members of the Botryosphaeriaceae family, were isolated with high frequency. Other pathogens, commonly associated with other GTDs, such as Phaeomoniella chlamydospora, Eutypa lata and Fomitiporia mediterranea, were found. Less-common species, including Paraconiothyrium brasiliense, Truncatella angustata, Seimatosporium vitis and Neocucurbitaria juglandicola were also identified in the same necrotic tissues/in the declining vines/in the symptomatic vines. Pathogenicity trials with representative strains were performed on 1-year-old potted grapevine cuttings of 'Barbera'. All strains caused brown wood symptoms i.e. necrotic vascular discoloration and were successfully re-isolated, except for N. juglandicola. The effect of the temperature on mycelial radial growth was also investigated and optimum growth temperatures of the isolated species ranged between 20 and 25 °C. Diplodia mutila, Paraconiothyrium brasiliense and Neocucurbitaria juglandicola were reported for the first time in association with symptomatic grapevine in Italy.

Key words: grapevine, Botryosphaeria dieback, Grapevine Trunk Diseases, Botryosphaeriaceae,

Evaluation of the risk related to *Halyomorpha halys* in the Libournais wine region

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Abstract

The presence of Halyomorpha halys, an invasive stink bug species, since 2020 in the Bordeaux vineyards has raised concerns among winegrowers in the Pomerol and Saint-Emilion AOC regions. These production areas are characterised by manual harvesting and the production of high value-added wines whose quality is likely to be altered by the aromatic deviations generated by this insect presents on grapes at harvest. To evaluate the risk incurred by these wine-growing areas, we set up a monitoring of the insect's abundance (nymphs and adults) in the plots. This monitoring carried out in 2021 on a few plots (n = 5), by beating technique of twenty vines per plot every two weeks from the beginning of the vegetative phase of vines in April to the end of harvest in September. It showed that the insect was present in the vineyard plots, that at least 1 generation was occurring on the vine and that adults were detected on the vine as soon as fruit set was reached, followed by nymphs from the bunch closure stage. In 2022, a much larger sampling (n = 26) was undertaken to characterise the abundance of the insect, taking into account the type of landscape surrounding the vineyard plot. Five beating sessions of twenty vines per plot, with a monthly schedule were planned from before fruit set to maturation. We found that 92% of the plots were infested. The insect was detected earlier and in higher abundance in plots with a border with a semi-natural habitat, significantly higher for nymphs in particular. In 12 plots at harvest time, an assessment in 3 transport crates per plot (several hundred bunches depending of the crates) showed a very low level of infestation per bunch, with an average of 2% of bunches with insects detected during the manual harvesting operation. After transport and during reception in the winery, this rate of infestation of these same bunches decreased to less than 1%. Finally, in the winery, after the grape processing line, no insect was observed at the optical sorting machine before these grapes were put into the fermentation tank.

These surveys show that the current population levels of *H. halys*, although present in the vineyards, may not necessarily cause any particular risk for the wine's quality of the Pomerol and Saint-Emilion AOCs. This study however only concerns this area of manual harvesting and does not take into account mechanical harvesting, which is also characterised by other equipment in the winery. An assessment of the risks in this case should be conducted. The quantitative evolution of populations must nevertheless be monitored in order to anticipate any new outbreak of this invasive insect that has recently arrived in Bordeaux.

Key words: brown marmorated stink bug, invasive species, population dynamic, grapevine, risk assessment

Cryptoblabes gnidiella and their natural enemies in Italian vineyards: an update

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Abstract

Native of the Mediterranean basin, the honeydew moth Cryptoblabes gnidiella (Milliére) recently became a primary pest, especially in vineyards located along the Italian, French and Spanish coastal areas. This moth is an extremely polyphagous species, feeding and developing on more than 60 plant species belonging to 30 different botanical families, including Daphne gnidium L. (Thymelaeaceae), considered its most common wild host plant. Cryptoblabes gnidiella females lay eggs mainly on the green parts of the cluster, such as racemes, pedicels and only seldom on the berries. The eggs and newly hatched larvae, which occur in the innermost parts of the bunches, are difficult to detect, so hampering a proper definition of intervention thresholds and reducing the effectiveness of insecticide strategies. Although this pest starts flying in the vineyards in April-May, finding eggs, larvae, or damage on the bunches in May-June is quite rare. The larvae mainly feed on the green parts of the ripening bunches, preferring the late compact-bunch varieties. The progressive decay and dryness of the bunch related to the larval trophic activity is further increased, in the presence of high humidity, by the proliferation of mold and the presence of saprophagous insects. Despite the increasing relevance of this moth pest, the studies performed until now failed to identify a reliable strategy for its sustainable management and little information is still available on the natural enemies associated to this moth species in the different areas where it occurs. In this research we analyzed the parasitoids associated to C. gnidiella, detected in recent studies performed in vineyards and natural areas in Tuscany and Apulia (Central and Southern Italy, respectively). Of note, we detected and identified some insect parasitoids of the moth, among which Phanerotoma leucobasis Kriechbaumer, a braconid of the subfamily Cheloninae, displayed high parasitisation rates both in the open field and in the laboratory. This species was newly recorded in Italy and could be eligible to become a possible biological control agent against C. gnidiella.

Key words: Hymenoptera, Braconidae, vine, biological control, honeydew moth, parasitoid.



Is the grape's microbiome responsible for *Drosophila suzukii* attraction to different grape varieties?

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Abstract

Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is an invasive polyphagous pest that can provoke huge yield losses in stone fruits and small fruits. On the other hand, grapes tend to be a poor substrate for SWD. In Germany only in years with rot problems some varieties are attacked before commercial harvest. In order to avoid unnecessary insecticide applications and to develop an alert system for growers, it is crucial to know the factors that make initially unsusceptible grapes susceptible to SWD. In addition to physical factors such as berry skin resistance, the microbiome of the grapes could play a major role here. SWD oviposits more eggs on yeast positive substrates than on yeast negative ones and rely on olfactory information associated with oviposition sites. Attractive odours cues are often related to distinct resources such as fungi, or ripening fruits. The aim of this study is to test if the changes of different grape varieties' microbiome combination during ripening process affect the attraction level of grapes to SWD. We expected that the microbiomes combination related to different grapes' varieties changes during the time and this changes during the ripening process changes the attraction of grapes to SWD.

