IOBC-WPRS

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Future IPM 3.0 towards a sustainable agriculture

IOBC-WPRS general assembly
Meeting of the WGs Integrated protection in viticulture, Induced resistance in plants against insects and diseases and Multitrophic interactions in soil

15-20 October 2017, Riva del Garda, Italy

Future IPM 3.0

BOOK OF ABSTRACTS

Sponsored by the OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems

The Conference was sponsored by the OECD Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems, whose financial support made it possible for most of/some of the invited speakers to participate in the Conference.

Local organizers

FONDAZIONE EDMUND MACH

C3A CENTRO AGRICOLTURA ALIMENTI AMBIENTE

UNIVERSITÀ DI TRENTO

FONDAZIONE EDMUND MACH

UNIVERSITY OF TRENTO - Italy

LAIMBURG
Land- und Forstwirtschaftliches Versuchszentrum
Centro di Sperimentazione Agraria e Forestale
Research Centre for Agriculture and Forestry

universität innsbruck

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Sponsors and exibitors

Scientific collaborations

Media partners

Patronage and local parners

Autonomous Province of Trento

Autonomous Province of Bolzano

Landwirtschaftliches Schulwesen | Land Tirol

European Region Tyrol-South Tyrol-Trentino

Riva del Garda municipality

Think global - eat local

Riva del Garda Fierecongressi

Trentino Catering

felicetti

SPEZIALBIER-BRAUEREI FORST BIRRA-BIÈRE-BEER-BIER

MARZADRO
Distillatori per passione dal 1949

MENZ & GASSER

APOT

ASSOMELA
ASSOCIAZIONE ITALIANA PRODUTTORI DI MELE

Südtiroler Speck g.g.A. SÜDTIROL Speck Alto Adige IGP

Qualità Alto Adige

CAVIT TRENTO

MEZZACORONA

CANTINA FRANTOIO DAL 1926
AGRARIA RIVA DEL GARDA

CANTINA TOBLINO

Hosteria TOBLINO selling point

TRENTINO TRENTINGRANA

ASTRO

Val di Gresta
CONSORZIO ORTOFRUTTICOLO

Scientific advisory boards

Workshop: Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

IOBC-WPRS Working Group Induced resistance in plants against insects and diseases

Annegret Schmitt, Brigitte Mauch-Mani, Corné M.J. Pieterse, Gerardo Puopolo, Ilaria Pertot, Markus Kelderer, Michele Perazzolli, Victor Flors

IOBC-WPRS Working Group and Multitrophic interactions in soil

Christian Steinberg, Gerardo Puopolo, Heribert Insam, Martin Thalheimer, Michele Perazzolli, Stefano Cesco, Tanja Mimmo, Yigal Elad, Youry Pii

Workshop: Novel tools and new challenges for IPM in viticulture

IOBC-WPRS Working Group Integrated protection in viticulture

Carlo Duso, Claudio Ioriatti, Denis Thiéry, Gianfranco Anfora, Gerd Innerebner, Ilaria Pertot, Klaus Marschall, Mauro Jermini, Michael Maixner, René Fuchs, Sergio Angeli, Silvia Schmidt, Tirtza Zahavi, Valerio Mazzoni

Welcome!

The main challenges for agriculture worldwide are sustainable production and the reduction of risks and impacts of pesticide use on human health and the environment. These challenges can be met through integrated pest management (IPM) and the use of sustainable approaches and techniques. All stakeholders, including citizens, are expecting production systems to become more sustainable, while supplying healthier and safer food and protecting the environment and its biodiversity. Research is progressing rapidly and farmers will have increased access to innovative tools in plant protection and production.

The aim of FutureIPM3.0 is to bring together stakeholders dealing with different aspects of sustainable production in agriculture with a special focus on plant protection to share scientific, technological and regulatory information to build the future strategies. The main objective is to promote knowledge exchange among scientists, companies, advisors, farmers, policy makers and stakeholders, to identify approaches, tools and techniques to meet the future needs of crop production and protection.

FutureIPM3.0 will offer the most advanced scientific and technical knowledge for advisors and growers, a unique opportunity for companies to present their innovation in IPM and to see the most recent scientific advances to further improve their products' portfolio. The conference will be also a stimulating arena for scientists to present their most advanced discoveries.

Since FutureIPM3.0 is about sustainable agriculture and development, we have proudly organized the event according to the principle waste avoidance and sustainable use of resource. All gadgets have been eliminated and the conference material and documents are either recyclable or available online. To avoid food waste during the conference, we collaborate with Trentino catering and Riva del Garda FiereCongressi within a "Food for Good" project. Thanks to this initiative, the surplus food from the meals during the conference will be delivered to charitable organizations such as family homes, soup kitchens and refugee centers. Moreover, we will provide meals with local ingredients.

The international conference on "Future Integrated Pest Management" will be not a mere scientific congress, but it will combine a list of events to share scientific, technical and market knowledge on the major pillars of sustainable crop production.

The plenary session 'FutureIPM3.0 towards a sustainable agriculture' sponsored by the OECD Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems will gather the most renowned experts worldwide on three relevant subjects: the role of policies and trends in sustainable integrated management in agriculture, the most advanced tools and innovation in crop protection and production, and the challenges that sustainable integrated production in agriculture will face and how to address them.

The conference FutureIPM3.0 is organized within the frame of the International Organization for Biological and Integrated Control (IOBC/WPRS) General Assembly and by three working groups (Induced resistance in plants against insects and diseases, Integrated protection in viticulture, Multitrophic interactions in soil) of the IOBC/WPRS. Two Parallel workshops will focus on three hot topics of IPM:

- Induced resistance in plants: the contribution of science to an effective field application
- Preserving soil quality and health for the future generations
- Novel tools and new challenges for IPM in viticulture

The week of events is open by a technical conference (in Italian) presenting the most recent innovations for growers (biopesticides and pheromones, biocontrol and beneficial arthropods, agronomic practices, physical and technical solutions to prevent and control pest and pathogens,

equipment and technologies, resistant and tolerant varieties, ICT to support a sustainable use of pesticides, web services, apps, decision support systems, digital solutions, etc.).

In addition, the week of events hosts a workshop of the COST Action FA1405 - Using Three-way Interactions Between Plants, Microbes and Arthropods to Enhance Crop Protection and Production – focusing on Bottlenecks and opportunities in utilizing crop-arthropod-microbe (CAMo) interactions. A BRt2oB and R2R event, an exhibition and some technical and cultural events complete the program.

FutureIPM3.0 is organised by International Organization for Biological and Integrated control (IOBC/WPRS), Fondazione Edmund Mach, Laimburg Research Centre, University of Trento, Free University of Bolzano-Bozen and University of Innsbruck, under the patronage of Euregio Tyrol-South Tyrol-Trentino, Landwirtschaftliches Schulwesen Land Tirol, Autonomous Province of Bozen/Bolzano, Autonomous Province of Trento. We wish to thank the scientific societies, the sponsors, the scientific advisory boards and all collaborators that made the event possible.

We welcome you and hope you will enjoy the meeting.

On behalf of the organizers
The convenors

Ilaria Pertot

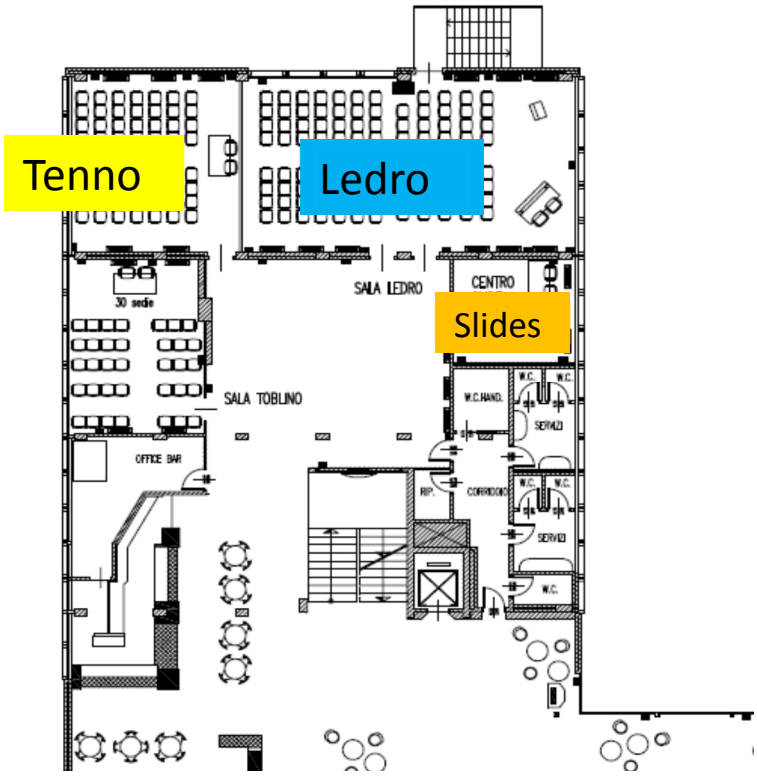
Claudio Ioriatti

PROGRAM

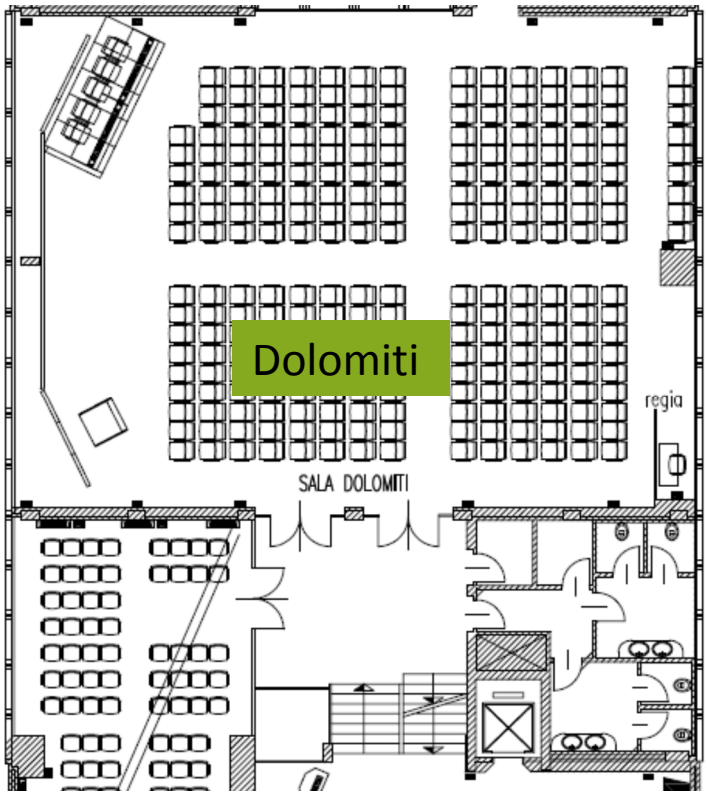
Program overview | Future IPM 3.0

Sunday 15 October, 2017	
IOBC-WPRS General Assembly	IOBC-WPRS members only
Monday 16 October, 2017	
Coniugare competitività e sostenibilità: la sfida dell'agricoltura	Plenary session, in Italian
IOBC-WPRS General Assembly	IOBC-WPRS members only
PROJECT INTERFUTURE MEETING - From microbial interactions to new-concept biopesticides and biofertilizers	INTERFUTURE project partners only
Tuesday 17 October, 2017	
Future IPM 3.0 towards a sustainable agriculture	Plenary session
Brokerage and Venturing Event on Research & Innovation on sustainable agriculture	B2B and R2B event
Bottlenecks and opportunities in utilizing crop-arthropod-microbe interactions	EU-COST Action FA1405 workshop
Wednesday 18 October, 2017	
Ecological perspectives of induced resistance in plants and multitrophic interactions in soil	IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"
Novel tools and new challenges for IPM in viticulture	IOBC-WPRS Working Group "Integrated protection in viticulture"
Thursday 19 October, 2017	
Ecological perspectives of induced resistance in plants and multitrophic interactions in soil	IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"
Novel tools and new challenges for IPM in viticulture	IOBC-WPRS Working Group "Integrated protection in viticulture"
Friday 20 October, 2017	
Ecological perspectives of induced resistance in plants and multitrophic interactions in soil	IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"
Novel tools and new challenges for IPM in viticulture	IOBC-WPRS Working Group "Integrated protection in viticulture"

1st Floor



2nd Floor



Rooms

Sunday 15 October	
IOBC-WPRS general assembly	**Room LEDRO**
Monday 16 October	
IOBC-WPRS general assembly	**Room LEDRO**
Plenary Session - Una produzione agricola competitiva e le sfide della produzione sostenibile	**Room DOLOMITI**
Project Meeting Interfuture (for project members only)	**Room TENNO**
Tuesday 17 October	
Plenary Session - Future IPM 3.0 towards a sustainable agriculture	**Room DOLOMITI**
B2B EVENT - Brokerage and Venturing Event on Research & Innovation on sustainable agriculture	**PALAVELA**
COST Action FA1405 workshop - Bottlenecks and opportunities in utilizing crop-arthropod-microbe interactions	**Room LEDRO**
Wednesday 18 October	
Parallel Workshop - Ecological perspectives of induced resistance in plants and multitrophic interactions in soil	**Room LEDRO**
Parallel Workshop - Novel tools and new challenges for IPM in viticulture	**Room DOLOMITI**
Thursday 19 October	
Parallel Workshop - Ecological perspectives of induced resistance in plants and multitrophic interactions in soil	**Room LEDRO**
Parallel Workshop - Novel tools and new challenges for IPM in viticulture	**Room DOLOMITI**
Friday 20 October	
Parallel Workshop - Ecological perspectives of induced resistance in plants and multitrophic interactions in soil	**Room LEDRO**
Parallel Workshop - Novel tools and new challenges for IPM in viticulture	**Room DOLOMITI**
Coffee breaks, lunches, dinners	**PALAVELA**
Poster room	**PALAVELA**

Presenter guidelines

Oral presentations

Oral presentations
Please, prepare your presentation in the Microsoft Office Power Point (PPT or PPTX) or PDF format.
Please, remember the time allocated for each presentation:
- **Plenary lectures:** 30 minutes presentation and discussion at the end of each session (17 October)
- **Key note lectures:** 25 minutes presentation plus 5 minutes of discussion (18-20 October)
- **Oral presentations:** 15 minutes presentation plus 5 minutes of discussion (18-20 October)

Presentations have to be loaded on one of the meeting's computers at least 1 hour before the start of the session.

Poster presentations

Please prepare your poster of maximum **90 cm width** and **120 cm height**.
Posters can be affixed to the assigned poster board from 16 to 20 October. The Organizing Committee will not be responsible for posters that are not removed by the end of conference.
You can find information regarding the number of the poster board allocated to you on the program here below. Please, use the board with the same number.

Poster flash talks

We kindly ask to all poster authors to shortly present their main results (maximum 2 minutes) during the **Poster flash talk sessions**. No Power Point presentation is required, we will project abstract title, authors and highlights to support your flash talk.
Please, check on the program here below your poster number and session.

Program | IOBC-WPRS general assembly

For IOBC-WPRS member only

Sunday 15 October, 2017			
9:00	**11:00**	**Administration, chair Philippe Nicot**	
		Presidential welcome address	Philippe Nicot
		Greetings from IOBC/EPRS	Milka Glavendekic
		Report of the President	Philippe Nicot
		Report of the Secretary General	Gerben Messelink
		Presentation of the web site improvements	Madeleine Bühler
		Financial report of the Treasurer	Sylvia Blümel
		Audit Commitee	Peter Esjberg
		Votes of the Assembly: approval of the financial and activity reports	
		Results of the election of the new Audit committee for 2017-2021	
		Presentation of the new Council to the Assembly	
		Address by new President	
11:00	11:30	Coffee break	
11:30	**13:30**	**14 Reports, chair Gerben Messelink**	
		Commission - IP & Biocontrol in North-African countries	Ahmed Mazih
		Commission - Guidelines for integrated production	Frank Wijnands
		Commission - Harmonized regulation of biological control agents	Josep Jaques Miret
		Discussion	
		WG Pheromones and other semio-chemicals in integrated production	Jürgen Gross
		WG Multitrophic interactions in soil	Christian Steinberg
		WG Integrated protection in viticulture	Carlo Duso
		WG Integrated control in oilseed crops	Malgorzata Jedryczka
		WG Integrated protection of field vegetables	Richard Meadow
		Discussion	
		WG Integrated control in protected crops, temperate climate	Bruno Gobin
		WG Integrated control in protected crops, Mediterranean climate	Carmelo Rapisarda
		WG Integrated protection in oak forest	Pino Angelo Ruiu
		WG Integrated protection of stored products	Pasquale Trematerra
		WG Integrated protection of olive crops	Dionyssios Perdikis
		Study group palm dates	Abdulaziz Mohamed
		Discussion	
13:30	14:30	Lunch	

14:30	**16:30**	**10 Reports, chair Ilaria Pertot**	
	WG Integrated protection of citrus crops		Ferran Garcia-Marí
	WG Integrated protection of fruit crops		Claudio Ioriatti
	WG Pesticides and beneficial organisms		Guy Smagghe
	WG Induced resistance in plants against insects and diseases		Brigitte Mauch-Mani
	WG Biological and integrated control of plant pathogens		Jürgen Köhl
	WG GMO's in integrated plant production		Michael Meissle
	Discussion		
	WG Microbial and nematode control of invertebrate pests		Eustachio Tarasco
	WG Integrated control of mite pests		George Broufas
	WG Benefits and risks of exotic biological agents		Olda Nedved
	WG Landscape management for functional biodiversity		Baerbel Gerowitt
	Discussion		
	General discussion on WGs		
16:30	17:00	Coffee break	
17:00	**19:00**	**Convenors Meeting** **Old & New Council Meeting**	
19:00	22:00	Dinner	

Monday 16 October, 2017

9:00	**11:00**	**Recommendations by the Assembly to the new Council** **chair Andrea Lucchi**
	Recommendations by previous general assembly and Council-Convenor meeting + actions taken	
	New Recommendations by the Assembly to the new Council	
	Amendments of Statutes / Byelaws	
	Debate by the Assembly	
	Closing of the General Assembly	
11:00	11:30	Coffee break
11:30	**13:30**	**Meeting of the New Council**
11:30	**13:30**	**Meeting of Convenors (room Toblino)**
13:30	14:30	Lunch break
14:30	**18:00**	**Meeting of the New Council**
19:00	21:00	Dinner

Program | Una produzione agricola competitiva e le sfide della produzione sostenibile

Key note, round tables (in Italian) – Sala Dolomiti (second floor)

Monday 16 October, 2017			
8:30	9:00	Registrazione dei partecipanti	
9:00	9:30	Saluti Autorità e apertura del Convegno	Dott. Michele Dallapiccola
9:30	10:00	Dialoghi tra un giornalista e un ricercatore: la sostenibilità in agricoltura	
10:00	10:30	Keynote lecture: Comportamento dei prodotti fitosanitari nell'ambiente, nelle produzioni e negli alimenti	Prof. Marco Trevisan
10:30	11:30	Dibattito - La sfida della sostenibilità in viticoltura e frutticoltura di montagna, fondovalle, e pianura.	Dott. Alessandro Dal Piaz Dott. Carlo Debiasi Dott. Claudio Mazzini Dott. Claudio Ioriatti Dott.Ssa Irene Holzmann Dott.Ssa Mariagrazia Tommasini
11:30	11:45	Coffee break	
11.45	12:45	Dibattito - Specie invasive e cambiamento climatico: l'impatto sull'agricoltura	Prof. Alberto Bellin Enologo Mario Pojer Dott. Andrea Berti Dott.Ssa Lara Maistrello Dott. Vincenzo Verrastro
12:45	14:00	Pranzo	
14:00	15:00	Dibattito - Agricoltura –società: informazione, sicurezza alimentare, convivenza	Dott.Ssa. Emanuela Bozzin Dott. Donatello Sandroni Dott. Alberto Dezza Dott. Antonio Frattarelli Prof. Geremia Gios
15:00	16:00	Dibattito - Il ruolo delle nuove soluzioni tecniche e sostanze attive microbiologiche o naturali nella difesa delle coltureProspettive relative ai prodotti fitosanitari a base biologica	Dott. Sandro Frati Dott. Vittorio Veronelli Dott. Gabriele Posenato Prof. Mario Pezzotti
16:30	17:00	Coffee break	
17:00	18:00	Dibattito - ICT e meccatronica in campo: sensori, app, droni, agricoltura di precisione	Dott. Steno Fontanari Dott. Antonio Manes Prof. Francesco Marinello Dott. Andrea Guarise Dott. Luca Pedron
18:00	18:15	Conclusioni	
18:15	18:30	Premiazione del 'Concorso Agricoltura Sostenibile per un Futuro Sostenibile'	
18:30	21:00	Apericena	

Program | Future IPM 3.0 towards a sustainable agriculture

Conference sponsored by the OECD (www.oecd.org/agriculture/crp)

Tuesday 17 October, 2017			
8:00	8:30	Registration	
8:30	8:40	Opening and chair of the sessions	Ilaria Pertot
8:40	9:00	OECD Co-operative Research Programme: Biological Resource Management for *Sustainable Agricultural Systems*	Gary Fit
9:00	**12:30**	**Policies and trends in sustainable integrated management in agriculture and organic production**	
9:00	9:30	Integrated pest management concepts and implementation: state of the art and opportunities for the future	Carlo Malavolta
9:30	10:00	Alternatives to contentious inputs in organic farming: regulatory and technical issues	Lucius Tamm
10:00	10:30	The importance of networking among stakeholders and cross-sector contamination for sustainable crop protection	Sylvia Blümel
10:30	11:00	Coffee break	
11:00	11:30	Sustainable biological and technical approaches in plant protection: overview of the market and a critical discussion on the present regulations	David Cary
11:30	12:00	The fresh fruit and vegetable sector from a sustainable supply chain perspective	Helene Deruwe
12:00	12:30	Discussion	
12:30	13:30	Lunch	
13:30	**16:30**	**Tools and innovation in crop protection and production**	
13:30	14:00	The challenge of developing microbial biocontrol products for disease control	Jürgen Köhl
14:00	14:30	Biocontrol with beneficials insects and new perspectives in insect biocontrol	Felix Wackers
14:30	15:00	Sustainable weed management, what's next after glyphosate?	Micheal D.K. Owen
15:00	15:30	Sensors, drones and precision agriculture for sustainable production	Rino Goller and Maurizio Barazzuol
15:30	16:00	Unearthing plant-beneficial traits of the root microbiome for sustainable crop protection	Corné Pieterse
16:00	16:30	Discussion	
16:30	17:00	Coffee break	
17:00	**18:30**	**Sustainable integrated production in agriculture: the challenges**	
17:00	17:30	Landscape management and preservation of biodiversity	John M. Holland
17:30	18:00	Sustainable production faces the challenge of invasive species	Max Suckling
18:00	18:30	General discussion and conclusion	
18:30	**19:00**	**Summary of conclusions chaired by a professional Journalist**	
19:00	21:00	Aperi-dinner	

Program | Bottlenecks and opportunities in utilizing crop-arthropod-microbe interactions

COST Action FA1405 workshop, Using Three-way Interactions Between Plants, Microbes and Arthropods to Enhance Crop Protection and Production

Tuesday 17 October, 2017			
15:00	**17:00**	**EU-COST Action FA1405 workshop**	
15:00	15:15	Information about COST and Introduction to COST Action FA1405	Richard Meadow
15:15	17:00	COST Action FA1405 Workshop	
		Panel member	Carolin Schneider
		Panel member	Edith Ladurner
		Panel member	Alexander Schouten
19:00	21:00	Aperi-dinner	

Program | Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil" – Sala Ledro (first floor)

Wednesday 18 October, 2017			
8:00	9:00	Registration	
9:00	9:10	Welcome to participants	Michele Perazzolli and Gerardo Puopolo
9:10	9:30	Opening and IOBC-WPRS presentation	Brigitte Mauch-Mani and Christian Steinberg (convenors), Ilaria Pertot and Yigal Elad (liason officers)
9:30	**13:00**	**Session 1: Novel and old players in plant-microbe interactions** **Chairs: Victor Flors and Philippe Nicot**	
9:30	10:00	Keynote lecture: An active starch degradation metabolism provides sugars for callose priming during *Plectosphaerella cucumerina* infection	Victor Flors
10:00	10:20	Effect of drench application of biocontrol preparations on tomato plants against *Botrytis cinerea* and *Oidium neolycopersici*	Marc Bardin
10:20	10:40	Sugar homeostasis mediates arbuscular mycorrhizal fungi-induced resistance against *Botrytis cinerea*	Neus Sanmartín Martínez
10:40	11:00	Arbuscular mycorrhizal fungi induced systemic biocontrol against root-knot nematode on chilli	Thanasan Khaosaad
11:00	11:30	Coffee break	
11:30	12:00	Keynote lecture: The effect of β-aminobutyric acid in the protection of tomato harvest against *Botrytis cinerea*	Estrella Luna
12:00	12:20	Systemic immunity in wheat is activated by the deployment of transcription factors	Sanjukta Dey
12:20	12:40	Fosetyl-aluminum improves defence against *Venturia inaequalis* in apple	Anze Svara
12:40	13:00	Characterisation of a broad-range, biologically active substance from *Pseudozyma aphidis* with a dual mode of action: antibiosis and induced resistance	Raviv Harris
13:00	14:30	Lunch	
14:30	**16:50**	**Session 2: Novel and old players in plant-microbe interactions** **Chairs: Xavier Daire and Franco Faoro**	
14:30	15:00	Keynote lecture: Two lysine motif receptor-like kinases (VvLYKs) participate in chitin-triggered immunity in grapevine	Benoit Poinssot
15:00	15:20	New defence metabolic pathways under the control of the hormonal peptide systemin	Victoria Pastor
15:20	15:40	The xyloglucans: are they new elicitors of *Arabidopsis thaliana* immunity?	Justine Claverie

15:40	16:00	Bio-based compounds inducing resistance against *Leptosphaeria maculans* in oilseed rape	Lenka Burketova
16:00	16:20	Molecular mechanisms of chemical immune priming without costs to plant growth	Willam Buswell
16:20	16:50	Keynote lecture: Plant produced β-aminobutyric acid: the immune system controls its accumulation	Ivan Baccelli
16:50	17:30	Poster Flash Talks Session 1: Novel and old players in plant defence regulations	
17:30	**19:00**	**Poster session 1 (with drinks): Novel and old players in plant defence regulations**	
19:00	21:00	Aperi-dinner	

Thursday 19 October, 2017

9:00	**13:00**	**Session 3: Functional ecology of microbial interactions in soil** **Chairs: Heribert Insam and Yigal Elad**	
9:00	9:30	Keynote lecture: Crosstalk effects of environment and vineyard soil management on soil microbial diversity and composition	Michaela Griesser
9:30	9:50	Linking transcriptomics to the rhizosphere microbiome - Interactions during clubroot development as a case study	Stefan Ciaghi
9:50	10:10	Spatial and temporal *in-situ* analyses of gene expression in complex host-pathogen-systems using the *Plasmodiophora brassicae*-cabbage-pathosystem as a model	Julia Badstöber
10:10	10:30	RNA-Seq unveiled the bacterial mycophagy mechanisms implemented by *Lysobacter capsici* AZ78 interacting with *Phytophthora infestans*	Gerardo Puopolo
10:30	10:50	Evaluation of the efficacy of a biocontrol agent, *Gliocladium catenulatum*, to colonise soils and to reduce *Fusarium graminearum* growth under microcosm and field conditions	Adeline Picot
11:00	11:30	Coffee break	
11:30	12:00	Keynote lecture: Weed microbiome characteristics and the development of bioherbicides	Friederike Trognitz
12:00	12:20	qPCR and NGS techniques for pathogen detection and monitoring of microbial communities in soil after application of broad spectrum fungal treatments in cucumber crop	Ana Belén López Santísima Trinidad
12:20	12:40	Influence of environmental factors and plant protection products on the growth and survival of *Trichoderma harzianum* strain INAT11	Edith Ladurner
12:40	13:00	Herbie 72®: a tool to standardise the adoption of anaerobic soil disinfestation for intensive cropping systems under realistic scenarios	Andrea Minuto
13:00	14:30	Lunch	
14:30	**16:50**	**Session 4: Ecology and factors affecting induced resistance** **Chairs: Gary W. Felton and A. Corina Vlot**	
14:30	15:00	Keynote lecture: Multitrophic regulation of induced defence responses	Gary W. Felton

15:00	15:20	Protein-based products as resistance inducers: disease control and mechanisms of action	Martina Cappelletti
15:20	15:40	Deciphering the impact of nutrient stress in mycorrhiza-induced resistance against *Botrytis cinerea* in tomato	Paloma Sanchez-Bel
15:40	16:00	Impact of agricultural practices on plant disease: what can we learn for resistance inducers optimisation?	Elsa Ballini
16:00	16:20	The age-dependent priming.	Diego Mateu Garcia
16:20	16:50	Keynote lecture: Monoterpenes in systemic acquired resistance within and between plants	A. Corina Vlot
16:50	17:20	Poster Flash Talks Session 2: Functional ecology of microbial interactions in soil	
17:20	17:30	New convenor election (Chair: Ialria Pertot)	
17:30	**19:00**	**Poster session 2 (with drinks): Functional ecology of microbial interactions in soil**	
19:00	22:00	Social dinner and poster awards	

Friday 20 October, 2017

9:00	**13:00**	**Session 5: Multitrophic interactions and plant defence** **Chairs: Annegret Schmitt, Erik Alexandersson and Michele Perazzolli**	
9:00	9:30	Keynote lecture: Cellular regulations of grapevine resistance induced by *Trichoderma* spp.	Michele Perazzolli
9:30	9:50	A new tool to assess the grapevine defence at the high-throughput :« Neovigen96» chip and Fluidigm® technology	Marie-Cécile Dufour
9:50	10:10	Resistance induction by hot water treatments to control apple postharvest diseases	Elena Baraldi
10:10	10:30	Volatile methyl salicylate induces systemic signalling in the phylloxerated root system of hybridised *Vitis* spp.	Markus Walter Eitle
10:30	10:50	Efficacy of elicitors on boosting insect natural enemies: the case of vineyard	Martina Parrilli
10:50	11:20	Coffee break	
11:20	11:40	Elicitation responses in cucumber plants after treatment with a fraction of a liquorice leaf extract from *Glycyrrhiza glabra*	Annegret Schmitt
11:40	12:00	Identification and functional characterisation of grapevine volatile organic compounds for the sustainable control of downy mildew	Valentina Lazazzara
12:00	12:20	Evaluation of the antifungal activity of the protein and non-protein extracts of *Trichoderma asperellum* and *Trichoderma atroviride* culture filtrates against *Phytophthora infestans*	Saida Messgo-Moumene
12:20	13:00	Closing remarks	Michele Perazzolli, Gerardo Puopolo and Ilaria Pertot
13:00	14:00	Lunch box	

14:00	**21:00**	**Excursion and dinner**

Poster sessions and Poster Flash Talks

Wednesday 18 October	**Poster session 1: Novel and old players in plant defence regulations**	
IR.1	Regulatory role of *SlyWRKY75* transcription factor in stress in tomato plants	Maria José López-Galiano
IR.2	Induction of resistance in wheat against leaf rust by application of biotic and abiotic inducers	Fares Bellameche
IR.3	Contrasting effects of the rhizobacterium *Pseudomonas simiae* on Diamondback moth and Cabbage root fly	Julia Friman
IR.4	First insights on the ability of a *Lysobacter capsici* member to induce resistance mechanisms in grapevine plants	Francesca Brescia
IR.5	Chitosan vs. chitosan nanoparticles in the control *of Fusarium graminearum*: a synergistic effect of fungitoxic activity and plant defence activation?	Franco Faoro
IR.6	Fructose and sucrose as priming molecules against pathogens and pests?	Marie Zimmermann
IR.7	The effect of phosphite on Phytophthora infestans and synergism with conventional fungicides in field-grown potato and tomato in Ethiopia	Erik Alexandersson
IR.8	Role of ferulic acid in Fusarium head blight resistance of wheat spikes	Charlotte Martin
IR.9	The *Reynoutria sachalinensis* knotweed leaf extract elicits defence responses in *Cucurbita pepo* plants against *Podosphaera xanthii*	Emilia Markellou
IR.10	The allelopathic potential of gramine in barley	Mauro Maver
IR.11	Allelopathic effects of *Crotalaria juncea* and dimethyl disulfide (DMDS) on tomato plants in the future development of a biocontrol method against root-knot nematodes	Ingrid Arnault
IR.12	Study of the antagonistic effect of *Trichoderma* spp. against *Fusarium* spp. Involved in Fusarium head blight and root rot of wheat	Renane Rachida
IR.13	Effects of β-aminobutyric acid on aphid stylet activities	Glen Powell
IR.14	Disease suppression in eggplant (*Solanum melongena* L.) nurseries carries over to reduced wilt and fruit rot in subsequent plantings	Naznin Nahar
IR.15	Synergising pest deterrence and plant defence induction: a novel integrated pest management system for *Trialeurodes vaporariorum* on glasshouse grown tomato	Niall Conboy

Thursday 19 October	**Poster session 2: Functional ecology of microbial interactions in soil**	
IR.16	Vineyard in-row and cover crop management affects mesofauna composition	Michaela Griesser
IR.17	Vineyard location and vineyard management effects on soil respiration measurements	Astrid Forneck
IR.18	The effect of cover crops in alleviating copper toxicity in grapevine plants	Laura Marastoni
IR.19	Evaluation of the effect of solid and liquid digestate produced in a biogas plant on soil quality and plant growth	Fabio Valentinuzzi

IR.20	Green manure as sustainable tool to microbial diversity in organic vineyards	Claudia Maria Oliveira Longa
IR.21	Assessing the impact of green manure on ecosystem functioning of soil microbial communities	Caroline Provost
IR.22	Soil biota from newly established orchards are more beneficial to early growth of cherry than biota from older orchards	Paige Munro
IR.23	The composition of apple and pear bark microbiota suggest microbial migrations from soil	Elena Arrigoni
IR.24	Effect of different oilseed rape management systems on earthworm community (Oligochaeta: Lumbricidae)	Ivan Juran
IR.25	The effects of plant growth-promoting rhizobacteria (PGPR) on the growth and quality of strawberries	Youry Pii
IR.26	The biological control agent *Pseudomonas chlororaphis* subspp. *aureofaciens* M71 originates natural mutants impaired in the ability to control *Fusarium oxysporum* f.sp. *radicis-lycopersici* on tomato	Gerardo Puopolo
IR.27	Search for plant-based biofungicides against toxigenic contaminants in barley (*Hordeum vulgare* L.) forage in hydroponics	Saida Messgo-Moumene
IR.28	Effect of natural nitrification inhibitors on nitrogen contents in soil and plant growth	Muhammad Aammar Tufail
IR.29	Evaluating the effect of slow releasing polymer coated urea on growth and yield of maize	Muhammad Aammar Tufail
IR.30	Combined use of organic biofumigant materials and a biological control agent: First experience in Switzerland	Vincent V. Michel

Program | Novel tools and new challenges for IPM in viticulture

Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture"

Wednesday 18 October, 2017

8:00	9:00	Registration	
9:00	9:10	Welcome to participants	Gianfranco Anfora and Valerio Mazzoni
9:10	9:30	Opening and IOBC-WPRS presentation	Carlo Duso (convenor), Mauro Jermini (liason officer), Denis Thiéry, Christoph Hoffmann, Michael Maixner, Agnès Calonnec, René Fuchs, Tirtza Zahavi (SB convenors)
9:30	**11:00**	**Session 1: New knowledge and solutions against viruses, phytoplasmas and their vectors: vectors** **Chairs: Mauro Jermini and Gerd Innerebner**	
9:30	10:00	Keynote speaker: Monitoring *Xylella fastidiosa* insect vectors in Northern and Southern California to understand recent Pierce's disease incidence	Lucia G. Varela
10:00	10:20	*Phlogotettix cyclops*: occurrence, infectivity with flavescence dorée and importance as vector in Austrian vineyards	Gudrun Strauss
10:20	10:40	The role of scale insects as vectors of grapevine viroses in German viticulture	Nadine Steinmetz
10:40	11:00	State and development of tools for the Flavescence dorée management in Switzerland	Mauro Jermini
11:00	11:30	Coffee break	
11:30	**13:00**	**Session 2: New knowledge and solutions against viruses, phytoplasmas and their vectors: pathogens and interactions with plant and vectors** **Chairs: Mauro Jermini and Gerd Innerbner**	
11:30	12:00	Keynote speaker: When a Palearctic bacterium meets a Nearctic insect vector: genetic and ecological insights into the emergence of the grapevine Flavescence dorée epidemics in Europe	Sylvie Malembic-Maher
12:00	12:20	The potential use of endosymbiont/endophytic bacteria to reduce yellows disease symptoms in wine grapes	Vered Naor
12:20	12:40	Contrasting susceptibilities to Flavescence Dorée in *Vitis vinifera* rootstocks and wild *Vitis* Species	Sandrine Eveillard
12:40	13:00	New insights into Pinot gris disease and the associated Grapevine Pinot gris Virus	Elisa Angelini
13:00	14:30	Lunch	
14:30	**17:00**	**Session 3: Classical and novel tools against arthropod pests: disruption of insect communications** **Chairs: Denis Thiéry and Sergio Angeli**	
14:30	15:00	Keynote speaker: Mating disruption of vine mealybug, *Planococcus ficus*, using sprayable microensapsulated pheromone in California table grapes	David R. Haviland
15:00	15:20	Year-round mating disruption in vineyards overcomes the vine mealybug (*Planococcus ficus*) population's build-up during the warming winters	Rakefet Sharon

15:20	15:40	Future sustainable IPM in viticulture: electrospun mesofibres as alternative approaches for *Lobesia botrana* pest management	Hans E. Hummel
15:40	16:00	Exploitation of genetically modified *Vitis vinifera* plants with altered kairomone emission ratio for the control of the European Grapevine Moth *Lobesia botrana*	Gianfranco Anfora
16:00	16:20	Vibrational mating disruption of glassy-winged sharpshooter *Homalodisca vitripennis*, vector of *Xylella fastidiosa* in California	Valerio Mazzoni
16:20	16:40	Open-field vibrational mating disruption: the effect on leafhopper pests and the vineyard ecosystem	Rachele Nieri
16:40	17:00	First characterisation of herbivore-induced volatiles released by grapevine (cv. Pinot noir) under attack of *Empoasca vitis* (Hemiptera: Cicadellidae)	Sergio Angeli
17:00	17:30	Poster Flash Talks session 1	
17:30	**19:00**	**Poster session 1** (with drinks) **New knowledge and solutions against pathogens and their vectors**	
19:00	21:00	Aperi-dinner	

Thursday 19 October, 2017

9:00	**13:00**	**Session 4: Classical and novel tools against arthropod pests: *Drosophila suzukii*** **Chairs: Claudio Ioriatti and Silvia Schmidt**	
9:00	9:30	Keynote speaker: Physical barriers against *Drosophila suzukii* in viticulture	Christian Linder
9:30	9:50	*Drosophila suzukii*: important differences in the susceptibility of grape cultivars	Patrik Kehrli
9:50	10:10	Influence of field margins containing blackberries on appearance of *Drosophila suzukii* and infestation of grape berries in adjacent vineyards	Lisa Weißinger
10:10	10:30	Biological control of *Drosophila suzukii* by means of the pupal parasitoid *Trichopria drosophilae*: field and semifield experiences	Marco Valerio Rossi Stacconi
10:30	10:50	Field testing of insecticides against spotted wing drosophila (SWD) in viticulture	Martina Falagiarda
11:00	11:30	Coffee break	
11:30	**13:00**	**Session 5: Ecology and multiple interactions in pest control** **Chairs: Carlo Duso and Christoph Hoffmann**	
11:30	12:00	Keynote speaker: Plant-herbivore interactions in the context of climate change: Effects of elevated CO_2 concentrations on grapevine and European grapevine moth (*Lobesia botrana*)	Annette Reineke
12:00	12:20	Cultural control of *Lobesia botrana* on grapevines	Fatemeh Kiaeian Moosavi
12:20	12:40	Influence of landscape complexity and vineyard management on leafhoppers abundance in North-Italian vineyards	Giulia Zanettin
12:40	13:00	Biological control of the mealybug *Planococcus ficus* in vineyards of North-eastern Italy	Alberto Pozzebon
13:00	14:30	Lunch	

14:30	**17:00**	**Session 6: IPM implementation and tools** **Chairs: Carlo Duso and Christoph Hoffmann**	
14:30	15:00	Keynote speaker: IPM Implementation benefits from the partnership between scientists and growers: a case study in a Tuscan wine-growing area	Andrea Lucchi
15:00	15:20	How spraying technology could reduce pesticides use in vineyards	Xavier Delpuech
15:20	15:40	New tools to improve the sustainability of the vineyards: scouting with 4GRAPES app, cloud processing and real-time visualisation on CARTO	Giovanni Bigot
15:40	16:00	BugMap, a citizen science approach to monitor the spread of the invasive Brown Marmorated Stink Bug *Halyomorpha halys* (Hemiptera: Pentatomidae)	Robert Malek
16:00	16:20	VitiMeteo in Europe - supporting IPM for practitioners	Ronald Krause
16:20	16:40	Forecasting flight activity of *Lobesia botrana*, in the Northeast of Portugal, using a degree-day model	Cristina Carlos
16:40	17:00	VIVA indicators: the global approach to assess, improve and communicate grape-wine sustainability in Italy	Gloria Luzzani
17:00	17:30	Poster Flash Talks session 2	
17:30	**19:00**	**Poster session 2 (with drinks)**	
19:00	22:00	Social dinner and poster awards	

Friday 20 October, 2017

9:00	**13:00**	**Session 7: Classical and novel tools about pathogens** **Chair: Agnes Calonnec and Tirtza Zahavi**	
9:00	9:30	Keynote speaker: Analysis of the predominant factors generating various epidemic profiles on resistant varieties	Agnes Calonnec
9:30	9:50	Anti-fungal capacities of ozone dissolved into water against fungi associated with Grapevine Trunk Diseases	Marielle Pagès
9:50	10:10	The FEM grapevine breeding program for pathogen resistances: towards a sustainable viticulture	Silvia Vezzulli
10:10	10:30	Microbial ecology of resistant grapevine genotypes sheds light on biocontrol prospects of *Plasmopara viticola*	Christina Morauf
10:30	10:50	Ontogenetic resistance of grapes: A chance to reduce fungicides in vineyard and residues in wine?	Karl Bleyer
10:50	11:20	Coffee break	
11:20	11:40	Biomarkers identification in 'Bianca' grapevine leaves after *Plasmopara viticola* infection	Giulia Chitarrini
11:40	12:00	Impact of elevated CO_2 concentration on interactions between *Vitis vinifera* L. and *Plasmopara viticola*, the causal agent of downy mildew	Moustafa Selim
12:00	12:20	Botrytis bunch rot control in South-West France vineyards using a novel bacterial biological control agent and two biocontrol registered products	Carlos Calvo-Garrido
12:20	12:40	Endophytic yeasts in shoots of healthy and esca diseased grapevines	Manuela Steiner