In a wind tunnel experiment during 12 weeks we investigated the response of SWD to different grape variety microbiomes during the ripening process. Microbiomes from different grapes varieties including Regent, Dornfelder and Rebberger were washed off grapes by an isotonic buffer. The centrifugation pellet was resuspendet in 5 ml buffer and used in wind tunnel experiments. In a dual-choice test within the wind tunnel, we used oviposition and fly location as a measure of microbiome attractiveness. In order to rule out physical causes for the oviposition intensity in the preference test, we used ripe cultivated blueberries, which - unlike grapes – are always susceptible to SWD oviposition. Two Petri dishes filled with blueberries (about 40 gr), one treated with 5 ml microbiome suspension and one with pure isotonic buffer as a control, were located in one side of wind tunnel with 30 cm distance. 40 females and 20 males of 7-10 days old SWD were released from the release point on the opposite side of wind tunnel. After 24 hours, the experiment ended. The number of flies in each petri dish as well as the eggs laid by SWD were counted. The composition of the microbiome of the grapes was checked for each sampling date using metabarcoding. Several species within the microbiome could be identified that quantitatively correlate with the oviposition of SWD. In addition, we checked which species appeared for the first time at the time berries and/or wash buffer became attractive.

Our egg laying results show that the attractiveness of the berry surface wash-off changes during the ripening process. SWD oviposition increased after calendar week 40 on Dornfelder- and after calendar week 41 on Rebberger wash-off. SWD oviposition on Regent demonstrate constant fluctuation during ripening. Several species of microorganisms were identified whose occurrence on the berries correlated with SWD oviposition. The current study suggests that grape microbiomes are attractive for SWD and this attraction changes during ripening process. The tipping point for attraction could be practical to set a warning system in veinyards against SWD crop loss.

Key words: Drosophila suzukii, SWD, Grape susceptibility, Alert system, Metabarcoding, grape microbiom

Protocol "AVELEDA DID - Do It Yourself "for determination of the risk of primary infection and the detection of oospores maturity of *Plasmopara viticola*

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Abstract

The decision-making basis for carrying out a preventive or curative treatment is related to the quantity and intensity of the presence of downy mildew fungus in its active form. In the Vinho Verde region, the level of infection that will manifest in the n+1 season is defined during the months of June to September of the previous year. Nights with high and constant relative humidity, and summers with frequent rainfall, lead to strong and potentially dangerous oospores germination, reflecting a higher risk of serious losses in grape production. Winter conditions (rain and temperatura) can influence the primary inoculum load in the vineyard. Cold and dry winters leading to poor maturation of oospores; while more moderate and wet winters will result in strong and early-maturing oospores. However, the Vinhos Verdes Region has an average rainfall of 1200 mm / year and determining the risk of this disease is crucial for the success of the crop. The economic and environmental impact of all treatments must be quantified beforehand. A protocol developed by Aveleda, S.A. named "Do it Yourself" can be utmost useful for a disease risk evaluation. The protocol recommends inducing the budding of a sensitive cultivar (in Vinho Verde region, cultivar Avesso) in a greenhouse in early February to promote the growth of shoots, before natural budbreak in the vineyard. In early March, the maturation of oospores is determined through a weekly application of a field-preserved inoculum solution on the leaves of the host plants, maintaining a relative humidity of 80% and temperature between 20°-25°C for 4-6 days until leaf contamination and spotting occur. Once primary infection is confirmed in the greenhouse plants, other plants are placed before a relevant precipitation period in field conditions and then again subjected to controlled environment conditions. The appearance and quantification of spots will be the crucial for deciding on the timing or need of the first treatment and the antifungal to be use, preventive or curative. These trials were carried out for several years always before natural budbreak of the vines, between phenological states B, C, or D and demonstrated potential to be a fundamental tool for disease decision support. On average, the use of this protocol did represent an effective reduction of two treatments per year due which is a considerable reduction of the impact of the use of phytosanitary products and fuel, soil compaction; and sustainability of the entire viticulture ecosystem. This protocol has been tested for 14 years and the results have been validated in the vineyard.

Key words: downy mildew diseases, innoculum preparation, simulation of field conditions, primary infection, real precipitation, disease, decision support, sustain viticulture.

Fungicide reduction enhances beneficial arthropods in grapevine

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Abstract

Pesticides are often considered to be among the main causes of arthropod decline, which could potentially affect ecosystem services such as natural pest control. Grapevine is one of the most pesticide-dependent crops, usually receiving ten fungicide sprays in three months of the growing season in the study area of the Palatinate. In both organic and conventional viticulture, each spray consists of at least two plant protection products with different toxicity to non-target organisms. However the use of fungicides can be reduced by about 70 % by growing fungus-resistant varieties,.

Under quasi insecticide-free growing conditions (mating disruption), we investigated the effects of different frequencies of fungicide applications in organic and conventional viticulture with regard to toxicity to non-target organisms. We worked with susceptible (frequently sprayed) and fungus-resistant grapevine varieties (rarely sprayed). We investigated the natural pest regulation of the main grapevine pest *Lobesia botrana*. The arthropod fauna of the canopy was studied with beet sheets. To demonstrate the potential of natural pest control, sentinel cards with eggs of *L. botrana* were exposed in the vine foliage to quantify predation. In addition, sentinel cards with eggs and pupae of *L. botrana* were exposed and observed with infrared cameras to identify the predators of grape berry moth that are common in our study region.

The drastic reduction of sprays on fungus-resistant varieties led to a reduced toxicity load in the canopy. The reduction potential was greater in organic vineyards than in conventional ones. The reduced sprays promoted predatory arthropods, especially spiders. However earwigs (Dermaptera) appearing to be the dominant and most voracious predators preying on our sentinel cards, were not affected by fungicide sprays. This could explain why the overall predation rate of *L. botrana* eggs was high (80 %) but not significantly affected by fungicide sprays.

Efficacy of recent active aerosol emitters versus conventional passive dispensers for the mating disruption of grapevine moths

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Abstract

The two grapevine moths Lobesia botrana and Eupoecilia ambiguella are the two major lepidopteran pests in European vineyards. In the past, these two moth species were controlled by insecticides, but over the last decades mating disruption has established itself as an environmentally friendly alternative all over Europe. Today this integrated pest management measure is implemented area-wide in nearly all Swiss vineyards covering probably more than 80% of the viticultural area. Until recently mating disruption relied on conventional passive-release dispensers, deployed in vineyards at high densities of 200-500 dispensers/ha. With the authorisation of active-release emitters by the Swiss government in 2019, more and more winegrowers switch to this less labour-intensive control method requiring the installation of only 2-5 emitters/ha. We were therefore interested to monitor if this new form of aerosol emitters, with considerably less sex pheromone sources per area, provides a similar protection against grapevine moth damages than conventional mating disruption. Over the last four years, we found no evidence that the novel active-release emitters CheckMate® Puffer LB/EA are less effective than the classical passive-release dispensers of the type Isonet® or RAK®. Damages remained for both types of aerosol emission stable and at a very low level in vineyards. Moreover, both provided a considerable better protection against grapevine moth damage than the traditional application of insecticides. Overall, our observations confirm that the deployment of less sex pheromone sources per area by the installation of active-release emitters does not reduce the efficacy of mating disruption at low pest population densities. It can therefore be an interesting alternative to conventional mating disruption with 200-500 passive-release dispensers per hectare. The installation of a few active active-release emitters is not only less labour-intensive, but sex pheromone release can also be programmed taking into account pest's actual life stage, flight activity periods of males as well as the phenology and sensibility of the crop. Last but not least, the deployment of a few active aerosol emitters might also reduce the accumulation of plastic dispensers in viticultural soils and the nearby environment thereby reducing environmental pollution.

Key words: Vitis vinifera, Lepidoptera, Tortricidae, IPM.



New multiple linear regression models for predicting the European grapevine moth (*Lobesia botrana*) in Austria

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Abstract

Climate change leads to a variety of new challenges for sustainable crop production. Increasing temperatures may promote the spread, developmental speed and overwintering of thermophilic insect pests and thus promote climate induced risks for regional crop production systems. In order to limit these future risks, improved or new forecasting models could help to optimize the timing of monitoring and control measures. In this context, prediction models for the European grapevine moth, Lobesia botrana (Denis & Schiffermüller) (Lepidoptera: Tortricidae) were generated using long-term monitoring data (1980 to 2021) from 60 selected monitoring sites in 4 federal provinces in Austria. Prediction models for the first seasonal occurrence of the different developmental stages (egg, larvae, adult) of the 1st and 2nd generation of *L. botrana* were generated by applying stepwise multiple linear regression (MLR) analysis. The input data for the MLR analysis comprised the DOY (day of year on which the first seasonal occurrence was observed), nine measured weather parameters (e. g. max/min/mean temperature, precipitation and global radiation), temperature sums, number of days with temperatures above/below lower and upper developmental threshold values and number of days with precipitation. Furthermore, a different number of monthly (2-11) and weekly (7-36) calculation periods were tested for the different developmental stages and generations. The performance evaluation of the generated MLR models resulted in an R² of 0.82, 0.76, and 0.77 and RMSE of 1.85, 2.49, and 2.74 for the prediction of the first seasonal occurrence of the 1st generation adult, egg and larvae, respectively. For the prediction of the first seasonal occurrence of the 2nd generation of adult, egg and larvae, the performance evaluation of the generated MLR models resulted in an R² of 0.89, 0.56, and 0.68 and RMSE of 2.33, 4.03, and 4.01, respectively. The validation of the generated MLR models was carried out with additional data including the observation data from 2022 and showed better prediction accuracy than the performance evaluation for almost all MLR models, only the prediction of the 2nd generation adult resulted in a lower prediction accuracy, with an R² of 0.80 and a RMSE of 2.63. On average, 61% of the 1st generation predictions and 53% of the 2^{nd} generation predictions were in the optimal prediction range (0 ± 2 days deviation between observed and predicted DOY). To further improve the prediction accuracy additional data sets, especially from years with extreme weather events, should be included into the analysis. This study is part of the ACRP-13th Call Project RIMPEST (KR20AC0K17957) including the development of similar MLR models for E. ambiguella.

Key words: Lobesia botrana, seasonal occurrence, prediction, multiple linear regression, climate change.



Diseases and arthropods susceptibility of *Vitis vinifera* in the grape growing regions of Quebec, Canada.

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Abstract

The production of the European grapevine *Vitis vinifera* comes with challenges such as high disease susceptibility and poor cold hardiness, and little information is available on its growth in the Quebec, Canada, climate. Recent studies have clearly demonstrated that the different genotypes of pathogenic fungi have a specialization towards their host. For example, four genetic groups (genotypes) of *Plasmopara viticola* have been identified by phylogenetic studies and the predominance of *P. viticola* genotypes is not the same in Europe as in America. It is therefore essential to assess the sensitivity of grape cultivars originating from other regions to the genotypes present in eastern Canada. This project aimed at characterizing the agronomic properties of the main *V. vinifera* cultivars in five viticultural areas of Quebec, Canada. The study was carried out in ten commercial vineyards over two full years (may 2021 to may 2023). In both years, diseases (powdery and downy mildew, black rot, Botrytis and anthracnose) and arthropods (e.g., leafhoppers, Japanese beetles) susceptibility was similar between cultivars and was associated to the site. Moreover, disease and arthropod incidence was different between conventionally and organically managed vineyards. From this study, cultivar suitability to certain Quebec, Canada, will help the sustainability of the industry by assisting in the decision-making process when establishing new vineyards.

Key words: grapevine, diseases, arthropods, Vitis vinifera.

The Use of Knotwood Extracts for Potential Bioprotection against Grapevine Trunk Diseases

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Abstract

Early grapevine decline is a growing issue for the worldwide vineyard in the climate change context. Among the decline diseases, Esca, Botryosphaeria and Eutypa diebacks are a serious challenge for viticulture. They cause significant economic losses on a national and international scale and are currently without reliable and effective control solutions for the wine industry. The only known efficient treatment, sodium arsenite, has been banned in 2001 in France and 2003 in Europe for toxicity reasons and today numerous alternatives are being studied, including natural substances.