12:40	13:00	Closing remarks	Carlo Duso, Denis Thiéry, Christoph Hoffman, Michael Maixner, Agnès Calonnec, René Fuchs, Tirtza Zahavi
13:00	14:00	Lunch box	
14:00	**21:00**	**Excursion and dinner**	

Poster sessions and Poster flash talks

Wednesday 18 October	**Poster session 1: New knowledge and solutions against pathogens and their vectors**	
VIT.1	Distribution of symptoms associated to GPGV and its potential vector *Colomerus vitis* in North-eastern Italy	Carlo Duso
VIT.2	Grapevine virus diseases in South Tyrol – a survey	Gerd Innerbner
VIT.3	New acquisition about the role of *Colomerus vitis* in the transmission of Grapevine Pinot gris virus	Valeria Malagnini
VIT.4	Three years experience in France using PreDiVine DSS analysing 25 vineyards for *Scaphoideus titanus* monitoring	Mauro Jermini
VIT.5	Longevity and reproductive profile of *Scaphoideus titanus* Ball adults reared under controlled conditions	Mauro Jermini
VIT.6	Comparative transcriptome profiling of two *Vitis vinifera* varieties with different level of resistance/susceptibility to Flavescence dorèe	Elisa Angelini
VIT.7	Development of a new strategy for the control of Flavescence dorée disease based on a multiannual model of *Scaphoideus titanus* and a landscape analysis	Mauro Jermini
VIT.8	Mating behavior and vibrational communication of the meadow spittlebug *Philaenus spumarius*	Sabina Avosani
VIT.9	Management of Downy Mildew (*Plasmopara viticola* Berk. & Curtis, Berk. & De Toni) by reducing copper applications through BCAs treatments and new copper formulations	Davide Mosetti
VIT.10	Insights into the genetic bases of downy mildew resistance and polyphenol induction in a grapevine inter-specific segregating population	Giulia Malacarne
VIT.11	Effect of weather on appearance of grape downy mildew in Israel	Tirtza Zahavi
VIT.12	Insight into the mechanism of conidial germination of the powdery mildew mycoparasite *Ampelomyces quisqualis*	Dario Angeli
VIT.13	Are Swiss fungal resistant varieties appropriate for growth under the soil and climatic conditions of Quebec, Canada?	Caroline Provost
VIT.14	How vineyard features and application time may affect the phosphonates residues in the bunches	Oscar Giovannini
VIT.15	VITIFUTUR – A transnational platform for applied research and further education in viticulture	Alexandra Wolf
VIT.16	Grapevine protection: from proof of concepts to pre-industrial biofungicides	Oscar Giovannini
VIT.17	Residents non-dietary pesticide exposure risk perception survey: knowledge gaps and challenges for targeted awareness-raising material development	Maura Calliera

VIT.18	The SUDOE "VINOVERT" project: potential of pesticide use reduction in three South-West European vineyards regions	Marc Fermaud
VIT.19	Roll-out of IPM in Belgian viticulture	Kris Vandenwyngaert
VIT.20	Grapevine trunk diseases: the relevance of disinfection of propagation material	Laura Mugnai

Thursday 19 October	**Poster session 2: IPM implementation and tools against arthropod pests**	
VIT.21	Emulpar' 940 EC as a mechanical natural product in two-spotted spider mite (*Tetranychus urticae* Koch.) control on berry crops	Wojciech Piotrowski
VIT.22	Impact of leaf removal, copper and kaolin on grape skin thickness in order to reduce *Drosophila suzukii* infestation	Michael McGeary
VIT.23	Entomopathogenic fungi as control agents for *Drosophila suzukii*	Elisabetta Gargani
VIT.24	Management of *Linepithema micans* and *Eurhizococcus brasiliensis* in new vineyards	Marcon Botton
VIT.25	Evaluation of arthropod biodiversity in the vineyards of the Centre-Val de Loire region	Ingrid Arnault
VIT.26	Efficacy of kaolin against *Empoasca vitis* in vineyards	Federico Tacoli
VIT.27	Berry skin resistance explains oviposition preferences of *Drosophila suzukii* best in different pre-damaged grapevine cultivars	Wiebke Entling
VIT.28	Compensation effects induced by grape phylloxera on root growth, leaf respiration and sink activity in *Vitis* ssp.	Markus Walter Eitle
VIT.29	Preliminary analysis of arthropod aggregation overwintering in vineyards with different management	Vincenzo Verrastro
VIT.30	*Drosophila suzukii* oviposition behavior on wine clusters	Valerio Mazzoni
VIT.31	Does *Drosophila suzukii* represent an additional factor of risk of sour rot disease development in wine grape?	Franca Ghidoni
VIT.32	Oviposition deterrent effects of particle films and entomopathogenic fungal strains against *Drosophila suzukii*: preliminary laboratory assays and field trials	Nuray Baser
VIT.33	Morphological study of the antennal sensilla of the invasive *Halyomorpha halys* Stål (Hemiptera: Pentatomidae)	Aya Ibrahim
VIT.34	The use of biorational compounds for vineyards protection against pests	Milka Glavendekic
VIT.35	Is *Drosophila suzukii* ovipositor involved in chemoreception?	Cristina Maria Crava
VIT.36	Assessment of capture efficacy of *Drosophila suzukii* (Matsumura) trapping devices in mass trapping and release recapture trials	Silvia Schmidt
VIT.37	Rearing *Campoplex capitator* in Italy and in Chile: preliminary achievements	Andrea Lucchi
VIT.38	Five-year analysis of population dynamics in *Drosophila suzukii*: usefulness of monitoring traps and their relevance for viticulture	Niklas Samuel
VIT.39	Beneficial insects associated with *Lobesia botrana* in vineyards	Milka Glavendekic
VIT.40	Circadian densities of mites on vine leaves in Montalcino area (Tuscany, Italy)	Sauro Simoni

VIT.41	Assessment of new trap designs and liquid baits improved with lactic acid bacteria for capturing *Drosophila suzukii* Matsumura	Giuseppe Maddalena
VIT.42	On the importance of the sister-species comparison in agricultural pest science and IPM: *Drosophila subpulchrella* as a case study	Omar Rota-Stabelli

ABSTRACTS

Conference sponsored by the OECD's Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems

Future IPM 3.0 towards a sustainable agriculture

OECD
BETTER POLICIES FOR BETTER LIVES

Integrated pest management concepts and implementation: state of the art and opportunities for the future

Carlo Malavolta

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Highlights

An analysis of Integrated pest management (IPM) and integrated production concepts and implementation is presented, based on state of the art in comparison with IOBC Integrated production principles and of some National action plans approved in application of Directive on sustainable use of pesticides.

Possible improvement steps in implementation are described and proposed, including possible support systems and opportunities for the future in terms of legislation and financial support.

The importance of networking among stakeholders and cross-sector contamination for sustainable crop protection

Sylvia Blümel

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Highlights

The successful implementation of sustainable crop production including integrated pest management should consider and bring together the perceptions and contributions of a broad range of different stakeholders, which can be especially achieved by appropriate networking. Although the identified pertinent innovation needs and challenges for crop production, product processing and marketing along the food chain are of primary importance, the significance of training, knowledge exchange and communication for a contemporary cross-sector transfer, which requires innovative multi-actor approaches, is well recognized. Particularly with regard to the Directive 2009/128/EC "Sustainable Use of Pesticides" several initiatives and projects within Europe, mainly granted by the EC (e.g. ENDURE http://www.endure-network.eu/, EIP-AGRI https://ec.europa.eu/eip/agriculture/ or C-IPM http://c-ipm.org/) were carried out or are in progress in order to connect in different ways and complexity the multitude stakeholders such as farmers, advisory/extension service and researchers, but also food processors, retailers, consumer protection organisations and NGOs. It should be mentioned that the first concept of a holistic Integrated Crop Production in Europe was developed in the late 1980s by the IOBC. The presentation will introduce and compare different types and levels of networking of some of the existing initiatives and projects.

The fresh fruit and vegetable sector from a sustainable supply chain perspective

Helene Deruwe

Agriculture and Research Policy Officer - Freshfel Europe, Belgium
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Highlights

Agriculture takes up 40% of all land surface and is the third largest emitter of total global greenhouse gases. Sustainability is not just a buzz word anymore, and it expanded beyond a merely political discussion. It is a reality for farmers and business along the whole food supply chain, and it becomes a license to produce and trade. The growing attention to sustainability from consumers, customers, NGOs, media, and politicians led to regulatory and non-regulatory tools and a diversity of rules, schemes, and certifications, in its turn leading to confusion and extra costs. Additionally, different stakeholders come up with different initiatives that target different difficulties and hotspots. In her presentation, Helene will talk about the different policy initiatives on European level, with 2 case studies from the Member States, the different certification schemes and standards, social and environmental compliance, sustainable lifestyles, and innovation and technology.

The challenge of developing microbial biocontrol products for disease control

Jürgen Köhl

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Highlights

Commercial biological control products for the use against plant diseases must be highly efficient against the targeted diseases. However, the used antagonists have to fulfil a broad range of additional requirements regarding market sizes for the envisaged products, ecological characteristics and production costs of the antagonists, safety, toxicological and eco-toxicological risks and protection of intellectual property rights. Consequently, a broad range of criteria has to be considered during the selection of new antagonists. Screening programs can use a stepwise approach to assess candidate antagonists for this broad range of criteria in order to exclude unwanted candidates in an early stage. Essential decisions at the beginning of new screening programs are to collaborate with biocontrol industries from the beginning, to include relevant commercial questions early during the screening program and to combine the expertise in plant pathology with expertise in biotechnology, agronomy, microbiology, toxicology, registration, marketing and product development. Examples of screening programs for the development of new biocontrol products against apple scab and powdery mildew (www.biocomes.eu) will be discussed.

The project BIOCOMES has received funding from the European Union's Seventh Framework Programme (grant agreement 612713).

Biocontrol with beneficials insects and new perspectives in insect biocontrol

Felix Wäckers

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Highlights

The concept and practice of biological pest control goes back almost two millenia. The largescale release of mass reared biocontrol agents to control agricultural pests was developed almost a century ago. As an effective alternative to chemical control it has increased steadily. Increasing demands from consumers, supermarkets and the food industry for healthy and safe food; decreasing availability of pesticides; and pesticide resistance issues are important drivers underpinning the strong growth of biological pest control.

The research focus has long been on the development of new organisms for the control of existing and novel pests. This has resulted in an ever increasing portfolio of natural enemies being available on the market.

Recently, research has started to also focus on developing new concepts and strategies to make existing biocontrol agents act more effectively. In my presentation, I will cover examples of these new strategies, including the use of food supplements to allow preventative establishment of biocontrol agents before the pest arrives; the use of fibers to provide oviposition substrates for predatory mites and "Flying Doctors", a system to utilize bumblebees to disperse antagonists for efficient biological control of flower associated pathogens and pests.

Sustainable weed management, what's next after glyphosate?

Micheal D.K. Owen

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Highlights

There is a critical need to adopt diverse tactics beyond herbicides, to manage weeds and mitigate herbicide-resistant weeds evolution. Herbicides have been the primary approach to weed management for decades and the burgeoning issues of evolved herbicide resistances in key weeds reflects agricultural systems where herbicides have been the principle control tactic. The inclusion of alternate strategies for weed control has declined steadily and the loss of weed management diversity resulted in evolved resistance to a number of herbicides. Herbicide resistance and weed management issues exist not only in the USA but also throughout the world. Glyphosate was used in a majority of the row crop acres in the USA. There are many reasons and justifications for this including time management efficiency, cost, effectiveness, and the simplicity and convenience of weed control. The ecologically narrow focus of this approach has resulted in widespread evolved resistance to glyphosate to the extent that it should be clear that weed management in row crops is not sustainable if based primarily on a single herbicide. However, herbicides will continue to play a significant role in the weed management. Cultural and biological tactics will be important components of successful weed management programs in the future.

Sensors, drones and precision agriculture for sustainable production

Rino Goller and Maurizio Barazzuol

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Highlights

Today we have the possibility to integrate a huge quantity of information, quantitative information and qualitative information, time series related to meteorological data, various type image (multispectral, thermal, etc), with UAV can extend the samples of territories portion analyzed. We can combine these pieces of information with the theoretical studies to create models to improve the decision about the entire cycle of production but the results are in any case static. Using the Artificial Intelligence (Neural Network, Deep Learning, Data Mining, etc) we can transform the static model in a dynamic model. The dynamic model can adapt the strategies during the environment change and not only after. We have in front of us a continuous change, climatic change, law change, genetic adaptation or resistance, with a static model we arrive every time late, we have to change the perspective.

Unearthing plant-beneficial traits of the root microbiome for sustainable crop protection

Corné Pieterse

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Highlights

Plants nurture a large community of plant growth-promoting rhizobacteria (PGPR) that provide them with essential services, such as enhanced mineral uptake, growth promotion, and protection from pathogens. These plant microbiota are predominantly hosted by the root and can be selected for by the plant, e.g. via root exudates. Our research is focused on understanding plant-beneficial functions that are encoded by the root microbiome and the role of plant genes that facilitate these functions. In recent years, we demonstrated that in response to foliar pathogen infection, Arabidopsis roots recruit a specific consortium of synergistic microbes to their rhizosphere that in turn trigger a broad-spectrum induced systemic resistance (ISR). We discovered that such PGPR-ISR is associated with priming for accelerated defense-related gene expression, which only becomes apparent after pathogen attack, providing a cost-effective mechanism of protection. In contrast to leaves, roots reprogram the expression of a large set of genes in response to colonization by PGPR. We identified the root-specific transcription factor MYB72 as a central regulator of the onset of ISR. Current research is focused on understanding early root-microbiome interactions with the ultimate goal to develop future crops that are better able to maximize profitable functions from their root microbiome.

Landscape management and preservation of biodiversity

John M. Holland

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Highlights

Birds and invertebrates are two taxa that have declined in recent decades attributed to intensive agricultural practices. The 47-year long Sussex study run by the Game and Wildlife Conservation Trust showed that many arthropod taxa have declined and this has been attributed mostly to the direct and indirect effects of pesticides. This has had consequences for animals higher up the food chain, especially so for birds. Agri-environment schemes have been deployed to some extent to help reverse declines. Assessments of individual agri-environment and semi-natural habitats show their value to beneficial invertebrates, which is typically driven by their plant composition, however their impact at local and landscape scales will depend on how they are deployed. Birds and pollinators respond positively to increases in the proportion of semi-natural habitats. Flower-rich habitats are highly attractive to pollinators and can enhance abundance at farm-scales, however, landscape maps generated in QuESSA reveal gaps in our landscapes. Likewise, pest natural enemies show heterogeneous distributions at local to landscape levels which may be attributed to lack of semi-natural habitats. Local management practices and the type and proportion of semi-natural habitats can be used to increase levels of biocontrol.

Sustainable production faces the challenge of invasive species

David Maxwell Suckling

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Highlights

nvasive species are a construct of our time, being the re-adjustment of a man-made biogeographical discontinuity, from plants moved around the world without some or most of their parasites, to a future with many more widely distributed herbivores. In fact, the all trophic levels above and below our valued plants are experiencing rapid change and stemming the tide of ecological change is proving very challenging to many countries. Arrival of new key pests can redefine whole IPM programs and consign years of research investment to history when new pests require reintroduction of broad-spectrum insecticides for control. Initial high crop losses from new alien invasive species, summed with historical pest damage from existing pests increasingly threatens the viability of food production. Adaptive responses to this changing landscape of increasing pest complexity in production ecosystems need to consider ecosystem services such as pollination and biological control, as well as biotechnical and cultural controls where needed, to reduce reliance on pesticides. More sustainable systems would be expected to have more sources of mortality for key pests, acting in concert. They would also be expected to mitigate biosecurity risks, which varies in complexity between jurisdictions, and is strongly influenced by neighbouring jurisdictions.

Meeting of the IOBC-WPRS Working Groups
"Induced resistance in plants against insects and diseases" and
"Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

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Oral Session 1

Novel and old players in plant-microbe interactions

An active starch degradation metabolism provides sugars for callose priming during *Plectosphaerella cucumerina* infection

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Highlights

- Plants soil drenched with indole-3-carboxylic acid display callose priming preceded with a more active starch catabolism.
- This process is mediated by the BAM1 amylase and impaired in the *bam1* mutant
- Vesicular trafficking directed by ATL21 and SYSP121 is also more active upon I3CA treatments
- This suggests that BAM1, ATL13 and SYP121 are relevant components of the pathway of callose priming

Introduction

Indolic derivatives mediate plant resistance against specific biotic challenges. Trp derivatives, such as indol-3-carboxyaldehyde (ICHO) and indol-3-carboxylic acid (I3CA; Gamir et al., 2014), increase upon infection by pathogens (Böttcher et al., 2014; Gamir et al., 2014).

Treatments with beta-aminobuyric acid primed the indole I3CA in Arabidopsis infected with *Plectosphaerella cucumerina* (Gamir et al., 2012). This compound followed a priming behavior when different priming stimuli were used, and application of this metabolite induces resistance against fungi (Gamir et al., 2014). However, I3CA may participate in defence signalling or activation since it does not display direct antifungal effect on *P. cucumerina*.

Material and methods

Experiments with adult plants (five-week old plants) were soil-drenched with 5 ml of water as a mock treatment (control). I3CA treatment was soil-drenched with a 150 µM final concentration 48 h prior infection. Plants were challenged by 6 µl drops of 5×10^6 spores/ml of *P. cucumerina*.

Aniline blue staining was used to determine callose levels as described in Luna et al. (2011). Callose was quantified in micrographs using GIMP (2.6.12) software.

Gene expression by quantitative real-time PCR (RT-Q-PCR) was performed using RNA samples extracted from leaf tissue using Trizol. The RT reaction was performed following reverse transcriptase instructions of the trademark kit (Takara).

The 35S:BAM1-YFP constructs were recombined into pDONR201/207 using BP ClonaseMix II kit (Invitrogen). After sequencing, all constructs were recombined into pEarleyGate101 destination vector using LR ClonaseMixII kit (Invitrogen) and introduced into Col-0 plants (Wild type) or *bam1* plants for complementation via *Agrobacterium* transformation.

Results and discussion

In the present study, we profile the pathway of callose priming. Following I3CA treatments abscisic acid (ABA) is accumulated, this activates a program of starch degradation that releases sugars that will be transported likely through the vesicular system to the cell wall. At the cell wall, these sugars will be assembled in a more efficient manner upon *P. cucumerina* infection providing an effective defense. This clearly states a link between ABA, starch degradation and callose deposition in defence priming. Using I3CA as a priming stimulus, we demonstrate that ABA positively regulates starch catabolism to trigger augmented callose deposition upon infection with *P. cucumerina*. The ABA deficient mutant *npq2* is impaired in I3CA-IR and callose priming. Another hormonal signal that may mediate I3CA could be the jasmonic acid (JA) and salicylic acid (SA). JA is linked before with ABA in callose accumulation following an infection. In our study, we found a simultaneous induction of both ABA and JA but not SA in I3CA-treated plants. After infection of control plants, both hormones reached the levels observed in I3CA-treated plants suggesting that I3CA is preparing the plant to react faster before the infection. Although both hormones are good candidates to mediate I3CA-IR, the protection by I3CA is intact in JA-impaired mutants. Following treatments with I3CA the *bam1* gene expression is induced. To test its relevance in the priming mechanisms, the mutant *bam1* that is impaired in a stress-related amylase, was treated with I3CA. The *bam1* mutant was impaired in I3CA-IR and accordingly did not display callose priming. The relevance of *bam1* as a key gene in I3CA-IR was demonstrated by complementation of the *bam1* mutant. The 35S:BAM1 expression lines in the *bam1* background demonstrated restored sensitivity and I3CA-IR, as well as showed elevated levels of callose upon infection. Confocal microscopy confirmed that BAM1 is located at the chloroplasts.

I3CA treatments activate *ATL31* and *SYP121* gene expression upon challenge, providing an appropriate cellular environment for faster callose accumulation and primed Arabidopsis defence against *P. cucumerina*.

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Effect of drench application of biocontrol preparations on tomato plants against *Botrytis cinerea* and *Oidium neolycopersici*

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Highlights

- Drench application of biocontrol products was evaluated for protection of tomato against *Botrytis cinerea* and *Oidium neolycopersici*
- Powdery mildew was significantly reduced by the biostimulant EUCLID-1-ANT and slightly reduced by sucrose and fructose
- At a higher dose, EUCLID-1-ANT significantly reduced the development of both pathogens but had negative side effects on plant growth

Introduction

Most fresh market tomato production in Europe is done in greenhouses equipped with drip irrigation systems. In such conditions, resistance-inducing preparations can be efficiently delivered to the root system to protect the aerial parts of the plants against pests and diseases. On tomato, two drench applications of *Trichoderma harzianum* or benzothiadiazole (BTH; Meller Harel et al., 2014) or one of β-aminobutyric acid (BABA; Bruce et al. 2017) were shown to reduce the development of *Botrytis cinerea* following its inoculation on detached leaves. These results raise interest in the use of such methods in greenhouse production for the protection of pruning wounds against *B. cinerea* and of the whole canopy against other foliar pathogens.

The objective of this study was to evaluate the protective potential of drench application on tomato plants in greenhouse conditions. Different preparations with putative resistance-inducing properties were tested for their effect against *B. cinerea* and *Oidium neolycopersici*.

Material and methods

Tomato plants var. Monalbo were produced during 7 weeks on rockwool cubes in a heated glasshouse. Plants were fertigated with a standard nutrient solution through a drip irrigation system. Sugars (fructose, sucrose, trehalose), plant extracts and microorganism-based products (Regalia, Serenade, Md-L13), a biostimulant (EUCLID-1-ANT) and a compost (EUCLID-2-ANT) were applied weekly as a drench (5 ml/treatment) for 5 weeks. Water was used as a control. Plant growth was assessed 7 weeks after sowing by measuring plant height, as well as stem and petiole diameters. To test the protective effect against *B. cinerea*, three leaves per plant were removed, leaving 10 mm petiole stubs on the stems. Petiole stubs were inoculated with 10 µl of a spore suspension of strain BC1 adjusted to 10^6 spores/ml. Lesion expansion on the stem was recorded from the 4^{th} to the 7^{th} day after inoculation and AUDPC was calculated. To test the efficacy of treatments against *O. neolycopersici*, a spore suspension adjusted to 2×10^3 spores/ml was sprayed on the plants. Disease severity (number of pustules/leaf area) was estimated 10 days after inoculation on two leaves per plant. Inoculated plants were randomly distributed in controlled growth chambers with climatic conditions conducive to the development of both pathogens (21°C, HR > 80%). Five plants per replicate were evaluated for each treatment and the whole experiment was repeated three times.

Results and discussion

With the exception of the biostimulant EUCLID-1-ANT, applied at a high (3%) concentration, no protective effect against *B. cinerea* was observed with any of the preparations.

A slightly but consistent (up to 20%) reduction in *O. neolycopersici* development was observed with sucrose, fructose and EUCLID-1-ANT applied at a low (0.1%) concentration. When applied at a high (3%) concentration, the efficacy of EUCLID-1-ANT reached 48% but strong negative side effects were observed on plant growth. No effect was observed with any of the other preparations.

Work is in progress to compare the efficacy of the preparations when applied as a foliar spray. Further experiments will also be carried out to explore possible differences among varieties in the efficacy of foliar- or drench-applied preparations against *B. cinerea* and *O. neolycopersici*, as well as the influence of N fertilisation of the plants. Field work is also needed to validate the present results for the biostimulant EUCLID-1-ANT and to evaluate possible effects on other diseases and pests, as well as on the yield and quality of the crop.

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Sugar homeostasis mediates arbuscular mycorrhizal fungi-induced resistance against *Botrytis cinerea*

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Highlights
- Callose deposition is a likely mechanism of defence mediating MIR
- AM plants showed a priming profile of callose deposition due to a more activated carbohydrate metabolism which enhance sugar homeostasis in these plants
- SNARE-mediated vesicular trafficking also play a key role in the callose deposition pathway

Introduction

Plant's immune system can be enhanced upon an appropriated stimulus. Beneficial microorganisms, as arbuscular mycorrhizal fungi (AMF), can stimulate the plant immune system, a process known as mycorrhiza induced resistance (MIR) that is considered a specialised induced systemic resistance (Mauch-Mani et al., 2017).

One of the first layers of plant defence against fungal attack is the formation of a polymer of callose which is accumulated in the cell wall to form together with other components the papillae. This is a physical barrier to prevent the penetration of fungal pathogens (Luna et al., 2011).

During MIR, the AMF produce changes in the plant's carbohydrate metabolism which allows the fungus to acquire nutrients. The aim of our investigation is to determine whether this mobilisation of sugars can be used by mycorrhized plants in a faster callose deposition upon fungus attack. Moreover, we hypothesise that vesicular trafficking is important in callose deposition (Maekawa et al., 2014).

Material and methods

In this research study, we have studied the effect of MIR in tomato plants (var. Better Boy) upon a necrotrophic fungus (*Botrytis cinerea*) infection. Two weeks after tomato plants germination, plants were mycorrhized with the arbuscular mycorrhiza fungus *Rhizophagus irregularis*. Four weeks later, when mycorrhiza was correctly established, plants were infected with *B. cinerea*. Samples for gene expression analyses and for callose quantification were collected after 72 h of infection.

RNA extraction and quantitative real-time PCR reactions were performed as described by Sánchez-Bel et al. (2016) using primers for the amplification of sugar transporters (*SUT2* and *SUT4*), sucrose synthases (*SUS*), invertases (*LIN6*), amylases (*BAM1*) and the callose synthase (*GLS5*). We have also studied the gene expression of *SYP121* and *ATL31*, both genes important in the fungal penetration resistance.

Two treatments were used one day before the infection to determine the importance of the callose synthase PMR4 (2-deoxy-D-glucose, 2DDG 1 mM) and to determine the relevance of vesicular

trafficking (Brefeldin A, BFA 100 µg/ml) in callose deposition. Inoculation was applied in a drop. Plants were sampled 72 h after infection to study callose content and lesion diameter.

Results and discussion

We hypothesise that starch can be a source of sugars for a faster callose deposition. Mycorrhized plants showed an enhanced β-amylase 1 gene expression (*BAM1*) which is the main responsible for starch degradation unrelated to circadian rhythm. After starch degradation, BAM1 releases manose that is subsequently transformed into free glucose. This free glucose can be used by the callose synthase (PMR4) in the callose deposition.

Accordingly, mycorrhized plants showed an enhanced expression of the genes codifying for the sucrose transporters SUTs and the sucrose synthases SUSs irrespective to infection. Interestingly, AM plants also showed an upregulated jasmonate (JA)-dependent invertase (*LIN6*), which hydrolyses the sucrose transported to the apoplast in the plant-fungus interface. All these results suggest that changes triggered by the fungus in the plant sugar metabolism may have an impact on defence by activating a more efficient starch hydrolysis that generates higher rates of free monosaccharides for a more efficient callose synthesis.

Vesicular trafficking was suggested to play a role in the callose deposition in Arabidopsis. The callose synthase and sugar monomers are transported within the cell by actin-dependent vesicle trafficking. The fusion of these vesicles with the plasma membrane is mediated by the SNARE complex. A Q-SNARE protein, SYP121, and a ubiquitin ligase, ATL31, are relevant in callose deposition trough the callose synthase PMR4 in the fungal penetration sites. In our study, we also profiled the gene expression of *SYP121* and *ATL31* during MIR. *ATL31* shows an enhanced expression in mycorrhized plants, however *SYP121* has only an enhanced expression in mycorrhized plants upon infection.

Regarding PMR4 gene expression, it was upregulated in mycorrhized plants compared to control plants with and without infection. Treatments with BFA and 2DDG showed a deformed callose, which indicates that vesicular trafficking and PMR4 are necessary for a correct callose deposition.

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Arbuscular mycorrhizal fungi induced systemic biocontrol against root-knot nematode on chilli

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Highlights

- Root-knot in chilli roots caused by nematode (*Meloidogyne incognita*) is a serious problem for chilli crops production in Thailand. Managing nematode by the chemical use is severely harmful either to farmers or other food chain consumers
- The search and use of microbial as biocontrol agents, especially arbuscular mycorrhizalfungi, could be an alternative for the nematode management

Introduction

Arbuscular mycorrhiza fungi (AMF) colonise roots of most plant species. AMF and plants live in a symbiotic relationship where both partners derive benefits from the association. Mycorrhizal root colonisation has great potential as a biocontrol agent against a broad range of soil-borne fungi and nematodes, however, only a few studies so far examined AMF-plant parasitic nematode interaction in a systemic bioprotective effect of mycorrhizal root colonisation. Therefore, the aims of this study were to investigate whether a systemic bioprotectional effect of AM on parasitic nematode depends on the degree of root colonisation by the AMF and whether this systemic bioprotectional effect can be linked to the accumulation of salicylic acid (SA).

Material and methods

In the experiments, 3 AMF inoculums; *Funneliformis mosseae*, *Rhizophagus irregularis* (Obtained from INOCULUMplus, France), and the mix strains inoculum (consisting of *Glomus* spp., *Acaulospora* spp., *Gigaspora* spp., and *Scultellospora* spp.) (Obtained from Department of Agriculture, Ministry of Agriculture of Thailand), were performed with chili split-root systems. The different strains of AMF inoculation were applied prior to or after or simultaneous to one side of the split-roots and the other side of split-roots was inoculated with 1000 eggs/ml of the egg nematode solution.

Results and discussion

Root infection by root-knot nematodes was systemically reduced when chili plants showed high degrees of mycorrhizal root colonisation, whereas a low mycorrhizal root colonisation exhibited no effect on root-knot nematode infestation. From the results, a clear systemic bioprotectional effect depending on the degree of root colonisation by the mycorrhizal fungus was shown. At a higher mycorrhizal colonisation rate, the concentration of SA was increased in roots colonised by the mycorrhizal fungus but no systemic increase of SA could be measured in non-mycorrhizal roots of mycorrhizal plants, indicating that the systemic bioprotectional effect against root knot nematodes is not mediated by SA.

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The effect of β-aminobutyric acid in the protection of tomato harvest against *Botrytis cinerea*

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Highlights
- Soil drenching seedlings with β-aminobutyric acid (BABA) induced post-harvest resistance in tomato fruit against *Botrytis cinerea*
- Yield was not reduced, but fruit ripening was delayed and metabolomic differences in fruit were found. Also, traces of BABA were identified in the fruit
- Abscisic acid (ABA) has a complex role in the BABA-induced resistance phenotype.

Introduction

Tomato, like other cropsn 2, suffers from substantial yield losses due to diseases. *Botrytis cinerea* (grey mould) can cause the loss of over 50% of the annual tomato harvest (Gianessi and Reigner, 2006). This pathogen is particularly devastating as in addition to green tissue it also infects fruit, resulting in extensive post-harvest losses. Novel disease control techniques are essential to achieve a sustainable tomato industry. Plants have sophisticated defence mechanisms which can be enhanced to better resist diseases (Mauch-Mani et al., 2017). Hence, research focused on the tomato immune system provides a potential source of novel control techniques. β-aminobutyric acid (BABA) effectively induces resistance against *B. cinerea* in tomato plants (Luna et al., 2014) and different application methods can provide long-lasting protection (Luna et al, 2016). Here, we have examined whether treatment of tomato plants with BABA at different developmental stages results in a durable induced resistance in tomato fruit.

Material and methods

Tomato plants of the variety micro-tom were grown under controlled environment conditions for 12 weeks. BABA treatments were administered by soil-drenching at different developmental stages: seedlings ("BABA Seedling"), following fruit production ("BABA Green") and when plants had ripened their fruit ("BABA Red"). Seedling treatments were executed with a concentration of BABA of 0.5 mM, whereas "BABA Green" and "BABA Red" treatments were with 1 mM of BABA. Fitness parameters (fruit number, ripening, size and water content) were assessed at different times during the 12 weeks of growth, as described in Wilkinson et al. (2017). *B. cinerea* infection was performed by drop inoculating tomatoes with 5 µl of inoculum at a concentration of 5×10^4 spores/ml. The disease was analysed by measuring lesion diameter and visual fungal colonisation, as described in Wilkinson et al. (2017). Untargeted metabolomics and targeted hormone analysis were performed as described in Wilkinson et al. (2017) and Petriacq et al. (2016), respectively. Abscisic acid (ABA) was applied to the fruit after harvest, with a concentration of 100 µM supplemented with 0.01% (v/v)

Silwet L-77 to ensure even application across the fruit. BABA quantification in the fruit was done as described in Wilkinson et al. (2017).

Results and discussion

Tomatoes produced by plants which had been treated with BABA at the seedling stage were more resistant to *B. cinerea* than those produced by the controls. Thus BABA-IR is capable of protecting tomato fruit post-harvest. Fruit from the "BABA Green" and "BABA Red" treatments did not show statistically significant differences in resistance compared to the water controls. This illustrates that BABA-IR in fruits is not effective when plants are treated after the onset of fruit production.

Costs to yield or other fitness parameters were investigated following treatment with BABA at different developmental stages. BABA treatments did not trigger an alteration in yield, size or water content. However, fruit from the "BABA Seedling" and "BABA Green" treatments were delayed in ripening. Also, we quantified BABA content in harvested red fruit from all treatments. BABA was not detected in the fruit of either water controls or the "BABA red" treatment. It was however detected in tomatoes from plants of the "BABA Seedling" and "BABA Green" treatments. Hence this indicates that not only is BABA translocated from the vegetative tissue into fruit but also that BABA is metabolised slowly.

Follow-up analysis focused on plants treated with BABA or water at the seedling stage. Untargeted metabolomics demonstrated a long-lasting re-orchestration of plant metabolic profiles in tomatoes after chemical treatment by BABA. Moreover, we performed a targeted analysis of key defence hormones. The only hormone that differed significantly between treatments was ABA, with double the amount accumulated in the fruit of "BABA Seedling" plants relative to that of the controls. To further the knowledge of the role of ABA in BABA-IR, fruit from plants treated with water or BABA at the seedling stage were sprayed post-harvest with water or ABA one day before infection. Interestingly, ABA induced susceptibility in the fruit of control plants. However, this susceptibility phenotype was absent in the fruit of "BABA Seedling" plants, therefore providing further evidence of the role of ABA in BABA-IR post-harvest. The BABA-dependent role of ABA in the induced resistance could arise from BABA's ability to prime multiple defence processes that are regulated by complex interacting signalling pathways.

Overall, we have demonstrated that BABA induces post-harvest resistance in tomato fruit against *B. cinerea* with no penalties in yield. Future work is required to dissect the exact role of ABA in BABA-IR in tomato fruit.

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Systemic immunity in wheat is activated by the deployment of transcription factors

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Highlights

- *Pseudomonas* spp. (Ps) infection in wheat induces systemic immunity against *Xanthomonas translucens* pv. *cerealis* (Xtc). RNA-seq analyses revealed that Ps potentiates defence by the accumulation of ERF and WRKY transcription factors (TFs)
- Additionally, Ps 'primes' a subset of genes regulated specifically when challenged by Xtc, including a *Triticum aestivum* Ps-primed (*TaPsP*) TF

Introduction

Defence priming is a plant response induced upon treatment of a priming stimulus, which maintains a state of immune readiness without sustaining the cost of an active defence response. On the event of a subsequent infection, primed plants surmount a faster and stronger defence response (Martinez-Medina et al., 2016). Of the different forms of primed responses known in plants, systemic acquired resistance (SAR) is characterised by global immunity in distal leaves of plants following a localised infection. The initial infection thus acts as the primary stimulus which triggers the emanation of long distance signals to distal plant parts. Here we show that *Pseudomonas* spp. (Ps) inoculation in first true leaves of wheat induces SAR-like systemic immunity against the subsequent challenge of *Xanthomonas translucens* pv. *cerealis* (Xtc). We aimed at defining the Ps triggered molecular components required for priming in wheat. Identification of molecular factors inducing priming will help in the designing of yield intensive crop protection measures in future.

Material and methods

All infection and immunity assays were performed according to Dey et al. (2014). Phloem exudates were collected according to Carella et al. (2016). Salicylic acid (SA) (Sigma-Aldrich was used at a final concentration of 100 µM or 1 mM in a solution containing 0.025% methanol in 10 mM $MgCl_2$. Benzothiadiazole (BTH), commercially available as BION (Ciba Geigy), was dissolved in water and used at a similar concentration of 100 µM or 1 mM in 10 mM $MgCl_2$. Plants infiltrated with 10 mM $MgCl_2$ were used as mock for BTH treatment.

RNA isolation and qRT-PCR was done according to Dey et al. (2014). To test for statistical significance of qPCR data, log transformed values of relative quantitation from at least three independent biological experiments were tested using the one sample t test. Statistical significance between two or more treatments was tested using one way ANOVA (Holm Sidak multiple testing test) (SigmaPlot ver. 12).

RNA-Seq was performed according to Dey et al. (2014). RNA-Seq data were mapped to the latest wheat assembly (TGACv1) and analysed using Kallisto. The differential gene expression was determined using the Bioconductor/EdgeR package in the R software.

Results and discussion

Here we show Ps inoculation in first true leaves of four week old wheat plants induces systemic immunity against Xtc, the causal agent of bacterial leaf streak. To deduce whether Ps induced systemic immunity represents a SAR-like phenomenon, we infiltrated phloem exudates collected from Ps infected wheat leaves into healthy Arabidopsis plants. Our results show accumulation of the SAR associated *PATHOGENESIS RELATED-1* (*PR1*), in Arabidopsis leaves that received the Ps induced phloem exudates in comparison to mock. Furthermore, infiltration of the first true leaves of wheat plants with the SAR associated phytohormone salicylic acid (SA) or its functional analogue BTH triggered systemic immunity against Xtc. Taken together our data reveals that wheat systemic immunity resembles a canonical SAR response.

To understand the molecular factors responsible for Ps induced priming in wheat, we performed a RNA-Seq analysis. Multifactorial analysis of Ps induced plants was compared with plants challenged with Xtc that received a Ps preinoculation and 'naïve' Xtc treated plants, all analysed in comparison to the respective mock treatments. Our results revealed that Ps infection induces accumulation of *ETHYLENE RESPONSIVE FACTOR* (*ERF*) and *WRKY* transcription factors (TFs) which also represents the most enriched family of TFs. qPCR analysis further confirmed systemic but not the local accumulation of two wheat *ERF* and *WRKY* TFs. In addition, RNA-Seq analysis revealed a subset of Ps primed genes, which were regulated specifically upon Xtc challenge in Ps preinoculated plants, which included a *Triticum aestivum* Ps-primed (*TaPsP*) TF. qPCR analysis further confirmed that the active transcription of the *TaPsP* TF occurs specifically upon Xtc challenge in Ps preinfected plants, and is not induced by defence response to Xtc. The *TaPsP* TF is neither expressed in systemic leaves of Ps treated plants due to active defence response against Ps. *In silico* analyses of the promoter of the *TaPsP* TF (using JASPAR) showed ERF and WRKY binding sites which suggests that the ERF and WRKYs may prime the *TaPsP* TF, by binding to the promoter region. Taken together, our data reveal that Ps infection primes defence response in wheat through the accumulation of *ERF* and *WRKY* TFs, thereby facilitating active transcription of *TaPsP* specifically upon challenge with Xtc. Systemic immunity in wheat thus exemplifies a monocotyledonous model of biologically primed immunity analogous to SAR and is activated by the interplay of transcription factors.

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Fosetyl-aluminum improves defence against *Venturia inaequalis* in apple

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Highlights

- Apple (*Malus* × *domestica* Borkh.) is continuously threatened by apple scab caused by *Venturia inaequalis*. The infection pressure is currently increasing
- We examined whether defence priming with fosetyl-aluminum in combination with polyploidy strengthens defence of the apple plants and what are the underlying transcriptomic mechanisms of such an improved defence

Introduction

Domesticated apple is continuously threatened by apple scab. The infection pressure is steadily increasing due to the favorable environmental conditions, in combination with strict Integrated Pest Management guidelines, Fungicide Resistance Action Committee restrictions (FRAC) and extra-legal residue minimization requirements from retail, which limit the number of fungicide applications with various modes of action and, therefore, alternatives are needed.

Such an alternative to improve current practice can be defence priming with fosetyl-aluminum in combination with polyploid plants. We aimed at unravelling the effect of such a priming on resistance to *Venturia inaequalis* in apple. In addition, we examined whether and how it alters the transcriptomic profile of diploid and tetraploid apple plants.

Material and methods

Tetraploid and diploid 'Gala' and G58 isoforms were used in the greenhouse trial. *Venturia inaequalis* strain 104 was used to inoculate the plants. Part of 'Gala' plants was primed with fosetyl-aluminum. Next, actively growing shoots (one per plant) from these plants were inoculated (Daniels et al., 2012). Control plants were mock inoculated by water. After the inoculation the visual evaluation was performed via the optimised protocol developed by Chevalier et al. (1991). Disease symptoms were observed in the four most susceptible leaves. Next, the real-time PCR was performed according to the in-house protocol developed by Torfs et al. (unpublished data).

Finally, total RNA was extracted from a pool of the four leaves per plant in four biological replicates. Construction of strand specific cDNA libraries with PolyA depletion was performed, followed by a Single End 100 bp Illumina HiSeq2500 RNA 2 lanes sequencing. Furthermore, the sequences were checked for their quality and the reads were mapped to a 'Golden Delicious' reference transcriptome ('Malus_x_domestica-CU_RNA_seq_genes-all.fa.gz'). Different comparisons were carried out by the use of expression values using false discovery rate *p*-value correction test between all the comparisons. The differentially expressed genes (DEGs) were mapped in MapMan software. The GO terms were loaded to AgriGO toolkit and compared to apple transcriptome, in order to assess the enriched molecular processes.

Results and discussion

Macroscopic symptoms evaluated during the inoculation experiment in 2017 indicated different degrees of susceptibility with the most severe symptoms in unprimed diploid 'Gala' plants (94.3% severity and 83.6% sporulation) and the lowest in tetraploid primed 'Gala' plants (76.7% severity and 42.4% sporulation). Moreover, the symptoms were almost completely reduced in G58 genotype (26.3% severity and 0.7% sporulation). The susceptibility of unprimed plants was in all treatments increased in comparison to primed ones as well as in diploid in comparison to tetraploid ones.

The highest relative amount of *V. inaequalis* DNA was detected in unprimed diploid 'Gala' plants (121.9 pg *V. inaequalis*/ng *M.* × *domestica* DNA) and the lowest in the tetraploid G58 plants (0.5 pg *V. inaequalis*/ng *M.* × *domestica* DNA). Susceptibility of unprimed plants was again in all treatments increased in comparison to primed ones. Therefore, quantitative molecular analysis confirmed visual evaluations.

Transcripts and their annotations from Mapman software were merged for target selection from 367,073,407 reads. After the bins and annotations were linked to the transcripts, 17.2% DEGs were unannotated. For the analysis of transcriptomic changes in different treatments, about 7-13 million 100 base pairs (bp) reads were obtained, depending on the obtained cDNA library. In the analysis of Partial Least Squares clustering of the reads data was the strongest in comparison of diploid (2x) and tetraploid (4x) inoculated and uninoculated 'Gala' genotype, while the effect of defence priming was lower. Enrichment analysis showed enriched bins belonging to various process among which are also defence responses to pathogens, synthesis of secondary metabolites and responses to oxidative stress, when GO IDs of DEGs were aligned with those from the apple transcriptome.