Trees develop strategies to fight infections and biodegradation from pathogens. Our objective is to get inspired from these defence mechanisms to find new environmentally friendly grapevine treatments, in a context of circular bioeconomy. Moreover, the raw material that provides interesting natural compounds (wood extractives) comes from wood industry by-products (i.e. waste).

Our aim in then two-fold: control grapevine diseases with natural compounds and create higher added values for the wood industries by-products.

So, we have evaluated new means of protecting pruning wounds and young grapevine plants coming from nurseries, through the use of secondary metabolites involved in tree's defence mechanisms.

In vitro assays with Douglas knots (*Pseudotsuga menziesii*) extracts demonstrated a fungal growth inhibition against two pathogenic fungi (*Neofusicoccum parvum* and *Fomitiporia mediterranea*) involved in grapevine trunk diseases (GTDs) with EC50 values of 0.5 to 1.0 mg/mL for both pathogens. Knots extracts showed non-toxic effect on grapevine callus of *V. vinifera* cv Gewurztraminer and a protective one on detached canes models when applied after inoculation of the pathogens. Additional *in planta* trials, in the greenhouse and then in the vineyard and nursery, will allow us to evaluate if Douglas knots extracts could be a good candidate to control GTDs.

Keywords: grapevine trunk diseases (GTDs), antifungal, plant defence stimulator, wood extracts, circular bioeconomy

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Bismuth subsalicylate: fungistatic and plant defence stimulator for grapevine trunk diseases management.

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Abstract

Since the use of sodium arsenite was banned in 2001, increasing numbers of grapevines affected by grapevine trunk diseases (GTDs) have been observed, and a lack of highly effective control products has led research on bismuth subsalicylate (BSS). This compound has been used in pharmaceuticals like PeptoBismol®, for decades. The antifungal capacity of BSS (which contains salicylic acid) was assessed against GTD pathogens, and its ability to stimulate plant defence genes.

An objective was to design an appropriate formulation for BSS which had water solubility. A suitable formulation based on a liquid polymer was developed, with small particle size which increased the bioavaibility of the compound, an extremely important feature for eventual developments. Antifungal activity of the formulated BSS against GTD pathogens was confirmed, through growth inhibition of *Neofusicoccum parvum* (isolates Bt 67 and Bourgogne), *Diplodia seriata* (98.1) and *Fomitiporia mediterranea* (PHCO36). Stimulation of defence genes was analysed by RT-qPCR on grapevine callus (*VvPAL, VvEDS1, VvHSR1* overexpressed), and the non toxicity of BSS on grapevine cells was confirmed by fluorescence microscopy. Tests on plants, in nursery, are currently in progress to confirm these results.

BSS was then evaluated *in planta* using vertical plant endotherapy technique, developed in our laboratory. BSS was injected directly into grapevine degraded wood where mycelium complexes are concentrated. Preliminary observations will be presented for symptomatic grapevines treated (n=100) with BSS for 2 years in Alsace, taking into account the complexity of GTD symptom expression.

Key words: grapevine trunk diseases, bismuth subsalicylate, antifungal, plant defence stimulator, vertical plant endotherapy.

Field investigation on phenology, ecology and integrated management of *Pseudococcus comstocki* in north-eastern Italy vineyards

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Abstract

Pseudococcus comstocki Kuwana (Hemiptera: Pseudococcidae) was reported for the first time in Italy in 2004 on mulberry; subsequently it spread to apple, pear and peach in the northeastern regions of Italy. Since 2018, heavy Comstock mealybug infestations have been reported on vineyard, where direct (large amount of sap depleting)) and indirect (honeydew and sooty mould fungi development) damages were observed. In this study, during the 2019-2021 vegetative seasons, fields surveys were carried out in order to investigate the phenology, ecology and the integrated management of *P comstocki* in some Veneto grape growing areas. The Comstock mealybug held three generations per year, overwintering as egg laid under the bark within waxy ovisacs with a cottony appearance. The eggs hatching takes place in April, in correspondence with leaves growing, where the juvenile stages feed. The females of the first generation appear between June and July. The females of the second generation are present from the beginning of August until September, while those of the third generation appear from October onwards. P. comstocki is a very mobile species; throughout the vegetative season juveniles and the pre-ovigerous females move from the woody plant organs to the vegetative ones, and vice versa, to feed and lay eggs. Ants contribute to mealybug diffusion, transporting them from the canopy to the ground, where small female mealybug colonies on the first roots are formed and covered with thin soil layer. Regarding males, after hatching the juveniles leave the vegetation and move to the woody parts to complete the development and then they die within a couple of days. For this reason, males are hardly observable in the open field, while their flights are identifiable with the use of pheromone traps. P. comstocki control rely on applications of synthetic insecticides, including tetramic acid derivates and neonicotinoids, at first- or second-generation nymphs migration. Considering that the efficacy of chemical control is variable and sometimes unsatisfactory an integration with biological control were tested. The releasing of 2,000 adults (CITRIPAR® - Koppert) per hectare of the parasitoid Anagyrus vladimiri Triapitsyn (Hymenoptera: Encyrtidae) showed an average parasitization of 16%. P. comstocki is becoming a serius pest in northeastern Italy vineyadrs. A prompt monitoring and a correct recognition respect the other two grapevine mealybugs (Planococcus ficus and Helicoccus bohemicus) is the pre-requisite to design a rational control management against the Comstock mealybug

Key words: Comstock mealybug, Anagyrus vladimiri, honeydew, chemical control, grapevine.