Defence priming with fosetyl-aluminum can strongly influence defence of apple against apple scab. Moreover, such a reaction can even be improved in polyploid plants. The studies of the use of fosetyl-aluminum in apple are rare, as well as studies of restriction of *V. inaequalis*. However, the disease restriction is not absolute as in resistant apple genotypes. Therefore, further research is needed in order to optimise the role of this plant enhancer in apple - *V. inaequalis* interactions.

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Characterisation of a broad-range, biologically active substance from *Pseudozyma aphidis* with a dual mode of action: antibiosis and induced resistance

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Highlights

- Isolated metabolites from the biocontrol agent *Pseudozyma aphidis* isolate L12 inhibit varied fungal and bacterial phytopathogens, both *in vitro* and *in planta*
- Isolated metabolites from the biocontrol agent *P. aphidis* isolate L12 activate the induced systemic resistance machinery in tomato plants

Introduction

Natural product-based pesticides may serve as an alternative to the traditional synthetic pesticides, which can have potentially damaging effects on both human health and the environment. Microorganisms are a prospective source of such biological pesticides (Balba, 2007).

A unique and active strain of *Pseudozyma aphidis* isolate L12, an epiphytic and non-pathogenic basidiomycete yeast, which was isolated in our lab, was found to have biocontrol ability against diverse fungal and bacterial phytopathogens with multiple modes of action: antibiosis, parasitism, competition and induced resistance (Barda et al., 2015, Buxdorf et al., 2013). Furthermore, *P. aphidis* isolate L12 secretions were found to inhibit a broad range of plant pathogens (Barda et al., 2015).

This work demonstrates that metabolites isolated from the biocontrol agent *P. aphidis* isolate L12 can inhibit varied fungal and bacterial phytopathogens, and in addition may activate the induced systemic resistance (ISR).

Material and methods

Biologically active metabolites were extracted from *P. aphidis* biomass, using the organic solvent Ethyl Acetate (EtAc), at 60°C. The EtAc fraction was collected, filtered and concentrated using a rotor evaporator (Buchhi, Flawil, Switzerland) at 42°C to a final volume of 80-100 ml, containing 100-200 mg/ml of dry weight.

In vitro germination inhibition of fungi and growth inhibition of bacteria were measured using the agar diffusion method. Bio-active extract, containing 1 mg in dry weight was aliquoted on Whatman paper discs (6 mm diameter). The discs were placed in the center of potato destrose agar (PDA) plates, embedded with plant pathogens. The diameter of the inhibition zone was measured after 24 h.

In planta antibiosis experiments were performed by inoculating tomato plant (cv. Micro-Tom) with a spore suspension of *Botrytis cinerea* (6,000 spores/per leaflet), mixed with increasing concentrations of *P. aphidis* extract. After 5 days, the areas of the lesions were measured and analysed using ASSESS 2.0 image analysis software.

The ability of the extract to prime ISR in tomato plants was demonstrated by applying the extract to three bottom leaflets of the plant 48 h before inoculation, and then inoculating the top leaves with *B. cinerea* (2,000 conidia/per leaflet). After 96 h, the lesions were analysed as described above. Gene

expression was monitored using qRT-PCR on RNA extracted from tissues harvested 72 h post inoculation.

Results and discussion

We tested the spore germination inhibitory effect of *P. aphidis* extract on three pathogens: *B. cinerea*, *Alternaria alternata* and *Fusarium oxysporum* f. sp. *lycopersici*. Using disk diffusion assays, the following inhibition zones were obtained: 5.8 cm^2 for *B. cinerea*, 5.3 cm^2 for *A. alternata* and 5.2 cm^2 for *F. oxysporum* f. sp. *lycopersici*. Additionally, strong inhibitory activity of the extract against fungi mycelial growth was established, with IC50 values of 606 µg/ml for *B. cinerea*, 221 µg/ml for *Pythium* spp., 519 µg/ml for *Rhizoctonia solani*, 455 µg/ml for *Sclerotinia sclerotiorum*, 2270 µg/ml for *F. oxysporum* f. sp. *lycopersici*, and 2038 µg/ml for *A. alternata*. Growth inhibition of bacteria was also measured using disk diffusion assays and the following inhibition zones were obtained: 43 cm^2 for *Pseudomonas syringae* pv. *tomato*, 28.5 cm^2 for *Xanthomonas campestris* pv. *vesicatoria*, 59 cm^2 for *Clavibacter michiganensis* subspp. *michiganensis*, 34 cm^2 for *Erwinia amylovora* and 34 cm^2 for *Agrobacterium tumefaciens*. The *in vitro* results demonstrated a strong activity of the extracted metabolites against all tested fungi and bacteria. These results are consistent with the results of Barda et al. (2015), who performed a similar bacteria inhibition experiments with an extraction of the L12 filtrate.

The results of the *in planta* experiments demonstrated a dose-dependent reduction in disease infection. A significant inhibition of *B. cinerea* lesions on tomato plants was obtained when a spore suspension of this pathogen was treated with extract concentrations higher than 4.2 mg/ml. A concentration of 7 mg/ml caused a reduction of over 95% in the lesion size of *B. cinerea* on tomato plants. Similar results were obtained when the extract was sprayed on the plants 2 h before inoculation with the spore suspension. Furthermore, preliminary results demonstrated that crude extract of *P. aphidis* can activate significant induced resistance and can reduce the lesion size of *B. cinerea* on the tomato leaflets by 20% 6 days post inoculation, in extract-treated versus untreated plants. When gene expression was monitored 72 h post infection with *B. cinerea*, we observed up-regulation of pathogenesis related genes such as: *PR1a* (2-fold), *LOX* (13-fold), *GlucA* (12-fold), *Chi3* (14-fold), *Chi9* (5-fold), *PIN1* (3-fold) and *AOS* (2-fold), in extract-treated versus untreated plants. These results suggest that the isolated metabolites from *P. aphidis* isolate L12 could serve as natural pesticides using a dual mode of action: antibiosis and induced resistance.

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Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Oral Session 2

Novel and old players in plant-microbe interactions

Two lysine motif receptor-like kinases (VvLYKs) participate in chitin-triggered immunity in grapevine

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Highlights
- Two Pattern Recognition Receptors (PRRs) VvLYK1-1 and VvLYK1-2 participate in the signaling of chito-oligosaccharides in grapevine
- VvLYK1-1 is involved in powdery mildew resistance

Introduction

In nature, plants are constantly exposed to potentially pathogenic microbes such as bacteria, fungi, oomycetes or viruses. However, plants have developed effective immune systems triggering various defence reactions against invading pathogens upon the perception of pathogen-associated molecular patterns (PAMPs; Dodds and Rathjen, 2010). The recognition of these conserved microbial signatures is ensured by Pattern Recognition Receptors (PRRs) which also detect plant endogenous molecules released during pathogen invasion, called damage-associated molecular patterns (DAMPs; Boller and Felix, 2009).

Chitin, a fungal cell wall component, is a well-known PAMP that triggers defence responses in many mammal and plant species. The aim of the study was to determine the effects of chito-oligosaccharides on grapevine's immunity and identify the receptor(s) involved in the perception of chito-oligosaccharides in grapevine.

Material and methods

Grapevine cells (*Vitis vinifera* cv Gamay) were cultivated as described in Gauthier et al. (2014). *Arabidopsis thaliana* plants from wild-type (WT) Columbia (Col-0), mutant and transgenic lines were grown *in vitro* for two weeks in controlled conditions for defence responses or in jiffy peat pellets in a controlled growth chamber for four weeks for protection assays. Grapevine cells or Arabidopsis plants were treated with water, chitin, chitosan (Elicityl, 0.1 g/l for cells and 1 g/l for plants) or flagelline (10 µM) taken as a positive control. ROS production and cytosolic Ca^{2+} variations ($[Ca^{2+}]$cyt) in grapevine cells were performed according to Dubreuil-Maurizi et al. (2010) after elicitor treatments, by measuring the chemiluminescence of luminol for H_2O_2 production and using apoaequorin expressing cells to detect variations of $[Ca^{2+}]$cyt. Protein extraction, SDS-PAGE and western blotting for MAPK phosphorylation analysis were carried out as previously described (Trdà et al., 2014). RNA extraction and quantitative real-time PCR were performed using primers for the

amplification of defence marker genes (*CHIT4C*, *STS1-2*, *PAL*, *RBOHD*, and *FRK1*). Two days after elicitor treatment, *Botrytis cinerea* and *Plasmopara viticola* infections were performed on grapevine plants. For protection assays to *Erysiphe necator*, leaves were infected, maintained on agar medium in the incubator and then sampled at 0, 4, 8, 12 and 24 hours post inoculation.

Results and discussion

In grapevine cells, chitin treatment induced a rapid and transient increase in free $[Ca^{2+}]$cyt that peaked at 2 min but not chitosan, even if the basal level remained higher during the whole experiment. Both chito-oligosaccharides did not trigger any H_2O_2 production contrary to the flagelline epitope flg-22. Chitin and chitosan treatment induced the phosphorylation of two MAPKs with relative molecular masses of 45 and 49 kDa in grapevine cells but chitosan activated the phosphorylation of these two MAPKs longer than the chitin treatment. The expression of defence marker genes activated by different elicitors was then followed by qPCR. Among them, both chito-oligosaccharides induced the expression of four grapevine defence genes encoding an acidic chitinase (CHIT4C), a stilbene synthase (STS1-2), a phenylalanine ammonia lyase (PAL) and a respiratory burst oxidase homolog D (RBOHD), 1 hour post-treatment (hpt).

The efficacy of chitin- and chitosan-induced immunity was investigated in *Vitis vinifera* leaf discs infected by the necrotrophic fungus *B. cinerea* or with the biotrophic oomycete *P. viticola*, the causal agents of gray mold and downy mildew, respectively. If chitin pretreatment induced a low but significant resistance against these pathogens, chitosan reduced very significantly the *B. cinerea* lesion diameter and the *P. viticola* sporulation.

Taken toghether, these results demonstrate that grapevine perceives chitin and chitosan suggesting that at least one PRR for chito-oligosaccharides perception exists.

To identify the receptor of chito-oligosaccharides in grapevine, the grapevine family of LysM receptor like kinases was characterised and three proteins, respectively named VvLYK1-1, VvLYK1-2 and VvLYK1-3, showed a close relation to Arabidopsis CERK1/LYK1 (Chitin-Elicitor Receptor Kinase 1) and the rice ortholog CERK1. By functional complementation of the Arabidopsis *cerk1/lyk1* mutant, impaired in chitin perception and signalling, we demonstrated that VvLYK1-1 and VvLYK1-2 are involved in the signalling of chito-oligosaccharides in *Vitis vinifera*. Moreover, VvLYK1-1 plays a key role in basal resistance against the grapevine powdery mildew causal agent *E. necator*.

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New defence metabolic pathways under the control of the hormonal peptide systemin

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Highlights

- Here we report a new, easy and sensitive method to quantify systemin in plants that can be applied for the measurements of other plant peptides
- The study of what systemin does in plants by metabolic means, shows that systemin has a relevant role in defence, and not only as a starting point of jasmonate synthesis

Introduction

Systemin (SYS) is a signal peptide recognised to be induced by wounding and seems to induce the same signalling pathway as methyl jasmonate (MeJA; Pearce et al., 1991). SYS is known to originate from the cleavage of the proto peptide prosystemin (PROSYS). Nevertheless, little is known about its role in plant defence and the real presence of this peptide in tissues, since all the studies have been made by determination of the PROSYS gene expression or protein abundance (McGurl et al., 1992; Narváez-Vásquez et al., 2007; Coppola et al., 2015).

In the present work, it is shown an easy method for SYS quantification, using an LC-MS/MS technique, and will allow to study its actual role in plant physiology and defence. A metabolomic study in plants that overexpress PROSYS and an antisense transgenic plant (PS+ and PS- respectively) shows that the role of SYS is more complex than expected in the one known as the initiation of jasmonic acid (JA) pathway.

Material and methods

Wild type tomato plants (*Solanum lycopersicum*) variety BetterBoy and the transgenic lines, which are, overexpressor 35S::PROSYS and the antisense of PROSYS gene lines were provided by Ryan's laboratory and referenced in McGurl et al. (1992). All plants were grown in a growth chamber under 16 h of light (300 $\mu E/m^2$ s) at 26ºC and 8 h of dark at 22ºC. Tomato seeds were sown in vermiculite, and when developed cotyledons, plantlets were transferred to a 300 ml pots with vermiculite:soil (1:1) mixture and watered three times a week with Long Ashton solution (Hewitt, 1966). After 6 weeks since germination, samples were harvested and placed in -80ºC until analysis. Metabolomic analysis has been done as in Pastor et al. (2014). Targeted analysis has been conducted as in Gamir et al. (2012). RTqPCR has been performed as previously in Sanchez-Bel et al. (2016).

Results and discussion

SYS quantification is important for unravelling the actual role for SYS in plant pathology and physiology. In the present work, an easy method for SYS quantification is described. For such purpose, it has been used a wild type cultivar of tomato BetterBoy, the overexpressor PS+ and the

antisense PS-. Although they display higher or no levels of PROSYS respectively respect to the control, a chromatographic tandem mass spectrometry has been used to finally detect the actual levels of the SYS. From fresh material of tomato leaves, a simple extraction of proteins was performed. After concentration of the extraction by evaporation in speed-vac, samples were resuspended in 500 µl of the initial chromatographic conditions. Then, the solution was injected in a TQS equipment (Waters) and the chromatographic separation was performed on a C18 column suitable for peptides (Phenomenex). The quantification of the basal levels allowed the determination that PS+ has higher levels of SYS than the wild type, while the PS- transgenic plants have no detectable levels. Nevertheless, the levels were not as high as expected for a transgenic plant that overexpresses a gene. This leads us to think that other post-translational modifications might take part in the process of cleavage releasing SYS.

Despite to be an "old" known peptide, there is very low information about its mode of synthesis, secretion and action in plant defence and even the basal levels in the plant. Some previous reports (El Oirdi et al., 2011) show that PS+ is more resistant to biotic stress, specifically against the necrotroph *Botrytis cinerea*. Moreover, transcriptional studies confirm that PS+ has a more active salicylic acid (SA)-dependent signalling responses, as well as the octadecanoid synthesis pathway (Coppola et al., 2015). Then, we performed a targeted and non-targeted chromatographical analysis by a UHPLC-TQD and UPLC-TOF respectively. Interestingly, we found that SYS is affecting more metabolic pathways than expected. The targeted analysis showed high accumulation of SA in PS+. At this point, it is worthy to say that due to the similarity in systemic responses given by SYS application, MeJA and wounding, which is to induce the wound-inducible Proteinase Inhibitor (PI) proteins in distal leaves (Pearce et al., 1991), it was proposed that SYS acts upstream of jasmonates synthesis. Then, PS+ might have higher levels of JA and less SA due to the known negative crosstalk between SA and JA (Reyes et al., 2008). But in our experiments PS+ show higher levels of SA and no differences between the three lines (wild type, PS+ and PS) in JA content. On the other hand, new defence pathways appear to be under the control of SYS. This open new line of research in basal and induced resistance depending on the study of what endogenous SYS does in plant or what the exogenous SYS can induce in plant defence upon *Botrytis cinerea* infection.

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The xyloglucans: are they new elicitors of *Arabidopsis thaliana* immunity?

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Highlights

- Fragments derived from plant cell wall xyloglucans induce *Arabidopsis thaliana* defence responses and protection against *Botrytis cinerea*
- Xyloglucan-triggered immunity against *B. cinerea* requires the phytoalexin, ethylene and jasmonic acid-dependent pathways

Introduction

Plant resistance is based on their ability to perceive microorganisms and induce immune responses to stop their invasion. This recognition is possible via the perception of eliciting molecules released during the plant/pathogen interaction. These elicitors, called PAMPs (Pathogen-Associated Molecular Patterns), gather conserved molecular patterns such as bacterial flagellin or fungal chitin and activate a set of defence-associated responses termed PAMP-triggered immunity (PTI; Newman et al., 2013). Plants are also able to distinguish fragments from plant cell wall such as oligogalacturonides (OGs; Ferrari et al., 2013) commonly called DAMPs (Damage-Associated Molecular Patterns).

Xyloglucans (Xh) are the main component of hemicellulose in eudicot primary cell walls and are composed of a β-1-4-glucan backbone with side chains of xylose, fucose or galactose. The first aim of this study was to investigate if Xh were new DAMPs of Arabidopsis immunity and characterised their mode of action.

Material and methods

Arabidopsis seeds of the WT Columbia (Col-0) and mutants in the same background were obtained from the Nottingham Arabidopsis Stock Center (NASC). Plants were grown in a controlled growth chamber for 4 weeks. Arabidopsis cells were cultivated as previously described (Trdá et al., 2014). Cells or plants were treated with water, Xh or OG taken as a positive control (both used at 1 g/l for defence responses and 2.5 g/l for protection assays). In cell suspensions, H_2O_2 production was determined using the chemiluminescence of luminol (Dubreuil-Maurizi et al., 2011). Cytosolic Ca^{2+} variation ($[Ca^{2+}]$cyt) measurements were carried out on Arabidopsis transformed plant expressing apoaequorin according to Manzoor et al. (2013). Trdà et al. (2014) previously described protein extraction, SDS-PAGE and western blotting for MAPK phosphorylation analysis. RNA extraction and quantitative real-time PCR reactions were performed as proposed by Manzoor et al. (2013) using primers for the amplification of defence marker genes (*PR-1*, *PAD3*, *ICS1* and *LOX3*). Callose deposition was revealed by aniline blue staining. Two days after treatment, *Botrytis cinerea* and *Hyaloperonospora arabidopsidis* infections were performed according to Manzoor et al. (2013).

Results and discussion

Xh treatment induced a dose-dependent MAPK phosphorylation in Arabidopsis cell suspensions. From 5 to 60 min, Xh treatment induced a rapid phosphorylation of two MAPKs with relative molecular masses of 43 and 47 kDa. Treatment with Xh did not induce any free $[Ca^{2+}]$cyt variations whereas OG treatment induced a rapid and transient increase in free $[Ca^{2+}]$cyt that peaked after 30 sec. Xh did not trigger any H_2O_2 production, as observed in control cells but OG treatment induced an oxidative burst with maximal H_2O_2 production detected at 10 min. To investigate late defence responses, we analysed callose deposition at the site of infection by *B. cinerea* after elicitor treatments. Xh and OG-treatment resulted in a significant increase of callose production 3 days post infection with the pathogen. The expression of different defence genes was analysed by qPCR. Xh triggered the accumulation of *PR-1*, *PAD3*, *LOX3* and *ICS1* transcripts. To further investigate the efficacy of xyloglucans to induce resistance, we performed protection assays against the necrotrophic fungi *B. cinerea* and the biotrophic oomycete *H. arabidopsidis*. Xh treatment applied 48 h before pathogen infection significantly reduced both the *B. cinerea* lesion diameter and the *H. arabidopsidis* sporulation on Arabidopsis leaves. Together, these results suggest that Xh are new elicitors of Arabidopsis immunity. Interestingly, some defence responses triggered by Xh are different from those induced by OG. As Arabidopsis responds to Xh treatment, we aimed to identify some signalling components. By using a genetic approach with T-DNA mutants in different defence responses, our data indicated that the Xh-triggered immunity against *B. cinerea* requires the phytoalexin (*cyp71A13*, *pad3*, *pad2*), ethylene (*etr1*, *ein2*) and jasmonic acid-dependent pathways (*dde2*, *lox3*, *coi1*).These results show that Xh are recognised by Arabidopsis. In order to identify a receptor involved in Xh perception or signalling, knock-out mutants of previously known *A. thaliana* receptors or candidate receptors up-regulated in microarray analysis have been tested. All these mutants will be tested by analysing MAPK activation assays after Xh treatment.

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Bio-based compounds inducing resistance against *Leptosphaeria maculans* in oilseed rape

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Highlights
- Hydrolyzates of food waste serve as disease suppressants in oilseed rape
- Animal protein hydrolyzates induce defence responses and resistance against *Leptosphaeria maculans*

Introduction

Biodegradable products capable of suppressing plant diseases are of great importance. Their effect can be based either on a direct antimicrobial activity, stimulation of plant fitness or resistance induction. It is supposed that such crop protecting preparations represent a valuable alternative to pesticides. To be economically interesting, the source material of resistance-inducing compounds has to be low-cost and available in sufficient quantity. On the grounds of these requirements, we were searching for resistance inducers in food wastes and their combinations with other efficient compounds of different origin.

Material and methods

Hydrolysed animal protein wastes, fermented urban kitchen and gardening wastes were fractionised and applied to oilseed rape by spraying. Relative expression of genes involved in main defence signalling pathways was monitored by RT-qPCR. The cotyledons of treated plants were inoculated by *Leptosphaeria maculans* spore suspensions by infiltration. The development of necroses was documented and evaluated by image analysis.

Results and discussion

We focused on protein hydrolysates prepared from food by-products and leather wastes, as well as water-soluble substances obtained by alkaline hydrolysis of urban biowastes and composts. The composition of the hydrolysates was analysed. Their effect on plant defence system activation was investigated in oilseed rape (*Brassica napus*) and their ability to induce resistance was monitored against *Leptosphaeria maculans* in cotyledon tests. The application of the hydrolysates reduced symptoms of the disease and induced the expression of defence genes implicated in signalling pathways regulated by salicylic acid and ethylene. The results indicate that food wastes can serve as a valuable source of compounds utilizable in plant protection.

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Molecular mechanisms of chemical immune priming without costs to plant growth

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Highlights

- A targeted screen of structural analogues of the immune priming agent (R)-β-aminobutyric acid revealed a novel resistance-inducing compound, (R)-β-homoserine (RBH)
- To identify the mode of plant perception of RBH, we are screening a large collection of confirmed homozygous T-DNA insertion lines for loss of RBH-induced resistance in Arabidopsis

Introduction

Specific chemicals can boost quantitative disease resistance by priming the plant's immune system to respond more quickly and/or strongly against attack. Priming agents thus represent a promising alternative to pesticides in crop protection. Unfortunately, the use of priming agents, such as the non-proteinogenic amino acid β-aminobutyric acid (BABA), is often accompanied by undesirable non-target effects on plant growth (Luna et al., 2014). We recently identified a structural analogue of BABA, R-β-homoserine (RBH), which primes partially different immune responses than BABA without repressing plant growth. However, the mechanism(s) by which plants perceive RBH remains unclear. To elucidate the molecular perception mechanisms, we designed a screen of confirmed homozygous T-DNA insertion lines of Arabidopsis for loss of RBH-induced resistance (RBH-IR) against the pathogenic oomycete *Hyaloperonospora arabidopsidis*.

Material and methods

Arabidopsis T-DNA insertion lines obtained from the European Arabidopsis Stock Centre were sown in multi-well trays containing a 2:1 (v/v) M3 soil/sand mixture and grown under standard Arabidopsis growth conditions [(8 h-day (21ºC) and 16 h-night (18ºC) cycle at ~60% relative humidity (RH)]. Two-week old seedlings were soil-drenched with RBH by pouring a 2 × concentrated solution at 50% of the well volume. Induced resistance in Arabidopsis was quantified against the biotrophic oomycete *H. arabidopsidis* (Hpa), strain WACO9. In-tray treated controls (Col-0) were used to evaluate effectiveness of RBH against Hpa in wild-type, RBH-IR-expressing plants. In-tray non-RBH-treated controls (Col-0) were used to confirm ability of Hpa inoculum to infect each successive tray. T-DNA lines showing visible sporulation were repeated in additional multi-well trays prior to confirmation and further analysis in pot assays.

Results and discussion

From the 2800 lines screened thus far, we have identified 19 putative impaired in RBH-IR (*iri*) mutants, including lines with T-DNA insertions in genes involved in iron homeostasis, lipid signalling and cell wall modification. Several of these genes have been shown to be significantly up-regulated in response to pathogen attack, suggesting roles in basal defence which are primed by RBH pre-treatment. Specifically, several putative mutants contain T-DNA insertions in genes encoding members of plant defence-associated PYK protein complexes, and proteins thought to interact with these complexes (Nagano et al., 2008). These β-glucosidase-containing complexes are important components of inducible broad-spectrum defence responses in plants. Interestingly, another putative *iri* mutant contains an insertion in a gene encoding a pectin methylesterase. This family of genes plays diverse roles in cell wall modification, but this finding supports our previous work demonstrating a role for cell-wall defence in RBH-induced resistance. As the T-DNA lines used are confirmed homozygous (O'Malley and Ecker, 2010), detecting mutant phenotypes is possible using a small number of plants per line (< 10) and is therefore possible at low cost and using minimal space. Furthermore, using two-week seedlings facilitates a rapid (6-9 month timescale) identification and confirmation of a range of putative mutants. Future work will focus on in-depth characterisation of confirmed *iri* mutants with respect to RBH perception and the downstream defence signalling network(s) by comparing metabolic priming responses between *iri* mutants and wild-type plants.

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Plant produced β-aminobutyric acid (BABA): the immune system controls its accumulation

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Highlights

- Endogenous β-aminobutyric acid (BABA) levels increase after the molecular recognition of pathogen presence
- BABA is accumulated differently during resistance or susceptibility to disease
- The Arabidopsis mutant constitutive expresser of pathogenesis-related genes 5 (*cpr5*) constitutively produces high basal levels of BABA

Introduction

β-aminobutyric acid (BABA) is a non-protein amino acid that, when applied to plants, can induce resistance through priming (Mauch-Mani et al., 2017). BABA was believed to be xenobiotic until recently, when its endogenous production was demonstrated to occur in Arabidopsis and some crops (Thevenet et al., 2017). Further analyses revealed that BABA levels increase both following infections with virulent pathogens and abiotic stress (Thevenet et al., 2017). What is the role of plant produced BABA during stress remains however to be established. In order to investigate the biological significance of endogenous BABA variations during plant-pathogen interactions, we analysed BABA levels in Arabidopsis plants after infections with virulent, avirulent *(AvrRpt2), and* non-pathogenic (*hrpA*) strains of *Pseudomonas syringae* pv. *tomato* (Pst) DC3000, and after treatment with defence elicitors (Flg22 and AtPep2). In addition, a number of mutants with altered defence phenotype were screened for basal and induced levels of BABA.

Material and methods

Experiments were performed on 5-week-old *Arabidopsis thaliana* plants accession Columbia (Col-0) or Wassilewskija (Ws), and their mutants. Infections and peptide treatments were performed by leaf infiltration. BABA was extracted, purified and analysed by ultra-high pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), as described in Thevenet et al. (2017).

Results and discussion

BABA significantly accumulated after 24 hours of infection with Pst DC3000 AvrRpt2, whereas infection with virulent Pst DC3000 led to significant BABA accumulation after 48 hours. Infections with a 10-fold lower inoculum performed with both strains confirmed the early induction of BABA occurring during infection with *AvrRpt2*. BABA was also induced after infection with the non-pathogenic Pst DC3000 *hrpA* mutant, which is defective in type-III secretion and thus unable to deliver effectors into the cell and to suppress PTI. Importantly, Flg22 infiltration led to a significant increase in endogenous BABA levels after 48 hours of treatment in Col-0 plants, but not in Ws plants,

which naturally possess a non-functional flagellin receptor. BABA was also induced after infiltration with a peptidic DAMP (Damage-Associated Molecular Pattern), the plant-elicitor peptide 2 (AtPep2). Finally, a mutant screening allowed to reveal that the Arabidopsis mutant constitutive expresser of pathogenesis-related genes 5 (*cpr5*) produces high basal levels of BABA. In the light of our results, it is possible to conclude that the accumulation of BABA is regulated by the plant's immune system.

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Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Oral Session 3

Functional ecology of microbial interactions in soil

Crosstalk effects of environment and vineyard soil management on soil microbial diversity and composition

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Highlights

- Metagenomics analyses of vineyards and treatment effects provide a complex picture of influences
- Single factors are difficult to determine, hierarchical effects have to be analysed and complex sampling in mechanistic approaches is needed in future

Introduction

Vineyard management practices and production systems influence soil properties and thereby affect soil microbial communities (Burns et al., 2016). Soil microbial communities contribute to soil quality and soil healthy both linked to cycling and stability of soil organic matter, pathogen suppression, mineralisation and aggregate stability among others. Recently, improved techniques as sequencing, metabolite and protein analyses made the use of omics methods applicable for eco-physiological studies. The number of omic studies in different agronomic areas increased the last years providing increased knowledge of below ground processes related to microbial activities. Results from vineyards give a complex picture of influencing factors and general assumptions are difficult to be drawn (Burns et al., 2016; Burns et al., 2015). We aimed to analyse effects of cover crop managements (permanent cover, alternating cover, bare ground) within nine Austrian vineyards with a metagenomics approach.

Material and methods

Samples for the determination of microflora composition and biodiversity were sampled in the frame of the BiodivERsA/FACCE-JPI joint project “PromESSinG” in nine Austrian vineyards in Lower Austria (Krems, Langenlois) and Burgenland (Großhöflein, Eisenstadt). In all vineyards three different practices for inter-row management were established in 2015: open soil, alternate soil cover, permanent soil cover. Sampling date was the 8.6.2016 and samples from three inter-row managements treatments (permanent cover, alternating cover, bare ground) were obtained as pooled samples from 10 core samples (0-10 cm depth and 2.5 cm diameter). Each vineyard × treatment combination was sampled twice obtaining a final number of 54 samples for analyses. DNA extraction was performed with the Power Soil DNA Extraction kit according to manufacturer's instructions. DNA was sent for sequencing with primers: 16S 515/806 and ITS4/ITS7 with Genome Quebec. Representative sequence OTUs (operative taxonomic units) with 97% sequence identity were obtained from all retrieved sequences. Bacterial 16S and fungal ITS sequences were counted for each sample and these matrices imported and further processed in R and Canoco 5 for multivariate analyses with soil parameters.

Results and discussion

In a first step, the effects of the independent variables landscape, vineyard and treatment on determined soil parameters, as pH, nutrients, C, N content were analysed and strong influences of the vineyards on actually all determined parameters were obtained, whereas small treatment effects were only observed for K_2O, Mg, C_tot, C_org and N_tot contents. This already points towards a strong influencing location factor on upcoming microbial analyses overlaying the effects of the inter-row management. A weighted MANOVA (Adonis) on generalised Unifrac distances matrices derived from bacterial and fungal communities confirmed that both communities are not significantly influenced by the treatments applied in our analyses. Previous studies observed an effect of cover crop mix on soil bacterial communities, but hierarchical effects influenced the results supporting more complex pictures with the need for mechanistic studies (Burns et al., 2016).

The Shannon diversity index was calculated for all treatments and vineyards giving a diverse picture. Values between 3-3.6 and 5-5.6 were obtained for fungi and bacteria respectively and vineyards differed substantially. Combining all vineyards no significant influence of treatments on the Shannon index was determined, but a tendency of lower values in permanent cover inter-rows was observed. Parallel-determined basal soil respiration was increased in this treatment indicating a higher biological biomass. Possible hierarchical influences and site effects will be characterised in further bioinformatics analyses. To detect the correlation of each OTU with the specific soil parameter detected in the vineyard, first the median of each soil parameter per vineyard was calculated. Then random forest models were built (2000 trees) using the OTUs as independent variables and landscape, vineyard, Cu, $CaCO_3$, pH or sand as the dependent variable. For each dependent variable the importance of each OTU for the random forest model was recorded. Heatmaps and the OTUs which have in at least one dependent variable a decrease in accuracy of more then 10%. Linear models and coefficients were calculated for each independent variable and respective OTUs to determine the most influencing factor on microbial community determined in this study. The results provide a complex picture and support the already mentioned need for mechanistic studies.

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Linking transcriptomics to the rhizosphere microbiome - Interactions during clubroot development as a case study

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Highlights

- Symptomless roots of clubroot infected plants show a higher expression of pathogen recognition genes than roots from uninfected plants collected in the field
- Pathogen recognition genes are heavily downregulated during disease development
- The role of the root microbiome is discussed

Introduction

The club root pathogen *Plasmodiophora brassicae* is one of the economically most important parasites of brassica crops. *P. brassica* is an obligate biotrophic protist reliant on a living host plant. Despite its importance, relatively little is known about infection strategies and host defence mechanisms compared to fungal and bacterial plant pathogens. Previous studies predominantly investigated host response during the initial infection stages. The aim of this study was to analyse the transcriptomic changes of the resting spore forming stages in field samples. The role of the root microbiome during clubroot disease has never been examined. To analyse the influence of the root microbiome, the fluorescent *in situ* hybridization (FISH) was used to identify and locate bacteria present in diseased and healthy roots. The aim of the combination of these two approaches was to identify key players and processes during the late gall development stages when the pathogen inoculum for the next year is formed.

Material and methods

Kohlrabi plants (*Brassica oleracea* var. *gongylodes*) with and without clubroot symptoms were collected in September 2016 in Tyrol (Austria). Galls and roots were washed with tap water and (i) stored in RNA later for RNA extraction or (ii) preserved in 4% paraformaldehyde (PFA) and dehydrated in an ascending ethanol series for FISH experiments. Total RNA extraction was performed using the Qiagen RNeasy Plant Mini Kit. Poly(A) selected RNA was sequenced on an Illumina HiSeq 2500 platform in 125 bp paired-end read mode at VBCF Vienna. All reads were quality checked and trimmed. Reads of at least 75 bp length were kept for further analyses. High quality reads were assembled de novo using Trinity and expression estimation was performed using RSEM. To separate kohlrabi and *P. brassicae* the transcripts were blasted against coding sequences (CDSs) of *B. oleracea* (Liu et al., 2014) and a custom database containing all available *P. brassicae* CDS data (Schwelm et al., 2015, Rolfe et al., 2016), respectively. Long ORFs were predicted using TransDecoder. Annotation was performed using InterProScan, Mercator for MapMan analysis, KAAS to obtain KEGG Orthology, and blasting sequences against NCBI nr database. Assembled transcripts were processed with edgeR to obtain $\log_2$-fold changes between disease stages. Thin sections were incubated with FISH-probes for Eubacteria, Proteobacteria, and Firmicutes according

to (Grube et al., 2009), counter stained with DAPI, and analysed using a Leica LSM SP5 confocal laser-scanning-microscope.

Results and discussion

In 2016, a heavy outbreak of clubroot disease was seen at the test site, although the site had not been used for growing brassicas during the previous years. A precipitation rich growing season further supported disease development by benefiting resting spore dispersion across the field. The soil-pH at the site was , despite being prepared by liming, very low with values ranging from 4.41 ± 0.08 at the areas where cabbage was grown to 5.65 ± 0.01 on the part of the field where a mixed set of broccoli and kohlrabi was grown. A low soil pH below pH 6 is known to be beneficial for disease development. *P. brassicae* is known for re-programming host metabolism (Ludwig-Müller et al., 2009). Here we analysed differences of expressed genes in different tissues of the same plant but also between uninfected and infected plants growing in close proximity to each other. Symptomless roots of infected plants generally showed an upregulation of pathogen recognition genes compared with clubroot tissue of the same plants. Most of the differentially expressed pathogen recognition genes (e.g. *RPS2*, *NPR1*) were downregulated during disease development. There were also differences between plants without visible infection (control) and symptomless roots of infected plants with pathogen recognition genes upregulated in the latter. In clubroots, pathogen recognition genes were downregulated as compared to the symptomless roots. Overall, this allows to speculate that not yet known factors decide about the success of an infection and the establishment of the parasite. Such factors could be for example root age and fitness, environmental stress (water, nutrients, pH), or could be linked to the microbiome of the host plant. The soil and rhizosphere microbiome has an important role in preventing and causing diseases, and the role of bacteria during clubroot development has been discussed since the early days of clubroot research (Karling, 1968). On the same plants that were used for transcriptomics, epiphytic bacteria were detected using FISH probes. Bacterial abundances increased from uninfected/symptomless roots to rotting galls. Almost all detected bacteria belonged to the phylum Proteobacteria. Firmicutes were detected only rarely and isolated on the root surface. On the surface of rotting galls also oomycete hyphae were found and it is likely that they contribute to clubroot decay. No endophytic bacteria were detected in any of the root or gall types, neither by FISH nor by using DAPI staining. These results show that FISH is suitable to analyse the spatial distribution of bacteria associated with clubroots, however, a more targeted sampling combined with high throughput sequencing will be needed for functional analyses of the microbiome and its link to the transcriptomic changes in the host plant.

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Spatial and temporal *in-situ* analyses of gene expression in complex host-pathogen-systems using the *Plasmodiophora brassicae*-cabbage-pathosystem as a model

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Highlights

- We demonstrate a method to monitor gene-expression at single-cell level in obligate microbe-plant interactions
- It is a sensitive and specific method to localise expressed genes of interest along spatiotemporal gradients
- This method has the potential to rapidly increase our understanding key processes involved in complex plant-microbe interactions on single cell level

Introduction

Plant pathogen interactions often follow a spatial and temporal development where the expression of genes in both, the pathogen and the host undergo crucial changes. Therefore, it is of great interest to find, detect, and localise key genes, to understand these processes and to break down the interactions to individual cells and the whole plant. One approach to target such processes is the use of highly sensitive, and gene specific fluorescent *in situ* hybridization (FISH) methods that target the mRNA. Such methods are especially promising in pathosystems that are complex and where the biological system does not allow a cultivation or synchronisation of the pathogen. The obligate biotrophic pathogen *Plasmodiophora brassicae*, which causes clubroot-disease, is characterised by a complex life cycle with six different stages, which are often present at the same time in the host (Schwelm et al., 2015). Aim of this study was to develop a FISH-based protocol, to link mRNA transcripts to specific life cycle stages and cells.

Material and methods

Chinese cabbage plants with root-galls were collected from Ranggen (Austria) and fresh Chinese cabbage seedlings were grown in the greenhouse in pathogen conductive soil (pH 5.7). After 12 days, we inoculated the seedlings with *P. brassicae* spores. Some plants were kept untreated as a control. After 6 weeks of growth, the root galls were collected, washed, fixed and cut with a cryotome. A rolling circle amplification (RCA)-FISH protocol was used according to Weibrecht et al. (2013). The fundamental technique for RCA-FISH is based on three specific designed probes, a primer containing LNAs (Locked Nucleic Acid) facilitating a better RNA/DNA hybrid formation, a loop-shaped padlock probe which is amplified by RCA, and a fluorescent labelled detection probe. RCA leads to an amplification of the signal, which is beneficial in plants with a high autofluorescent background and for genes with a low expression level. Probes were designed for a highly expressed chitin synthase (Genbank: CEP00011.1) and actin (Genbank: AY452179.1) of *P. brassicae*. Negative controls were included in every experiment and samples were analysed using the TYPE Leica LSM SP5, a confocal laser-scanning-microscope.

Results and discussion

We could detect and localise mRNAs of all selected genes. It was possible to link the transcript to specific life-cycle stages. Actin mRNA could be detected in young plasmodia of the parasite, as well as when the resting spores were formed. We tested this method on a housekeeping actin gene, because it should be present in all developmental stages of the parasite. Chitin synthase encoding mRNAs of *P. brassicae* could only be detected when the resting spores were formed, which are, to our current knowledge, the only parasite structures containing chitin. No signals were detected in the negative controls. Adapting the RCA-FISH method from their original use in human cells to plant cells was tricky. Fixation of the RNA as well as cutting had to be optimised, to facilitate a good accessibility of the mRNA to the different probes and enzymes. The cutting and thus the size and the thickness of the sample sections are very important for a successful experiment. Also detection settings and parameters of confocal laser scanning microscopy need to be adapted for each pathosystem. Currently, we are in the process of evaluating this method for large-scale analyses. The aim is the development of an automated mRNA counting image analysis workflow for quantitative analyses of genes of interest. Our results confirm that this method is a very sensitive and specific way to detect and also to localise specific genes in the cells of the pathogen, as well as in the host cells on the µm-scale. The successful establishment of this method in a complex host-pathogen-system with *P. brassicae* and cabbage highlights the potential of this method, because this method can easily be expanded to other pathosystems. Linking the expression of certain genes to host cells along a spatiotemporal gradient improves our understanding of the biology of the parasites, but also of the scale (i.e. individual cells *vs* systemic response) and the timing of processes in the host. Understanding the biology behind host-pathogen-interactions is the fundamental step for the development of new plant breeding systems and a yield increase of crops and the here presented method can be applied to different questions involving microscale processes in the plant and the rhizosphere.

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RNA-Seq unveiled the bacterial mycophagy mechanisms implemented by *Lysobacter capsici* AZ78 interacting with *Phytophthora infestans*

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Highlights

- *Lysobacter capsici* AZ78 cells spread on *Phytopthora infestans* mycelium through the disaggregation of biofilms and the formation of type IV pili
- *Lysobacter capsici* AZ78 cells kill *P. infestans* mycelium through the biosynthesis of nonribosomal peptide antibiotics and, then, degrade the cell wall releasing cellulases and glucanases

Introduction

The rhizosphere is a nutrient-rich ecological niche occupied by large numbers of different microorganisms and it is where interactions between antagonistic bacteria and phytopathogenic microorganisms occur (Raaijmakers et al., 2009). Next generation sequencing techniques have significantly helped in extending our knowledge of the molecular patterns characterising these interactions (Massart et al., 2015).

The bacterial genus *Lysobacter* encompasses antagonistic bacterial strains particularly effective against phytopathogenic oomycetes (Hayward et al., 2010). However little is known about the molecular mechanisms implemented by *Lysobacter* spp. during the interaction with phytopathogenic oomycetes. Based on this, our aim was to investigate the bacterial molecular mechanisms underlying the interaction of *L. capsici* AZ78 (AZ78; Puopolo et al., 2014) with *Phytophthora infestans* (Pi) using a RNA-Seq approach.

Material and methods

The AZ78 and Pi strains were stored and routinely grown according to Puopolo et al. (2016). To analyse the interaction, Pi plugs (5 mm) were transferred onto cellophane film overlying PAM dishes and grown for 96 h at 20°C. The Pi mycelium was then inoculated with 80 µl of AZ78 cell suspension (1×10^9 CFU/ml) and incubated at 25°C. Samples consisting of Pi mycelium with AZ78 cells were collected at 6 and 24 hoi, immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. Three treatments were analysed: AZ78 and Pi interacting for 6 h and 24 h at 25°C (Lc6, Lc24) and a control consisting in AZ78 incubated alone for 6 h at 25°C (Lcc). For each treatment, three replicates of six dishes each were processed. Once total RNA was extracted, RNA samples (50 ng/µl) were processed with Illumina sequencing and the resulting merged reads of treatment samples were aligned to the AZ78 genome (Puopolo et al., 2016) using the Subread aligner with default parameters. Changes in gene expression level were analysed with the Voom method and differentially expressed genes (DEGs) were selected using Volcano Plot. DEGs were annotated on the basis of the

NCBI gene description and Blast2GO description and qRT-PCR reactions were carried out to validate the RNA-Seq results.

Results and discussion

To our knowledge this is the first time that RNA-Seq has been applied to study the molecular changes occurring in a biocontrol *Lysobacter* strain interacting with a phytopathogenic microorganism. The Lc6 and Lc24 samples resulted in 290 (5.5% of genes in AZ78 genome) and 548 (10.4% of genes in AZ78 genome) DEGs, as compared with Lcc using a P-value of ≤ 0.001 and $\log_2$ Fold Change ($\log_2$FC) ≥ 2 respectively. The proportion of up-regulated genes was 55.5 and 63.7% in Lc6 and Lc24 respectively.

The functional annotation of DEGs allowed to dissect the molecular patterns modulated in AZ78. The transcriptional profiling of AZ78 was characterised by the up-regulation of 15 and eight genes involved in the biogenesis of type IV pilus (T4P) at 6 h and 24 h respectively. Likewise, genes responsible for biofilm disaggregation were up-regulated at 24 h. Based on these data, it is conceivable that, during the interaction, AZ78 cells released from the disaggregating biofilm structures spread over Pi mycelium by using the T4P machinery.

Once entered in contact with Pi mycelium, genes related to the biosynthesis of nonribosomal peptide antibiotics were upregulated at 24 h. Interestingly, the gene AZ78_1098, encoding a non-ribosomal peptide synthase/polyketide synthase responsible for the production of a polycyclic tetramate macrolactam was also up-regulated at 6 h of interaction. Concurrently, an entire genome region (AZ78_4515-AZ78_4522) encoding several metallocarboxypeptidases was up-regulated. Other genes encoding lytic enzymes such as glucanases and cellulases were instead up-regulated at 24 h similarly to genes involved in the catabolism of galactose, a monosaccharide associated with the oomycete cell-wall. The concurrent up-regulation of these genes suggest that AZ78 cells may first kill Pi cells through the release of antibiotics, then can start degrading dead Pi mycelium releasing extracellular lytic enzymes and finally feed on the by-products deriving from the degradation of Pi cell wall.

Results also showed that Pi responded to these attacks since AZ78 cells activated defence mechanisms by up-regulating genes encoding a catalase (AZ78_1116), putative oxidoreductases (AZ78_1581, AZ78_1588 and AZ78_1600) and permeases of major facilitator superfamily (AZ78_3401, AZ78_5171) at 6 h and 24 h.

These results were validated analysing the relative expression level of 11 selected AZ78 genes using qRT-PCR and a close correlation (Pearson's $r = 0.89$) was observed between $\log_2$FC measured with RNA-Seq and qRT-PCR.

In conclusion, the attack strategy implemented by AZ78 could be ascribed to the necrotrophic interaction of bacterial mycophagy, associated to the transcriptional activation of T4P machinery, antibiotic and lytic activities.

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Evaluation of the efficacy of a biocontrol agent, *Gliocladium catenulatum*, to colonise soils and to reduce *Fusarium graminearum* growth under microcosm and field conditions

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Highlights

- The efficacy of *Gliocladium catenulatum* to colonise soils and to reduce *Fusarium graminearum* growth was evaluated under both microcosm and field conditions
- We gave evidence that *G. catenulatum* has competitive advantages over *F. graminearum* in soils
- However, its efficacy is reduced when confronted to the soil native microbiota

Introduction

Fusarium graminearum (Fg) is one of the main causal agent responsible for Fusarium Head Blight (FHB) of cereals. Besides yield losses, FHB represents a threat to human and animal health due to the possible production of mycotoxins. To face the lack of effective strategies, new control strategies must be developed including the use of biocontrol agents (BCAs).

The primary source of Fg inoculum originates from infected crop residues on which the pathogen can survive over the winter. A reduction of the primary inoculum should ultimately turn into a reduction of the infection pressure at anthesis which is the most susceptible stage of infection. This could be achieved by treating soils with antagonistic organisms against Fg.

The aim of this study was to evaluate the efficacy of a BCA, *Gliocladium catenulatum* (Gc), to colonise soils and to reduce Fg growth in soils under both microcosm and field conditions. The influence of maize residues on the pathogen growth was also studied.

Material and methods

A 1st experiment was carried out to monitor both Fg and Gc in soils which had been autoclaved to suppress the soil biota and focus on the pathogen-BCA interactions. Samples were collected from fields in Brittany. Each hole of a seeding tray was filled with 20 g of autoclaved soil and inoculated either with Fg, Gc or both. Fg inoculum consisted in contaminated ground maize kernels at 0.4 and 0.04 g per hole. Gc was prepared by diluting Prestop®, a commercialised formulation of Gc, in sterile distilled water and sprayed directly on the soil at approximately 10^6 and 10^7 colony forming units (CFU) per hole. The tray was incubated in controlled conditions and watered every two days. A similar experiment was conducted on autoclaved maize residues (kernels). 20 g of kernels were inoculated with either Fg, Gc or both. qPCR analysis using Fg specific primer (Nicholson et al., 1998) and Gc specific primers (Paavanen-Huhtala et al., 2000) were then performed on total DNA extracted from soil or kernels at 0 and 7 or 15 days. A similar experiment was conducted using living soils supplemented or not with maize residues. Soils were inoculated with Fg (at 0.4 g of contaminated ground maize kernels), Gc (at 10^7 CFU per hole) or both simultaneously. Three soils and their corresponding maize residues, collected during fall 2016 after maize harvest in Brittany, were studied.

A 2-year field experiment was also conducted to evaluate the efficacy of Gc to reduce FHB and mycotoxin production in triticale.

Results and discussion

Under autoclaved-soil conditions, Fg growth was always significantly lower when Gc was applied to the soil, whatever the dose. In contrast, Gc growth was never decreased in mixed inoculations with Fg compared to single Gc inoculations. The competitive advantages of Gc over Fg were also confirmed on autoclaved maize kernels. Indeed, Fg was only able to grow in single Fg inoculations while the levels of Gc DNA remained unaffected by the presence of Fg.

Unsurprisingly, results using livings soils were not that striking. First, Fg growth was significantly reduced, if not null, in living soils compared to autoclaved soils, suggesting that the native microbiota restrict or even impede the pathogen growth. Soil treatment with Gc was able to significantly reduce Fg growth in one of the three soils. In addition, the levels of Gc DNA remained similar during the 15 days of the experiment, suggesting that the BCA was not able to grow but persisted under living soil conditions.

Maize residues, added to the soil samples, helped increase, decrease or had no significant impact on Fg growth compared to soils without residues. These results suggest that the microbiota associated with residues also plays a key role in the net soil suppressiveness or conduciveness to the pathogen growth. Metabarcoding data is currently being processed to describe the fungal and bacterial communities associated with those soil and maize residues. Such knowledge may contribute to identify consortia of microorganisms responsible for the suppressiveness or conduciveness of soils and maize residues to Fg growth. The climatic conditions at flowering during the 1st year field experiment did not allow the development of FHB. During the 2nd year experiment, results showed no reduction of the disease symptom, evaluated at flowering, and mycotoxin content in mature triticale kernels in the plots treated with the BCA compared to the untreated condition. Yet, treatments with half dose of fungicide (Kestrel) and BCA allowed to significantly reduce the mycotoxin content (by 80% compared to the untreated condition), similarly to the treatment with 100% fungicide. In addition, the BCA was not persistent in soils, as determined by the levels of BCA DNA in soils collected at harvest which were below the limit of quantification. The colonisation success of the BCA could probably be optimised by applying the BCA several times.

In conclusion, our results gave evidence that Gc has antagonistic activities against Fg. Application of the BCA to soils seems therefore a promising way to control soilborne diseases such as FHB. However, its efficacy depends on its ability to persist in soils, which partly depends on the microbiota to which it is confronted.

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Weed microbiome characteristics and the development of bioherbicides

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Highlights

- Grapevine and weeds share partially microbiome components when grown in the same soil, particularly in the rhizosphere
- A much higher degree of specificity was encountered when comparing microbiomes in the root interior
- In the rhizosphere, weed microbiomes showed a higher species richness, and generally perennial plants hosted more diverse microbiomes

Introduction

Crop and weeds growing side by side acquire their associated microorganisms from the same soil source, but every plant species selects its own microbiome, and this influences plant competitiveness, health and productivity. This specificity is mediated through specific exudates which serve as nutrients and signalling molecules for microorganisms. Microorganisms being able to respond to these substances are enriched in the root environment. Furthermore, the plant microflora may be affected by agricultural management practices. Besides causing changes in the microbial community structure, agricultural management may also affect microbiome functions. Weeds are undesirable from an agricultural management point of view, but little understanding exists on their contribution to soil diversity and functioning. We assessed diversity and functional characteristics of grapevine- and weed-associated microbiota by employing a cultivation-independent approach and by analysing microbial isolates.

Material and methods

Rhizosphere and root material was collected in vineyard in Illmitz (Austria) from grapevine and four weeds (*Lepidium draba* L., *Stellaria media* L., *Lamium amplexicaule* L., *Veronica arvensis* L.) in the immediate surroundings of the grapevine plants. Each plant species and plant compartment was sampled on five sites in the vineyard with three replications. The DNA was isolated from the rhizosphere soil and surface sterilised roots. Partial 16S rRNA genes were amplified and subsequently sequenced using the Miseq sequencer (Samad et al. 2017). The reads were analysed using the bioinformatics pipeline described by Samad et al. (2017). Additionally, 500 bacterial strains were isolated from the rhizosphere and surface sterilised roots of grapevine and *L. draba*. The strains were functionally characterised for plant growth-promoting characteristics. To test for herbicidal functions the strains were tested for growth inhibition against *L. draba*, lettuce and *Arabidopsis thaliana*.

Results and discussion

The rhizosphere and root microbiome showed significantly different numbers of observed operational taxonomic units (OTUs) among all plant species, whereas the Simpson index values were significantly different among the plant species only in the root microbiomes. The diversity of the

weed rhizosphere was higher compared to the rhizosphere of grapevine. To find shared OTUs between all plant species, plant compartment and sampling sites, the OTU had to be present at least in two out of three replicates and three out of five sampling points. In the rhizosphere, 52% of the OTUs were shared among the plant species. Among root endophytes, only 13% were common among all plant species. Seven abundant OTUs were present in all plant species and compartments. The shared OTUs could be also found in the strains isolated from grapevine and *L. draba* rhizosphere and soil.

Each plant species had unique OTUs in the rhizosphere as well in the root. All weed species hosted a higher number of specific OTUs than grapevine.

To obtain information on functional characteristics of bacterial strains obtained from grapevine and the weed *L. draba*, 250 strains from each plant species were isolated, 125 from the rhizosphere and 125 from roots. The strains were assigned to seven different classes and 35 genera. *Pseudomonas* was the most prevalent genus among all isolates counting for 35%. The strains isolated from *L. draba* comprised a higher percentage of strains producing hydrogen cyanide, siderophores and indole-3 acetic acid and solubilizing phosphate, whereas the strains from grapevine showed a higher percentage of ACC deaminase production and antifungal activity against *Cylindrocarpon destructans in vitro*.

To identify bacterial strains, which could inhibit the growth of the weed *L. draba*, a total of 98 strains were tested on seeds of *L. draba* and *A. thaliana*. Seven strains showed a phytotoxic effect on *L. draba*, but none of them were phytotoxic to *A. thaliana*. The effects on *L. draba* were different depending on the strain. Some strains caused a die back of seedlings, whereas others caused a reduction in radicle or seedling biomass. The most promising strains were also tested on *L. draba* in the greenhouse. Three of them significantly reduced root or shoot length. All this strains showed *in vitro* IAA production.

To understand the mode of action of the most promising strain, *Pseudomonas viridiflava* strain Cdrtc14 obtained from *L. draba* surface-sterilised roots, was sequenced (Samad et al. 2016). No complete pathogenicity island or pathogenicity related effector genes were found. The strain is equipped with several genes related to heavy metal resistance, stress response and auxin biosynthesis. We found that the effect of the strain is dosage dependent and the cell free supernatant did not cause any growth reduction of the seedlings.

Our results revealed that weeds are a potential source for bioherbicides with new modes of action.

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qPCR and NGS techniques for pathogen detection and monitoring of microbial communities in soil after application of broad spectrum fungal treatments in cucumber crop

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Highlights

- Nowadays, the study of the fungicides effects on non-target microorganisms has become important because of biodiversity losses
- It was analysed the impact of two broad spectrum fungicides (fluopyram and penthiopyrad) on soil microorganisms
- Bacterial and fungal communities were affected by fungicides two months after the end of the application indicating the persistence of fungicides in soil

Introduction

Excessive application of fungicides in horticultural crops have been raised as public concerns recently worldwide, since it has been demonstrated that pesticides have an impact on non-target microorganisms in rhizosphere (Singh el al., 2015). Fluopyram and penthiopyrad are new broad spectrum fungicides, both recommended to control powdery and downy mildew (Proffer et al., 2012), and its objective is the succinate dehydrogenase enzyme related with fungal respiration (Zhang et al., 2014). At this regard, it is reasonable to assume that foliar applications of these fungicides could accumulate in the soil affecting target and non-target microorganisms.

In this work, we analysed by qPCR the effect that foliar applications of fluopyram and penthiopyrad had in pathogens which cause downy and powdery mildew, as well as common foliar and soil pathogens affecting cucumber crop. In addition, we checked if this effect is extended to microbial community by means of next generation sequencing (NGS) techniques.

Material and methods

Three plots of 150 m^2 (25 × 6 m) with a separation among them of 50 m^2 were set out: two for fungicide treatments and one as control. A total of 100 cucumber plants were sown per plot. Plants were treated (at leaf level) with two commercial fungicides: Luna Devotion with Fluopyram (FL) and diadimenol (Bayer Crop) and Frontelis (Dupont) with Penthiopyrad (PE). Plots were sprayed for six times with an interval of 7 days for both fungicides. Eight samples per treatment from rhizospheric soil and leaves were collected in three different times: a) after first fungicide treatment, b) at the end of treatment applications and c) two months after treatments. qPCR was performed on leaf and soil samples to analyse pathogens that affected cucumber crop using Vegalert qPCR Taqman® quantitative kits (Microgaia Biotech S.L, Murcia, Spain). Illumina MiSeq sequencer was used to analyse microbial communities in soil. In addition, fungicide residues were analysed by gas chromatography (GC) in soil one day after first treatment dose, and two months after the end of treatment period.

Results and discussion

Analysis performed by qPCR detected four of the six pathogen analyzsed in leaves: *Alternaria* spp., *Botrytis cinerea*, *Pseudoperonospora cubensis* in all samplings, and *Didymella bryoniae* only in the first sampling. In the first sampling, no significant differences were found in pathogen abundance between fungicide treatments and control. In the second sampling a significant increment of *P. cubensis* was detected in control samples in comparison with fungicide treatments. Despite differences in *Alternaria* spp. and *Botrytis* spp. abundance were not statistically significant between treated and untreated leaves, a clear tendency was observed in fungicide treated samples, where the abundance of both pathogens was reduced. In the last sampling, significant differences were not found in pathogen abundance between fungicide treated samples and control. In soil, eight pathogens of the thirty analysed were detected: *Fusarium* spp, *Alternaria* spp., *Pythium* spp., *Olpidium bornovanus*, *Monosporascus cannonballus*, *B. cinerea*, *P. cubensis* in all treatments and samplings, and *F. oxysporum* only in the first sampling. No significant differences in pathogen abundance were observed between treatments until the last sampling, where six of eight pathogens showed significantly less abundance in PE-treated samples compared with FL-treated or control samples. In case of FL-treated samples, only three pathogens showed significant differences with control. Fungicide residues analysis of soil showed the presence of both fungicides only one day after first treatment, increasing the residue quantity two months after the end of treatment period.

Fungal community composition was significantly different between fungicide treated and untreated samples, varying across samplings. The influence of fungicide treatments in these changes analysed by PERMANOVA revealed that fungal community was influenced by the addition of fungicides in the second sampling (after fungicide treatments) and in the third sampling. Nevertheless, fungal diversity was only significantly affected by PE-treated samples two months after the end of the treatment (third sampling). These conclusions were in agreement with the results obtained from qPCR and soil residual fungicide analysis, and could disclose that both fungicides (especially PE) could be accumulated and remain in soil, acting against target and non-target microorganisms. Bacterial community composition was characterised by Proteobacteria, Bacteroidetes and Firmicutes phyla in all treatments and samplings. At order level, *Alteromonadales*, *Pseudomonadales* and *Bacillales* were predominant in the first and second sampling in all treatments and control. Analysis of fungicide effect in bacterial community performed by PERMANOVA, showed that there were not influence of fungicides in bacterial community composition nor in the first neither in the second sampling. However, the effect of fungicides was significant in the third sampling. No significant changes were found in diversity index along the experiment.

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Influence of environmental factors and plant protection products on the growth and survival of *Trichoderma harzianum* strain INAT11

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Highlights

- Interactions among plant species, soil pH, water availability, pathogens and *Trichoderma harzianum* strain INAT11 are extremely complex
- Root colonization by strain INAT11 is influenced by a combination of factors and not just one single factor

Introduction

Fusarium head blight of wheat (*Fusarium graminearum*, henceforth Fg) and Pink Ear Rot of maize (*Fusarium verticillioides*, henceforth Fv) are diseases caused by fungi of the genus *Fusarium* causing severe yield losses and mycotoxins contamination. In preliminary studies conducted by University of Padova (Italy), *Trichoderma harzianum* strain INAT11 (deposit number DSM25764) showed promising activity against Fg and Fv. Within the European research project BIOCOMES, it was therefore decided to investigate whether strain INAT11, applied as a seed treatment (i.e. below-ground), could actually control Fg and Fv in aerial plant parts (i.e. above-ground).

To evaluate whether seed treatments with strain INAT11 could constitute a feasible plant protection tool, the effects of environmental factors and conventional plant protection products, commonly applied in wheat and maize, on the growth of the antagonist were investigated.

Material and methods

The influence of environmental factors, such as temperature (tested range: 5 - 35°C), pH (tested range: 4.0 - 8.0) and water availability (AW; tested range: 0.995 - 0.910), on the development of strain INAT11 was investigated in *in vitro* studies on buffered growth media. The survival, development, root colonisation and disease control potential of strain INAT11 applied as seed treatment may vary considerably depending on abiotic factors (e.g. soil pH, water availability) and biotic factors (e.g. plant species, presence/absence of pathogens), as well as on the interaction of these factors. Thus, we investigated the effects of soil pH on in-soil survival and development of strain INAT11, Fv and/or Fg on potted plants and in field studies. Moreover, soil pH, water availability, plant species and presence/absence of strain INAT11, Fv and/or Fg on root colonisation by strain INAT11, Fv and/or Fg were also investigated. Finally, 22 pesticides (9 fungicides, 4 insecticides and 9 herbicides) selected among those commonly applied to cereals, were tested for their compatibility with strain INAT11 in laboratory studies.

Results and discussion

The results of the *in vitro* studies on buffered growth media showed that the effects of temperature, pH and AW on strain INAT11 were similar to those on other *T. harzianum* strains (Jackson et al., 1991). Temperatures above 30°C and below 10-15°C affected growth and survival of the strain. When temperatures were close to optimal (25-30 °C), they seemed to slightly attenuate the negative effects of alkaline pH and low AW. Growth and survival of strain INAT11 were reduced at increasing pH values (negative effect of alkaline pH). Dry conditions (low AW) were also unfavourable to the growth of strain INAT11 in the *in vitro* tests. Based on the *in vitro* studies, optimal conditions for the growth of strain INAT11 are warm temperatures, sub-acidic pH values and high water activity.

However, in the subsequent studies on potted plants and field studies, extremely complex interactions among plant species, soil pH, water availability, pathogens and strain INAT11 emerged, and root colonisation resulted to be influenced by a combination of factors and not just one single factor. Furthermore, under open-field conditions, the effects of alkaline pH values on strain INAT11 were considerably less pronounced than those expected based on the studies conducted on buffered growth media.

As far as the compatibility among conventional plant protection products and strain INAT11 is concerned, the antagonist resulted to be negatively affected especially by fungicides, and therefore the combined use of fungicides with a INAT11 seed treatment should be avoided. Most of the tested herbicides and insecticides, instead, did not negatively affect strain INAT11, and could thus be used in combination with the microbial biocontrol agent without any special concern. It must be pointed out, that also some of the tested herbicides and insecticides showed toxic effects to strain INAT11, and therefore compatibility studies should always be performed prior to applying a not yet investigated active substance in combination with the antagonist.

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Herbie 72®: a tool to standardise the adoption of anaerobic soil disinfestation (ASD) for intensive cropping systems under realistic scenarios

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Highlights
- Herbie products, included the tested formulation, are produced in food and feed factories based on 100% vegetable raw materials
- The product is highly and fast biodegredable and enhance the effects of anaerobic soil disinfestation (ASD)
- The use of this product inside a specific working protocol, standardises the ASD process enhancing the adoption of this soil disinfestation technique

Introduction

Anaerobic soil disinfestation (ASD) is a soil disinfesting process based on anaerobic soil conditions after the incorporation of decomposable amendments into a water saturated soil immediately covered with plastic mulch for a period variable from two- to six-weeks. ASD mechanism is based on the increased oxidative respiration. As a consequence, thanks to the soil porosity water saturated and to the presence of plastic mulch, anaerobic conditions persist until the carbon source is utilised or soil moisture content drops. Furthermore anaerobic decomposition of the added soil amendment allows many toxic by-products to accumulate such as organic acids and other volatile compounds that finally decrease soilborne pests and disease density. The objective of this study was to develop a standardised approach to improve the practical adoption of ASD under realistic scenarios. To aim such objective, three trials were performed against five soilborne pathogens.

Material and methods

A 2-year field study was established in 2016 and 2017 at the Centro di Sperimentazione ed Assistenza Agricola of Albenga (SV – Northern Italy) to determine the effectiveness of ASD as an alternative to conventional soil disinfestation. Three separate trials were organised respectively in summer 2016, autumn 2016 and spring 2017. In order to standardise the disinfestation process a specific patented soil amendant, provided by Thatchtec bv., Wageningen (NL) and registered as Herbie 72®, was incorporated into the soil throughout a spading machine till 25 cm depth, followed by soil compaction, soil irrigation aimed at saturate the soil porosity in the soil layer of 20 cm depth (60-70 % WHC) and soil mulching with a barrier film able to strongly limit oxygen diffusion from air to soil atmosphere. Rates of Herbie 72 ranged from 4 to 25 T/ha and mulching periods varied from 1 week to 6 weeks. Untreated mulched (solarised) and unmulched controls were established for comparison to ASD treatments. In order to evaluate the effectiveness of each combination of soil amendant and covering period, artificially prepared biomasses of five soilborne pathogens (T-bags)

were incorporated at 15 cm of depth inside a plastic net after both the amendant application and the soil compaction, but before both soil irrigation and plastic covering. At the plot uncovering such biomasses were recovered, brought to the laboratory and tested for the quantification of still viable propagules.

Results and discussion

The proposed improvement of ASD showed a significant ability to strongly decrease the viability of *Rhizoctonia* spp., *Fusarium oxysporum* f.sp. *lycopersici*, *Sclerotinia sclerotiorum*, *Phytophthora* spp. and *Verticillium dahliae*. Moreover, particularly when Herbie 72 was applied at 12.5 t/ha, a significant reduction of weed emergence was observed. Regarding the percentage of plot covered by weeds, Herbie applied mulched at 25 t/ha showed best results, but also reduced rates gave acceptable soilborne disease control.

Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Oral Session 4

Ecology and factors affecting induced resistance

Multitrophic regulation of induced defence responses

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Highlights

- Our findings indicate that microbes are "hidden" players in mediating plant-herbivore interactions
- Our evidence from several plant-herbivore systems indicates that insect-associated microbes can have a profound effect on the ability of a plant to perceive herbivores and thus trigger plant defences

Introduction

Plants are subject to attack by an onslaught of microbes and herbivores, yet are able to specifically perceive the threat and mount appropriate defences. Plants have evolved two primary defence pathways: one regulated by jasmonic acid (JA), which defends against herbivorous insects, the other by salicylic acid (SA), which responds to microbial pathogens and is frequently antagonistic with JA. Chewing herbivores cause massive damage when crushing plant tissues with their mandibles, thus releasing an array of specific cues that may be perceived by the plant, which mobilises plant defences.

The aim of our study is to investigate the role of higher trophic levels in modifying the oral cues of herbivores that are perceived by plants.

Material and methods

We are using noctuid caterpillars and the host plant tomato to investigate these interactions. In addition we are including bacteria, viruses, and parasitoids as representatives of the higher trophic levels. We are using a variety of molecular and biochemical approaches to investigate these multitrophic interactions.

Results and discussion

While specific cues in the oral secretions of herbivores such as caterpillars and beetles trigger plant defences, we have found that bacteria associated with these secretions can trigger the SA pathway, which benefits the herbivore by suppressing JA regulated defences. These results reveal a new strategy for how herbivores evade plant defences by using symbiotic bacteria that deceive the plant into perceiving a herbivore threat as microbial, thus resulting in suppression of plant defences against herbivores.

In another recent study, we have found that insect parasitoids that parasitise caterpillars may indirectly have a strong impact on plant defences. Along with injecting an egg inside the caterpillar, the parasitoid injects symbiotic polydnaviruses, which disable the caterpillar's immune system. As part of this immunosuppression, one component in the caterpillar's saliva known to trigger plant defences is nearly completely suppressed. These striking findings indicate that a symbiotic virus produced in parasitoids not only causes a massive suppression of the caterpillar's immune system, but also suppresses the plant's immunity or defences against herbivores.

Acknowledgements

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Protein-based products as resistance inducers: disease control and mechanisms of action

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Highlights

- Leaf treatments with a protein derivative represent a sustainable strategy in plant protection, because they induce grapevine resistance, and change the structure of leaf microbial communities on grapevine
- Plant-protein hydrolysates reduce powdery mildew severity, and their biocontrol activity is affected by the protein source, degree of hydrolysis and peptide composition

Introduction

Grapevine (*Vitis vinifera*) is one of the major fruit crops in the world, and downy mildew (caused by the oomycete *Plasmopara viticola*) is a serious disease that requires frequent fungicide applications. Increasing concerns about the negative impacts of pesticides on human health and the environment encourage the development of harmless alternatives to synthetic chemicals, such as resistance inducers (Delaunois et al., 2014). Proteins and peptides represent a wide category of plant elicitors (Albert, 2013), and the protein derivative called Nutrient Broth (NB) showed a high efficacy in controlling powdery mildew under field conditions (Nesler et al., 2015). This study aimed to dissect the mechanisms of action of NB against grapevine downy mildew caused by the oomycete *P. viticola* and to develop low-cost protein hydrolysates from agro-industrial by-products.

Material and methods

Grapevine plants (Pinot noir ENTAV115) grown under greenhouse conditions or *in vitro* (Nesler et al., 2015) were kept untreated (UNT) or treated with water (H_2O), 3.0 g/l NB (Nesler et al., 2015), or with a commercial product based on laminarins (LAM, 0.75 ml/l Vacciplant, Belchim Crop Protection). RNA extraction and quantitative real-time PCR reactions were carried out for the amplification of pathogenesis-related genes (*PR-1*, *PR-2*, and *PR-4*), osmotins (*OSM-1* and *OSM-2*) and chitinase (*CHIT-3*) (Nesler et al., 2015). Collection of phyllosphere microorganisms, DNA extraction and amplification of bacterial (V6-V8 of the 16S rRNA) and fungal (ITS3-ITS4 of the internal transcribed spacer, ITS) fragments were performed as described by Cappelletti et al. (2016).

Soybean, rapeseed and guar meals were subjected to enzymatic (Alcalase or Flavourzyme at 1% or 50% E/S) or chemical (6N sulfuric acid, H_2SO_4; condition A: 121°C, 15 min, condition B: 100°C, 8 h) hydrolysis (Cappelletti et al., 2017). Courgettes (*Cucurbita pepo*) and powdery mildew caused by *Podosphaera xanthii* were selected as easy-to-handle study pathosystem. Courgette plants (cv Nero Milano) grown in greenhouse (Nesler et al., 2015) were sprayed with protein hydrolysates (1

g/l), water (H_2O) or non-hydrolysed protein sources (N-H), and for the acid hydrolysis with a potassium sulfate (K_2SO_4) solution. The identification of peptides and amino acids was performed by an external service company (ISB Srl, Italy).

Results and discussion

The preventive foliar application of NB reduced downy mildew severity as compared with control plants (UNT and H_2O-treated), and the efficacy was higher in NB- than in LAM-treated plants. The expression levels of *PR-1*, *PR-2*, *PR-4*, *OSM-1*, *OSM-2* and *CHIT-3* genes were upregulated by NB before *P. viticola* inoculation, demonstrating the induction of grapevine resistance. Although the expression level of *CHIT-3*, *OSM-1*, *OSM-2* and *PR-4* was higher in LAM- as compared with NB-treated plants, LAM showed lower efficacy than NB against downy mildew, suggesting that multiple mechanisms of action are involved in the biocontrol activity of NB.

Indeed, NB changed the structure of phyllosphere bacterial and fungal populations as compared with control plants (UNT and H_2O-treated), and these modifications were affected by the composition of the originally residing microbiome. The NB treatment increased the proportion of some genera (e.g. *Exiguobacterium*, *Pseudomonas*, *Serratia*, *Lysobacter*) that potentially include biocontrol strains, suggesting that these changes may contribute to disease control. Furthermore, experiments using *in vitro* grown plants, in the absence of phyllosphere microorganisms, showed that the NB reduced downy mildew symptoms as compared with H_2O-treated plants, and induced the expression of *PR-2*, *PR-4*, *CHIT-3*, *OSM-1* and *OSM-2* before *P. viticola* inoculation. In conclusion, NB reduced downy mildew symptoms mainly by the induction of defence mechanisms in grapevine, and changed proportions of some microbial taxa linked to the biological control of plant pathogens, possibly providing a partial contribution to the control of downy mildew and to the activation of defence signalling pathways.

In order to develop cheaper and environmental-friendly protein-based products to control grapevine diseases, courgette powdery mildew was used as preliminary model pathosystem. Protein hydrolysates obtained by agro-industrial by-products were obtained, and guar hydrolysates significantly reduced powdery mildew symptoms. Particularly, two specific hydrolysis methods led to the formation of bioactive products (guar enzymatic hydrolysate Alcalase 50% and guar acid hydrolysate condition B). The biocontrol activity of hydrolysates was affected by the original protein source, the method and the degree of hydrolysis, namely the percentage of cleaved peptide bonds. The composition in free amino acids and peptide fragment could regulate plant responses to the pathogen infection. However, the use of strong acids during the hydrolysis causes an increase of salinity (K_2SO_4) of protein hydrolysates, which contributes to the disease control. The foliar application of low-cost protein hydrolysates represents an innovative approach to control crop diseases, and further studies are required to fully clarify their mechanisms of action and the effects on phyllosphere microorganisms.

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Deciphering the impact of nutrient stress in mycorrhiza-induced resistance against *Botrytis cinerea* in tomato

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Highlights

- *Rhizophagus irregularis* colonization of tomato roots results in a defence priming increasing resistance of the shoots to *Botrytys cinerea*
- Perception of a nitrogen starvation has a negative impact in the plant resistance to pathogen
- Arbusular mycorrhizal plants antagonize this negative impact of N starvation perceiving more efficiently N depletion than non-mycorrhizal plants and activate the hormone regulation responses faster and stronger

Introduction

Arbuscular mycorrhizal (AM) symbiosis is one of the most widespread mutualistic associations worldwide stablished. Mycorrhizal plants are more resistant not only to root attackers but also to foliar pathogens and pest conferring the plant a mycorrhiza-induced resistance (MIR) (Song et al., 2015). Among the mechanisms operating in MIR, increased plant nutrition, and induction of defence mechanisms have been reported (Jung et al, 2012). Mycorrhizal associations and their benefits for plant health are affected by environmental conditions; thus nutrient availability can have a strong impact on symbiosis and plant defences (Pastor et al., 2014). In this sense, recent discoveries may suggest a link between MIR and nitrate transporters (Gamir et al., 2014).

In this study, we investigated the effectiveness of MIR against *Botrytis cinerea* in tomato, the mechanisms behind it and whether nitrogen sensing in the root environment has an impact on its functionality.

Material and methods

Tomato seeds (*Solanum lycopersicum* L. cv. Better Boy) were inoculated with the mycorrhizal fungus *Rhizophagus irregularis* and maintained in a 25% phosphorous Long Ashtom liquid solution with continuous aeration. A set of both non-mycorrhizal (NM) and mycorrhizal (AM) plants received a Long Ashton modified free-N solution for 48 h prior to *B. cinerea* infection; 3rd and 4rd leaves were collected 72 h post infection to perform the analysis. Cell death was detected by lactophenol - trypan ylue staining; for *B. cinerea* quantification, quantitative PCR was performed on plant extracts as described by Gamir et al., (2014), and fungal genomic DNA was extracted and quantified by comparing the expression of the housekeeping gene of the fungus *BcActin* as described by Sanchez-Vallet et al., (2010).

RNA extraction and RT-qPCR analysis was performed as previously described (Pastor et al., 2014) using the tomato elongation factor 1α (*LeEF1α*, acc. AB061263) as a housekeeping gene. Relative expression data were calculated from the difference in threshold cycle (ΔCt) between the studied genes and DNA amplified by primers specific for each gene. Hormonal extraction was carried

out in freeze dried and powdered leaf samples as described by Pastor et al. (2014). Targeted hormonal analysis was performed as previously described (Gamir et al., 2012). Non-targeted metabolic extraction and analysis were carried out as described by Pastor et al. (2014).

Results and discussion

AM plants displayed significantly lower levels of damage upon *B. cinerea* infection than NM plants without N starvation. Upon N starvation, both NM and AM tomato plants became more susceptible compared to normally fertilised ones. Although the disease rate assessed by trypan blue staining showed that N depletion fully abolished MIR, the determination of fungal biomass showed that AM plants still were significantly more resistant than non-mycorrhizal plants.

Jasmonic acid (JA)-related genes, *LOXD* and *PROSYS*, were significantly induced during MIR. In agreement with this enhanced activation of JA biosynthetic genes, levels of JA and its precursor (cis-12-oxo-phytodienoic acid, OPDA) were higher in infected AM plants although N starvation did not affected the expression of these genes.

Metabolic data showed that MIR against *B. cinerea* occurs through defence priming. PCA analysis of untargeted metabolome indicated that although a certain effect in the leaf metabolome is associated with mycorrhisation in the absence of challenge, changes were particularly pronounced when plants were under pathogen attack or perceiving nutritional depletion. Searching in the metabolome compounds that showed enhanced accumulation during MIR against *B. cinerea*, we found the amino acid Trp and its derivatives which were over-accumulated in infected AM tomato compared NM plants. Met, Tyr, Gln and Glu were also induced in infected plants. The induction of most of these metabolites follows a profile in which their accumulation during MIR was abolished under N depletion. Although these compounds are likely to contribute to the resistant phenotype of AM tomato plants because most of them have been previously linked to plant resistance against pathogens, they cannot be responsible for that part of MIR which was still functional upon transient N depletion because all of them display the same levels of NM plants under nutrient stress conditions

In conclusion, *R. irregularis* colonisation of tomato roots results in an increased resistance of the shoots to *B. cinerea*, likely through defence priming involving the induction of a set of secondary metabolites that appears to participate as an integral part of a complex tuning mechanism of the immune system in AM plants. However, plants that sense potential N starvation reorganise their metabolism to prepare for a nutritional battle antagonizing defence responses against biotic stress but this defence repression observed in NM plants is partially antagonised in AM plants that maintain an active part of the N-independent phytometabolome changes related to resistance, mounting a less effective but still functional MIR against *B. cinerea.*

Acknowledgements

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Impact of agricultural practices on plant disease: what can we learn for resistance inducers optimisation?

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Highlights

- Understanding why nitrogen fertilization increase the impact of diseases may explain the lack of efficiency of resistance inducers
- We propose that in plants supplied with elevated nitrogen fertilization, the observed enhanced induction of plant defence is over-passed by an increase in the expression of the fungal pathogenicity program, thus leading to enhanced susceptibility

Introduction

The Chair Agrosys "Engineering for sustainable agrosystems", supported by Montpellier SupAgro, aims to bring together stakeholders in R&D (public and private), businesses, farmers around a platform to develop technical and scientific guidance. One of our goal is to better integrate biocontrol solutions in farming systems. For this purpose, we organised a workshop in May 2017 gathering all the actors of biocontrol experiment at the field level. Some participants focused particularly on resistance inducers. At this occasion, many scientific questions raised but one of the factor that has a strong impact on these products efficiency in the field is the agricultural practices. Indeed agricultural factors such as nitrogen fertilisers and drought are known to increase the level of many plant diseases. Here we will focus on one of our research project and the impact of nitrogen on the susceptibility of rice to the blast fungus *Magnaporthe oryzae*.

Material and methods

Standard fertilisation solution was supplied every Monday for 3 weeks. Twenty-six days after sowing, we supplied on Monday either a fertilisation solution containing a nitrogen source (1N condition), or the same solution without nitrogen source and corresponding to the 0N condition (Ballini et al., 2013). This fourth fertilisation was done 1 day before inoculation. We also used a mock treatment corresponding to the solution into which spores are re-suspended (i.e., 0.5% gelatin solution). Five to seven days after inoculation, symptoms were analysed using ImageJ software. Tissues sample were collected 2 days after fungal inoculation for dual RNA sequencing and were sent to BGI Tech (Huang et al., 2017). The RNA sequencing depth allowed a good coverage of rice and *M. oryzae* genes. Differential expression between all repetitions was performed for each of the four conditions 0N mock inoculated, 0N *M. oryzae* inoculated, 1N mock inoculated and 1N *M. oryzae* inoculated. *M. oryzae* differentially expressed genes were confirmed by quantitative PCR experiments.

Results and discussion

Agrosys chair organised a workshop in May 2017 gathering all the actors of biocontrol field experimentation. The participants have submitted to the research community four main family of questions on induced resistance. The first one is about the application, its quality, its timing and the new ways to reach the target. The second one is about pathogens, diversity of response to the products and durability of induced resistance challenged by evolving pathogen effectors. The third one was about varietal response to plant inducers and new breeding programs for a more efficient induced resistance. The last one was about the impact of the environment and in particular, the agricultural practices on these products efficiency in the field. Here we will focus on one of our research project and the interaction between the blast fungus *M. oryzae* and rice. Our objectives were to understand the mechanisms by which nitrogen is inducing blast susceptibility (NIS) and drought is inducing blast susceptibility (DIS) (Ballini et al., 2013; Bidsinski et al., 2016). In order to understand these mechanisms, we have conducted a dual RNA-Seq experiment on rice-infected tissues (Huang et al., 2017).

At least four hypotheses can be proposed to explain NIS: an indirect effect via plant growth, an increase in nutrient availability for the fungus, a regulation of plant immunity by nutrients like amino acids and a direct, positive regulation of fungal pathogenicity functions by nutrient availability. Using the interaction between rice and the blast fungus *M. oryzae*, we provide some elements to these different hypotheses and propose a model for the possible molecular mechanisms triggering NIS. Similarly, to previous results, our dataset clearly indicates that N fertilisation increases susceptibility despite an increase in the expression of several defence genes. Therefore, there is no clear-cut indication that a weakened defence system could be responsible for the NIS phenomenon. This enhanced defence may be directly or indirectly due to an increase in some metabolites (e.g., Glutamine). On the other hand, the fungus perceived small differences after penetration and N fertilisation seems to fuel pathogen growth. Moreover, we have highlighted that the pathogen modified the expression of effectors and pathogenicity related proteins. We propose that after N fertilisation, despite an increase in defence, the host does not succeed in facing a concomitant increase in pathogenicity of the fungus, leading to enhanced susceptibility. These results allow us to conclude that agricultural practices may affect defence inducers efficiency more likely indirectly through a modification of pathogen aggressivity rather than after a breakdown of the immune system.

Acknowledgements

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The age-dependent priming

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Highlights

- Indol-3-carboxylic acid induces resistance in adults and in seedlings plants, but the mechanisms behind are different
- In adult plants it is due to faster callose accumulation, but in the seedlings this doesn't occur
- Starch degradation mediates the priming of callose against *Plectosphaerella cucumerina*

Introduction

Plant defences are highly dependent on the developmental stage. It was termed age-dependent resistance or age-related resistance (ARR). One of the first layers of plant defence against pathogens is the accumulation of callose around the infection site. It has been proved to be boosted in priming (Flors et al., 2008) by the chemical β-aminobutyric acid and we also have evidences that is triggered by the new indolic compound indole-3-carboxylic acid (I3CA) against pathogens (Gamir et al., 2014). Both chemical treatments are effective protecting Arabidopsis plants against the necrotrophic pathogen *Plectosphaerella cucumerina*, however the mechanisms by which defence priming is expressed in young seedlings and adult plants differs significantly.

We propose that the molecular mechanisms in defence priming are age-dependent and mostly related to the availability of carbohydrates provided by the starch accumulated in leaves, that strongly depends on the developmental stage of the plant.

Material and methods

Seeds of Arabidopsis accessions Col-0, the *pmr4.1* mutant (encodes a callose synthase that is required for wound and papillary callose formation in response to fungal pathogens), *bam1* mutant (Beta-amylase activity for starch breakdown) and 35S::BAM1-YFP, were cultivated at 20ºC day/18ºC night with 8.5 h light per 24 h and 60% of relative humidity. The experiments with seedlings (three-weeks old plants) were made with 5 ml of water as a control treatment and 150 µM final concentration of I3CA. Treatments were made 48 h before infection. Plants were infected with spray-inoculation of 5×10^5 spores/ml of *P. cucumerina* and *Botrytis cinerea*. Phenotypes were determined by disease rate and fungal biomass were quantified by qPCR.

Callose levels were determined using aniline blue staining as described in Luna et al. (2011). Callose has been quantified in micrographs using GIMP (2.6.12) software.

Results and discussion

We have demonstrated that I3CA induces resistance in adults (5 weeks old plants) and in seedlings (3 weeks old plants), but the mechanisms behind induced resistance are different. While in adult plants it is mostly due to faster callose accumulation, this resistance is independent to an increase of callose accumulation in the seedlings. This phenomenon has been shown in Arabidopsis plants

against *P. cucumerina* and *B. cinerea*, suggesting that the age-dependent priming is not pathogen-dependent.

The metabolomic studies have determined a clear separation in a Principal Component Analysis of the Arabidopsis responses to *P. cucumerina* at different developmental stages. Therefore the basal mechanisms of resistance against this pathogen are largely dependent on the plant age.

Treatments with I3CA in the *bam1* mutant (impaired in starch degradation) didn't show an induced resistance against *P. cucumerina*. Callose accumulation in seedlings of *bam1* mutant was not higher than the callose accumulated in control plants, like it was seen in adults plants. These results demonstrate that starch degradation mediates the priming of callose against *P. cucumerina*.

Both treatments of *pmr4.1* didn't show callose accumulation after infection, as expected. I3CA didn't enhance resistance of *pmr4.1* against *P. cucumerina*, due to the basal resistance of this mutant is higher than the basal resistance of the controls. Using I3CA as a priming stimulus, we have demonstrated that priming is highly dependent on the developmental stage of the plant.