Development of High-Resolution Melting assay for detection of Grapevine Trunk Disease fungal pathogens

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Abstract

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Grapevine Trunk Diseases (GTDs) are a denomination for a set of several fungal diseases that vastly colonize grapevine trunks, reducing vines' production potential and stunting their growth. Infection of grapevines by GTD-causing pathogens is a preponderant factor for production loss and increased vineyard maintenance costs, thus leading to a greater economic effort made by wine producers. Nowadays, most methods to avoid vine infections by GTDs pathogens in vineyards are preventive, through pruning wound protection by biological or chemical treatments, and removal and destruction of pruning debris and infected plants, which are identified by visual symptomatology. The main goal of this work is to develop a faster and more sensible method for the detection of GTD-causing pathogens by a High-Resolution Melting (HRM) assay, for further application in field samples.

A set of 303 infected grapevines were assessed for GTD symptomology and fungal species were isolated and purified from necrotic wood tissues in PDA medium amended with chloramphenicol. DNA was extracted using the CTAB method from purified fungal isolates and PCR was performed to amplify *loci* – ITS, TUB2, TEF1. Sanger sequencing of the amplicons was performed, and sequences were aligned to identify the Single Nucleotide Polymorphisms (SNPs) species-specific. HRM assays, with MeltDoctor[™] chemistry (Applied Biosystems[™]), were performed to obtain specific melting curves allowing species distinction.

One of the HRM assays produced melting curves able to distinguish among species belonging to the family Botryosphaeriaceae, causal agents of Botryosphaeria dieback. This approach will improve the detection of latent inoculum of the pathogens responsible for GTDs, allowing earlier detection of the diseases and consequently avoiding big outbreaks.

Key words: Granevine Trunk Diseases. High Resolution Melting. Single Nucleotide Polymorphism.

Effects of *Trichoderma atroviride* SC1 on nursery vines and GTDs pathogens

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Abstract

Grapevine trunk diseases (GTDs) are very important diseases affecting the plant at all stages of life, from nursery to mature vineyard, and are caused by several pathogens affecting the grapevine at all stages of life, from nursery to mature vineyard. Although an effective control strategy is not available, protocols to manage the production of nursery material, including the use of biocontrol agents, are suggested. This two-year study aimed to assess, in a commercial nursery, the activity of different applications of *Trichoderma atroviride* SC1 on GTDs natural infections, and the effects on vegetative parameters of nursery vines. *Trichoderma was* applied at pre-grafting hydration, callusing, rooting, or as a combination of the three stages. The vegetative conditions of plants were evaluated after the callusing, during the growth in the nursery field, and at the assessment of certifiable plants. At the transplanting in the field, further plants becoming from each treatment have been potted in order to evaluate the development of the root apparatus. At the end of the first year of trials, certifiable plants of each treatment were collected to assess the incidence of *Trichoderma* and fungal pathogens. Colonies were isolated in the laboratory from five sections of the wood from the trunk. *Trichoderma* proved to exert different effects depending on the different timing of applications. In particular, rooting application led to an increase of certifiable plants, while pre-grafting hydration provided the best activity in the reduction of the incidence of GTDs pathogens.

Key words: Trichoderma atroviride, control, nursery production, GTDs natural infections, nursery conditions.

Reasoned approach to *Botrytis cinerea* biocontrol in grapevine

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Abstract

Biocontrol agents (BCAs) are a promising alternative to chemical fungicides for the control of Botrytis Bunch Rot (BBR) in vineyards. Compared to synthetic fungicides, however, BCAs often show lower and more variable efficacy under field conditions. Here we describe an innovative, multi-criteria approach for the BBR control in vineyards based on 10-year studies that encompass the following: disease epidemiology and modeling; a meta-analysis of previous research on disease control; ecology of different BCAs (*Bacillus amyloliquefaciens*, *B. amyloliquefaciens* subsp. *plantarum*, *B. subtilis, Aureobasidium pullulans*, *Metschnikowia fructicola*, *Trichoderma atroviride*, *T. gamsii*, *T. asperellum*, and *Pythium oligandrum*); controlled-condition and field experiments on BCA efficacy.

Our reasoned approach leads to deciding whether a BBR control is needed and which BCA should be used based on three steps: (1) evaluation of the risk of infection at critical host growth stages through a weatherbased, mechanistic model; (2) consideration of the target infection pathway of *B. cinerea* at the time of intervention, and the consequent plant substrate on which the BCA should grow (e.g., flowers for preventing latent infection, bunch trash for reducing colonization and sporulation, berries at different ripening stages for controlling conidial and berry-to-berry infection) and the type of activity the microorganisms should primarily express (e.g., competitive colonization of the substrate, antagonistic activity, induced resistance); and (3) consideration of weather conditions (temperature, humidity and rainfall) at the time of application and in the days after, and their effect on BCA growth, and efficacy.

First field experiments in which our approach was compared with a conventional BBR control at the four critical stages (i.e., flowering, pre-bunch closure, veraison, and pre-harvest) with chemical fungicides were very promising.

Key words: biological control, biological control agents, gray mold, *Botrytis cinerea*, weather conditions.



Portrait of the distribution of grapevine trunk diseases in Quebec, Canada, and consideration of the microbiome in their epidemiology

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Abstract

Grapevine trunk diseases (GTD) are considered very damaging to the sustainability of the wine heritage in all the world's major wine regions. Many fungi are responsible for these diseases and attack the perennial organs of the vine, which causes the death of the plant in the short or medium term. GTD can affect young plantations and ageing vineyards; in both cases, the risks of these diseases are increasing in vineyards in Quebec, Canada. In addition, with the climatic changes anticipated over the years, new grape varieties will be established in Quebec, thus promoting the introduction of vine varieties more susceptible to GTD in our vineyards. Climate change can promote the spread of these diseases, including higher temperatures and heavy rainfall. The main objective of this project was to acquire knowledge on the etiology and epidemiology of grapevine trunk diseases in the vineyards of Quebec, Canada. The project was divided into two parts: 1) an assessment of the presence and distribution of GTD across Quebec was carried out according to various criteria (region, grape variety, age of the vineyard, and part of the plant); and 2) the characterization of microbiome related to the epidemiology of GTD. We sampled a hundred sample in several vineyards, and detection of these GTD were executed: Esca (Phaeomoniella chlamydospora., Phaeoacremonium sp.), Botryosphaeria dieback (Botryosphaeriaceae sp., Diplodia sp., Neofusicoccum sp.), excoriosis (Diaporthe ampelina), Eutypa dieback (Eutypa lapa). The results demonstrated the presence of several GTDs in Quebec vinevards. Fungi causing excoriose, Eutypa dieback, Botryosphaeria dieback, esca and black foot have been identified. In addition, the characterization of the microbiome related to the presence of specific GTD was done. The knowledge acquired will make it possible to establish the epidemiology of GTD under Quebec's climatic conditions. In addition, it will be possible to suggest cultural practices to reduce the spread of GTD in Quebec, Canada.