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Monoterpenes in systemic acquired resistance within and between plants

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Highlights

- This work focuses on the role of volatile organic compounds in systemic acquired resistance (SAR) in the model plant *Arabidopsis thaliana*
- We report that the monoterpenes α- and β-pinene are essential for SAR and also act as infochemicals propagating SAR-like immunity between plants
- This work underlines a possible ecological importance of SAR via air-borne signalling

Introduction

Salicylic acid (SA) and its associated local and systemic defence responses are important pillars in plant innate immunity. Local infections of plants with (hemi-)biotrophic pathogens result in pathogen-associated molecular pattern (PAMP)-Triggered Immunity (PTI) or effector-triggered immunity (ETI). Both PTI and ETI are characterised by the emission of long-distance signals that enhance the resistance of systemic, yet uninfected tissues against subsequent pathogen attack (systemic acquired resistance; SAR). Signalling molecules or intermediates that have been associated with SAR include (among others) the C9 dicarboxylic acid azelaic acid (AZA) and the putative lipid transfer proteins AZELAIC ACID INDUCED1 (AZI1) and EARLY ARABIDOPSIS ALUMINUM-INDUCED1 (EARLI1) (Jung et al., 2009; Cecchini et al., 2015). Here, we identified volatile monoterpenes that are essential for SAR and for plant-to-plant propagation of SAR-like immunity upstream of AZI1 and EARLI1 (Riedlmeier et al., 2017).

Material and methods

All methods and the associated materials are described in detail in Riedlmeier et al. (2017). We used *Arabidopsis thaliana* cultivar Columbia-0 (Col-0) for all experiments and included wild type (wt), *eds1-2* (enhanced disease susceptibility1-2), and *ggr1-1* (geranyl geranyl reductase1-1) mutant plants as sender plants in plant-to-plant communication experiments. For these experiments, plants were grown in stainless steel pots (2-3 plants per pot). At the start of each experiment 12 sender plants were sprayed with 10^8 colony forming units (CFU)/ml of *Pseudomonas syringae* pathovar *tomato* (Pst) carrying the effector AvrRpm1 (Pst AvrRpm1) in 10 mM $MgCl_2$ and 0.01% Tween-20 (v:v). The infection elicited signal emission and was compared to a mock treatment of sender plants with 10 mM $MgCl_2$ in 0.01% Tween-20 (v:v). One hour after the respective spray treatments, each group of sender plants was co-incubated with 8 receiver plants in 5.5 L vaccuum desiccators. Co-incubation was performed for three consecutive days. Once per day, the desiccators were opened and flushed with fresh air from the inlet of the growth chamber. After three days, fully expanded leaves of the receiver plants were syringe-infiltrated with 10^5 CFU/ml of Pst and the resulting *in planta* Pst titers were monitored at 4 days post-inoculation (dpi).

Results and discussion

Initial gas chromatography coupled to mass spectrometry analyses of SAR-related emissions of wild type and non-SAR-signal-producing *eds1-2* mutant plants associated SAR with monoterpene emissions (Riedlmeier et al., 2017). Four monoterpenes were found in the emissions of SAR signal-emitting Col-0 wt plants and three of these, the bicyclic monoterpenes α-pinene, β-pinene, and camphene, were undetectable in the emissions of similarly treated *eds1-2* mutants. Headspace exposure of *A. thaliana* to a mixture of α- and β-pinene enhanced the resistance of Col-0 wt plants to Pst growth. The same was observed if plants were exposed to camphene. Pinene-induced resistance was further associated with accumulation of reactive oxygen species. Also, full transcriptome analysis of pinene-treated plants strongly linked pinene-induced resistance to SAR with SA-related and in particular also SAR-specific genes among the most robustly induced genes in the response to pinene. These included AZI1, EARLI1, and two additional paralogs of AZI1 and EARLI1 (Riedlmeier et al., 2017). Concomitantly, pinene-induced resistance was dependent on AZI1, as well as on SA biosynthesis and signalling.

A. thaliana geranylgerany reductase mutants displayed reduced monoterpene biosynthesis in response to Pst AvrRpm1 and were SAR-defective (Riedlmeier et al., 2017). Normal local resistance to Pst growth in these mutants suggested that monoterpenes act specifically in systemic rather than local resistance. Strikingly, the volatile emissions from SAR signal-emitting wt plants induced resistance to Pst growth in neighboring wt plants. Because the low monoterpene emitters *eds1-2* and *ggr1-1* when used as sender plants did not induce the same response in wt receiver plants, plant-to-plant propagation of defence was associated with the presence of monoterpenes, including α-pinene, β-pinene, and camphene, in the emissions of the 'sender' plants (Riedlmeier et al., 2017). Our data suggest that monoterpenes, in particular pinenes, promote SAR, acting through ROS and AZI1. In addition, these volatiles appear to function as infochemicals in plant-to-plant signalling, thus suggesting a possible ecological function of volatile signalling in SAR propagating defence between neighboring plants.

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Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Oral Session 5

Multitrophic interactions and plant defence

Cellular regulations of grapevine resistance induced by *Trichoderma* spp.

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Highlights
- *Trichoderma*-induced resistance is mediated by transcriptional, translational and post-translational regulations
- *Trichoderma*-induced resistance is affected by the plant genotype, environment and fungal strain

Introduction

The *Trichoderma* genus is one of most studied biocontrol agents and some strains were able to induce systemic resistance in grapevine (Perazzolli et al., 2008). Grapevine is one of the most important fruit crops and is affected by several diseases, such as downy mildew caused by *Plasmopara viticola* (Gessler et al., 2011). The aim of this project was to identify transcriptional, translational and post-translational regulations of the *Trichoderma*-induced resistance in grapevine and to better understand genetic, environmental and chemical factors affecting the efficacy against downy mildew.

Material and methods

Grapevine plants were grown under controlled greenhouse conditions, treatments and inoculations were carried out as previously described (Perazzolli et al., 2008). A RNA-Seq approach (Perazzolli et al., 2012), an eight-plex iTRAQ protocol (Palmieri et al., 2012) and SIMAC purification (Perazzolli et al., 2016) were used to study global transcriptional, proteomic and phospho-proteomic changes of *Trichoderma*-induced resistance. Trichoderma species were tested (namely *T. harzianum* T39 and *T. atroviride* SC1) and the efficacy against downy mildew, modulation of plant defence genes and profiles of volatile organic compounds were analysed.

Results and discussion

Complex transcriptional (7024 differentially expressed genes) and proteomic (218 differentially expressed proteins) changes occurred in grapevine leaves during *Trichoderma*-induced resistance (Palmieri et al., 2012; Perazzolli et al., 2012). Moreover, the 45 and 49 kDa grapevine kinases were phosphorylated by the *Trichoderma* treatment and their activation was maintained after *P. viticola* inoculation. The *Trichoderma*-stimulated phosphorylation cascades included 103 proteins with significant changes in phosphorylation in response to beneficial and pathogenic interactions (Perazzolli et al., 2016) and they were involved in cellular processes of response to stimuli, signal transduction, transcription regulation and defence response. Particularly, proteins involved in

pathogen recognition, hormone signalling, gene expression regulation and defence response showed *P. viticola*-dependent phosphorylation changes exclusively in *Trichoderma*-treated plants and they may represent key regulators of priming mechanisms. The *Trichoderma*-induced resistance was affected by the plant genotype and exposure to heat and drought stresses. Moreover, the modulation of defence-related genes and the efficacy against downy mildew varied according to the *Trichoderma* species, indicating that specific elicitors and/or chemical determinants are recognised by the host plant as key stimulators of induced resistance mechanisms.

Acknowledgements

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A new tool to assess the grapevine defence at the high-throughput :« Neovigen96» chip and Fluidigm® technology

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Highlights

- We have developed a pioneering tool ("NeoViGen96" chip) based on microfluidic dynamic array platform for gene expression profiling to follow the spatio-temporal status of grapevine defences to set up alternative or complementary pest management methods with plant defence stimulator, associated or not with other pest management methods (biological control, plant breeding)

Introduction

Despite considerable progress in understanding the activity of elicitors and their reproducible effects in controlled laboratory conditions, their application on crops, such as grapevine, has been rather disappointing (Walters et al., 2013). In view of this situation, greater insight is needed into grapevine immune responses in relation to the genetic background of the plant, pathogen diversity and environmental conditions. The "NeoVigen96" chip enables to monitor the expression level of a selected-defence gene set which covers widely the various defence ways described in grapevine [salicylic acid (SA), jasmonic acid (JA), ethylene (ET) dependent signal transduction, pathogenesis related (PR) proteins, phytoalexins production and the cell wall reinforcement]. This tool has been used to assess the efficacy of potential inducers on susceptible cultivars and/on grapevine hybrids partially or totally resistant to downy and powdery mildew, in controlled conditions but also in field experiments.

Material and methods

The new "NeoVigen96" chip has been conceived by various strategies in order to obtain the most recent molecular data and find homologs to the already known responsive gene sequences and find new targets. The gene set included reference genes (N=11), PR proteins (N=28), some genes involved in secondary metabolites (phenylpropanoids, N=15) and indole pathway (N=5), others involved in the oxido-reduction system (N=5), in the ethylene or oxylipine/JA pathways (N=4), cell wall reinforcement (N=13) and others involved in pathogen detection-signalling and transcription signalling (N=15) (Dufour et al., 2016).This technology allows gene expression assessment on 95 cDNA preparations in a single run.

The Relative Expression (RE) of interest genes was calculated with the 2 -ΔΔCq method for every sample where ΔΔCq was the ΔCq difference between two samples. Genes were observed as differentially expressed for a *p*-value <0.05 in rank-based nonparametric multiple comparisons with the "nparcomp" package in the R statistical software.

Results and discussion

This new flexible high-throughput Q-PCR methodology is well adapted to monitor grape defence responses with a throughput 60 to 70 times higher than conventional assays and the samples and reagents used are approximately 6 times cheaper. Furthermore, amounts of cDNA required are 70 to 150 times smaller.

The "NeoViGen96" chip allowed us to demonstrate the defence-stimulating effect of benzothiadiazole (BTH), a well-known elicitor, in the vineyard, leading to a partial but significant protection against downy mildew. With fosethyl aluminium (FOS), a phosphonate known to have a double mode of action (as elicitor or as fungicide), the grapevine protection obtained against downy mildew in the vineyard could not be explained by weak elicitor activity so this suggests that it has a strong fungicide action in our hands. It is now possible to obtain better and easier understanding of grapevine responses to elicitation in the field. The potential of elicitors can be exploited by combining them in innovative pest management programs in association or in alternation with conventional fungicides in order to reduce the use of fungicides.

We then tested the usefulness of this tool to evaluate the state of defence of different cultivars or resistant genotypes having introgressed quantitative trait loci (QTL) of resistance against downy and powdery mildews and the interest of the association with plant resistance inducers in an innovative and sustainable program of pest management. Even if polygenic resistance is much less efficient, it postulated as a more durable alternative than monogenic resistance with major resistance because of the number of mutations needed for overcome is higher.

Thus, with this tool, we can show a strong correlation between gene expression and grapevine genotype resistance level and the application of BTH on these genotypes or cultivars induces changes at the molecular level in the vine leaves but differently depending on the genetic background of the plant.

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Resistance induction by hot water treatments to control apple postharvest diseases

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Highlights

- Hot water dipping (45°C, 10 min) of apple controlled fruit postharvest pathogens as *Penicillium expansum* (blue mould) and *Neofabraea vagabunda* (bull's eye rot), in artificially infected fruits
- The results indicated a significant inhibition of both diseases when fruit were inoculated after 4 and 3 hours respectively, from the treatment, supporting the hypothesis of resistance induction exerted by hot water

Introduction

Fungicide treatments remain one of the most effective methods to reduce postharvest decays protecting the fruit from infections occurring before treatment, including quiescent infections, as well as from infections during storage, handling and marketing. The repeated and continuous use of fungicides has led to a strong selection pressure in pathogen population and has increased the concerns about the effect of chemicals on human health and the environment. In this context, considerable efforts have been dedicated to finding safer methods for disease control. Among the alternative strategies, heat treatments have received a great attention, showing significant reduction of *Monilinia* spp. (Spadoni et al., 2013), *Penicillium expansum* (Barkai-Golan and Phillips, 1991) and *Neofabraea alba* (Neri et al., 2009) infections.

The aim of this study was to investigate on the possible involvement of resistance induction in fruit treated with hot water before the pathogen inoculation.

Material and methods

'Gala' apple harvested at commercial maturity, were heat treated by dipping in pre-wormed (45°C) water for 10 min, wounded with a nail (2 × 2 × 2 mm) and inoculated with 20 µl of a 10^4 conidia/ml of a *P. expansum* conidia suspension, after 1, 4, and 24 h from treatment. Incidence of disease was recorded after 6 days of incubation at 20°C.

'Golden Delicious' apple were treated with hot water and wounded as described above, subsequently they were artificially inoculated with *Neofabraea vagabunda* conidia suspension (10^5 conidia/ml) 0, 3, 6 and 24 h after treatment. Disease severity was assessed by measuring the diameter of lesions (mm) after 6 days at 20°C. In both experiments, control fruit were represented by fruit dipped in water at 20°C and inoculated with pathogens. The sample unit was represented by 4 replicates of 6 fruits each, and the experiment was performed three times.

In the first case, an apple microarray was used to conduct a global transcriptional analysis of gene expression in treated apple. In the second, crude protein extracts (CPEs) derived from the hot water treated apples were assayed on conidia germination and on the pathogenesis enzymes activities of *N. vagabunda*

Results and discussion

No visual symptoms of heat damage were observed on fruit treated with hot water for 10 min at 45°C. Additionally, no off-flavours or anomalous softening were detected by sensory evaluation in treated fruit, any significant differences were not found in sweetness, acidity, or fruitiness, in comparison to the control (data not shown). Fruit heat treated and then inoculated with *P. expansum* at 1 and 4 h after treatment, showed a significant disease reduction of 30%. No reduction was observed when the apples were inoculated 24 h after treatment. In previous trials, the exposure conditions of 45°C for 10 min had no effect on viability of *P. expansum* conidia (Spadoni et al., 2015b), consequently these data indicate the potential role of induced defence responses, resulting from the hot water treatment, in the resistance of harvested apples to pathogen infection. In contrast, on peach fruit, a hot water treatment, before *M. fructicola* inoculation provided a stimulation of conidia germination and no control of brown rot (Spadoni et al., 2015a).

Abiotic and biotic stresses often induce or modify signalling pathways. The microarray data (validated by RT-qPCR of 9 differentially expressed genes) revealed the predominant induction of heat shock proteins (HSPs), increasing in number and type, at all the analysed time points (0 – 24 h). Only a small number of genes were suppressed in hot water treated apples as compared to the number of induced genes. The reduction in the number of differentially expressed genes at 8 and 24 h after heat treatment suggests that the response to a high temperature treatment is temporary. The heat tolerant pathogen *P. expansum* is able to grow in apple tissues treated with hot water, but its reduced incidence in apple inoculated after 1 and 4 h from the treatment suggests an induced resistance response in fruit.

Apple fruit inoculated with conidia of *N. vagabunda* after 3 h from treatment showed a reduction of disease severity of 52% towards control. Crude proteins, extracted from fruit 0, 3, 6 and 24 h after treatment, significantly inhibited conidia germination of pathogen, although the best control was observed with CPE derived from fruit 3 h after hot water treatment (83%). In addition, a significant reduction of pathogenesis enzyme activities of the pathogen was detected when pathogens were exposed to CPEs derived from hot water treated apples. As asserted by Maxin et al. (2012), there are at least two components which may contribute to the mode of action of hot water: i) a direct and lethal effect of heat on fungal inoculum within or outside the fruit, and ii) an indirect effect mediated by a stress-induced physiological response of the fruit. In the case of peach, probably there is only a direct effect on the pathogen conidia, while our results on apple support the hypothesis of the induced resistance against tested pathogens, even if it cannot be also excluded a direct effect as already reported by Spadoni et al.(2015a).

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Volatile methyl salicylate induces systemic signalling in the phylloxerated root system of hybridised *Vitis* spp.

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Highlights

- Root phylloxeration stimulates the biosynthesis of methyl salicylate in root gall tissue belowground
- The salicylic acid signaling pathway, efficient against host pathogens, is activated locally in phylloxera root galls and systemically within root tips in the vicinity

Introduction

Since its introduction to Europe in the mid of the 19th century grape phylloxera (*Daktulosphaira vitifoliae*) is among the most dangerous pest in worldwide viticulture. The obligate biotroph parasite causes root gall (nodosity) formation on rootstocks of hybridised American *Vitis* spp. Nodosities and root tips of infested plants represent strong sink organs and contain elevated levels of carbohydrates and amino acids depicting them as attractive feeding tissues for secondary, soil-derived pathogens. Lawo et al. (2011) detected increased levels of methyl salicylate (MeSA) in the volatile root gall metabolome.

We aim to analyse whether volatile MeSA induces systemic resistance mechanisms in the root system. We hypothesise that the released MeSA activates the salycilic acid (SA) signalling pathway demonstrated by the regulations of salycilic acid (SA) responsive genes locally in nodosities (H1) and systemically in root tips of infested plants (H2).

Material and methods

Dormant cuttings of Teleki 5C Gm 6-52 (*V. berlandieri* × *V. riparia*) were propagated for 1.5 months under greenhouse conditions (25 ± 5°C, 60% rH, 16 h photoperiod) for root and shoot development. Two weeks after transfer into plastic containers containing a 1:1 perlite:seramis substrate, 24 rooted cuttings were inoculated with 300 grape phylloxera eggs collected from a phylloxera single founder lineage belonging to biotype C in isolated climate chambers (26 ± 4°C, 45% rH, 16 h photoperiod 125-140 W/m^2). Control root tips of not infested as well as nodosities of L4/5 (adult larval stage) grape phylloxera individuals and root tips of infested plants were collected 50 dai (2nd insect generation). RNA extraction, reverse transcription and subsequent qRT-PCR analyses were done according to Lawo et al. (2013). Microarray data was extracted from Griesser et al. (2013) confirming differentially expressed genes of pooled L2-L5 root galls compared to the mean of non-infested control root tips of Teleki 5C.

Results and discussion

Lawo et al. (2011) detected 38 increased volatile metabolites within the metabolome of phylloxera nodosities. Among them volatile MeSA, known to be an efficient activator of induced systemic resistance, was found to be significantly increased upon phylloxeration. In the present study

expression patterns of MeSA biosynthetic genes were upregulated in L4/5 nodosity tissue (*VviSAMT1* 2.94 log_2FC; *VviSAMT2* 2.80 log_2FC) providing evidence that nodosity formation triggered MeSA biosynthesis by the host plant via transcriptional stimulation. Microarray data (Griesser et al., 2013) confirmed the upregulations of *VviSAMT1* 3.24 log_2FC and *VviSAMT2* 3.02 log_2FC. MeSA is reported to activate the SA signalling pathway resulting in effective host defence mechanisms against pathogens.

Expression patterns of SA responsive marker genes were upregulated in L4/5 nodosity tissue (*VviPR2* 1.94 log_2FC; *VviPR5* 4.71 log_2FC and *VviSTS* 2.28 log_2FC) possibly indicating that grape phylloxera involves the SA defence pathway to protect nutrient rich nodosities against secondary soil-borne infections. Previous studies of soil-nodosity cross infections with fungal agents were difficult to compare and yielded in partially contrasting results, indicating an underlying host defence mechanism protecting vulnerable and nutrient rich galls. MeSA is reported to be the primary transport molecule to spatially transmit the SA signal among infested and not infested parts of the host within the phloem as a volatile (Jayakannan et al., 2015). We detected upregulated gene expression patterns of SA responsive marker genes (*VviPR2* 4.12 log_2FC; *VviPR5* 1.47 log_2FC and *VviSTS* 2.87 log_2FC) in not infested root tips of infested plants revealing that phylloxeration induced a systemic defence signal in the whole root system. Summarizing the present study demonstrated that grape phylloxera triggered MeSA biosynthesis by the host, resulting in the activation of the defensive SA signalling pathway in the global root system.

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Efficacy of elicitors on boosting insect natural enemies: the case of vineyard

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Highlights
- Low dosage silicon foliar treatments stimulate plants defence and improve the abundance of natural enemies

Introduction

The strategical use of chemical ecology in conservation biological control can enhance the regulation of pests by improving biological control agents and antagonists (Simpson et al., 2011). Some elicitors can affect the release of jasmonic acid (JA), a phyto-hormone that induces the emission of induced plant volatiles (HIPVs), leading to the attraction of natural enemies of pests (Dicke, 2009). For this reason, the elicitors activating in the plant a multiple response pattern could be used to increase the indirect resistance to pest infestations.

The study presents one of the first field experiment focused on the influence of elicitors on beneficial insects. This experimental activity was carried out in a vineyard in the province of Bologna, where silicon (a resistance inducer) was used for treatments also in combination with *Trichoderma* to evaluate effects on diseases and pests.

Material and methods

In order to achieve the aims of this work, a single silicon application was performed at inflorescences swelling (BBCH 55) using 12 g of silica gel per hl. After choosing the best and most efficient sampling method, sticky traps were arranged in the vineyard. They were positioned after 20 days from the treatment of silicon and replaced weekly for 3 times over a 3 week period (7, 14, and 21 June), beginning in early June. Finally, collected traps were analysed in the laboratory in order to evaluate the arthropods captured.

According to obtained data, the most abundant taxa were chosen (Ichneumonoidea, Chalcidoidea, Mimaridae, Nematocera, Phoridae) for statistical analysis and a multifactorial analysis of variance (ANOVA) was carried out, using treatments (silicon) and time (three sampling dates) as factors in testing the main effects of these as well as their interactions.

Production of JA by leaves was assessed in the laboratory by Solid Phase Micro Extraction (Zadra et al., 2006).

Results and discussion

During the sampling period of the experiment, 41,456 individual insects were captured. Silicon treated plants showed to attract Mymaridae, an important family of egg parasitoids in vineyard, in

comparison with control and also a repellent effect on Phoridae, a family that includes several herbivorous organisms. For Nematocera, Ichneumonoidea and Chalcidoidea, no significant effects were found. The study showed that silicon seems to be very efficient as resistance inducer. These results are associated to a greater JA production induced by this elicitor. JA production proved to be constantly and significantly higher in treated plants with respect to untreated ones. The experimental activity could be considered reliable due to the robust experimental plan and a sufficiently extended vineyard, which minimise interferences. Moreover, the sampling method is efficient according to other field experiments in Australia (Simpson et al., 2011). This field study showed a potential for applying a combination of chemical ecology (elicitors) and agroecology in order to increase conservation biological control of pests (attract and reward approach). Overall, this field experiment, whether confirmed in other contexts, could represent an interesting strategy in the modern approach of integrated pest management.

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Elicitation responses in cucumber plants after treatment with a fraction of a liquorice leaf extract from *Glycyrrhiza glabra*

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Highlights

- Liquorice leaf extract and fraction 4 elicit early responses in treated, non-infected cucumber plants
- Fraction 4 seems to induce self defence reactions against *Pseudoperonospora cubensis*, which depend on the cucumber cultivar

Introduction

Liquorice (*Glycyrrhiza glabra*) leaf extract effectively controls cucumber downy mildew, caused by *Pseudoperonospora cubensis*. Fraction 6 of the crude extract resembled to a high extent the efficacy and direct effect of the extract (Scherf et al., 2012). Fraction 4 (terpenoids and sterols) had a moderate efficacy but no direct effects *in vitro*, hinting the possible activation of the plant's self defence. Accumulation of H_2O_2 was found in cucumber leaf discs treated with fraction 4 and the crude extract (own studies).

The aim of this study was to get first insight in the potential of liquorice extract and fraction 4 to elicit responses in cucumber related to the plant's self defence. The trials were done on the cultivar (cv.) 'Agnes', a cv. moderately susceptible to downy mildew. Investigations on H_2O_2 accumulation, molecular analysis of the expression of peroxidase (*POD*) and efficacy trials were conducted in parallel.

Material and methods

Cultivation of plants and pathogen, fractionation of the liquorice extract (exception: CH_2Cl_2 was substituted by tBME), and the general set-up of trials on efficacy were done as described in Scherf et al. (2012). The detection of H_2O_2 in treated cucumber leaf discs followed the protocol of Thordal-Christensen et al. (1997).

Cucumber plants were treated with liquorice crude extract (2%), fraction 4 (2%), fraction 6 (2%), β-aminobutyric acid (BABA; 0.1%, POD trial), ethanol (EtOH; 2%, efficacy trial) or dimethyl sulfoxide (DMSO; 1%, POD trial) as negative control. For qPCR, samples of 3 plants per treatment were pooled (total 100 mg), harvested 1.5 h, 3 h, 6 h and 12 h after treatment and frozen in liquid nitrogen. RNA extraction was conducted with Direct-zol RNA MiniPrep Kit (Zymo Research) after manufacturer instructions. The cDNA synthesis was done with iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.) following the manufacturer's protocol. The qPCR was run following the protocol and program described in the manuals of Maxima SYBER Green qPCR Kit (Sigma-Aldrich) in a C1000 touch thermal cycler (BioRad) and the normalised relative expression of genes was calculated with Bio-Rad CFX Manager 2.1. The target gene was *POD* and *actin* was chosen as standard gene.

Results and discussion

In own former studies, an accumulation of H_2O_2 in non-infected cucumber leaf discs of the susceptible cv. 'Chinese Slange' 6 - 8 h after the treatment with liquorice extract and its fraction 4 was found (not published). We now observed the same reaction after treatment of leaf discs of the cucumber cv. 'Agnes' with liquorice extract and its fraction 4.

Reactive oxygen species (ROS) like H_2O_2, play a key role in the defence reaction of plants attacked by a pathogen (Lin and Ishii 2009) and are first reactions after elicitation. Thus, the accumulation of ROS in the tissue of cucumber treated with crude liquorice extract or its fractions, point to the involvement of an indirect effect in the plants. As being toxic for the plant tissue, the plant protects itself, e.g. by depletion of ROS with the help of POD (Lin and Ishii 2009).

The relative expression of *POD* in 'Agnes' was approx. eight times higher in BABA and fraction 4 treated plants [6 h post treatment (hpt) and 12 hpt, respectively], compared to the untreated control. We also found a peak of *POD* in liquorice treated, non-infected cucumber plants (cv. 'Agnes') 6 hpt, which was 2 times higher than untreated plants. A similar peak occurred in untreated, infected plants 6 h post inoculation. In untreated, infected plants, there was a second peak five times the height of the first one, 6 days post inoculation. These results suggest that in liquorice treated plants a second peak may occur after inoculation as well. This will be tested later.

The effect of liquorice crude extract and fraction 4 on the cucumber plants was remarkable, since the efficacy especially of fraction 4 after the protective treatment appeared to depend highly on the chosen cucumber cv. responsiveness. Whereas fraction 4 moderately controlled the infection with *P. cubensis* (efficacy = 17 %) in 'Chinese Slange', it here seemed to promote the infection in 'Agnes' (disease severity: EtOH control = 51%, fraction 4 = 70%). Moreover, the efficacy of the crude liquorice extract against *P. cubensis* was somewhat negatively affected in 'Agnes' (efficacy: 'Chinese Slange'= 90%; 'Agnes' = 82%)

The results clearly indicate an elicitation of defence and hint towards a mode of action that is partly build on the mechanism of induced defence especially caused by fraction 4. However, the final effect on disease control seemed to be cv. dependent. We already found first indications of different molecular responses of the cvs. 'Chinese Slange' and 'Agnes' upon infection with *P. cubensis*.

To verify the results of this preliminary study, further trials on the expression of other pathogenesis related genes, beyond *POD*, in non-infected and infected plants are foreseen. By comparing the expression of different target genes in cucumber cvs. with varying resistance levels and defence mechanisms, the interaction of the direct and indirect effect of liquorice leaf extract and it's fractions in controlling oomycetes should become clearer.

Acknowledgements

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Identification and functional characterisation of grapevine volatile organic compounds for the sustainable control of downy mildew

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Highlights
- Volatile Organic Compound (VOC) emissions differed between resistant and susceptible grapevine genotypes following *Plasmopara viticola* infection
- VOCs of resistant grapevines significantly inhibit downy mildew severity on leaf disk assays

Introduction

Grapevine (*Vitis vinifera*) is one of the most widely cultivated fruit crops and is susceptible to a large spectrum of pathogens, such as *Plasmopara viticola* that causes downy mildew (Gessler et al., 2011). Wild grapevine species are resistant to *P. viticola* and breeding programs have introduced resistance traits to susceptible cultivars. Plant defence responses are based on different mechanisms and volatile organic compounds (VOCs) play a crucial role in the communication between plants and other organisms. Although the emission of VOCs upon *P. viticola* inoculation was shown in resistant grapevine genotypes (Algarra Alarcon et al., 2015), the functional role of these molecules in the grapevine defence mechanisms was not yet investigated. The aim of this study was to identify and functionally characterise VOCs produced by resistant and susceptible grapevine genotypes in response to *P. viticola* in order to further develop innovative methods for the sustainable control of downy mildew.

Material and methods

The susceptible *V. vinifera* cultivar Pinot noir and four resistant genotypes (Kober 5BB, SO4, BC4 and Solaris) were grown for three months under greenhouse conditions. Plants were inoculated with a suspension of *P. viticola* sporangia as previously described (Perazzolli et al., 2012). Downy mildew severity was assessed at seven days after inoculation according to the OIV-452 descriptor and scores from 1 (the most susceptible) to 9 (the totally resistant) were assigned (Bellin et al., 2009). Leaf samples were collected before (T0) and six days (T1) after *P. viticola* inoculation and five replicates (plants) were analysed for each genotype at each time point and the experiment was carried out twice. Each sample was frozen in liquid nitrogen and ground to a fine powder. Leaf powder was weighed into 20 ml headspace vials and analysed by headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME/GC-MS; Weingart et al., 2012). Eight identified

VOCs were selected according to their emission profiles and pure compounds were tested against *P. viticola* by leaf disks assays. Downy mildew development was assessed on leaf disks at one, two and six days post inoculation (dpi) by aniline blue staining.

Results and discussion

VOC profiles measured by HS-SPME/GC-MS analysis revealed a high reproducibility of the two experiments. Terpenes, isoprenoids, aldehydes, alcohols, esters and heterocyclic compounds were found in both experiments in all the five genotypes tested and their abundance was generally greater in resistant genotypes as compared with Pinot noir at T1. Differences in terms of VOC abundance were found in resistant genotypes at T1 as compared to T0, whereas small changes were found in Pinot noir VOCs between the two time points. The abundance of two sesquiterpenes was higher in all resistant genotypes as compared with Pinot noir at T1. Moreover, other three sesquiterpenes showed a higher abundance in three resistant genotypes (BC4, Kober 5BB and Solaris) as compared with Pinot noir at T1. Kober 5BB and Solaris showed also a higher abundance of one heterocyclic compound and one isoprenoid as compared with Pinot noir at T1. Finally, the abundance of a C5 aldehyde was higher in Kober 5BB as compared with Pinot noir at T1. These eight pure VOCs were tested against *P. viticola* in liquid suspension and in air volume. The eight VOCs impaired the development of downy mildew symptoms at dosages that ranged from 0.1 to 10.0 g/l in liquid suspension. However, five of them caused severe phytotoxic effects on leaf disks at the dosage of 10.0 g/l. Four pure VOCs (one isoprenoid, one alcohol, one C5 aldehyde and one heterocyclic compound) significantly reduced downy mildew symptoms at the dosage of 20.0 mg/l in air volume, when each VOC was applied to a filter paper disk and placed on the lid of the Petri dish.

Microscope observations with aniline blue staining revealed marked differences between control and VOC-treated leaf disks after *P. viticola* inoculation. The number of pathogen structures was reduced in leaf disks treated with one isoprenoid, one alcohol and one heterocyclic compound as compared to control disks at one, two and six dpi. Moreover, no *P. viticola* structures were visible on leaf disks treated with the C5 aldehyde. This aldehyde and one isoprenoid were also able to reduce the diameter of *P. viticola* sporangia.

In conclusion, downy mildew increased the production of VOCs (terpenes, isoprenoid, alcohols, aldehydes and heterocyclic compounds) in resistant and not in susceptible genotypes and these molecules are associated to the activation of grapevine defence mechanisms. Moreover, VOCs of resistant genotypes play a major role in the grapevine resistance and significantly reduced downy mildew symptoms on susceptible leaf disks, indicating that they can be further developed as sustainable control molecules.

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Evaluation of the antifungal activity of the protein and non-protein extracts of *Trichoderma asperellum* and *Trichoderma atroviride* culture filtrates against *Phytophthora infestans*.

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Highlights

- The methanolic extracts of *Trichoderma asperellum* culture filtrates revealed an important inhibition on mycelial growth, sporulation, germination and on the disease reduction on leaf discs of potato inoculated with each of the two isolates of *Phytophthora infestans*
- The 2H-Pyran-2-one-6-pentyl compound could be responsible for the fungicidal activity

Introduction

The biocontrol is an innovative approach in the management of strategic crop diseases. According to several authors, some *Trichoderma* spp. have demonstrated their efficiency as biological control agents due to their antagonistic and hyperparasitic power. The present study aims to test, *in vitro* and *in vivo*, the efficiency of protein and non-protein parts extracted from an isolate of *T. atroviride* and twelve isolates of *T. asperellum* taken from the rhizosphere of Algerian potato culture zones on the two isolates A1 and A2 of *Phytophthora infestans*, in order to identify the active ingredient fungicide against a late blight of potato.

Material and methods

This work aims to study the *in vitro* and *in vivo* antifungal power of protein and non-protein extracts of the culture filtrates of 12 isolates of *T. asperellum* (TA, TB, TC, TE ,TF , TG, TH, TI, TJ, TK, TL and TM) and one isolate of *T. atroviride* (TD) issued rom Algeria rhizosphere and tested against the A1 and A2 of *P. infestans* isolates.

Results and discussion

The protein assay revealed significant concentrations (25.36 µg/ml) of extracellular enzyme extract of the TK isolate of *T. asperellum* and intracellular enzyme extract (46.30 µg/ml) of the TM isolate of *T. asperellum*. The study of enzyme activity highlighted proteolytic activity for all antagonistic isolates with the largest area proteolysis (10.3 mm) recorded for the TH and TM isolates of *T. asperellum*. However, no chitinolytic activity was present in all isolates

However, the protein extracts of all isolates showed a low inhibitory ability on mycelial growth, but moderate on sporulation and germination of the two pathogenic isolates. It is worth mentioning

the absence of the mycoparasitism effect on morphology and the absence of inhibition on the pathogenicity of *P. infestans* isolates.Furthermore, chemical analysis by FTIR of butanol extracts of all *Trichoderma* spp. isolates culture filtrates revealed 16 chemical groups with some similarities between antagonists isolates and the dominance of acids, alkanes, aromatic groups and alcohols. Chemical analysis by GC-MS of the methanol and hexane extracts of antagonists cultures filtrates revealed 32 metabolites with the dominance of 2H-Pyran-2-one-6-pentyl component (6PP). The antioxidant activity by UV spectroscopy using the method of trapping of the free radical DPPH showed a moderate reducing power compared to ascorbic acid for methanol extracts of all isolates of Trichoderma spp. culture filtrates.

The antifungal activity of the two types of previous extracts has confirmed *in vitro* and *in vivo* significant fungicidal activities, on the A1 and A2 *P. infestans* isolates. The methanolic extracts of culture filtrates of the TC, TE, TG and TK *T. asperellum* isolates confirmed the complete inhibition of mycelial growth, sporulation, germination and the pathogenicity of *P. infestans* isolates. What prompts us to propose their use in the biocontrol field trials for their formulation as biofungicides in the management of late blight of potato caused by both A1 and A2 *P. infestans* isolates.

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Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Poster Session 1

Multitrophic interactions and plant defence

Regulatory role of *SlyWRKY75* transcription factor in stress in tomato plants

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Highlights

- *SlyWRKY75* gene expression is induced in response to biotic stress, especially in *Botrytis cinerea* infected tomato plants
- SlymiR1127-3p might be a putative regulator of *SlyWRKY75* gene expression in tomato plants undergoing *B. cinerea* infection
- Epigenetic markers that will allow studies of transgenerational inheritance of *SlyWRKY75* stress biomarker were detected

Introduction

WRKY transcriptional regulators are involved in defence to diverse plant stress conditions and are induced in response to several phytohormones [jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA)] and pathogen attack (Shankar et al., 2013). MicroRNAs (miRNAs) are endogenous small non-coding RNAs that regulate expression of their target genes in plants and perform key roles in response to a wide array of biotic and abiotic stresses, targeting a vast range of transcription factors, including WRKY factors, and defence-related genes (Kamthan et al., 2015). WRKY75, described to be involved in inorganic phospate (Pi) stress response, has been recently found also induced in the defence against necrotrophic pathogens in Arabidopsis, but little is known about the functional roles of specific miRNAs with WRKY75 proteins, particularly in non-model plants. In this study we analysed the expression of *SlyWRKY75* gene in tomato plants in response to different stresses and the underlying molecular mechanisms at the level of miRNAs and epigenetic regulation.

Material and methods

Tomato plants (*Solanum lycopersicum* Mill cv. Ailsa Craig) were grown under greenhouse conditions at 26/18°C (day/night) temperature, with 16/8 h (light/darkness) and RH = 60%. Four-week-old plants were kept untreated (control) or subjected to drought stress (deprived of water during 1 week), or temperature stress (5ºC temperature increase), or *Botrytis cinerea* infection (applying to the plants droplets containing spores and sampling 24 hours post inoculation; hpi), or *Pseudomonas syringae* pv *tomato* DC3000 infection (inoculation by dipping plant leaves into the bacterial suspension and sampling 48 hpi), or *Leptinotarsa decemlineata* (Colorado potato beetle, CPB) infestation (larvae of different developmental stages) or Hexanoic acid (Hx) inducer treatment as in Finiti et al. (2014).

Total RNA was isolated from leaves of control and treated tomato plants, 1st cDNA was synthesised, and *SlyWRKY75* gene expression was assessed by qRT-PCR, using Power SYBR Green PCR Master Mix (Applied Biosystems) and gene specific forward and reverse primers. For each sample, 3 biological replicates were analysed using the mean values of 3 technical replicates. Relative-fold calculations were made using *RPS18* (ribosomal protein S18) gene to normalise gene expression. miRNA expression was also analysed by qRT-PCR.

Plant hormones][ABA, SA, cis-12-oxo-phytodienoic acid (OPDA), JA, JA conjugated with isoleucine (JA-Ile)] were determined from fresh plant tissue by reverse-phase HPLC-quadrupole-hexapolequadrupole mass spectrometry (Micromass spectrometer).

Results and discussion

Previously, we found that *WRKY75* gene expression was induced in tomato plants in response to *B. cinerea* infection, as well as upon Hx (a plant priming inducer) treatment (Finiti et al., 2014). To investigate the role of *SlyWRKY75* in response to stress conditions, we first analysed by qRT-PCR differential gene expression profiles in tomato plants under biotic or abiotic stresses compared to control plants. Results showed induction only in plants undergoing biotic stresses (*B. cinerea* or *P. syringae* infection and CPB infestation), but not in response to abiotic stresses (drought or temperature) or Hx treatment. *SlyWRKY75* gene expression was considerably higher in response to *B. cinerea* (55-fold) than that detected in *P. syringae* infection (31-fold) or CPB infestation (2-fold).

Levels of SA, JA, JA-Ile, OPDA and ABA hormones in response to stress conditions were also determined. Tomato plants undergoing biotic stresses (*B. cinerea* or *P. syringae* infection, CPB infestation) showed higher levels of JA, JA-Ile and SA than control plants, whereas ABA levels were increased under drought stress and SA in response to drought and temperature.

As previously described in Arabidopsis plants infected with *Sclerotinia sclerotiorum* and *Pectobacterium carotovorum* (Chen et al., 2013), in the present work gene expression profiling in tomato plants indicated that SlyWRKY75 might be a transcriptional regulator of SA-dependent defence signalling pathways in response to plant stresses, and JA pathway to defend against *B. cinerea*, *P. syringae* and CPB stresses, or ABA pathway against drought stress.

To assess the role of miRNAs in controlling the expression of *SlyWRKY75* associated with biotic stress conditions, putative miRNA targeting SlyWRKY75 were predicted (Dai and Zhao, 2011) as well as the corresponding secondary structure fold (RNAfold web server). We found only one predicted target site in the 3′-UTR of *SlyWRKY75* mRNA with substantial sequence complementarity to *SlymiR1127-3p* miRNA. In plants, most target mRNAs only contain one single miRNA complementary site which can exist anywhere along the target mRNA rather than at the 3'-UTR as in animals (Zhang et al., 2006).

SlymiR1127-3p expression levels were determined under biotic stresses and were only repressed in *B. cinerea* infected tomato plants (0.4-fold), as it generally occurs in plants, which show a negative correlation between the expression levels of miRNAs and their target genes (Xin et al. 2010). Histone modifications analysis by ChIP demonstrated the presence of the activator histone modification H3K4me3 on the promoter and on the gene body of *SlyWRKY75* at 24 hpi. The induction of this gene in response to *B. cinerea* correlates with the presence of an activator mark.

In conclusion, *SlyWRKY75* gene expression was upregulated in response to biotic stresses, especially in *B. cinerea* infected tomato plants, and may be regulated by miRNA-mediated pathways modulating the activation of defence signalling responses.

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Induction of resistance in wheat against leaf rust by application of biotic and abiotic inducers

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Highlights

- The exogenous application of β-Aminobutyric acid (BABA) as a soil drench reduced brown rust efficiently
- Seed dressing with *Pseudomonas protegens* CHA0 reduced the number of sporulating pustules on the leave significantly. The induction of resistance was visible as necrotic or chlorotic flecks

Introduction

Leaf rust of wheat, caused by *Puccinia triticina*, has always been one of the major constraints in wheat production. The use of synthetic fungicides for disease control may have negative effects on human and animal health as well as for the environment. The high degree of virulence variation in time and space within the pathogen population is a major constraint for breeding of stable and durable leaf rust resistance in wheat (Kolmer and Hughes, 2013). An environmental friendly alternative procedure to protect plants against disease could be the activation or the re-enforcement of proper plant defences using specific biotic or abiotic elicitors.

This study seeks to determine the capacity and the degree of induction of resistance against leaf rust in wheat by the known resistance inducer β-Aminobutyric acid (BABA) and the biocontrol bacterium *Pseudomonas protegens* strain CHA0.

Material and methods

Experiments were done with the highly susceptible bread wheat cultivar Arina (Agroscope/DSP). For sterilisation, wheat seeds were rinsed in 70% ethanol, incubated for 5 min in 5% sodium hypochlorite and washed three times with sterile distilled water, seeds were then soaked overnight either in the resistance inducer (bacterial suspension or BABA solution) or in sterilised distilled water (mock inoculation). Thereafter, seeds were pre-germinated on 1% water agar, planted in a standard potting mixture (soil/sand, 3:1, vol/vol) and placed in a growth chamber with 16 h day at 22°C, 8 h night at 18°C.