Key words: grapevine, grapevine trunk diseases, epidemiology, microbiome.



Validation of a physiologically based demographic model (PBDM) to predict adults and immature stages of *Lobesia botrana* in Spain

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Abstract

The European grapevine moth (EGVM) Lobesia botrana is one of the main insect pests of grapevine that causes important losses to vineyards worldwide. IPM programs for L. botrana are based on monitoring populations and applying control measures based on monitoring data. Predictive models are an important tool for complementing monitoring data and support decision making about crop protection. The physiologically based demographic model (PBDM) developed by Gilioli et al. (2016) simulates the population dynamics over time as a function of environmental data. Gilioli et al. (2016) validated the model against monitoring data on adults and immature stages collected in Veneto region (Northern Italy); as the model provided reliable phenology and rational dynamics of the abundance of all stages of the EGVM, it was implemented in the Decision Support System (DSS) vite.net® - a real-time web platform used to support decision making for integrated pest management of grapevines. In this work, the model was further validated by using monitoring data of adults collected at weekly intervals in 15 locations in 2021 and 2022 in the provinces of Valencia, Rioja and Aragon (eastern and northern Spain); in 2021, data on eggs and larvae were also collected in 5 locations in Valencia. PBDM simulations were also compared with those provided by the model of Touzeau (1981), an empirical model developed in Toulouse (France) that is used in some European areas. PBDM simulations of adults and immature stages agreed with the field observations in all the vineyards and were more accurate than the simulation provided by the Touzeau model.

Key words: Lobesia botrana, IPM, pest modelling

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Fungicide sensitivity and resistance assessment of *Plasmopara viticola* populations from Basque Country vineyards

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Abstract: Humid and temperate climate conditions in the Basque Country make the effective control of grapevine downy mildew dependent on numerous fungicide applications along the grapevine growing season. Quinone Outside Inhibitor (QoI) and Carboxilic Acid Amide (CAA) fungicides sensitivity loss in *Plasmopara viticola* has been reported in several European countries, including Spain. To have knowledge of the situation of QoI and CAA sensitivity in the Basque winegrowing regions, *P. viticola* isolates were collected and analyzed. The G1105S mutation and the G143A mutation, conferring resistance to CAAs and QoIs, respectively, were detected by PCR-RFLP analysis. 39% of the samples was found to be resistant to CAAs whereas 60% of the samples was found to be resistant to QoIs. Results suggest a reduced effectiveness of both groups of fungicides in Basque Country vineyards, and thus measures for resistance management are needed to achieve an effective disease control.

Key words: grapevine downy mildew, Plasmopara viticola, fungicide resistance

Climate-resilient disease management in viticulture using Al-based Decision Support System (DSS)

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Abstract

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Climate change is affecting viticulture worldwide, making it a challenge for farmers to adapt to unpredictable and irregular growing seasons. In Switzerland, growers suffered severe losses in yield due to hailstorms and mildew infection in 2021, while 2022 was a favorable year for grapevine cultivation. Accurate disease predictions are critical in rapidly changing climate conditions to protect the harvest from pathogens. Classical mechanistic models for mildew prediction have limitations in integrating new abiotic and biotic factors.

Al methodologies can provide novel, automated solutions for precision agriculture and disease management. We present an Al-based decision support system (DSS) that uses field disease pressure data from various locations in Switzerland along with microclimate data to build a robust self-learning model that continuously learns from new field inputs. Moreover, the prediction model is integrated with the fungicide database, enabling farmers to choose fungicides and optimize dosage according to disease pressure. Overall, our system supports farmers' decisions by visualizing the predictions of the AI model in an easy-to-use online platform.

In summary, our AI-based decision support system improves upon existing disease prediction models by incorporating additional variables and considering their interdependencies at various timescales. Importantly, our research is aligned with the sustainability goals of the United Nations Development Programme (UNDP), as our model promises to guide farmers towards a minimal, but efficient, application of fungicides, thus reducing the load on the ecosystem.

Keywords: Downy mildew, disease management, artificial intelligence, AI, viticulture, integrated pest management, IPM, forecast, modelling, decision support system, DSS, climate change, climate resilient viticulture.


The importance of Rural Extension and Advisory Services to achieve a Sustainable Viticulture in a Climate Change Scenario

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Abstract

The success of sustainable rural development depends on developing and implementing comprehensive strategies for dealing with climate change, drought, territorial desertification and natural disasters. Improving access to information, education, extension services and learning resources, will lead to a strong and more resilient rural community.

Climate change is one of the main challenges of the viticulture sector. The European Green Deal and "Farm to Fork" Strategies are ambitious, innovative and they demand a decreasing of 50% on the use of conventional active substances by 2030. This could be considered an opportunity but is also a very demanding challenge for farmers.

Considering the availability of active substances in the EU, in 2011 existed 556 substances against the 454 in March 2023, while the number of not renewed substances in 2018 were 27 and in March 2023 the number is 118. (Anne Chapelle & Jean-Claude Malet (DGAL, FR))

For example, in Portugal, since 1997 there has been a reduction of active substances used in vineyards between 13%-26% in fungicides and 33%-52% in insecticides. The number of new biopesticides increased around 20%. These numbers, represent the need of a deep adaptation by farmers. (VI Jornadas de Homologação de Produtos Fitofarmacêuticos / April2023)

The rural extension and advisory services play a huge role in supporting farmers to achieve all the demands. Through a face-to-face presence in the field, but also with the help of digitalization, precision farming, AI and data analysis, it's possible to create methodologies enabling farmers to deal with the new goals.