The bacterial suspension was prepared with an overnight culture of *P. protegens* strain CHA0 at the concentration of either 2.8×10^6 CFU/ml or 2.8×10^8 CFU/ml.

The BABA (Sigma-Aldrich, Germany) solution, consisting of two concentrations 5 mM and 50 mM, was used to drench the soil 48 h before the infection with leaf rust on mock-inoculated seeds.

Infections with leaf rust were done at the 2 leaf stage of the plants (BBCH 12). For this, fresh harvested urediospores of *P. triticina* (isolates from Switzerland) were mixed with talcum powder (ratio 1:9) to obtain a concentration of 2.5×10^5 spores/plant and rubbed smoothly by hand along the leaves. The response of the seedlings was scored 12 days after infection based on the infection types expressed on each plant described by Johnston and Browder (1966).

Results and discussion

BABA is a well-recognised inducer of resistance against a broad spectrum of pathogens such as fungi, bacteria, virus and nematodes (Baccelli and Mauch-Mani, 2016). BABA is often applied as a soil drench (Amzalek and Cohen 2007). In this work, BABA treatments as soil drench reduced leaf rust significantly in wheat. Drenching at a concentration of 50 mM was able to protect completely wheat seedlings against leaf rust similar to results obtained with other rust species (Amzalek and Cohen 2007; Barilli et al., 2012).

Inoculation with *P. protegens* strain CHA0 gave a somewhat different result. While mock-inoculated plants showed lots of sporulating uredia (high infection type), plants with the bacterial treatment on the seeds showed a mix sporulating uredia and chlorotic and necrotic flecks. The concentration of the bacterial inoculum was not decisive for this effect. Overnight soaking of seeds in a BABA solution did not yield any protective activity against *P. triticina* even at 50 mM (results not shown). While bacteria establish on the roots, their protective effect is arguably weaker, but lasts longer. In this study, BABA was an efficient inducer only when applied 2 days before infection with leaf rust. Several studies demonstrate that BABA can also be used 1-3 day post-infection against a large spectrum of pathogens (Justyna and Ewa 2013).

This study shows that it is possible to induce resistance in wheat against leaf rust with both BABA and beneficial bacteria. We are now pursuing with histological experiments to clarify the mechanisms underlying the observed resistance.

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Contrasting effects of the rhizobacterium *Pseudomonas simiae* on Diamondback moth and Cabbage root fly

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Highlights

- Knowledge of plant growth promoting rhizobacteria (PGPR) and their relation to insects is lacking. Here, the aim was to study insect performance on PGPR inoculated plants
- No plant promotion was found, however insect weight was significantly altered. Insects may respond in a positive manner in terms of performance to rhizobacterial colonization which may lead to total higher crop loss

Introduction

Plant growth promoting rhizobacteria (PGPR) are considered to have potential to not only increase agricultural yields by plant growth promotion, but also through priming of plant defence via induced systemic resistance (ISR) defence priming. PGPR, via plant-mediated effects, are generally regarded to have negative impact on the performance of chewing insects. However, there are also examples of insects that are benefited by the colonisation of rhizobacteria. To be able to utilise the plant defence potential of these bacteria, we need a greater understanding of the interactions and factors affecting the outcome of rhizobacterial inoculation of plants.

The aim of this study was to investigate insect performance on plants inoculated with PGPR on both an aboveground and a belowground insect species.

Material and methods

In a greenhouse experiment, we grew white cabbage plants (*Brassica oleracea* var. Christmas Drumhead) in a sterilised perlite-soil mix, together with the ISR inducer *Pseudomonas simiae* (formerly *fluorescens*) WSC417r. After 5 weeks, we infested the plants with Diamondback moth (*Plutella xylostella*) and cabbage root fly (*Delia radicum*) neonate larvae, which were allowed to freely feed from the leaves and roots respectively. Insect and plant biomass were recorded.

Results and discussion

We found no increase in plant biomass in *P. simiae*-inoculated control plants, however, adult biomass of *D. radicum* was enhanced, whereas biomass of *P. xylostella* was significantly reduced. Plant biomass was not significantly reduced, but showed a trend of biomass reduction in insect-infested plants. Our findings demonstrate that below-ground insects may respond in a positive manner in terms of performance to rhizobacterial colonisation unlike many leaf feeders, which subsequently may lead to higher crop loss from these below-ground insects.

First insights on the ability of a *Lysobacter capsici* member to induce resistance mechanisms in grapevine plants

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Highlights

- Viable and heat-killed cells of the biocontrol agent *Lysobacter capsici* AZ78 are as effective as copper hydroxide in controlling grapevine downy mildew
- Application of viable and heat-killed *L. capsici* AZ78 cells induces the deposition of callose in grapevine leaves

Introduction

The bacterial genus *Lysobacter* is regarded as a valuable source of novel biocontrol agents against phytopathogenic oomycetes (Hayward et al., 2010). Application of *L. capsici* AZ78 (AZ78) cells on grapevine plants drastically reduced attacks of *Plasmopara viticola*, the causal agent of grapevine downy mildew, and antibiotics produced by AZ78 were shown to be toxic against *P. viticola* sporangia (Puopolo et al., 2014). However, the high level of disease reduction suggested that more than one AZ78 mechanism could be involved in the plant protection. Since biocontrol *Lysobacter* strains can also induce plant resistance (Kilic-Ekici and Yuen 2004), our aim was to assess the ability of AZ78 to trigger in grapevine molecular mechanisms related to plant resistance. We tested the expression pattern of plant pathogenesis-related genes and the deposition of callose in grapevine leaves treated with viable (vAZ78) and heat-killed (hkAZ78) AZ78 cells.

Material and methods

Lysobacter capsici AZ78 was routinely grown at 27°C onto Luria-Bertani agar. The efficacy of AZ78 against *P. viticola* was evaluated on susceptible plants of *Vitis vinifera* cv. Pinot Noir, grown under controlled greenhouse conditions (25 ± 1°C, 60 ± 10% RH) for two months. Plants were treated with suspensions of 10^8 CFU/ml of vAZ78 and hkAZ78 (90°C for 10 min) cells 24 h and 6 h before *P. viticola* inoculum. Control plants were treated 6 h before the *P. viticola* inoculum with distilled water (DW) and copper [$Cu(OH)_2$; 2.5 g/l; Kocide 3000, Du Pont de Nemours, USA]. *Plasmopara viticola* was propagated onto Pinot Noir plants; the inoculum suspension (5×10^5 sporangia/ml) was prepared according to Puopolo et al. (2014). One hour before the *P. viticola* inoculation, AZ78 populations residing onto leaves treated with vAZ78, hkAZ78, DW and $Cu(OH)_2$ were quantified through dilution plating method. The percentage of leaf area covered by sporulating lesions (disease severity) was evaluated 120 h after *P. viticola* inoculum. Five plants were used for each treatment; the experiments were repeated three times.

Deposition of callose in leaves treated with DW, vAZ78 and hkAZ78 cells was evaluated 1 h before *P. viticola* inoculum and 24 h after *P. viticola* inoculum according to Diez-Navajas et al.

(2007). At the same time points, modulation of *PR-1* and *PR-4* genes in leaves treated with DW, DW, vAZ78 and hkAZ78 cells was assessed through quantitative Real Time-PCR (qRT-PCR) according to Nesler et al. (2015).

Results and discussion

Understanding how the biocontrol agent AZ78 can effectively control *P. viticola* is an important step for its development as a novel biofungicide. In this study, we evaluated the ability of this biocontrol agent to reduce *P. viticola* infections through the activation of plant resistance mechanisms.

Greenhouse trials clearly showed that the application of both vAZ78 and hkAZ78 cells was effective in reducing *P. viticola* attacks similarly to $Cu(OH)_2$. Indeed, DW treated plants showed a disease severity of 51 ± 12%, whereas disease severity reached not significantly different values of 11 ± 12%, 8 ± 9% and 11 ± 15% on plants treated with $Cu(OH)_2$, vAZ78 and hkAZ78 cells, respectively. No AZ78 cells were recovered from plants treated with $Cu(OH)_2$, DW and hkAZ78 cells, demonstrating that the heat-treatment was effective in killing AZ78 cells. On the other hand, an AZ78 population of 4.30 ± 0.16 $\log_{10}$ CFU/g of leaf was recovered from plants treated with vAZ78 cells, confirming the AZ78 ability to persist on grapevine leaves (Puopolo et al., 2014).

The deposition of callose is one of the quickest responses of the plant to the invasion of pathogens. Before *P. viticola* inoculum, callose deposition was observed in leaves treated with vAZ78 cells whereas no reaction was observed in DW- and hkAZ78-treated leaves. However, 24 h after the *P. viticola* inoculum, callose deposition was observed in the stomata of both vAZ78- and hkAZ78-treated leaves, while different zoospores nearby the stomata were registered in DW-treated leaves. Interestingly, callose deposition increased during the incubation period: an extended and intense callose deposition around the stomata was observed 120 h after *P. viticola* inoculation both in vAZ78- and hkAZ78-treated leaves. On the othe rhand, many sporangiophores emerged from stomata in DW treated leaves. The increase in callose deposition in vAZ78- and hkAZ78-treated leaves was not associated with the modulation of genes related to plant resistance. In fact, qRT-PCR analysis revealed no differences in relative expression level of *PR-1* and *PR-4* between DW-treated leaves and leaves treated with vA78 and hkAZ78 cell at 6 h and 24 h after *P. viticola* inoculation.

Overall our results confirmed the ability of AZ78 to effectively control *P. viticola* on grapevine plants under controlled conditions. Since hkAZ78 cells were effective in reducing the disease similarly to vAZ78 cells and $Cu(OH)_2$, it is conceivable that AZ78 may induce some defence strategy in grapevine plants. Although *PR-1* and *PR-4* genes were not induced by the application of vAZ78 and hkAZ78 cells, we showed that AZ78 cells trigger the deposition of callose in correspondence of the stomata, reducing the grapevine downy mildew severity.

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Chitosan vs. chitosan nanoparticles in the control of *Fusarium graminearum*: A synergistic effect of fungitoxic activity and plant defence activation?

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Highlights

- Chitosan and chitosan nanoparticles are able to inhibit the germination and mycelium growth of *Fusarium graminearum* and to control wheat head blight caused by this dangerous fungus
- When loaded with the antioxidant N-acetyl cysteine chitosan nanoparticles lose their activity *in planta*, suggesting that the control of head blight by nanoparticles is mediated by an oxidative burst

Introduction

Chitosan (Cs), a deacetylated chitin derivative, exerts its activity both as plant resistance activator and fungitoxic compound. The former property is due to the induction of localised micro-oxidative bursts in the treated plants (Faoro et al., 2008), while the fungal toxicity is possibly the consequence of an increased membrane permeability, together with the chelation of essential nutrients and the binding to DNA (Hadwiger, 2013). Chitosan nanoparticles (Cs-NPs) are fungitoxic as Cs (Kashyap et al., 2015) and they can penetrate more easily into plant tissues, thus getting in contact with the invading fungus. However, it is not known if they are also able to induce plant defence mechanisms. In this study, we attempted to control a *Fusarium graminearum* GFP- engineered strain, both *in vitro* and *in planta* (durum wheat), by treatment with Cs, Cs-NPs and Cs-NPs loaded with the antioxidant N-acetyl cysteine (NAC), with the aim to shed light on the mechanisms of Cs-NP activity.

Material and methods

Cs (161 kDa, 90% N-deacetylation) was acquired from Biobasic (Canada). GFP-engineered *F. graminearum*, wt strain 8/1, was kindly provided by Prof. Wilhelm Schäfer (Biocenter Klein Flottbek, Hamburg, Germany). Cs (0.5 mg/ml) was dissolved in 0.05% acetic acid and adjusted to pH 5.3 with NaOH. Cs-NPs were prepared with the ionotropic gelation method detailed in Rampino et al. (2013), that produced 150-200 NPs, as assessed by dynamic light scattering and transmission electron microscopy (TEM). NAC loaded NPs were prepared adding 0.5 mg/ml NAC to the Cs solution before ionotropic gelation with Tripolyphosphate (TTP). The actual load of NAC in Cs-NPs was 1 mg/ml, as assessed by HPLC. Inhibition rate of radial mycelial growth was recorded after 5 d culture on PDA medium added with 0.25 µm filter-sterilised Cs, Cs-NPs, Cs-NPs-NAC, 0.05% acetic acid or with a fungicide (Scenic, Bayer). Conidia germination assays were carried out by mixing 1.5 ml of each of the above compounds with 0.5 ml of conidia suspension (10^5/ml) and examining the presence of germ tubes after 2 and 5 d. For *in planta* experiments, durum wheat plants, cv. Colombo, were kept in growth chambers at 22°C, 60% RH and 16 h day light. Inoculation was done at anthesis by injecting 10 µl of conidia suspension (10^5/ml) in medial spikelets, 24-48 h after treatment of the ears with Cs,

Cs-NPs, Cs-NPs-NAC, acetic acid or Scenic. Infection results were assessed either visually or microscopically 3-4 weeks after inoculations.

Results and discussion

In the *in vitro* experiments, Cs and Cs-NPs but not Cs-NPs-NAC showed a significant reduction of radial mycelial growth of *F. graminearum*. In the case of Cs-NPs-NAC, a reduction was also observed but was not significant in respect to control (acetic acid) because of the variability of the single experiments. In contrast, both types of Cs-NPS were more effective than Cs in reducing conidia germination. In fact, only 8% of conidia germinated after 5 dd incubation with Cs-NPs, against 15% in Cs alone and 30% in acetic acid (negative control). The fungicide (positive control) showed a similar inhibition rate as Cs-NP.

In growth chamber experiments, wheat spikelets treated with Cs and Cs-NPs before inoculation, showed a significant delay of symptom appearance in respect to control in which the whole ears were blight after 3 weeks. Instead, in Cs and Cs-NP during the same time lapse the infection was still localised in the inoculated spikelets. The microscopic observation of these spikelets by UV light to detect the GFP engineered fungus, confirmed the limited spread of the mycelium over paleas lemmas and glumes. Interestingly, in Cs-NPs-NAC treated spikelets the infection spread to the whole ear which appeared blight at the same time as control. Thus, it seems that the antioxidant activity of these NPs hampers the capacity of unloaded Cs-NPs to control the fungus. A possible explanation could be that their ros reactive oxygen species (ROS) scavenger activity is able to neutralise the micro-oxidative burst induced by Cs, in turn responsible for the activation of plant defence (Faoro et al., 2008).

In conclusion, besides confirming the already reported ability of Cs-NPs in controlling *F. graminearum* (Kheiri et al., 2016), this study indicates that this control *in vivo* is likely to be due to a synergistic effect of the fungitoxic properties of Cs-NPs and their ability to activate plant defence mechanisms as Cs.

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Fructose and sucrose as priming molecules against pathogens and pests?

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Highlights

- In vineyards, fructose allowed to reduce the doses of copper against *Plasmopara viticola*
- Fructose showed the same efficacy as the natural pyrethrum against *Scaphoideus titanus*
- In corn production, sucrose and fructose reduced the number of *Ostrinia nubilalis* larvae
- The sucrose reduced the frequency of attack of *Helicoverpa armigera*

Introduction

Sugars could act as "priming" molecules inducing preparation of plants to defend in case of micro-organisms attacks. Theses knowledges led to the new concept of "sweet immunity" where sugars are widely accepted as players in plant innate immunity (Bolouri Moghaddam and Van Den Ende, 2012; Trouvelot et al., 2014). The exogenous foliar application of sucrose and D-fructose can induce resistance by antixenosis to the insect egg-laying codling moth (*Cydia pomonella*). In apple orchards, the application of sucrose at 0.01 g/l reduced the means of infested fruits by 41.0 ± 10.0 % (Arnault et al., 2016). USAGE and SWEET frameworks contributed to explore the efficacy of sugars against pathogens and pests. Here, we reported new interesting results of field trials of the use of fructose and sucrose against downy mildew (*Plasmopara viticola*) and the leafhopper (*Scaphoideus titanus*) in vineyards and, against corn borer (*Ostrinia nubilalis*) and corn earworm (*Helicoverpa armigera*) in corn productions.

Material and methods

For the "downy mildew field trials", several treatments were applied in organic vineyards in 4 experiments between 2012 and 2014 (cultivars Gamay and Côt). The aim was to test the efficacy of fructose at 10 mg/l in combination with reduced copper dose (100 g/ha or 150 g/ha) compared to the reference copper dose (400 g/ha to 600 g/ha). Each bioassay was randomised in block. The downy mildew assessment was done with the disease severity on fruits and leaves (percentage of organs covered by sporulating lesions).

For the "leafhopper field trials", the objective was to compare the applications of sucrose and fructose at 10 mg/l on larvae (3 applications before the larvae stage) or associated with natural pyrethrum. One experiment was conducted in Vaucluse in 2016 on Sauvignon cultivar. Larvae were counted on 50 leaves per block.

Concerning the corn borer and the "corn earworm field trials", the objectives were to test the effect of sucrose and fructose at 100 mg/l and 1 g/l. The two field trials located in Landes and in Bouches-du-Rhône were randomised in block. The first application of sugar was carried out in the seed line at the time of sowing and then the two following applications were carried out in the treatment of the aerial parts on maize (stages 2-3 leaves and 4-5 leaves).

Results and discussion

In the Gamay vineyards, the reduced copper modality combined with fructose at 0.01 g/l was intermediate between the modality of reduced copper and the maximal dose of copper. In the Côt vineyard, the modality of reduced copper with fructose at 0.01 g/l was as effective than the treatment with maximal dose of copper.

On grapevine, the sucrose at 0.01 g/l seemed to increase the action of pyrethrum on the populations of leafhoppers *S. titanus*. Fructose, used alone, has a comparative or even better efficacy than the one of pyrethrum only. The application of sucrose at 1 g/l and 10 g/l or sucrose at 1 g/l associated with fructose at 1 g/l reduced the number of corn borer larva per plant with an efficacy up to 50%. The association of sucrose + fructose at 1 g/l provided the best efficacy.The applications of sucrose at 1 g/l and 100 g/l made it possible to reduce the frequency of the attacked ears by corn earworm larvae with efficacy of 15 and 23% respectively.

In conclusion, the applications of sugars were first tested with success to control the codling moth (*C. pomonella*) in apple trees and opened a door to the development of new strategies. This work brings new interesting results in organic vineyard for the biocontrol of the leafhopper and the downy mildew and in maize productions against the corn borer and the corn earworm.

Foliar applications of sugars are presented as methods of stimulating plant immunity to control pathogens and insects but the mechanisms were not yet elucidated. Several hypotheses can be advanced. A single sugar applied on leaves without any injury can induce a plant response. The output and input of the sugars through the cuticle follow the photosynthesis rhythm. One might think that a basic natural immunity (innate immunity) should be partially maintained by this mechanism. The application of sugar could be at the origin of a stress or a self-damage signal. The sugar should be present at the wrong time somewhere in the apoplast, in the plasma membrane or within the cell in the cytosol. At this occasion the immunity could be magnified. The host-specific non-pathogen associated epiphytic microorganisms can induce leaking of metabolites from plants and/or produce them. Their possible contributions to chemical signals given by the leaf surface is an issue that should not be ignored. The role of epiphytic microorganisms and genes involved in the plant-defence system (apple and vine) are explored in the framework SWEET (CAS DAR 2016-2019). Furthermore, sucrose and fructose have been approved for the control of European corn borer and codling moth as basic substances (EC implementing Regulations No 916/2014 and 2015/1392 respectively).

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The effect of phosphite on *Phytophthora infestans* and synergism with conventional fungicides in field-grown potato and tomato in Ethiopia

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Highlights

- Spraying with phosphite reduces late blight in potato and tomato in a highland tropical climate
- Phosphite can reduce the use of conventional fungicides with 75% without loss in protection level or crop yield

Introduction

Phytophthora infestans causing late blight is one of the most destructive plant pathogens affecting both potato and tomato cultivation worldwide. Therefore production still depends on high fungicide use. In this study, field trails were carried out for three consecutive years in Ethiopia to investigate the efficiency of phosphite, an inorganic salt with direct and indirect toxicity on oomycetes, and its combinations with conventional fungicide Ridomil against potato and tomato late blight.

Material and methods

Two potato cultivars, Belete and Jalene, moderate resistant and susceptible to late blight, respectively, and a moderate resistant tomato cultivar, Melkasholla, were used. With the appearance of the first late blight symptom plants were treated weekly with recommended or doubled dose of phosphite, Ridomil, combination of half the recommended dose of phosphite and Ridomil, or in a combination of 75% of the recommended dose of phosphite and 25% of the recommended dose of Ridomil. Plants from the inner rows were examined on a weekly basis for late blight severity and relative area under the disease progress curve (rAUDPC) was calculated. At maturity, yield from inner row plants was recorded and sorted as marketable or unmarketable and weighted. Natural infestation was relied on during all the experimental years. However, in a separate experiment we tested the phosphite sensitivity for a number of European and Ethiopian *P. infestans* strains *in vitro*.

Results and discussion

The results showed that potassium phosphite combined with reduced dose of Ridomil had the same effective suppression of potato and tomato late blight as the full recommended dose of Ridomil. In the moderate resistant potato cultivar, Belete, phosphite alone had adequate foliar protection. In

the susceptible potato cultivar, Jalene, phosphite treatment resulted in higher foliar infection compared to full dose of Ridomil and the other phosphite and Ridomil combinations. However, phosphite provided a clear protection against foliar infection than the untreated control. In tomato, phosphite was as effective as the recommended dose of Ridomil and combination of phosphite and Ridomil. Yield also increased with phosphite and phosphite and Ridomil synergism. However, phosphite treatment alone led to less yield than the recommended dose of Ridomil and the combination between Ridomil and phosphite. Whilst the tomato yield obtained in phosphite treated plots was virtually the same as with Ridomil and the combined treatments. *In vitro* plate assays showed that sensitivity against phosphite varied between *P. infestans* strains. These findings suggested that phosphite could be used against potato and tomato late blight alone or combined with reduced dose of fungicides in Ethiopia. The amount of fungicide use could also be reduced by 75 percent.

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Role of ferulic acid in Fusarium head blight resistance of wheat spikes

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Highlights

- Fusarium pathogens infect wheat florets at anthesis causing Fusarium Head Bligh. Ferulic acid, an antioxidant compound, is already present in flower tissues at flowering
- Wheat varieties with high FA content in flower tissues showed an elevated spike resistance. FA might play an important role in resistance of spikes against F. graminearum infection

Introduction

Fusarium head blight is one of the most noxious wheat diseases causing not only severe yield losses but also results in contamination of grains with mycotoxins. The pathogen infects the spike during flowering, first by penetrating into the florets and subsequently by spreading throughout the spike via the rachis. Ferulic acid (FA) is the most abundant phenolic compound in plants and present in the wheat flower (Zhou et al, 2005). The high antioxidant activity is a well-recognised element in the resistance cascade against fungal infections and insect pests. Preliminary *in vitro* studies showed an inhibitory effect of FA on *Fusarium* growth and mycotoxin synthesis (Boutigny et al, 2010). The aim of this study was to link the concentration of FA in wheat florets at flowering in different wheat varieties and their resistance against *Fusarium graminearum* in the field.

Material and methods

Eight wheat varieties were grown in the field in Nyon (VD), Switzerland. Three plots of each variety were artificially inoculated at flowering with a *F. graminearum* strain FG 13 (available at: mycoscope.bcis.ch). A forth plot was not inoculated and served as a control. Symptoms of FHB were rated as described in Mascher et al. (2005) and spike resistance of varieties was compared using the area under disease progression curve (AUDPC).

FA content in flower tissues without husks was determined at flowering and 10 days post flowering (p.f.) with high-performance-liquid-chromatography (HPLC). Analyses were carried out on single plants with three replicates for the two growth stages.

Results and discussion

FA analyses revealed that the antioxidant compound is present in various concentrations in flower tissues at flowering, when *Fusarium* pathogen attempts to penetrate the florets. Average contents of FA in flower tissue increased between flowering and 10 days p.f. from 100 to 125 mg/kg.

Significant differences in the resistance were observed between varieties. Varieties were clustered by the resistance level, showing that florets of susceptible varieties contained significantly less FA than resistant varieties both at flowering and a fortiori at 10-days p.f. Interestingly, the local

variety “Munstertaler” possessing high spike resistance, contained the highest content of FA 10 days p.f. However, FA contents in florets and AUDPC showed no correlation.

The results suggest that FA is already present in florets and might play an important role in resistance of spikes against *F. graminearum* infection. The underlying interactions between FA and *Fusarium* pathogens remain to be investigated. Yet, it is conceivable that a certain content of FA in the floret is necessary to impede primary infections by *F. graminearum*. Studies including a larger number of varieties are in progress to investigate the role of ferulic acid in the different resistance mechanisms of wheat spikes and grains against *Fusarium* pathogens.

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The *Reynoutria sachalinensis* knotweed leaf extract elicits defence responses in *Cucurbita pepo* plants against *Podosphaera xanthii*

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Highlights

- Courgette susceptible and resistant to *Podosphaera xanthii* genotypes respond differently to *Reynoutria sachalinensis* extract
- The extract induced a more localized resistance response compared to chitin on susceptible plants
- Mode of action is possibly mediated by Salicylic Acid induced genes

Introduction

Podosphaera xanthii is the causative agent of courgette (*Cucurbita pepo*) powdery mildew. It is an obligate biotroph causing severe yield and commercial losses by reducing the photosynthetic activity and the consumption of carbohydrates and other nutrients in the host plants (Elad et al., 1996). The *Reynoutria sachalinensis* knotweed leaf total extract, available under the commercial names of Milsana® and Regalia®, has been tested against powdery mildew of many plant species, e.g. cucumber, tomatoes, grapes and wheat. Field studies have reported high efficacy of Milsana® against cucumber powdery mildew (Konstantinidou and Schmitt, 1998) and reduction of mildew disease severity on both cucumber and courgette (La Torre et al., 2004; Bokshi et al., 2008).

The aim of this study was to investigate the effect of *R. sachalinensis* extract on crop performance and disease development on hybrids with variant susceptibility to the pathogen and to dissect the mechanisms of action as resistance inducer.

Material and methods

Courgette seeds of a resistant and a susceptible hybrid to *P. xanthii* were grown either in glasshouse conditions in soil amended with and without chitin (in a split-split plot design), or in growth rooms. Disease intensity on leaves, yield and fruit quality parameters were assessed in the former case. In the latter case, plants were treated with the elicitor two days prior to artificial inoculation. Conidia germination and fluorescing callose cell wall deposition was observed by means of UV/vis microscopy. For microscopy experiments, samples were collected 1, 2, 3 and 4 day post inoculation for observation of spore germination on leaf surfaces, and the 7th day post infection for observation of callose deposition. Leaf samples for liquid chromatography–mass spectrometry (LC-MS) analysis were collected before (day 0) and after inoculation (days 1, 2 and 4). For the gene expression study, leaf material was collected two days after *R. sachalinensis* extract application and prior to inoculation with *P. xanthii* conidia (day 0), and for four consecutive days post inoculation (days 1 to 4). The identification and semi-quantification of phenolic compounds was performed by LC-MS. The relative gene expression of *PR1*, *PR2*, *PAL* and *LOX* genes was determined by real time

PCR. Primers were designed after homology testing using selected courgette, cucumber and watermelon unigene and EST sequences available in the Cucurbit Genomics Database.

Results and discussion

The effect of using less susceptible genotypes (lsg), and soil or foliar applied elicitor treatments (chitin, Milsana®) on powdery mildew severity and crop performance showed that *R. sachalinensis* (Milsana®) significantly increased the number of fruits produced per plant. Plants of the lsg had the highest yield when treated with the reference fungicide and the lowest yield when treated with twice the standard rate of Milsana®. In contrast, susceptible plants reached the highest yield when treated with standard and twice the standard rate of Milsana® and the lowest yield when untreated. These results indicate that although the lsg did not appear to significantly control powdery mildew under high disease pressure, resulted in higher total yield and number of fruits compared to the susceptible genotype which is generally preferred by growers for its high yield potential. In conclusion, this study allows us to suggest that the use of elicitors on resistant genotypes needs to be further tested to elucidate possible allocation cost effects.

Total (free and conjugated) ferrulic and cumaric acids concentrations increased compared to water treated controls on day 2, when susceptible plants were treated with the elicitor. No differences were observed on day 4 between treated and control plants. All treatments (chitin soil amendment and foliar Milsana® applications) inhibited conidia germination, and increased callose deposition in susceptible plants. Results indicate that chitin in soil induced systemic resistance (ISR) in courgette plants and that Milsana® induced a more localised induced resistance response.

The relative gene expression of salicylic acid (SA) and jasmonic acid (JA) marker genes was studied in susceptible plants treated or not with the elicitor and under biotic stress pressure by *P. xanthii*, for a time interval of four consecutive days. Gene expression showed that treatment of plants with *R. sachalinensis* prior to pathogen infection, resulted to augmentation of the transcript levels of the SA-related *PR1*, *PR2* and *PAL* genes during the first four days post inoculation, though with different patterns of response through time. The maximum effect was observed in the upregulation of the *PR1* transcript during the first day, suggesting a possible priming effect of *R. sachalinensis* to *C. pepo* plants. *PR2* and *PAL*, although they follow a different pattern of expression respect to *PR1*, were upregulated in treated samples respect to controls, showing a peak during the fourth and the third day respectively. The complete downregulation of *LOX* gene expression post pathogen inoculation, indicated that the defence responses against *P. xanthii* are probably mediated through the SA pathway. The downregulation of *PR1*, *PR2* and *PAL* gene expression in control samples during the first two days of infection, could suggest a suppression of plant defence responses, possibly by the pathogens effector proteins.

Acknowledgements

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The allelopathic potential of gramine in barley

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Highlights

- Gramine content and accumulation were influenced and induced by abiotic stresses like iron (Fe) deficiency
- Gramine accumulation in barley enhances its allelopathic potential against weeds

Introduction

Barley is the fourth most important cereal crop in the world and, besides its importance in food production, for centuries it has been known for its allelopathic proprieties. Several studies demonstrated that this peculiar feature could be due to the synthesis and release of gramine. Gramine is produced by members of the genus *Hordeum* and mainly allocated in leaves; its accumulation in plants could be constitutive or induced by both abiotic and biotic stresses, suggesting an additional role in defence mechanisms. Yet, experimental evidence suggest that the processes of domestication and diversification counter-selected for gramine accumulation and therefore for the allelopathic potential of barley.

This study will focus on the evaluation and comparison of gramine content in wild and cultivated barley at different development stages as well as in different tissues. In addition, different gramine biosynthesis induced by abiotic and/or biotic stress will be investigated.

Material and methods

Barley plants were hydroponically grown for three weeks in a climate chamber either in a full nutrient solution or in Fe starvation. Samples of leaves, from the first to the fourth leaf, and roots were collected every 2 days following a time-course approach and immediately stored at -20°C for further analysis. The first step consisted in the extraction of gramine in pure methanol of the sampled tissues, using the method previously described (Velozo et al., 1999). Then gramine concentration was determined by high pressure liquid chromatography (HPLC) using the method described by (Zhou et al., 2006).

Results and discussion

Preliminary analyses proved the reliability of the HPLC method to quantify the concentration of gramine in extracts of barley leaves and roots. Calibration curves showed a good linearity with an average correlation coefficient (R^2) of 0.996 and a limit of detection (LOD) equal to 0.025 mM (4.44 mg/l) and a limit of quantification (LOQ) of 0.085 mM (14.81 mg/l).

Leaves of a local variety of barley sampled 4 days after germination (DAG) exhibited a gramine content of 0.27 mg/g fresh weight (FW), suggesting that this barley variety belongs to gramine-low content barley. At 6 DAG a relevant accumulation of gramine (0.83 m/g FW) was observed in the first leaf of Fe deficient barley plants whereas leaves sampled from control plants showed only 0.5 mg/g FW. The second leaves showed such a strong accumulation (1.87 mg/g FW) in Fe deficient

conditions at 10 DAG. This variation of gramine content in barley leaves during plant development due to Fe deficiency, suggests an additional induction of its biosynthesis by stress condition.

The other barley cultivar analysed, i.e. Solist, revealed the total absence of gramine both in leaves and roots during the plant development; therefore Solist as gramine-free cultivars (such as Proctor or Morex (Leland et al., 1985) has been chosen as control cvultivar

Further studies will involve several wild barley accessions to assess their potential to synthesise gramine. The best performing in terms of gramine concentration will be subjected to different abiotic and biotic stresses.

Acknowledgements

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Allelopathic effects of *Crotalaria juncea* and dimethyl disulfide (DMDS) on tomato plants in the future development of a biocontrol method against root-knot nematodes

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Highlights

- Plants treated with *Crotalaria juncea* and dimethyl disulfide (DMDS) showed higher growth than control plants
- Total soluble sugars were higher in the roots of DMDS treated plants as compared to the levels measured in the roots of plants treated with *C. juncea* or control plants
- Plant defence gene expression -salicylic acid, jasmonic acid and ethylene pathways- was higher in plants treated with DMDS and *C. juncea*

Introduction

Tomato is one of the most consumed vegetable products in the world and the optimisation of yields is hampered by the ravages of pests, including root-knot nematodes (RKN), which have a major agro-economic impact on a global scale (FAOSTAT, 2014). RKNs *Meloidogyne* spp. induce the formation of root galls, symptoms of a dysfunction of the vascular system of the plant. Their economic importance is increasing as most chemical control agents for RKNs have been prohibited for environmental and health reasons.

SERUM aims to develop a biocontrol strategy, based on the use of plants known for their sanitizing effects against *Meloidogyne*, the development of an alternative method to chemical fumigants is now a necessity to fight against these phytoparasites. We tested under greenhouse conditions the biostimulatory effects of *Crotalaria juncea* and dimethyl disulfide (DMDS) on the growth of tomato plants (21 days after treatment) by molecular and biochemical approaches.

Material and methods

Tomato plants (*Solanum esculentum* cv. St Pierre) were grown for three weeks under greenhouse conditions. Plants were kept either treated with water, 2.5 g/l of extracts of freeze-dried *C. juncea* or DMDS (at a concentration of 10^{-4} mol/l).

The biostimulatory effect was first estimated using a phenotypic approach by growth measurements (via image analysis software and determination of fresh masses and dry masses). In addition, a biochemical approach was carried out for a finer characterisation of the biostimulatory effect via a determination of soluble sugars, starch, soluble proteins, chlorophylls and the Carbon/Nitrogen ratio (C/N). In order to confirm the biochemical approach, we measured by quantitative PCR the level of expression of plant defence genes and sugar transporter genes. Total

RNA were therefore extracted from the foliar and root parts of plants treated with *C. juncea* and with DMDS (dose 10^{-4} mol/l). We measured the level of expression of genes involved in salicylic acid, jasmonic acid and ethylene pathway, which are three of the main pathways activated by plants after recognition of a pathogen and three genes encoding sucrose transporters (SUTs) and two genes encoding sucrose transport facilitators (SWEETs, "Sugars Will Eventually Be Exported Transporters").

Results and discussion

Phenotypic effect: twenty-one days post-treatment (D + 21), plants treated with *C. juncea* and DMDS showed an increased growth as compared to the control plants. Indeed, their root and foliar dry masses and their leaf areas were superior to the control plants. These results suggest that plant growth is stimulated by these two treatments.

R/S ratio analysis: the biostimulating effect of DMDS (at 10^{-2} mol/l) suggested by Arnaud et al. (2003) on the vegetative growth of cucumber seems to be confirmed in tomatoes. However, the mode of action of these biostimulants seems to be different because *C. juncea* favor mainly the root growth whereas the DMDS stimulates more the foliar growth.

This is corroborated by the observation of the R/S ratio, which is minimal for plants treated with DMDS at 10^{-4} mol/l and maximum for plants treated with *C. juncea* (stimulating effect mainly focused on leaf and root growth, respectively). In addition, the root system of plants treated with DMDS at 10^{-4} mol/l has more ramifications than plants of other modalities. This demonstrates structural changes induced by DMDS treatment at a dose of 10^{-4} mol/l, which can lead to a better assimilation of soil nutrients, and thus ultimately explain increased growth compared to control plants. Moreover, the high R/S ratio generated by the treatment with extracts of *C. juncea* seems to favor leaf growth, which could indicate a greater investment of the elements synthesised by the plant in this organ.

Total sugar content: The incorporation of *C. juncea* in the soil is associated with an increased content of total soluble sugars within the roots. The injection of DMDS at 10^{-4} mol/l is associated with an increased content of soluble sugars in the plant as a whole (root parts + aerial parts), resulting from an increase in the root compartment as compared to the control plants. This result should be compared with the phenotypic observation of plants treated with DMDS at 10^{-4} mol/l characterised by an increased foliar growth, which may be linked to a greater number of photosynthetically active leaves.

Gene expression level: The molecular analyses showed that the treatment of tomatoes by DMDS stimulates the gene expression with a strong overexpression of defence genes, as well as genes encoding sugar transport facilitators of the SWEETs family.

To conclude, a stimulating effect on the growth of the tomato was demonstrated at 21 days after the incorporation of *C. juncea* and DMDS at 10^{-4} mol/l. For *C. juncea*, these stimulating effects are mainly observed at the root level, while for the DMDS it is mainly a foliar growth. Treatments with crotalaria and DMDS induced biostimulation associated with an increase in total soluble foliar and root sugars respectively and a strong overexpression of defence genes and sugar transport genes.

Acknowledgements

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Study of the antagonistic effect of *Trichoderma* spp. against *Fusarium* spp. involved in Fusarium head blight and root rot of wheat

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Highlights

- Evaluation of the *in vitro* and *in vivo* efficacy of some species of *Trichoderma* toward some *Fusarium* sp. isolates in the protection of wheat against Fusarium wilt
- Seeds treated with isolate *T. harzianum* Th.15 showed the highest degree of resistance

Introduction

Fusarium head blight is an important disease of wheat. It is caused by several species of *Fusarium* at flowering stage of the plant. It causes important losses in yield quality and quantity because of the accumulation of the mycotoxines. (Agrios, 2005). Through our study, an identification of *Fusarium* species was made from the wheat collected in: INA (Institut national agronomique), ITGC (Institut Technique des Grandes Cultures).

Biological control assay against *Fusarium* spp. was carried out by using different isolates of *T. longibrachiiatum*, *T harzianum* and *T. atroviride* that previously showed an antagonist activity *in vitro* and *in vivo*.

Material and methods

Study of the growth and aggressiveness of isolates *Fusarium* spp. *in vitro*. Evaluation of the pathogenicity of *Fusarium* spp. isolates by estimating their effect on the growth of coleoptile. *In vitro* study of the antagonistic activity of *Trichoderma* spp. againts *Fusarium* isolates. Effect of *Trichoderma* spp. isolates on mycelial growth of *Fusarium* spp. Direct and remotely (indirect) confrontation. Effect of culture filtrates of *Trichoderma* spp. on mycelial growth of *Fusarium* spp. Study of the effect of *Trichoderma* spp. isolates on disease development. *In vivo* study of *Trichoderma* spp. antagonist activity against *Fusarium* spp.

Results and discussion

Five isolates belonging to the species: *T. atroviride* (Ta.7, Ta.13), *T. harzianum* (Th.6, Th.15) and *T. longibrachiatum* (TL.9) were tested against four *Fusarium* species (*F. culmorum*, *F. avenaceum*, *F. moniliforome* and *F. solani*). Tests were carried out using *in vitro* and *in vivo* based bioassays and the evaluation of antagonistic activity *in vitro* was performed using two techniques: direct and indirect confrontation. In the case of direct confrontation, a net reduction of the pathogen growth was observed with variability in the sensitivity of *Fusarium* spp. towards *Trichoderma* species tested. Their effectiveness was evaluated by the percentage of the pathogen colony growth reduction which varied from 4 to 92%. The highest percentage growth reduction of all *Fusarium* species was obtained with the isolate *T. longibrachiatum* TL.9 where a percentage of 92% was obtained with *F. solani*.

Once more, in direct confrontation pathogen isolate colonies were invaded by *Trichoderma* with a variability of this behavior which varied from total recovery, partial or no recovery by the antagonist. In the case of *Fusarium* species, total or partial recovery with the species *T. atroviride* and *T. longibrachiatum* and no recovery with the species *T. harzianum* were observed.

In indirect confrontation (no direct contact) between the pathogen and the antagonist, where inhibition occurs only as a result of volatile antifungal substances produced by the antagonist, significant reductions on the pathogen growth compared to the control were obtained percentage of reduction varied between 4 and 81% and the highest percentages within *Fusarium* species (*F. avenaceum*, *F. culmorum* and *F. solani*) were obtained with *T. longibrachiatum* TL.9 but for *F. solani* the highest percentage was obtained with *T. harzianum* Th.15.

By *in vivo* bioassay, *T. atroviride* isolates which have been proved to be most effective *in vitro* test was assessed against the species *F. culmorum* by seed treatment before sowing wheat in soil infested with *F. culmorum* as result, a percentage of inhibition of disease severity of 90% was obtained with *T. atroviride* Ta.13 and 52% with *T. atroviride* Ta.7 showing the effectiveness of this species in wheat protection against root rot and grown rot.

In this study, it was also shown the production of antifungal volatile 6pp (6-pentyl–α–pyrone) by Ta.13 and that this isolate is a major producer of 6 pp.

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Effects of β-aminobutyric acid on aphid stylet activities

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Highlights

- Electrical penetration graph (EPG) recordings of pea aphid feeding on tic bean plants showed that β-aminobutyric (BABA), applied as a root drench, had no detectable impact on insect stylet activities
- When BABA treatment was combined with prior aphid infestation, phloem sap ingestion by EPG-recorded aphids was significantly reduced.
- BABA may prime plants to enhance aphid-induced resistance

Introduction

Aphids are one of the major groups of insect pests of crop plants. Control of these insects is still primarily achieved using conventional pesticides. β-aminobutyric acid (BABA) is a non-protein amino acid that enhances basal plant defence mechanisms in a range of plant species against a variety of plant pathogens. It has also been demonstrated that BABA applied to host plants as a root drench suppresses the performance of insect herbivores such as aphids and caterpillars. BABA application to plants has been demonstrated to inhibit aphid growth, reproduction and survival on several crop plant species (Hodge et al., 2005; Cao et al., 2014; Zhong et al., 2014), but the mechanisms of action remain unclear. In this investigation, the electrical penetration graph (EPG) technique was used to investigate stylet activities of aphids on plants previously exposed to BABA (applied as a root drench) and the results compared with aphids on water-treated plants.

Material and methods

The aphids used in this study were clone JF01/29 of the pea aphid, *Acyrthosiphon pisum* Harris. Aphids were cultured at low density on seedlings of tic bean (*Vicia faba* var. minor L.) grown in pots of damp sand. Plants used in experiments were grown in an environment-controlled glasshouse with a 16:8 hour day: night cycle, a minimum day time temperature range of 15-18°C and a minimum night time temperature of 12-15°C. Plants were grown in compost with the addition of Perlite and Vermiculite (10:1:1 by volume) in 8 cm plastic pots and were watered as required with untreated water. Tic beans were maintained in the glasshouse until 14 days after sowing (=day zero in the BABA induction experiment), when the roots were treated with 25 ml of either distilled water (controls) or 25 mM BABA solution (DL-β-aminobutyric acid, purity > 95%, obtained from Sigma-Aldrich Ltd., Poole, UK) solution applied as a soil drench. On day 7, plants were used in EPG experiments, where the stylet activities of a single apterous adult pea aphid (previously starved for 2 hours) were recorded continuously over a 6-hour period (one aphid per plant, tethered using a 3 cm gold wire of 20 µm diameter as required for the EPG technique). In some treatments, plants were also exposed to a group of feeding aphids (10 immature aphid nymphs per plant, restricted to plants by enclosing in perforated plastic bags fastened around the pot using an elastic band) for a 48 h period (between days 4 and 6).

Results and discussion

In previous experiments (Hodge et al., 2005) we have demonstrated that BABA applied as a root drench to legumes reduces the performance of the pea aphid. On tic bean, BABA caused a dose-related reduction in the mean relative growth rate (MRGR) of individual aphids and their intrinsic rate of population increase (rm). These reductions in aphid performance may be linked with BABA-induced phytotoxic stress or direct toxicity to aphids, but a series of previous experiments have not provided consistent evidence for these two possibilities in the *A. pisum*/*V. faba* insect/host plant system. Our results instead point to the possibility of a BABA-induced aphid resistance mechanism. In the present experiments, initial 6-hour EPG recordings on *V. faba* showed no differences in recorded stylets activities, including the electrical waveform (E2) associated with phloem sap ingestion. Aphids ingested phloem sap for similar periods of time (approximately 4 hours of the 6-hour experiment), whether plants had been previously exposed to BABA or water control treatments. However, significant differences in stylet activities emerged when plants had been exposed to a combination of BABA and previous feeding by aphids. EPG-recorded aphids spent less time ingesting phloem sap on test plants previously exposed to BABA and a group of inducing aphids, compared to aphids feeding on plants exposed to water and aphids. On plants exposed to BABA and prior aphid induction, EPG recorded aphids initially located phloem sieve elements as quickly as those aphids feeding on control plants. However, phloem sap ingestion was subsequently disrupted on BABA/aphid – treated plants. By the end of the experiment, only 50% of aphids were showing the E2 waveform on BABA/aphid – treated plants, compared with 95% of aphids on water/aphid – treated plants (n=20). These results suggest that sieve element defences may be enhanced on BABA-treated plants, leading to disrupted phloem sap ingestion by *A. pisum.* However, the onset of effective BABA-enhanced plant defence may require extended exposure to aphid feeding. While there was no evidence for enhanced plant resistance to single aphids feeding for 6 hours during the EPG experiment, a group of 10 aphids previously feeding for up to 48 hours may have been sufficient to trigger a resistance mechanism that was augmented by BABA. Further experiments will be necessary to elucidate potential mechanisms of aphid resistance that are enhanced by BABA. In a different legume/aphid system (Aphis glycines feeding on soybean), induction of several plant defence-related genes was augmented following BABA root drench treatment (Zhong et al., 2014). Since aphid resistance genes often operate via phloem-specific mechanisms, it is possible that resistant-gene-mediated resistance and BABA-induced resistance share common features. We therefore plan further EPG experiments to investigating interactions/commonalities between these two types of aphid resistance.

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Disease suppression in eggplant (*Solanum melongena* L.) nurseries carries over to reduced wilt and fruit rot in subsequent plantings

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Highlights

- *Trichoderma harzianum* was soil applied to eggplant nursery, eggplant field or both in a participatory trial in Bangladesh
- In the nursery the fraction of healthy seedlings per sown seed increased, transplanted seedlings showed less wilt and fruit rot
- Field application alone or in combination with nursery reduced wilt and fruit rot
- *T. harzianum* provides feasible low cost disease control

Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crops in Bangladesh as in other Asian countries because of its high consumption and economic value to small land-holders. However, sustainability is greatly compromised by diseases and the use of chemical pesticides, which most often show ineffective. As much land is flooded by the start of the eggplant growing season, farmers nurseries are confined to limited land. When seedlings are transplanted they are likely to carry diseases from nurseries to field.

Attempts were made to develop more integrated techniques, but many remained at the experimental level. *Trichoderma spp.* are considered environment friendly disease control agents and as stimulants of plant growth (Harman, 2000). Hot water treatment is known to reduce seed borne pathogens (Mancini and Romanazzi, 2014). Here we tested, together with farmers, whether the combination of above methods could control wilt and fruit rot problems better than their conventional practices.

Material and methods

A farmer nursery, with reported damping off problems, was used to test three treatments: (1) a no intervention control; (2) a farmer control (i.e. spraying when damping off occurred) and (3) a combination of soil treatment with *Trichoderma harzianum* and hot water treatment of seeds. The experiment used a randomized complete block design (2m × 2m plots) with seven replicates, where the seven participating farmers each managed one replicate and donated their seeds for their own replicate.

Cultured *T. harzianum* suspension was obtained from Bangladesh Agricultural University IPM laboratory (BAU-IPM). 25 ml of the suspension containing 12×10^6 CFU/ml was added per kg of peat soil: black gram bran (1/1) mixture. This mixture was applied at 8 g/m^2 nursery or field soil 7 days before sowing or transplanting. Seeds were treated with a machine immersing seeds 15 minutes at 50-55 °C designed by BAU-IPM. Emergence and damping-off were recorded until transplanting.

At transplanting seedling growth parameters were recorded and farmers anonymously scored each other's seedlings based on overall growth of seedlings.

In farmers' fields, a split-plot experiment was laid out using farmers as replicates and within each replicate a *Trichoderma*-treated and an untreated main plot each with two subplots, planted to seedlings of nursery treatments (2) and (3). The following 5 months number of wilted plants and rotten and healthy fruits were recorded.

Results and discussion

Nursery inoculation with *T. harzianum* and seed treatment with hot water increased the number of healthy seedlings at transplanting through both increased emergence and decreased post-emergence damping-off. It also improved seedling quality compared to farmer's conventional practice. Overall vigour index, root length, seedling height and number of lateral roots were almost doubled and seedling girth ratio were more balanced. Unbalanced girth ratio between diameter at soil level and at 5 cm above the soil indicates diseased seedlings. Farmers consider number of lateral roots and balanced girth ratio as most important criteria. Farmers in Bangladesh have restricted time to sow nurseries and have limited available land to produce seedlings quickly after sudden crop loss like the 2017 late floods. Soil amendment with *Trichoderma* will be of good use because it both improved seedling health and growth rate allowing earlier transplanting. Moreover, these vigorous and healthy seedlings showed more resistant against soil borne pathogens after transplanting.

Transplantation of the improved seedlings significantly reduced incidence of wilt and fruit rot compared to farmer's conventional practice both when fields were amended with *Trichoderma* or not amended. Farmers practice led to 50% wilted plants and 30% fruit rot. Using *Trichoderma* both in the nursery and the field combined with hot water treatment of seeds led to 11% wilt and 6% fruit rot. Only treating the field led to 27% wilt and 14% fruit rot, while only treating the nursery soil and the seeds reduce wilt to 22% and fruit rot to 11%. Hence, farmers could improve their business by only treating nursery or only treating field while most effect will be obtained when combining both. From an economic view point treating nursery will be more feasible because it requires less input and labour than treating field. However, farmers may treat both nursery and field because it seemed to be more effective and incur less cost than conventional weekly spraying.

Sustainability of integrated pest management (IPM) greatly depends on involvement of farmers in the research process and helping them to generate solutions suitable in their farming systems and integrating components that are ecologically sound and readily available (El Khoury and Makkouk, 2010). In Bangladesh conversion from chemicals as major means of disease control to more environmental sustainable practices is needed. Our study showed that biological control improved seedling health and fitness reducing disease incidence in the field and increased yield. However, a further economic assessment is needed to assess suitability of *Trichoderma* in terms of cost and labour. Also, training of farmer trainers and progressive farmers seems logical so that they can prepare their own products at village level when it is difficult to travel to buy *Trichoderma* due to flood. Bangladesh Agricultural University has the capacity to carry out such work.

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Synergising pest deterrence and plant defence induction: a novel integrated pest management system for *Trialeurodes vaporariorum* on glasshouse grown tomato

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Highlights

- Pre-treatment with methyl salicylate (MeSA) reduces whitefly performance on tomato under glasshouse conditions only initially
- A combination of MeSA sprayed plants and slow release bottles of limonene is the most effective at controlling whitefly populations

Introduction

The glasshouse whitefly (*Trialeurodes vaporariorum*) is a prevalent and persistent pest of tomato crop worldwide. With the many negative implications of pesticide use well known, combined with the fact that glasshouse whitefly are now known to be resistant to a number of insecticides (Du et al. 2016), the need for alternative control procedures is clear. Two methods of pest management are here synergised to prolong efficiency and work against the pests on two separate fronts. Methyl salicylate (MeSA) was applied to tomato as a plant defence elicitor and D-limonene was employed in the form of a slow release bottle to repel the whitefly from the crop.

MeSA is produced by tomato in response to whitefly infestation (Lopez et al., 2012) and is critical for defence against pathogens and phloem feeding insects (Rowen et al., 2017). A previous study from our lab group (manuscript in preparation) found that companion plants French marigold (*Tagetes patula)* pushed whitefly away from tomato crop. Air entrainment/GC-MS analysis found D-limonene to be the main component of *T. patula* volatile emissions and was therefore employed in the form of a slow-release bottle to repel the whitefly in a similar way.

Material and methods

Hybrid tomato variety “Elegance” were grown from seed in a pest free propagation glasshouse at Stockbridge Technology Centre. As the first leaves began to appear (after 7 days), half of the 288 seedlings were sprayed with volatile MeSA dissolved in 50% ethanol at a concentration to deliver 140 µg of MeSA per seedling. This process was repeated every day for a period of 5 days. At the 3-4 leaf stage (day 21 from seed) the plants were introduced to a glasshouse containing 9 aubergine plants heavily infested with *T. vaporariorum.* The plants were arranged into blocks of four different treatments with 8 plants in each, this four treatment block was replicated 9 times. The Control treatment had 8 untreated tomato, the limonene treatment had 5 D-limonene slow release bottles placed amongst 8 untreated tomato, the MeSA treatment had 8 MeSA treated plants and the synergised treatment had 8 MeSA treated plants and the slow release bottles also. Whitefly performance was assessed by selecting one leaf at random from each plant in the glasshouse and counting any visible settling adult insects. These leaves were then removed and placed in sealed

plastic bags and stored overnight at 4°C for examination under low power microscopy (4× magnification) the next day for whitefly (and other pest) larvae and eggs. The abundance of all pest insects present was recorded, to test the effect of each treatment on other pest species as well as the target pest *T. vaporariorum.* Sampling was conducted weekly for a period of 10 weeks, at this point the fresh weight and yield from each plant was recorded.

Results and discussion

All treatments proved to have a negative effect on whitefly performance at various stages of the experiment. The MeSA sprayed treatment had significantly less *(p=<0.05)* settling adult whitefly compared to the control for the first and third weeks of sampling. This treatment also had significantly less eggs on the third and fourth weeks of sampling. Both the limonene and synergised treatments were more effective at reducing whitefly performance with settling and eggs were significantly less than the control for the first four weeks of sampling. After this time point, both treatments retained lower settling and egg values than the control. Interestingly, the MeSA treatment did not reduce the amount of whitefly nymphs as compared to the control. However the limonene treatment significantly reduced nymphs at weeks 5 and 6 and the synergised treatment significantly reduced nymphs at weeks 5, 6 and 7 (note that nymphs only became visible at week 4 of the experiment). From week 7, all treatments showed no significant decrease in whitefly numbers (settling, eggs and nymphs). Two-spotted spider mites (*Tetranychus urticae*) melon thrips *(Thrips palmi)* and tomato hornworm *(Manduca quinquemaculata)* were all found to be present in the glasshouse but only in comparatively small numbers and showing no preference between the treatments.

Whilst none of the treatments show a sustained decrease in whitefly numbers, the results from the limonene and synergised treatments is certainly promising. The use of these slow release limonene bottles is effective at initially deterring the whitefly from tomato, translating to a reduction in eggs laid and subsequently less nymphs forming in the latter weeks of the experiment. Whilst the effect was not as amplified as could be expected, the inclusion of MeSA treated plants with the slow release bottles in the synergised treatment was marginally more effective at reducing whitefly performance. One key issue with this experiment is that the results of pest performance hinge on the not yet acquired yield of each treatment. We suspect an overall “cost” incurred by MeSA defence activation in tomato. Whilst fruit numbers (which were counted each week as they formed) are not currently significantly different between the treatments, there may well be a difference in fresh weight of the fruit from each treatment. If of course there is no difference in yield, then this could suggest that MeSA is acting as a priming agent, only increasing the inherent defence responses of the tomato once the insects arrive on the plants. Further experiments assessing defensive enzyme activity and expression of key defence related genes will be done to understand for certain whether MeSA has primed these plants or merely induce an immediate defence response.

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Meeting of the IOBC-WPRS Working Groups "Induced resistance in plants against insects and diseases" and "Multitrophic interactions in soil"

Ecological perspectives of induced resistance in plants and multitrophic interactions in soil

Poster Session 2

Functional ecology of microbial interactions in soil

Vineyard in-row and cover crop management affects mesofauna composition

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Highlights

- Mesofauna abundance and composition is affected by different soil management in vineyard inter-rows
- Vegetation soil cover provides higher populations and increased biodiversity also reflected in QBS-ar index assuming higher ecological values

Introduction

Grapevine as crop of high economic value has due to its perennial nature also the possibility to establish sustainable production system with lower external inputs and increased biodiversity. Biodiversity especially in the soil is influenced by environmental factors as well as management practices. Knowledge about the linkage of between biodiversity, ecosystem services and agronomic practices is of increasing importance. The presented study focuses on the biodiversity of the mesofauna in nine Austrian vineyards and three different inter-row management systems established in each vineyard. Samples from the inter-row, as well as in-rows of selected vineyards were collected. The mesofauna reacts on changing environments very fast making them interesting candidates as bioindicators for soil quality (Cardoso et al., 2013). Their contribution to important ecosystem services as decomposition, nutrient cycles and soil structure is well known (Sauvadet et al., 2017).

Material and methods

Samples for the determination of mesofauna composition and biodiversity were in the frame of the BiodivERsA/FACCE-JPI joint project "PromESSinG" in nine Austrian vineyards in Lower Austria (Krems, Langenlois) and Burgenland (Großhöflein, Eisenstadt). In all vineyards three different practices for inter-row management were established in 2015: open soil, alternate soil cover, permanent soil cover. Pooled soils core samples (10 times 0-10 cm and 2.5 cm diameter) were used for mesofauna extraction with the Berlese-Tullgren method. Three sampling periods were conducted: May, June and September. Mesofauna families were determined using a simplified key provided by project partners. Data analyses were performed with SPSS and Canoco 5.

Results and discussion

In 2016, twenty different taxa could be observed in the experimental vineyards used in this study. Among them the groups Acari, Collembola and Enchyträe gave highest abundances. These groups contribute to litter decomposition and have therefore high ecological importance. In total 10,629 individuals were collected whereas equal numbers with 1,583 and 1,567 were obtained in May and June. Highest amounts were collected in September with 7,479 individuals. Abundances have to be related to traits to evaluate ecological functions. Indices like QBS-ar as well as abundance of the mesofauna were significantly increased in inter-rows with permanent soil cover as compared to bare

ground. Similar observation were observed for the comparison between littler layer and below soil samples, higher abundances and values within the littler layer. No influence of weed management methods in in-rows were determined in our 1-year experiment. Mechanical weed control and herbicide spraying gave similar results, most probably due to the short time of the establishment of the treatments. Long-term observations would be needed to evaluate the effects of these different methods. To further evaluate influencing factors on mesofauna composition and biodiversity multivariate statistical methods with Canoco 5 were conducted to determine soil and environmental parameters with had the biggest influence on our observations. These tests are ongoing. Nevertheless specific experimental setups are needed to precisely determine the effects of management practices and production systems on soil parameters and soil biodiversity on different trophic levels.

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Vineyard location and vineyard management effects on soil respiration measurements

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Highlights

- Basal respiration differs between vineyards and correlates with total N and soil potassium content
- Permanent vegetation cover in vineyards inter-rows increases basal respiration in six out of nine vineyards in comparison with bare ground inter-rows

Introduction

Soil respiration is a key ecosystem process leading to the production and release of carbon dioxide from the soil by plants, bacteria, fungi and animals. The process is part of the carbon cycling, where CO_2 from the atmosphere is converted into organic compounds by photosynthesis, which are used to build structural components or to gain energy through respiration. These components are further decomposed by microorganisms thereby closing nutrient cycles. Environmental factors as temperature, soil moisture and nitrogen can strongly influence the carbon conversion rate. Vineyards are intensive agronomic systems with different management practices. Especially soil cover management affects the amount of organic matter being incorporated in the upper soil part. Within a three years project we aim to analyse the factors influencing soil microbial community and microbial activity in different vineyards and soil management treatments

Material and methods

Samples for the determination of root respiration were sampled in June 2016 in the frame of the BiodivERsA/FACCE-JPI joint project "PromESSinG" in nine Austrian vineyards in Lower Austria (Krems, Langenlois) and Burgenland (Großhöflein, Eisenstadt). In all vineyards three different practices for inter-row management were established in 2015: open soil, alternate soil cover, permanent soil cover. Soil samples were collected from all treatments and vineyards in duplicates, whereas each sample represents a pool of 10 core borer (0 – 10 cm) samples collected. The samples were shipped frozen to Fribourg and the basal respiration of 3.5 g water-saturated soil samples was measured during 20 hat 22°C with an automated electrolytic micro-respirometer (Scheu 1992).

Results and discussion

The basal respiration of soil microorganism was determined with samples obtained from the upper soil part (0 - 10 cm). Samples were collected from inter-rows with three different cover crop management treatments in nine vineyards in Austria. Basal respiration (µg O_2/h g soil dry weight) was substantially different between some vineyards assume strong environmental effects. This could be shown with a principal component analysis (PCA) and correlation analyses between determined

soil parameters and basal respiration, revealed strong positive influence of total N content and soil potassium concentration with coefficients of 0.720 and 0.618 respectively. The determined correlation between the basal respiration and the content of soil organic matter was much lower, with a coefficient of 0.430. In a next step, environmental conditions will be included in the analyses. Normalised data were used to differentiate between treatments. A strong increase in basal respiration in inter-rows with permanent ground cover was determined in six out of nine vineyards. These observations correspond with results from other authors find in previous studies in vineyards and other crops (Burns et al., 2016; Mbuthia et al., 2015). The clustering in the corresponding PCA was visible, but not as clear as expected. Therefore, additional multivariate analyses will be conducted to include additional factors as temperature and rainfall and other soil characteristics. By these analyses the interaction between vineyard, environment and inter-row soil cover management will be detected and the main influencing factors will be determined. In conclusion, the basal respiration in vineyard inter-rows is influenced strongly by the environmental conditions of the vineyard like soil and climate, and the cover crop management. In our study the influence of the vineyard itself was stronger as the cover crop management treatments analysed.

Acknowledgements

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The effect of cover crops in alleviating copper toxicity in grapevine plants

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Highlights

- Copper (Cu) concentration in plant tissues increases with increasing Cu concentration in nutrient solution being the root the main target (both simplast and apoplast)
- Intercropping with oat decreases the Cu concentration in grapevine plants indicating that the use of cover crops represents a promising tool to alleviate Cu toxicity in grapevines

Introduction

The intense use of Cu-based fungicides has led to an increase in Cu soil levels in vineyard soils worldwide. The main effects of plant Cu toxicity are the reduction of root growth and darkening, a reduction in fruit yield and chlorophyll content and a modification of chloroplast development. Moreover, Cu toxicity influences the uptake of other nutrients and, consequently, the ionome of leaves. In presence of high levels of Cu, plants release exudates and the exudation pattern is peculiar of the plant species. Copper tolerant plants release a range of organic molecules that are able to complex Cu altering its availability for plants. A possible solution in alleviating Cu toxicity in agricultural soils could be the use of cover crops. In particular, in vineyards intercropping could be a valuable option.

Thus, the aim of this research is to assess rhizosphere processes involved to alleviate Cu toxicity in grapevine plants either grown alone or intercropped with oat.

Material and methods

Two oat species and two grapevine rootstocks were used. *Avena sativa* L. cv Perona and cv Fronteira were at first characterised for their tolerance towards Cu to define the cultivar to intercrop with the rootstocks Fercal and 196.17. Subsequently, the effect of Cu toxicity on the grapevines grown alone and on the rootstocks grown with oat were investigated. The experiments were performed in a hydroponic system in controlled climatic conditions with Cu concentrations ranging from 0 to 50 µM. The morphological root parameters, the chlorophyll content (expressed as SPAD index) and shoot-root ratio was measured at the end of the growing period (4 weeks). Moreover, the root exudate release were monitored during the experimental period as described by Valentinuzzi et al. (2015) and characterised for their total phenolic compound content, flavonoid and flavonol compound content. The root morphology was assessed by Winrhizo (EPSON1680, WinRHIZO Pro2003b, Regent Instruments Inc., Quebec, Canada) and the mineral tissues composition of both shoots and roots by ICP-OES.

Results and discussion

The results showed that *A. sativa* L. cv. Fronteira exhibited the typical mechanisms of excluder plants, while the cv. Perona most likely detoxified Cu with an internal detoxification strategy. Consequently *A. sativa* L. cv Fronteira was chosen for the intercropping study.

The shoot-to-root ratio of grapevine plants decreased significantly only in Fercal rootstocks grown alone and in the intercropped 196.17 rootstocks with increasing Cu concentration. The shoot-to-root ratio of oat plants changed depending on the intercropped rootstock. Generally, oat plants intercropped with Fercal exhibited significantly higher shoot-to-root-ratios than oat plants intercropped with 196.17.

As expected, Cu increased with increasing Cu concentration in the nutrient solution in all plant tissues analysed (root simplast and apoplast, shoots) and in all growing conditions of both Fercal and 196.17 rootstocks and oat plants. The roots of grapevines were the main target for Cu accumulation, yet without differences between apoplast and simplast. In fact, Cu translocation to the shoots was very limited being the Cu concentration up to 100 fold lower than in the grapevine roots.

Comparing the two growing conditions, i.e. grapevine rootstocks grown alone or grapevine rootstocks intercropped with oat, we generally observed a reduction of Cu absorption. Yet, for the Fercal plants, the reduction resulted significant only in the root tissues (apoplast and simplast) and only for the intermediate Cu concentrations applied (5 and 25 µM): -45% and -44% in the root apoplast of 5 and 25 µM Cu treated rootstocks; -56% and -46% in the root simplast of 5 and 25 µM Cu treated rootstocks.

Even oat plants showed an increase of the Cu concentration in the different plant tissues with increasing Cu concentrations in the nutrient solution, although, the rate of the increase varies depending on the rootstock they are intercropped with.

Root exudate pattern varied during the cultivation period depending on the presence of the cover crop as particularly observed in the case of phenolic compounds: when intercropped the release is reduced, showing an exudation burst only at the highest Cu concentration.

Intercropping with oat seems thus a promising tool to alleviate Cu toxicity in grapevines. Future studies in soil environment are needed to fully elucidate the effect of the interaction between the cover crop and the vine pants.

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Evaluation of the effect of solid and liquid digestate produced in a biogas plant on soil quality and plant growth

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Highlights

- Reducing the nitrogen load and emissions of cultivated land using sub-products from a biogas plant as organic-mineral fertilizers in vineyards and orchards
- An increase in the quality and variety of eco-friendly fertilisers available to local farmers to improve soil quality parameters and crop performance

Introduction

Spreading of manure on agricultural soil is a main source of ammonia emissions and/or nitrate leaching. Thus, this issue is addressed by the Directives 2001/81/EC and 91/676/EEC to ensure greater protection of the environment and human health. The disposal of manure became an economic challenge for farmers, as the amount of waste produced is often greater than the limit allowed. Converting animal manure in a biogas plant could be an alternative solution. The by-products produced by the biogas plant – i.e. solid and liquid digestate - could be used as fertiliser and/or soil amendments, however, depending on the feedstock and the process, products might have different characteristics. Therefore, experimental activities and evaluation of these are required. The present study aimed at assessing the effect of the digestates obtained from a local biogas plant (Biogas Wipptal Gmbh), first on the quality parameters of the soil and afterwards on the growth of different plant species.

Material and methods

A first incubation experiment was aimed at evaluating the mineralisation and release of Nitrogen (N) in soils. Soils obtained from a vineyard located in Termeno (BZ) were fertilised with 100 mg N/kg of soil using the following N sources: solid digestate, commercial manure (4% N), urea (46% N). Control soil did not receive any N addition. Soils were then incubated at 20°C at 50% water holding capacity (WHC) for seven weeks. Nitrate and ammonium were extracted weekly with KCl 2M (1:10) and analysed colorimetrically. Furthermore, the effect of digestate on soil quality was evaluated by measuring soil pH, extractable organic carbon, extractable N and available phosphorus (Olsen). Afterwards, a pot experiment with different plant species (cucumber, maize and forage grass) was set up using five different treatments: control (no addition), solid digestate (pellets) 75 mg N/ kgof soil dry weight (DW), solid digestate 300 mg N/kg of soil DW, liquid digestate 37.5 mg N/kg and liquid fertiliser 75 mg N/kg. Plants were grown in a climate chamber under controlled conditions (14 h, 24°C, 70% RH during the day; 10 h, 19°C, 70% RH during the night), for at least 30 days depending on the plant species. Soils were periodically sampled and analysed for pH, N and available P as described in Cavani et al. (2016). Plant growth was monitored by measuring SPAD index and shoot biomass. At the end of the experiment plant elemental concentration was also evaluated by ICP-OES.

Results and discussion

The incubation experiment showed as expected that the highest release of nitrate in soils was observed in the treatments with urea. On the contrary, soils treated with the other N sources showed an immobilisation rather than a mineralisation. Soil quality parameters, e.g. pH, extractabale C/N, microbial biomass were not negatively affected by the different N sources. Furthermore, the pellets have led to a significantly higher available phosphorus content in soils.

First results of the pot experiment aimed at evaluating the effects of the liquid and solid digestates on plants; in the case of cucumber, a significant increase in biomass and SPAD index was observed only in the plants treated with liquid fertiliser and especially in the first phase of the experiment. A recovery of the SPAD index was, however, observed in the plants treated with the pellets at the end of the experiment. Analyses are ongoing for the evaluation of the effect of digestate on both the quality parameters of soil and the availability and uptake of nutrients by plants.

Long-term field experiments are however needed to fully understand the influence of the digestates on plant growth and soil fertility considering different agronomic practices and different crops.

Acknowledgements

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Green manure as sustainable tool to microbial diversity in organic vineyards

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Highlights

- No significant difference was detected in the diversity and composition of the microbial communities found in biodynamic and organic managed soils
- Green manure application affects the soil microbial composition, stimulates the growth of bacteria involved in the soil nutrient cycle and represents a potential strategy for enhance soil microbial biodiversity in organic viticulture

Introduction

Soil is a non-renewable resource, therefore, the use of more sustainable agricultural practices is essential to reverse the trend of soil degradation and to ensure its preservation. In organic viticulture systems, green manure represents a safe and non-polluting way to bring large quantities of organic matter into the soil, furthermore, by using green manure, soil erosion is reduced to tolerable levels and the process of washing away nitrate in vineyards is prevented (Rotaru et al., 2011). The microbiological diversity of soil is used as indicator of soil quality, considering the major role played by microorganisms in organic matter decomposition and nutrient cycling. In this work, a microbiological characterisation of soil from vineyards organically managed was performed with the objective to compare the soil microbial structure in organic and biodynamic vineyards and to determine the influence of green manure application on the soil microbiota under biodynamic vineyards.

Material and methods

The study site was located in the Trentino-South Tyrol region in northern Italy. Two vineyards were selected (Field 1 and Field 2), which were then divided into replicated plots (n=12). Starting from the autumn of 2011, each plot was managed according to organic (O), biodynamic (BD) or biodynamic with green manure (BDGM) principles. Soil sampling was carried out in autumn 2012. Three sampling points were chosen along two grapevine rows in each field and for each type of vineyard management. A total of 18 soil samples were collected from the topsoil per field (n= 36). The soil samples were sieved at < 2 mm particle size, lyophilised and the total genomic DNA was extracted using a FastDNA Spin kit (MP Biomedicals, France), following the manufacturer's instructions. The V1-V3 region of the bacteria 16S rRNA gene and the ITS1 region of fungi were amplified and sequenced using 454 pyrosequencing (GS FLX+ system).

Results and discussion

Our results indicate that diversity and composition of microbial communities associated with biodynamic and organic farming systems are mostly similar, while the effects of the green manure were significant on soil microbiota richness and diversity. The bacterial phyla Actinobacteria, Proteobacteria, Acidobacteria and Gemmatimonadetes and the fungal Ascomycota, Basidiomycota and Zygomycota dominated in soil under all management system. The alpha-diversity found in bacterial communities in BDGM samples was significantly higher of that in O and BD soils. On the other hand, the different soil managements did not influence the fungal alpha diversity. When beta-diversity was analysed using PERMANOVA, both fungal and bacterial communities were significantly different according to the soil management. With permutational pairwise comparisons, it was ascertained that the microbiome of BDGM soils was significantly different from those in O and BD soils. Regarding fungi, in each soil management, indicator species were mainly saprobic. Four black yeasts (*Cladorrhinum* spp., *Capnobotryella* spp., *Cystofilobasidium capitatum*, and *Exophiala* spp.) were the fungal indicator OTUs in BDGM soils. In BDGM and BD soils the bacterial indicator species were mainly genera associated with the soil nitrogen cycle. *Microvirga* spp. and *Pontibacter* spp., two nitrogen fixing bacterial genera, were found as significantly more abundant in BDGM soils compared to O and BD soils. On the other hand, the genus *Terrimonas*, involved in the S cycling in soil, was significantly more abundant in O soils than in BD and BDGM ones. The results of our study suggest that the use of green manures can significantly enhance the population of bacteria active in the soil nutrient cycle and increased also the presence of fungal OTUs with different ecological roles (saprobic, antagonist, pathogen). Evidence of increased nitrogen-fixing and nitrite-oxidizing bacteria populations as a response to green manure incorporation suggests their potential use to increase nitrogen availability in soil.

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Assessing the impact of green manure on ecosystem functioning of soil microbial communities

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Highlights

- Increase green manure biomass (sowing in July) results in a later yield date but no impact on lettuce weight
- Soil microbiome is mainly affected by soil compaction, carbon and nitrogen content and green manure biomass

Introduction

The negative effects of intensive agriculture are felt on ecosystem services and in particular on diversity (Matson et al., 1997). Soil organisms play a crucial role in biogeochemical cycles and interact with vegetation through nutrient transfer and water retention (Coleman et al., 2004). Soil disturbances influence the structure of the soil microbial composition (Elfstrand et al., 2007, Peck et al., 2016). Green manure is a crop that is implanted before or after a main crop and then incorporated into the soil at a given stage of growth. Green manures are used in cultivation practices to meet different objectives: i) improve soil fertility; ii) improve soil structure and organic matter content; ii) reduce soil erosion; iv) weed control; and v) interrupt pest cycles (Cherr et al., 2006). The objective of the project is to demonstrate that the seeding period of green manure may influence, through stochastic and niche processes, both α- and β-diversity of soil microbial communities.

Material and methods

A green manure (oat) was implanted on different dates in the fall 2015 and the impact on soil and main crop was observed during the 2016 growing season. The comparative treatments were oat sowing at: i) the end of July and incorporated in the spring of 2016; ii) the end of July and incorporated in the fall of 2015; iii) at mid-September and incorporated in the spring of 2016; and iv) a control (without green manure). Several parameters were noted: dry biomass and root depth of the green manure, soil temperature, apparent volumetric mass, soil compaction, hydraulic conductivity, vegetative development of lettuce and crop yield. Soil samples were taken in the spring of 2016 following the incorporation of green manure and prior to the establishment of the main crop. Soil samples were analysed in the laboratory for their nutrient content (C, N, P) and other physicochemical properties, including the retention capacity of atmospheric H_2, CO and CH_4, and the production of CO_2 as a result of microbial activity. At the level of the microbiome, the taxonomic profile of the bacteria, fungi and archaea of the soil was obtained by amplification. The abundance and taxonomic affiliation of the microorganisms associated with each sample was used for the calculation of diversity. In addition, through a main component analysis, links between soil physicochemical changes and changes in the organisational structure of the microbial community have been established.

Results and discussion

The results show that it is preferable to plant oats before September under Quebec conditions in order to obtain an appropriate biomass. Presence of high oat biomass may have partially favored later harvests and the impact on the weight of lettuce seems rather small. Incorporation of green manure in autumn reduced soil compaction for the two soil types. The soil microbiome analysis shows a great variety of bacteria and fungi. In terms of soil microbial diversity, a total of 6,684 different bacterial and fungal taxonomic units (OTUs) were obtained. Bacterial diversity is much greater than that of fungi. Four classes have been identified and are linked to major components, including soil compaction (24.5%), carbon and nitrogen content (16.1%) and green manure biomass (15.8%). These results are very interesting as they show that microbial genetic indicators can be associated with multifactorial classification. Thus, based on these data, some indicator species could predict whether optimum conditions are met for lettuce production. Despite the absence of significant differences between plots, genetic indicators suggest that Class 4 (oat sowing in July and incorporated in the autumn of 2015) resulted in greater yields. A study is currently underway to validate this model.

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Soil biota from newly established orchards are more beneficial to early growth of cherry than biota from older orchards

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Highlights

- Due to climate change, it is predicted that the area suitable for cherry production will expand into northern and higher elevation areas, which now have warmer temperatures and a longer growing season
- We tested if soils that had not previously grown tree fruits were more 'biologically suitable' for sweet cherry (*Prunus avium* L.) production than soils used for orchard production for more than 10 years

Introduction

Growth of young fruit trees replanted into old orchard soil is often poor and thought to be due to a consortium of plant parasitic nematodes and fungi. In the Okanagan Valley of British Columbia, cherry has traditionally been replanted into soil that previously supported tree fruits. Due to climate change, cherry production is expanding into northern and higher elevation areas that now have warmer temperatures and longer growing seasons (Neilsen et al., 2013). Models have considered how climate and soil physiochemical properties will influence cherry range expansion, but they have not considered soil biology (Neilsen et al., 2014). The objectives of this study were to compare soil from old orchards, newly planted orchards and non-cultivated soils with respect to the influence of soil biology on cherry growth, by measuring plant growth response to sterilisation, and to determine which biological or physicochemical properties best predict cherry growth among this array of orchard soils.

Material and methods

In October 2015, field soil was collected from 18 orchard sites, which differed in soil type, geographic region within the Okanagan Valley of British Columbia, Canada, and orchard status. The orchard status was defined as ‘old’ if it was previously cropped to a *Malus* or *Prunus* species for over 10 years, ‘new’ if it was a recently established sweet cherry orchard (< 10 years), or 'non-cultivated' if the soil was not previously cropped to any type of fruit tree. To determine the influence of soil biology on plant growth among the sites, a subsample of soil from each site was sterilised by microwaving, and another subsample was left untreated. Micro-propagated ‘Crimson’ sour cherry (*Prunus cerasus*) explants were planted into five pots (9.5 cm diameter and 10.7 cm height) filled with the untreated soil and five pots of the microwaved soil from each site. The explants were arranged in a complete randomised block design and grown for 10 weeks in a growth chamber. Before planting, soil physicochemical parameters were assessed for all 18 soils. At harvest, total shoot extension, shoot weight, and root weight were determined, as well as fluorescein diacetate (FDA) hydrolysis, which is a general indicator of gross microbial activity (Green et al., 2006). Root-lesion

nematodes (*Pratylenchus* spp.) were extracted from the soil and roots of each explant, using the Baermann pan and petri-plate techniques, respectively, and nematodes were quantified using an inverted compound microscope.

Results and discussion

Non-cultivated and new orchard soils did not significantly differ from each other for most variables, so the values were pooled, and the orchard soil types were subsequently defined as 'new' (n=6 orchards) and 'old'(n=12 orchards). The percentage increase in plant biomass after soil sterilisation was greatest in old soil (one-sample t-test; t=3.9; P <0.001), while sterilisation decreased plant biomass in new orchard soil (one-sample t-test; t= -3.9; P <0.001), indicating that soil biota in older orchards tended to be more harmful to cherry explants than biota in new soils. Even though populations of *Pratylenchus* spp. in the soil were not significantly different in old compared to new soil (one-way analysis of variance (ANOVA); F=1.2; P=0.29), there was greater root colonisation by *Pratylenchus* spp. for explants grown in old soils (one-way ANOVA; F=8.1; P=0.01) and the population density of *Pratylenchus* spp. in roots was negatively correlated with plant growth (Pearson correlation; r= -0.3; P=0.002). New orchard soils had greater FDA hydrolysis activity relative to old soils (one-way ANOVA; F=11.6; P=0.004). Higher overall microbial activity may be indicative of a soil food web suppressive to *Pratylenchus* and, potentially, other root pathogens. Overall, new orchard soils were more 'biologically suitable' for planting sweet cherry than old orchard soils, suggesting the importance of management practices that maintain soil health in new, and old orchard soils, so that biological transformations that allow for the development of root lesion nematode populations and root disease can be mitigated. To determine which key indicators significantly predicted plant growth in non-sterilised new and old orchard soils, all available biotic and abiotic variables were analysed using principal components analysis (PCA). After eliminating any variables with loading values less than 0.5, the number of indicators influencing plant growth were further narrowed using step-wise regression analysis. The model (R2 = 0.9) that included the fewest indicators and best described variation in shoot height in new soils was shoot height = 283 + 3.2 (sodium) + 4.6 (pH) – 0.01 (electrical conductivity) + 10 (organic carbon) + 111(FDA hydrolysis). In old soil, the equation of the regression line (R2=0.6) was shoot height = – 6332 + 34 (cation exchange capacity) + 2.1(sodium) + 121 (latitude) + 0.2 (calcium) – 0.04 (electrical conductivity) + 20 (organic carbon) + 52 (pH) + 89 (FDA hydrolysis). The variables FDA hydrolysis, total organic carbon, pH, and sodium were the common positive predictors of plant growth for both new and old soils. Electrical conductivity was a negative predictor common to both orchard types. Other variables were specific to orchard type. These findings suggest orchard management practices that maintain organic carbon levels and stimulate an active microbial community will benefit growth of cherry trees in both new and old soils.

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The composition of apple and pear bark microbiota suggest microbial migrations from soil

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Highlights
- The migration of soil microbial communities possibly define the bark microbiota
- The bark microbiota is affected by the bark age and plant species

Introduction

The study of plant-associated microbial communities has mainly been focused on soil and rhizosphere habitats, rather than the aerial part of the plant (Vorholt, 2012). Soil represents a reservoir of microorganisms (Martins et al., 2013) that may migrate to the plant phyllosphere trough rain splash, wind or agricultural practices (Zarraonaindia et al., 2015), but scarce information is available on the relations between the bark and soil microbiota. Despite the importance of bark as a potential habitat of plant pathogens and biocontrol agents (Buck et al., 1998), knowledges on composition and dynamics of its microbial communities are lacking. The aim of this work was to optimise a method for the analysis of the bark-associated fungal and bacterial microbiota and to assess the influence of plant genotypes and bark age on its composition.

Material and methods

Bark samples were collected using a fire-sterilised scalpel from one year-old shoots (new) and 3-4 years-old branches (old) of Abate and Williams pear varieties and Golden and Gala apple varieties before budding. Each sample consisted of a pool of five plants and three replicates were collected for each variety. Bark samples were processed and the viability of culturable fungi and bacteria was assessed using the classical plating method to determine the number of colony forming units (CFU) per unit of bark fresh weight (CFU/g). DNA was extracted from the ground samples using the FastDNA spin kit for soil (MP Biomedicals). The internal transcribed spacer 2 (ITS2) and the V5-V7 region of 16S rDNA were amplified and libraries were sequenced using the Illumina MiSeq technology in order to identify fungi and bacteria, respectively. A PERMANOVA analysis was carried out in order to assess the influence of bark age, plant variety and plant species on the composition of fungal and bacterial communities. Pear and apple bark microbiota was screened for the presence of potential plant pathogenic and beneficial genera.

Results and discussion

The amount of culturable fungi and bacteria was higher in new as compared with old barks. In addition to the bark age, the number of fungal CFU was also affected by the plant species and apple

variety, while the number of bacterial CFUs was affected by the apple variety. After quality filtering, detection of chimeric, singleton and plant sequences, a total of 2,050,096 and 2,757,400 sequences and a total of 430 and 824 operational taxonomic units (OTU) were detected for fungi and bacteria, respectively. A PERMANOVA analysis revealed that the diversity of fungal and bacterial communities was influenced by the bark age, plant variety and plant species. The dominant fungal microbiota was composed by *Alternaria* and *Cryptococcus* with consistent abundance among bark samples. Conversely, the abundance of *Aureobasidium* and *Sporobolomyces* was higher in new as compared with old barks, while that of *Cystobasidium* and *Rhodotorula* was lower. Moreover, the dominant genera *Phaeosclera* was more abundant in apple barks as compared with pear barks. The bacterial microbiota was mainly composed by *Deinococcus* and *Frondinhabitans* that showed consistent abundance among bark samples. Moreover, the abundance of *Amnibacterium*, *Curtobacterium* and *Hymenobacter* was higher in new as compared with old barks, while that of *Massilia*, *Modestobacter* and *Sphingomonas* was lower.

Soil-derived fungal (*Alternaria*, *Cryptococcus*) and bacterial (*Massilia*, *Microbacterium*, *Solirubrobacter*, *Terrimonas*) genera (O' Brien et al., 2005; Nicola et al., 2017) were found on apple and pear barks, demonstrating that the bark microbiota possibly originated from soil microbiota. Particularly, genera that include potential pathogens for pear and apple were found, such as fungal agents of bark (*Diplodia*), root (*Rosellinia*), leaf (*Alternaria* and *Taphrina*) and fruit diseases (*Gibberella*, *Peltaster*, *Penicillium*, and *Stemphylium*). However, beneficial genera with potential biocontrol or plant growth promotion activities were found both for fungi (*Aureobasidium*, *Coniothyrium*, *Cryptococcus*, *Rhodotorula,* and *Sporobolomyces*) and bacteria (*Arthrobacter*, *Deinococcus*, *Lactobacillus*, *Pedobacter*, *Cohnella*, and *Promicromonospora*) on apple and pear barks.

This method allowed to study the viability and the structure of fungal and bacterial communities of bark and to assess factors that affect the microbiota composition. The presence of fungal and bacterial genera typically belonging to the soil microbiota suggests that bark communities are possibly influenced by migration of soil microorganisms. Moreover, bark could represent a reservoir of plant pathogens and beneficial microorganisms.