According to the 2019 data, Portugal has 114 220Ha of vineyards for wine, on which 75% was under 1Ha and 20% between 1 - 5Ha. The area under Organic production represented only 1,8%. (GPP – Dec 2021). The viticulture sector is characterized by farmers with more than 60 years old, small to medium areas and a low educational degree. This situation leads to more difficulties in the implementation of an effective consultancy.

AVIPE is a wine grape growers association, founded in 1984, located in the Península de Setúbal wine region, 40 km south of Lisbon. It works with around 350 farmers, covering an area of 4000Ha of vineyards. Its main activity is on-field consultancy, supporting farmers in decision- making according to the principles of Integrated Pest Management and Organic farming. Irrigation management; good practices on plant protection products sprayings, nutrition and soil health preservation, training farmers along with bureaucratic work are also include on the services available to AVIPE's farmers.

So, to have a higher impact, the solution was the creation of living-labs/ demo-farms. It's considered crucial to enable farmers to see "*in loco*" the implementation of new technologies and practices.

AVIPE's dermo-farm, with an area of 40Ha of vineyards, is used to develop an applied research center that could give response to farmers difficulties and future challenges by implementing several trials in the different vineyard's plots:

NDVI and NDWI's vineyard mapping using satellite images, mapping soils by electrical conductivity, plant water potential measurement using pressure chamber in 22 different varieties for 16 consecutive weeks; plots exclusively treated in Organic; plots treated according to the LWA; weed mechanical control instead of herbicides use; cover crops; effectiveness tests of techniques to reduce sunburn phenomena; installation of functional margins and ecological infrastructures, notably according to the Miyawaki forest concept.

All these trials have allowed the development of several dissemination/awareness actions reaching more than 300 winegrape growers, in a pathway to a more sustainable and resilience viticulture.

Keywords: Advisory Services, Sustainable Viticulture, Regenerative Agriculture, Demo-farm.



Integrated Grape Production in Trentino (Northern Italy): where are we after thirty years since its first implementation?

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Abstract: The interest in a generalized reduction of the environmental impact of Trentino viticulture dates back to the early Nineties, when the adoption of the first integrated production program was promoted. Since then, the Trentino wine sector has embarked on a virtuous path that has recently led more than 5,000 winegrowers to join the national quality system for integrated production (SQNPI). In the 30 years since these early pioneering experiences on IPM, virtuous practices aimed at safeguarding individual and collective health have generally increased, as well as the sensitivity of producers and consumers, towards the environmental impact of agricultural activity. One of the outcomes of this process is the significant increase in organic production, which has now reached 13.5% of the wine-growing area. The introduction of technological innovations in phytosanitary strategies, and the impact of climate change on the vineyard ecosystems, have led to significant variations in the relative importance of the biotic adversities in Trentino wine growing. Analysing five of the most important problems, grape moths, green leafhopper, mites, botrytis and weeds, concrete and significant progress was made in the direction of a higher level of sustainability. Mating disruption (MD) for grape moth control is applied to the entire vineyard area (10,000 ha), and dedicated insecticide treatments have not been necessary since the beginning of 2000. The use of selective pesticides has favoured the conservative biocontrol of mite and green leafhopper whose chemical control is now limited to very few vineyards. The generalised implementation of the mechanical leaf removal allowed a significant reduction of the grey mold infection, which now requires one single chemical treatment on the 25% of the vineyards. However, the development of more environmentally friendly plant protection strategies has led to the emergence of pests previously considered secondary, such as the mealybug Planococcus ficus. Having experienced the lack of efficacy and sustainability of chemical control, the focus was again on the development and adoption of MD, combined with releases of the parasitoid Anagyrus vladimiri in spring, and targeted releases of the predator Cryptolaemus montrouzieri in summer. After having reached 1,500 ha of P. ficus MD in 2020, the area treated with this method is currently shrinking slightly, due both to the lower pressure of the pest, and to the drawback of the registered dispensers that are frequently removed by pneumatic defoliation machines. On the other hand, the effective management of downy mildew (DM), esca and, above all, Flavescence dorèe (FD) diseases, in a framework of sustainable IPM still meet several hindrances. Regulatory obstacles prevent a widespread of the already well performing DM resistant varieties, while no real solutions seem to be available for slowing down the constant increase of the esca disease. Despite the intensive chemical control and uprooting of symptomatic vines, FD is constantly spreading throughout the region. One of the most promising lines of research aimed at controlling the FD vector Scaphoideus titanus is the development of a control technique based on 'vibrational MD' which will be experimentally applied on 50 ha this year.

Key words: IPM guidelines, sustainable production, technological innovations, biocontrol

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Different grapevine cultivar responses to black rot attack in Romania-Crăciunelu de Jos vineyard

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Abstract

Before 2006, black rot infections in Romanian vineyards were relatively rare and of minimal economic relevance. In recent years, as a result of climate change, the disease's incidence and severity have steadily increased, particularly in the vineyards of central Transylvania, causing significant production losses.

The present study is based on field observations and aims to investigate how climatic conditions and treatment strategies from 2021 and 2022 have influenced the response, of different grapevine cultivars, to black rot attack. The studied cultivars were: Fetească neagră, Fetească regală, Fetească albă, Sauvignon blanc, Italian Riesling, Brumăriu, Radames and Rubin, all located in Crăciunelu de Jos vineyard, Romania`s viticultural zone 1 – the Transylvanian plateau. From the climatic point of view, the year 2021 was warmer (annual average temperature of 11.5°C) and rainier (788 mm sum of the annual precipitations) than the year 2022 (annual average temperature of 10.89°C and 581.4 mm sum of the annual precipitations). The treatment strategies in 2021 included 13 treatments, 3 of which were specific for black rot treatment and in 2022, 12 treatments were applied, 4 of which were specific for black rot treatment. The first signs of black rot attack were observed on the leaves on 16.06.2021 and 13.06.2022 and then on the grapes on 07.07.2021 and 17.07.2022. The assessment of the attack degree (AD [%]) of black rot was carried out in August, before the grapevines entered the veraison phenophase (BBCH 81-83). Statistically significant differences of black rot AD were observed in the studied years. Overall, the 2021 AD was significantly lower than the 2022 AD. Also, the AD observed on the leaves was lower than the AD observed on grapes. The most severe black rot AD was recorded for the Fetească regală cultivar (9.63 % AD). During the studied period Brumăriu cultivar did not show specific symptoms of black rot attack. The results of the study show that the influence of climatic conditions on black rot is significant, but by choosing tolerant/resistant cultivars and using the right treatment strategies, healthy harvests with good yields can be obtained.

Key words: black rot, grapevine cultivars, field evaluation, disease management.

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Field evaluation of sensitivity of disease resistant grape varieties to invertebrate pests

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Abstract

Powdery and Downy mildew are two foliar diseases that were introduced to Europe on American Vitis species in the 19th century. Those two diseases are the cause of most of the sprays applied in vineyards during the growing season. As there was no co-evolution between those pathogens and the European Vitis, all Vinifera cultivars are susceptible whereas American and Asian Vitis species differ in their susceptibility. Several breeding programs worldwide work on introducing resistance factors and creating European-like resistant cultivars. On the other hand, V. vinifera co-evolved with several "local" pests including pathogens and invertebrates. For instance, there is a big difference in susceptibility to the pathogen Phomopsis viticola between Flam seedless and Merlot and in the incidence of galls caused by Erineum mite (Colomerus vitis) between Sauvignon blanc and Syra vines. In 2020 we imported four wine grapes hybrids from STAATLICHES WEINBAUINSTITUT FREIBURG and in 2021 they were planted in commercial vineyards in four climatic regions in the north of Israel. Beside documenting the Powdery and Downy mildew development on these vines, we evaluated the incidence and severity of Erineum mites galls and the population of thrips. Both pests deform the leaves and with high population, the mites can cause early leaves senescence and delay in sugar accumulation. Mites incidence was significantly lower in Cabernet carbon compared with Souvignier gris, Prior and Tempranillo. In one of the plots, thrips population was highest in Tempranillo but this could be due to the higher vigour (more young leaves that attract thrips) in those vines.

Before recommending new cultivars to the growers it is important to define all their characteristics. Beside looking at their agronomic traits it is important to define the interactions with other possible pests that might need additional, sometimes more toxic, control measures.

Key words: Hybrid grape cultivars, Erineum mites, thrips

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The entomopathogenic fungus *Metarhizium robertsii* and its endophytic potential in grapevine to regulate radicicole grape phylloxera populations

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Abstract

Grape phylloxera *Daktulosphaira vitifoliae* destroyed 30% of European vineyards by the end of the 19th century, attacking the woody roots of grapevine *Vitis vinifera* (L.). To date, grafting grapevine on American resistant rootstocks is the leading solution to control the pest. However, grape phylloxera is present in most wine-growing soils and remains a threat in regions planted with non-grafted vines. Thus, alternative control solutions are investigated. The entomopathogenic fungus *Metarhizium robertsii* is known for its capacity to parasitize insects. Besides, the fungus can colonize plant rhizosphere and establish as an endophyte (i.e., within plant tissues) in many plant species. This study aimed to investigate if *M. robertsii* could persistently associate with the rhizosphere and as an endophyte in non-grafted grapevine *V. vinifera* without harming its growth while preventing the development of the grape phylloxera radicicole forms.

M. robertsii strain EF3.5(2) native to Bordeaux vineyard soils and a laboratory strain non-native to the vineyard (ARSEF-2575-GFP) were assessed to characterize and compare their establishment in the rhizosphere and as endophyte in the grapevine. We hypothesized that the vineyard-native strain had a more durable colonization compared with the non-native one. Droplet Digital PCR (ddPCR), a culture-based method, and confocal imaging of root segments were used to characterize the associations. The strain EF3.5(2) had a higher establishment in the rhizosphere and root-endosphere of non-grafted grapevine than the strain ARSEF-2575-GFP. Both strains established for up to 96-98 days post-inoculation (dpi) in both compartments. In addition, the effect of direct parasitism of the strain EF3.5(2) on the survival and development of radicicole grape phylloxera clone 'Pcr7' was evaluated in a woody root section bioassay. Depending on the method used to infect the insect, a significant reduction of 90 %, 70 %, and 86% in survival probability was found, and 92%, 89%, and 93 % in reduction of adults' development compared with control treatments. Also, the symptoms induced by grape phylloxera on non-grafted grapevines co-inoculated with M. robertsii were reduced by 91% (mean of 4.8 ± 8.4 nodosities) compared with the phylloxera infestedplants (mean of 52.7 ± 50.7 nodosities). After 24 h, qPCR analysis was performed with grapevines inoculated with the strain EF3.5(2) and showed, an up- regulation of the VvWRKY-75, VvPR3.2, VvPR4, and VvG1 genes, which are phylloxera-responsive defense genes involved in the resistance against phylloxera.

In conclusion, *M. robertsii*'s persistent association with non-grafted grapevine represents an alternative protection strategy against radicicole grape phylloxera, especially for wine-growing regions planted with non-grafted vines. Further field experiments should be carried out to determine the environmental factors affecting the effectiveness and persistence of *M. robertsii* associated with grapevine.

Keywords: Biological Control, non-grafted *Vitis vinifera*, *Metarhizium robertsii*, grape phylloxera, endophytism

Venue and Contact

CONFERENCE VENUE

Riojaforum Calle San Millán, 23 - 25, 26004 Logroño, La Rioja

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

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SOCIAL PROGRAM Monday, 2nd October | 20:00

Cocktail reception

Espacio Lagares Address: Ruavieja, 18-20, 26001 Logroño, La Rioja

Tuesday, 3rd October | 18:00-20:00

Poster Viewing session and cocktail Riojaforum Conference Centre

Address: Calle San Millán, 23 - 25, 26004 Logroño, La Rioja

Wednesday, 4th October | 16:00-20:00 Congress Tour

Vivanco Winery and Museum of Wine Culture Departure from Riojaforum

Thursday, 5th October | 20:00

Gala dinner

Bodegas Franco Españolas Address: Cabo Noval, 2, 26009 Logroño, La Rioja Includes a winery free visit. Dinner starts at 21:00

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