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Effect of different oilseed rape management systems on earthworm community (Oligochaeta: Lumbricidae)

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Highlights

- The aim of this study was to assess three different oilseed rape management strategies with regard to their effects on decomposers
- The results showed a significant reduction in earthworm density in systems using winter wheat as a subsequent crop, especially in the conventional production system, whereas integrated and organic cropping systems enhanced the density of the total earthworm community

Introduction

The number of earthworms in different habitats may vary from less than 10 to hundreds per m^2 but rarely exceed 400/m^2. The densities of earthworms can be significantly higher in pastures or soils that are rich in organic materials (1000 - 2000 earthworms/m^2). In cultivated areas, the average number of earthworms is between 70/m^2 and 80/m^2 (Paoletti et al., 1999). The most important factors that affect earthworm distribution are soil moisture and the quality and quantity of organic material, which are directly associated with the type of habitat (Edwards and Bohlen, 1996). The diversity of earthworm communities is dependent on an extensive range of factors, including the soil type, soil pH values, soil moisture capacity, precipitation, and present and past soil utilisation, as well as the degree of disruption. With the increasing interest in alternative crop management systems, earthworms play a central role in the ecological functioning of agroecosystems (Chan, 2001).

Material and methods

Field data were collected between 2010 and 2012 in trials located 10 km of Zagreb, in Šašinovečki Lug (N 45° 51′ E 16° 10′). The implementation of the field experiment during the first year was as follows: i) conventional oilseed rape production with 3-course crop rotation with intensive (standardised) use of pesticides, and intensive tillage and fertilisation according to current agricultural practices; ii) a highly integrated system with a 4-course crop rotation in which all measures known to enhance biodiversity (e.g., mulching, which is known to enhance predator abundance and earthworm activity, pesticide application only if unavoidable, wider row spaces to enable mechanical weed control, and lower fertiliser input to prevent leaching) were applied; and iii) an organic with an 8-course crop rotation, ploughing, no input of pesticides and mineral fertilisers, wide row spaces, mechanical weeding, and a 3 m turnip rape trap crop strip to distract pests from OSR plants and as a “beetle bank” for overwintering predators. In each management system, eight samples were prepared; in each trap crop (and former trap crop strips during the winter wheat growth season), four samples of 0.25 m² were prepared. Earthworms were assessed by formalin extraction

combined with hand sorting according to the guidelines ISO/TC 190/SC 4 WG 2 NO 22 of 11/03/2005.

Results and discussion

In oilseed rape, 714 earthworms were collected; in winter wheat, only 265 earthworms were collected. A total of six earthworm species were recorded. The dominant species were *Lumbricus terrestris*, *Allolobophora chlorotica* and *Aporrectodea rosea*. The number of earthworms species identified in this investigation is similar to the results of other studies, in which one to nine species were observed (Pfiffner and Mäder, 1998; Schmidt et al., 2001; Prescher et al., 2014). No significant differences in the diversity indices were noted among the management systems. Although the diversity indices among systems were similar, the values of the Shannon-Wiener index were higher in the conventional system than in the other two systems but lower than the results of other studies (Jones et al., 2001; Pelosi et al., 2009). The total earthworm density ranged from 8.4 ind/m^2 (integrated) to 10.2 ind/m^2 (conventional) in the oilseed rape growing season, whereas the total earthworm density in the winter wheat growing season ranged from 2 ind/m^2 (conventional) to 4.9 ind/m^2 in the former trap crop of the integrated management system. These findings are significantly lower than the findings of Hole et al. (2005) and Pelosi et al. (2009) but are similar to the results obtained by Bachelier (1979) and Gerard and Hay (1979) cit. Peres et al. (2006), who reported that the earthworm density in cultivated soils is usually less than 50 ind/m^2 but can be less than 10 ind/m^2 and even reaching zero. Within the same project and in the same management systems but with different soil types in Germany have recorded between 48 and 99 earthworms/m^2, which is significantly higher than our results. In both growing seasons (oilseed rape and winter wheat), no significant differences in the density of the endogeic group of earthworms between management systems were detected. The highest earthworms biomass in oilseed rape was recorded in the organic system, whereas the lowest was recorded in the conventional system, which is consistent with Arden-Clarke and Hodges (1988) and Hole et al. (2005). The biomass of the total earthworm community exhibited an increasing trend in the conventional winter wheat production system from previous crops despite intensive tillage and high pesticide and fertiliser input, which contrasts with Edwards and Lofty (1982) (Riley et al., 2008). This study revealed a significant density reduction of total earthworm communities during the winter wheat growing period following oilseed rape as a previous crop. Management systems with lower agrochemical inputs (integrated and organic) promote higher levels of earthworm activity and biomass compared with conventional management systems. Trap crop strips can enhance earthworm populations and can be employed as a bank (reservoir) for future crops.

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The effects of plant growth-promoting rhizobacteria (PGPR) on the growth and quality of strawberries

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Highlights

- Plant growth-promoting rhizobacteria (PGPR)-treated plants delivered larger strawberry fruits.
- PGPR can enhance the content of nutraceutical compounds in strawberry fruits

Introduction

Several studies have shown the benefits of plant growth-promoting rhizobacteria (PGPR) on plant mineral nutrition suggesting their application as biofertilisers (Pii et al., 2015). PGPR can stimulate plant growth, increase plant resistance to abiotic and biotic stresses and might thus have a positive effect also on fruit quality. The aim of this work was therefore to evaluate and compare the effects of beneficial microorganisms, supplied either as pure culture (*Azospirillum brasilense*) or as a commercial mixture (Effective Microorganisms, EM), on the growth and quality of strawberry (*Fragaria ananassa* cv. Elsanta) fruits. Strawberries are in fact among the most popular fruits, because of their unique taste and health benefits for humans, due to a high content of micronutrients, phytochemicals and antioxidants.

Material and methods

Strawberry plants were hydroponically grown either in a complete nutrient solution, or in a nutrient solution inoculated with *A. brasilense* or with EM for 10 weeks, as previously described (Pii et al., 2016). Strawberry fruits were harvested once at least 80% of the fruit surface showed a red coloration. Fresh weight (FW), yield per plant (g FW per plant), average fruit yield (g FW), average number of strawberry fruits per plant were assessed. At harvest, shoots and roots were separated assessing fresh weight (FW) and dry weight (DW) of the tissues together with the root to shoot ratios. Titratable acidity, total soluble solid content and firmness of fresh strawberry fruits were determined as previously described (Valentinuzzi et al., 2015). In addition, freeze-dried strawberry samples were homogenised and 100 mg of strawberry powder were extracted with 1 ml methanol (HPLC grade, Merck, Darmstadt, Germany). The mixture underwent sonication for 30 min at 4°C and the extracts were centrifuged at 14,000 × g for 30 min at 0°C; afterwards, the supernatant was collected and filtered through a 0.2 µm nylon filter. The content of total phenols of strawberry fruit extracts was determined following the Folin-Ciocalteau method, whilst the concentration of flavonoids and flavonols was determined by a pharmacopeia method, using rutin hydrate as reference compound (Valentinuzzi et al., 2015).

Results and discussion

The growth parameters were not affected by the PGPR treatment, with the exception of the sample treated with *A. brasilense* that showed lower values in the shoot growth. In terms of yield, the control plants had significantly higher yields than the PGPR-treated plants; however, these latter

delivered in average larger fruit. The color measurement showed significant differences between the samples, in fact strawberries treated with *A. brasilense* displayed higher values as compared with those supplied with EM and control plants.

The total sugar content was not affected by PGPR treatments, whilst the titratable acidity resulted higher in control samples. These features had an impact on the sweetness index, defined as the ratio between the sugar content and the acidity, which resulted increased in the PGPR-treated strawberries.

The content of total phenols showed no significant difference between the different samples, whilst flavonoids and flavonols resulted more concentrated in the samples supplied with *A. brasilense*. In addition, also the mineral composition of the strawberry fruits was influenced by the PGPR treatment. For instance, copper and zinc were accumulated in *A. brasilense*-treated strawberry fruits.

Based on these observations and results, it was shown that PGPR can play a role in improving fruit quality without negatively affecting the growth of plants; although the yield per plant was lower in the PGPR variant, the weight of harvested fruits was higher.

The PGPR also increase other important quality parameters such as micronutrient levels as well as health-promoting substances such as flavonoids and flavonols. The latter are not only important antioxidants for the plants but also for humans.

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The biocontrol agent *Pseudomonas chlororaphis* subsp. aureofaciens M71 originates natural mutants impaired in the ability to control *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato

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Highlights

- *Pseudomonas chlororaphis* subsp. *aureofaciens* M71 differentiated three natural mutants distinguishable for morphological traits
- *P. chlororaphis* subsp. *aureofaciens* M71 mutants were impaired in persisting on tomato roots and controlling tomato crown rot
- Mutants were characterised by a reduced ability in production of autoinducer signals and antibiotics

Introduction

Fluorescent pseudomonads are able to control plant diseases by effectively colonizing plant roots and releasing secondary metabolites toxic to phytopathogenic microorganisms. However, once applied on plant roots, mutants lacking these abilities may arise in biocontrol fluorescent pseudomonads impairing their success in controlling plant diseases (Chancey et al., 1999, 2002).

Pseudomonas chlororaphis subsp. *aureofaciens* M71 (M71) effectively controlled phytopathogenic fungi *in vivo* due to the production of phenazine-1- carboxylic acid (PCA; Puopolo et al., 2011; Raio et al., 2017). Three classes of M71 mutants, named M71a, M71b and M71c, were isolated from the rhizosphere of tomato plants treated with M71. The aim of this study was to evaluate how the occurrence of mutations may affect the M71 biocontrol performances and characterise the three mutants through a biochemical and microbiological approach.

Material and methods

The rhizosphere competence and antagonistic performance of M71 and its three mutants against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) were tested *in vivo* on tomato plants. Bacteria were re-isolated from roots of 20 days old tomato plantlets and colony phenotype was recorded. The stability of each strain phenotype was determined *in vitro* by treating tomato seeds with cell suspensions of rifampicin resistant marked strains. *In vitro* antagonistic activity of the four strains was tested against *F. o.* f. sp. *lycopersici*, Forl, *Pyrenochaeta lycopersici*, *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The four strains were tested for the ability to produce PCA according to Raio et al. (2017) and the release of N-acyl homoserine lactones (AHLs) was assessed using the biosensor strain *Chromobacterium violaceum* CV026. M71 and its three mutants were tested for proteolytic activity on skim milk agar, while production of siderophore was tested on Chrome Azurol S medium.

Results and discussion

The three M71 mutants had a colony morphology different from M71 morphology (bright orange and mucoid). Indeed, M71a colony was translucent and mucoid; the M71b colony was pale orange and mucoid while M71c showed a bright orange and rough colony. M71 and M71c efficiently colonised tomato rhizosphere and determined a significant reduction in disease incidence caused by Forl (38 to 50% vs. 85% control) *in vivo*. In contrast, M71a and M71b were not able to effectively colonise tomato rhizosphere and, as a consequence, were not able to control Forl. In the *in vitro* test, 40% of the colonies originated from tomato plants treated with M71 showed the M71a phenotype. M71a and M71b did not originate different colony phenotypes, demonstrating that these may be considered stable mutations. In contrast, 42% of the colonies deriving from tomato plants treated with M71c showed wild-type phenotype suggesting that the mutation occurred in M71c is a reversible mutation. *In vitro* antagonistic activity of M71c against fungi was very similar to M71. Both were active against all fungal species tested and induced the highest reduction of mycelial growth. M71a was active against *P. lycopersici* and *P. ultimum* only. M71b was less active than M71 against Fol and *P. lycopersici* and had no effect against *R. solani*. M71 was the most active PCA producer while M71a and M71b produced the lowest amounts. The reduced PCA production was associated with a drastic reduction of AHL biosynthesis in M71a and M71b. Indeed, these two mutants were not able to restore violacein production in *C. violaceum* CV026. M71c was able to produce amounts of PCA and AHLs similar to the wild type strain. The three mutants showed a reduced proteolytic activity compared to M71 whereas siderophore production was significantly higher in M71a and M71b.

Results indicated that three classes of mutants might derive from M71 when this bacterial biocontrol agent is applied on tomato roots. One of these three (M71c) is probably due to a reversible mutation and it is not impaired in the colonisation of tomato rhizosphere and in controlling Forl since the characteristics of the wild type are restored during the experiments. In contrast, the M71a and M71b phenotypes are attributable to stable mutations that cause an increase in siderophore production and the loss of the ability to release proteases and produce PCA and AHLs. The outcome of these mutations is a significant impairment in the efficacy of M71 against Forl on tomato plants. In *P. chlororaphis* 30-84, these features are due to the lack of functional *gacA*/*gacS* genes encoding a two-component transcriptional system that positively controls AHL and PCA production (Chancey et al., 1999, 2002). Future works will be aimed to better identify the molecular mechanisms underlying the occurrence of these mutations in M71 to reduce the risk of not reliable results when applied in the field.

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Search for plant-based biofungicides against toxigenic contaminants in barley (*Hordeum vulgare* L.) forage in hydroponics

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Highlights

- Mentha essential oil and crude cloves aqueous extract confirmed the *in vitro* fungicidal activity on *Microdochium nivale* var. *majus*, *Fusarium avenaceum* and *F. culmorum*

Introduction

The production of hydroponic barley can ensure great development in the fields of agriculture, livestock, environment, economy and health. On the other hand, toxinogenic molds, particularly those of *Fusarum* spp. represent tremendous constraints for this crop.

Material and methods

Our study focused on the research of plants with antifungal potency against the three isolates: *Microdochium nivale* var. *majus, F. avenaceum* and *F. culmorum*, which are toxinogenic contaminants from the hydroponic samples of feed barley. It consists of the use of aqueous extracts prepared by decoction from a range of plants composed of: *Eugenia caryphyllata*, *Lavandula stoekas*, *Allium satvum*, *Punica granatum*, *Aloysia citrodora*, *Cinnamom verum*, *Pistacia lentiscus* and raw test (150 mg/ml) and concentrations of: 102.5, 75, and 37 mg/ml, according to the direct contact method. In addition, crude hydrosols and emulsions (4% w/w water-agar) prepared from essential oils of Pennyroyal Mint (*Mentha pulegium*) obtained by hydrodistillation and by the Alambic method were also tested according to direct contact method and, at dosages of 7.5, 15, 30 and 60 µl, according to the micro-atmosphere method.

Results and discussion

All tested plant extracts showed a variability in the inhibition of mycelial growth and sporulation of the studied fungal isolates. Only the aqueous extracts based on crude cloves and the emulsion at 4% based on Pennyroyal Mint essential oil obtained by hydrodistillation showed complete inhibition of mycelial growth and sporulation (100%) of all isolates with respect to the aqueous extract of cloves, but with the exception of *F. culmorum* for the emulsion of the essential oil, according to the direct contact method. However, the total inhibition of the two parameters was recorded for this same

emulsion and at the concentrations of 30 and 60 µl for the three isolates of *Fusarium* spp., according to the micro-atmosphere method. Thus, the essential oil of *M. pulegium* extracted by hydrodistillation and the aqueous extract based on cloves can be proposed as biofungicides against *M. nivale* var. *majus* and *F. avenaceum*. It would therefore be interesting to test them in hydroponic cultures and identify their active ingredients. As, it would also be important to follow our research on this topic to find a solution against the isolate of *F. culmorum*.

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Effect of natural nitrification inhibitors on nitrogen contents in soil and plant growth

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Highlights

- Use of neem and moringa oil as natural nitrification inhibitors for increasing use efficiency of urea
- To study the effect of these natural substances on nitrogen contents in soil and plant growth

Introduction

Nitrification inhibitors are the compounds that delay bacterial oxidation of the ammonium (NH_4) to nitrate over a certain period of time. Nitrification inhibitor stabilizes the contents of NH_4, so that plants can easily uptake the nitrogen in NH_4 form (Singh and Verma, 2007). The uptake of NH_4 by plants during protein metabolism has a positive effect on the production of plant hormones like gibberellins, cytokinins (Pasda et al., 2001). Nitrification inhibitors are natural or synthetic compound. Natural nitrification inhibitors, when compared to synthetic, are less persistence, more biodegradable, easily available, cheaper and eco-friendly, while synthetic one are very expensive and unstable (Patra et al., 2006). It has been reported that various herbal products or by products such as Neem (*Azadirachta indica*) oil and cake inhibit nitrification (Prasad et al., 1999). Nitrification inhibition mainly occurs by blocking the enzyme ammonia monooxygenase (AMO) which is present in the *Nitrosomonas* spp. (Subbarao *et al*., 2006). Moringa oil has the nearly similar composition to Neem oil, so the nitrification inhibition of moringa oil would be more or less similar to neem oil.

Material and methods

A pot experiment was conducted to improve the nitrogen use efficiency in *Brassica napus* L. (test crop) in the wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. The experiment was designed in three factorial completely randomized design (CRD) with three levels and three replications for each level. Eight seeds of *Brassica napus* L. were planted in 7 kg soil of each pot and, after germination, five seeds were left after thinning. The treatments applied were: T_1: control (zero urea), T_2: 3% neem oil coated urea (NOCU); three levels of T_2: T_2L_1 (half dose of N : 0.055 g kg^{-1} of soil), T_2L_2 (recomended dose of N: 0.111 g kg^{-1} of soil) and T_2L_3 (full dose of N: 0.222 g kg^{-1} of soil); T_3: 3% moringa oil coated urea (MOCU) same levels used as in NOCU; T_4: ordinary urea (without coating) same levels used as in NOCU and MOCU

without coating. The rate of nitrogen application was different for all levels [L_1: 0.055 g/kg (Half dose of N); L_2: 0.111g/kg (recommended dose of N); L_3:0.222g/kg (full dose of N)]. These treatments were evaluated as potential nitrification inhibitors. Urea was coated as neem oil coated urea, moringa oil coated urea on w/v basis. The coated urea was prepared under laboratory conditions and applied at the time of sowing. The source of nitrogen, phosphorus and potassium were urea, single superphosphate and potassium sulphate, respectively. The rate of NPK was applied at 90 kg/acre, 60 kg/acre and 75 kg/acre, respectively. There were two harvests of *Brassica napus* L. for the equal interval of time such as after seven weeks and maturity (fifteen weeks) respectively. Data regarding crop growth and physiological parameters and crop yield were recorded. Soil physical and chemical properties and NO_3-N and NH_4^+-N, were analysed according to Soil and Plant Analysis Laboratory Manual (Ryan et al., 2001).

Results and discussion

Plant height significantly increased in T_2L_1 with the application of 3% neem oil coated urea with L_1 (Half dose of N: 0.055g/kg) at this level plant height was 99.5 cm as compared with 72 cm in the control with no urea. The moringa oil coated urea and ordinary urea were close in T_3L_3 and T_4L_3 with 87 cm and 89 cm plant heights, respectively. Overall neem oil coated urea was best for plant height.

Shoot fresh weight also increased significantly with 3% neem oil coated urea and the maximum fresh weight was 40.6 g, found in teh case of neem oil coated urea with level-1 (L_1: half dose of N: 0.055 g/kg). This was the highest fresh shoot weight, while lowest was in case of control (no urea).

Shoot dry weight, plant moisture content and number of leaves per plant were observed, which were significantly increased with the application of coated fertilizer as compared to ordinary urea (without coating). Chlorophyll contents were also higher in coated urea treatment than in ordinary urea and control treatments. However, maximum chlorophyll contents were observed in 3% neem oil coated urea followed by 3% moringa oil coated urea and ordinary urea (without coating).

Relative growth rate was increased in coated treatments, the maximum relative growth rate (33.25) was observed in T_2L_1 where 3% neem oil coated urea was used. There was not significant difference between 3% neem oil coated urea and 3% moringa oil coated urea in terms of relative growth rate.

Total grain yield per pot of brassica in coated treatments was higher than non-coated treatments. The maximum grain yield (0.41g) was observed in neem oil coated urea T_2L_1 with half dose of the N (0.055g) than the other two levels T_2L_2 and T_2L_3.

After harvesting of *Brassica napus* L., soil was analysed to assess the NH_4-N and NO_3-N content. The nitrification inhibition properties were identified in neem oil coated urea and moringa oil coated urea. However, results showed that: 3% neem oil coated urea and 3% moringa oil coated urea were most effective for inhibition of nitrification than ordinary urea (without coating). Overall results indicate that neem oil coated urea has potential to inhibit nitrification and to increase the fresh shoot weight, dry shoot weight, chlorophyll content by improving the nitrogen use efficiency, followed by moringa oil coated urea and ordinary urea.

Acknowledgements

This experiment was performed in wire house under Soil and Plant Nutrition Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan, for research.

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Evaluating the effect of slow releasing polymer coated urea on growth and yield of maize

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Highlights

- Polymer coated fertilizers can improve nitrogen use efficiency of maize crop by slow release of nitrogen from coated urea
- Slow release fertilizers reduce the leaching and volatilization losses of nitrogen and ensure its availability to the plant which not only improve the growth but also reduce environmental issues

Introduction

Nitrogen is one of growth limiting nutrients because it is involved in most of the biochemical processes of plants. Due to its high mobility, nitrogen is harmful to the environment (Chatterjee, 2012). Nitrogen deficiency reduces the production of chlorophyll and amino acid, consequently there is less growth and more susceptibility to pest (Mullen, 2011).

Before the uptake of soil nitrogen by plants, it is possibly lost through volatilization, immobilization, leaching and denitrification. The pathways and quantity which has to be lost from the soil depend upon environmental conditions, such as temperature and moisture. Controlled release fertilizers are being developed to improve nutrient use efficiency (NUE) while reducing environmental hazards. These types of fertilizers can provide many advantages to agriculture, such as high fertilizer use efficiency, reduced nutrient losses via fixation, leaching, denitrification, and reduction of soil chemical process that decreases the availability of nutrients (Lunt, 1991; Sharma, 2002).

Material and methods

Treatment Plan: T1= Control (without any fertilizer), T2= Recommended dose of N, P and K, T3 = Polymer coated urea at 100% of recommended dose with basal application, T4 = Polymer coated urea at 75% of recommended dose with the basal application, T5 = Polymer coated urea at 100% of recommended dose with the split application, T6 = Polymer coated urea at 75% of recommended dose with the split application.

In the treatments except for control, recommended doses of phosphorus (P) and potassium (K) (150 kg and 100 kg per hectare respectively) were applied. Seed rate was 10 kg per acre. N, P and K was used at the rate of 175-150-100 kg per hectare, respectively. Full doses of P and K was applied

before sowing while urea was applied in three splits, one before sowing and other two with first and second irrigation. Commercial urea was used for coating in Soil Fertility and Plant Nutrition Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. For coating with polymer, 1% solution of polymer was prepared in distilled water then was used for the coating of urea. For the second layer of coating, after drying, one more coating of urea with the solution was carried out. All the procedure was done under a controlled environment to avoid any contamination. The coated urea grains were dried and then stored under laboratory conditions till their use.

Results and discussion

It was investigated that treatments in which coated urea was applied, showed dramatic results both in biological production and nitrogen use efficiency (NUE).

Treatment where polymer coated urea was applied with 100% recommended dose in the basal application showed a remarkable increase in biological yield, followed by the treatment where we applied the same dose of polymer coated urea in splits. When both these treatments were compared with uncoated fertilizer applied treatment (T2) it revealed that maximum biological yield recorded in T6 which was 31,106 kg ha^{-1}.

When we compared the nutrient use efficiency of all the treatments, it was demonstrated that treatment with 100% polymer coated urea with split application showed maximum nitrogen uptake by plant along with other nutrients. In treatment T6, maximum nitrogen, recorded in grain and shoot was 2.74% and 1.71% respectively,

Nitrogenous fertilizers like urea may face nitrogen loss due to leaching in the form of nitrate or volatilization as ammonia. Under such circumstances, it is necessary to improve the effectiveness of nitrogenous fertilizers. Different approaches like the split application of urea, slicing, liquid fertilizers are in practice but still, NUE is not more than 50%. Nitrogen loss in the form of nitrates affects human and animal health by polluting the environment via leaching and volatilization. Controlled release fertilizers are the fertilizers which extend the duration of nitrogen supply to the plant as the plant needs nitrogen through its all growth stages and also these controlled release fertilizers reduce the nitrogen loss to the environment.

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This experiment was performed in research fields, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. Soil and Plant analysis were performed in Soil Fertility and Plant Nutrition Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan.

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Combined use of organic biofumigant materials and a biological control agent: First experience in Switzerland

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Highlights

- Organic biofumigant materials had a negative effect on the *Streptomyces* populations in the soil, but increased the dry matter production of young tomato plants
- The combination of a *Streptomyces* spp. as biocontrol agent with biofumigation should not be done simultaneously but sequentially

Introduction

Soilborne pathogens are a major threat to the production of horticultural crops. The phasing-out of methyl-bromide, a powerful fumigant, in 2005 (Gullino et al., 2003), triggered a rush in the development of alternative control methods. Among them is the replacement of synthetic soil fumigants by natural produced biocidal volatile molecules. This approach uses in first line *Brassica* species rich in specific glucosinolates which during their decomposition release volatile and toxic isothiocyanates (ITCs). This relatively new method was coinded with the term biofumigation (Kirkegaard, 2009). Another approach is the use of microbial agents antagonistic to soilborne pathogens. Among them are *Streptomyces* (Wiggins & Kinkel, 2005), and a product containing *Streptomyces griseoviridis* as biological control agent (BCA) is commericialized in Europe. The aim of this study was to test the combination of these two methods to improve the growth of tomato in a soil infested with *Verticillium dahliae.*

Material and methods

Biofumigant materials consisted of dried leaves and stems of two brown mustard (*Brassica juncea*) cultivars; with a high glucosinolate (GSL) content (cv. ISCI-99) and a low GSL content (cv. Arid). They were grown for six weeks in a greenhouse, then the above-ground biomass was dried at 35°C. The third organic material was defatted *Brassica carinata* seedmeal pellets (brand Biofence). The material was added to a sandy loam soil, naturally infested with *V. dahliae*, *Colletotrichum coccodes* and *Pyrenochaeta lycopersici*, at a rate of 5 kg FM/m^2 for the plants and 0.25 kg/m^2 for the pellets. After mixing, 0.4 L of soil were placed in 0.5 l plastic pots. Half of the pots were then irrigated with a 0.01% suspension of Mycostop, a commercial product containing *S. griseoviridis*. The other pots were irrigated with water. For each treatment, four pots were prepared and incubated at 20°C in the dark. After two weeks, the number of *V. dahliae* microsclerotia was determined by dry-plating on NP-10 medium (Kabir et al. 2004). The number of *Streptomyces* spp. was determined by serial dilutions on WA-SCA medium (Wiggins and Kinkel, 2005). The soil microbial activity was measured with the FDA-method (Schnürer and Rosswall, 1982). The major part of the soil was mixed with sterile clay granules (1:1, v:v), and then placed in two 0.4 l pots. Two weeks old tomato seedlings (cvs. Bonny Best and De Berao) were transplanted in these pots. After one month in the greenhouse, above-ground biomass was determined.

Results and discussion

Adding biofumigant material to the soil significantly increased the soil microbial activity compared to the non-amended control. The two brown mustard had the strongest effect, but also the Biofence pellets induced increase of the microbial activity was significant. In contrast, the *Streptomyces* populations were negatively influenced by the addition of the biofumigant materials. The two GSL-rich treatments i.e., the high GSL-content brown mustard cultivar ISCI-99 and the Biofence pellets, reduced significantly the number of *Streptomyces* spp. in the soil. The low GSL content cultivar Arid resulted in an intermediate effect. Adding the *S. griseoviridis* containing Mycostop to the soil did not influence the number of *Streptomyces* spp. The biofumigant materials had no effect on the number of *V. dahliae* microsclerotia in the soil but influenced the tomato dry matter production. Both cultivars had significant higher dry matter when grown in the soil amended with Biofence and ISCI-99. The low GSL content Arid increased significantly the dry matter of cultivar De Berao but not of Bonny Best compared to the non-amended control.

The negative effect of the biofumigant materials on the *Streptomyces* populations showed the fumigation potential of this technique. Unfortunately, the targeted pathogen *V. dahliae* was more resistant to ITCs generated by the organic amendments. The reason is most probably the insufficient ITCs concentration generated by Brassica amendments to kill *V. dahliae* microsclerotia (Neubauer et al., 2014).

The higher tomato yield of both cultivars caused by Biofence and the high GSL cultivar ISCI-99 indicated however a positive biofumigation effect on the plants. One reason might be a detrimental effect on the soil populations of the two other soilborne pathogens, *C. coccodes* and *P. lycopersici*. Another possibility might be a nutritional effect of the organic amendments on the tomato growth. However, this is less probable as the tomato plants were irrigated with a fertilizer solution to counterbalance such an effect.

In conclusion, the combination of a *Streptomyces* BCA with the biofumigation technique is not indicated. Other BCAs might be more resistant to ITCs and could eventually be used for a combined application. For the combination of *Streptomyces* spp. with biofumigation, we suggest an application of the BCA at the planting date when the ITCs generated by the biofumigation are no more present in the soil (Kirkegaard, 2009).

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Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture"

Novel tools and new challenges for IPM in viticulture

Novel tools and new challenges for IPM in viticulture

Oral Session 1

New knowledge and solutions against viruses, phytoplasmas and their vectors: vectors

Monitoring *Xylella fastidiosa* insect vectors in Northern and Southern California to understand recent Pierce's disease incidence

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Highlights

- Pierce's disease of grapevine has reemerged in some regions of Northern and Southern California
- Recent disease incidence in Northern California does not match the historical pattern associated with the vector *Graphocephala atropunctata*
- In Kern County an increase of the vector *Homalodisca vitripennis* is thought to be due to reduced insecticide efficacy in combination with changes in climate

Introduction

Pierce's disease (PD) epidemics recur periodically in different regions of California. In the North Coast disease incidence is associated with blue-green sharpshooter (BGSS), *Graphocephala atropunctata* while in Southern California with Glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*. The last PD epidemic in the North Coast occurred in the late 1990's when historically PD was closely associated with riparian zones, BGSS habitat. Since 2014 disease incidence has increased and it is not always associated with riparian areas. The introduction of GWSS into Southern California in the late 1990's caused PD epidemics in Kern County. Since 2001, area wide treatment programs coordinated by USDA have been in place to reduce GWSS populations. Spray programs to citrus where GWSS overwinter, coupled with removal of infected vines are key management components. Recently, despite annual areawide treatments, high population levels of GWSS have returned with a resurgence of PD.

Material and methods

To evaluate potential shifts in Pierce's disease epidemiology in the North Coast thirty-two vineyard blocks in Napa and Sonoma Counties are being monitored year-round with yellow sticky cards and sweep net sampling for all potential vector populations. Vector natural infectivity will be experimentally determined for collected samples. PD incidence for each vine in all study blocks was surveyed each fall. Every vine is geo-referenced on geographic information systems maps and subjected to spatial statistics. The plant communities adjacent to surveyed vineyard blocks were characterised to determine vector and bacterial sources. In Southern California, the success of areawide treatment programs in Kern County is evaluated by monitoring GWSS populations and PD incidence. GWSS monitoring is conducted by California Department of Food and Agriculture with yellow sticky traps. Each year in the fall, University of California personnel survey between 39 and 43 vineyards. Vineyards are chosen based on past history of surveyed sites, knowledge of PD distribution and GWSS trap catches. Surveyors looked for vines that expressed PD symptoms such as leaf scorch, persistent petioles, cane irregular maturity, stunted shoot growth and shrivelled fruit.

Samples were collected from all symptomatic vines to confirm presence of *Xylella fastidiosa* using an enzyme-linked immunosorbent assay (ELISA).

Results and discussion

In North Coast vineyard BGSS were collected on yellow sticky cards and from the canopy. *Draeculacephala minerva*, *Pagaronia* sp., *Philaenus spumarius*, and *Aphrophora* sp. were collected from sweeping vegetation at the edge of the vineyard blocks and *Carneocephala fulgida* from Bermuda grass. The BGSS monitoring program initiated in 2016 indicates vector populations in vineyards are low, with the highest densities in some Sonoma County vineyard blocks between the end of March and mid-May. BGSS migrate from riparian zones to adjacent vineyards in the spring, inoculating vines in a chronic manner. Late season infections occur, often away from riparian zones, but those plants do not result in chronic PD because the vine recovers from infection during the winter, although the specific mechanism remains unidentified. Elimination of reservoir host plant species of *X. fastidiosa* and BGSS from riparian corridors significantly lowers PD prevalence and has been the management tools used in this region. Historically prevalence of PD in the North Coast is below 2%; surveys of the 32 vineyard blocks indicate that several vineyards have over 25% symptomatic vines and PD incidence is found in vineyards away from riparian areas. Further studies are needed to determine the role other vectors have in disease epidemiology in the North Coast.

During the first year of the areawide treatment program in 2001 there were more than 140,000 sharpshooters caught in traps in Kern County. This included GWSS from citrus and vineyards before and after treatments were made. During 2002 and 2003 the areawide program were expanded to include all areas in Kern County where GWSS was detected. From 2004 to 2008 the program was highly successful. Once an area had participated in an areawide treatment program, follow-up treatments to the entire area were applied every two to three years and GWSS populations could be maintained at low levels with localised spot treatments as needed based on trap captures. In 2009 GWSS captures increased to nearly 40,000. Treatments increased; however 2010/11 captures remained at levels close to 40,000. Beginning in 2012 population increased to over 100,000 GWSS; poor control continues despite treatments every year. The two most prevalent theories for recent GWSS population increase are climate change and insecticide resistance. With regard to climate change, overwintering GWSS can tolerate very cold temperatures but require a minimum temperature in order to feed. In Kern County during winter months, thick fog can remain for long periods such that GWSS are unable to feed, causing them to desiccate and die. However, during the recent 5-year drought, the lack of rain did not create the inversion conditions required to have thick fog events, resulting in greater GWSS overwintering survival. Over the past seven years there has been a consistent increase in the incidence and distribution of PD present in Kern County.

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Phlogotettix cyclops: occurrence, infectivity with flavescence dorée and importance as vector in Austrian vineyards

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Highlights

- The Flavescence dorée phytoplasma is endemic in Europe, existing in several wild plant species and is transmitted by the introduced nearctic leafhopper *Scaphoideus titanus* (Ball)
- It was shown that *Dictyophara europaea* (L.) and *Orientus ishidae* (Matsumura) are able to transmit FDp to grapevine
- The question whether other vector species play a role in the epidemiology of FD is still an issue

Introduction

Flavescence dorée (FD) is a major grapevine yellows disease in Europe caused by phytoplasmas (FDp) of the taxonomic group 16SrV-C and 16SrV-D (Bertaccini and Duduk, 2009). In Austria, FD was recorded for the first time in Styria in 2009 and 2015 in Burgenland. In general the population of *Scaphoideus titanus* is low and only single grapevines were infected with the 16SrV-C strain in recent years.

During surveys conducted to search for potential vector species of FD in Austria, the non-native leafhopper *Phlogotettix cyclops* has been repeatedly found. Although this species is spreading and has been recorded in several European countries during the last years, little is known about its biology (Chuche et al., 2010).The aim of the study was to study the occurrence and biology of *P. cyclops* and its relevance in the epidemiology of FD in Austria.

Material and methods

The occurrence of *P. cyclops* was surveyed for several years in different winegrowing areas of Austria by using yellow sticky traps and beat sampling. To determine the infestation level with FDp in nature, the collected *P. cyclops* specimens were analysed for phytoplasma presence using real time PCR (Hren et. al., 2007) and strain differentiation according EPPO standard PM7/79.

Results and discussion

P. cyclops occurs in all winegrowing areas surveyed but in different densities. For the first time *P. cyclops* was detected as carrier of 16SrV phytoplasmas in 2013 in Austria, showing repeatedly strong FDp positive signals. The distribution data, host plants and the molecular genetic analysis of *P. cyclops* with regard to FD incidence as well as the on-going experimental work to test the transmission ability of this species to grapevine will be presented.

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The role of scale insects as vectors of grapevine viroses in German viticulture

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Highlights

- Grapevine leafroll associated viruses were detected in several winegrowing regions of Germany
- Three young vineyards were chosen for epidemiological analyses
- Different plant protection products (PPPs) were tested for effects on scale insects and their natural enemies

Introduction

Leaf roll is one of the most widespread grapevine diseases worldwide. Different filamentous viruses (GLRaV) are associated with the disease. Leaves of symptomatic vines show a progressing interveinal discoloration and often roll downwards. Sugar content of infected vines is often lowered and their yield can decrease dramatically depending on the variety. Scale insects are vectors of different viruses, including the grapevine leaf roll associated Ampeloviruses (mainly GLRaV-1, -3) as well as the Vitiviruses 'Grapevine virus' (mainly GVA) (Herrbach et al., 2016). The following scale insect vectors of these viruses are known to be generally present on grapevine in Germany: *Phenacoccus aceris*, *Heliococcus bohemicus*, *Pulvinaria vitis* and *Parthenolecanium corni*. The aim of this study is a re-evaluation of the significance of scale insects as virus vectors in the German viticulture. Additionally, disease spread is monitored in three vineyards.

Material and methods

For virus diagnostics 50-100 mg of phloem was scraped from collected wood samples. Total RNA was extracted from the phloem using a modified extraction method according to Rott and Jelkmann (2001). After reverse transcription (RT) followed by multiplex PCR we identified the viruses GLRaV-1, -2, -3 and GVA via agarose gel electrophoresis.

Wood samples from eight different wine growing regions in Germany were taken from vineyards with and without scale insects. Mostly ten samples per vineyard were taken from November to December 2016 for random analyses.

Three vineyard plots (planted between 1994- 2011) infested by scale insects, were selected in three different wine growing regions for epidemiological studies. Each of approximately 700 vines per plot was tested for virus infection.

Possible effects of PPPs on non-target organism were tested. We used the greenhouse mealybug *Pseudococcus viburni* as model organism and its parasitoid *Leptomastix epona*. Both were reared on pumpkins. For the tests non parasitised adult mealybugs and parasitised adult mealybugs were used. To test the effect of PPPs on the scale insects themselves non parasitised and parasitised adult mealybugs were put in petri-dishes (Ø 90mm, n=10) filled with Agar-culture solution (Peschiutta et al., 2017). These mealybugs were sprayed with PPPs in a LAB sprayer (Comp. Schachtner, Ludwigsburg) in the recommended dose. Petri dishes were checked daily for two weeks and the number of survived mealybugs was recorded.

Results and discussion

Virus positive vines were found in six (Baden, Moselle, Nahe, Pfalz, Wuerttemberg, Rheinhessen) of eight wine growing regions analysed. All of the viruses included in the tests appeared in these regions. Only the samples from Saxony and Middle Rhine revealed no positive results. In the wine growing region of Baden vineyards with (n=3) and without scale insects (n=3) could be compared. The proportion of virus positive vines was clearly higher in plots infested by scale insects compared to vineyards without vectors. These results refer to a potential roll of scale insects as virus vector in German viticulture. Further comparisons including more wine growing regions and different winegrowers will be carried out to confirm these preliminary results.

Epidemiological analyses were carried out in the wine growing regions Nahe, Wuerttemberg and Moselle. The first vineyard in the wine growing region Nahe was tested in 2015, 2016 and 2017. The virus infestation rose from 0.14 % (n=735) to 3.3 % (n=763, some vines were replanted). The second vineyard in the region Wuerttemberg was tested the first time in 2017. In this vineyard 2.59 % of the vines (n=695) were tested positive for viruses. In a third vineyard located in the region of Moselle, 5.69 % of the vines were tested positive. About half of the vines (n=350) in this plot were planted in 1994.Their infection rate was significantly higher ($Chi2= 19.72$, $df =2$, $p<0.05$) than the 1.33 % of infected younger vines (n=300) that were planted in 2009. Pattern of infection of all three vineyards will be tested with the program Patchy to investigate whether distribution of viruses is clustered or random.

The preliminary results hint at an important role of scale insects as virus vectors in German viticulture. The three vineyards will be studied further over the next years to monitor the spread of scale insect transmissible viruses. It could be interesting to compare disease progress rates related to different scale insect species. Analysing a wider variety of vineyards from different winegrowing regions should also help finding other parameters that influence the rate of virus spread.

Regarding the studies on side effects of plant protection products on scale insects and their natural enemies, a reliable test protocol needs to be established including highly effective products as positive control. For these analyses we will check common registered fungicides, herbicides or insecticides.

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State and development of tools for the Flavescence dorée management in Switzerland

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Highlights
- The Flavescence dorée complex is high in uncertainties and to solve this complexity we applied an Adaptive Management (AM) framework
- The AM framework has permitted to develop tools for the management of *Scaphoideus titanus* and the basis for a landscape analysis

Introduction

In Switzerland, *Scaphoideus titanus* was recorded first in Canton of Ticino (Swiss canton in the South of the Alps) in the 1967 and only in 1996 in the Canton Geneva, from where it began to colonise the vineyards along the Leman Lake, reaching today the vineyards of the Valais Canton. Flavescence dorée (FD) was first recorded in 2004 in Ticino. In 2015, the disease was found for the first time in the Canton of Vaud and in 2016 in the Canton of Wallis. The vineyards of the North site of the Leman Lake and in the West part of Switzerland are still free of *S. titanus* and FD. FD complex is high in uncertainties and to solve this complexity we applied an Adaptive Management (AM) framework, which is appropriate for complex problems high in uncertainties. This approach allows to increase the knowledge for *S. titanus* management and to learn how a system works and it permitted to develop the necessary tools for *S. titanus* management and for the landscape analysis.

Material and methods

The tools consisted in: i) sampling plans for estimating nymph densities of *S. titanus* in the canopy and improving techniques and methodology in population system study and management. ii) a phenology model, based on time-varying distributed delays, integrated in a Decision Support System (DSS), called PreDiVine. The model is the entry point into the AM system for improving its explanatory and predictive capabilities. It operates at the interface between research and pest management, establishes a close-loop between monitoring, management and analysis of systems and automatically improves the reliability of pest control relevant predictions as soon as additional information becomes available, iii) an age-structured multigenerational model of *S. titanus* populations to increase the knowledge into the dynamic of its populations. This model is useful to organise monitoring activities, to follow the stability of multi-annual occupancy of the vineyard, to control the colonisation potential and to develop control strategies, iv) a landscape analysis tool to quantify alternative host plant and vectors as basis for the development of a risk analysis system.

Results and discussion

PreDiVine has proved to be a robust DSS able to provide accurate predictions already starting from a zero-knowledge condition of the site and, at the same time, being flexible by re-using the data collected every year to self-improve the system calibration for future real-time predictions (see abstract and poster of Prevostini et al.). The development of the multigenerational model of *S. titanus* has showed how many aspects of its biology are still poorly known. The first step was to quantify the longevity, fecundity, egg oviposition duration and rate of females. The results suggest that *S. titanus* prefers relatively mild climates and they could contribute to explain its distribution area in Europe (see abstract and poster of Rigamonti et al.). The landscape analysis associated with a molecular epidemiology approach has shown that the FD complex could be considered as an open system. In such a situation, we have the necessity to develop a risk analysis system and to move from a concept of eradication of *S. titanus* to one of sustainability (see abstract of Jermini et al.). In conclusion, the AM framework was adequate to deal with high uncertainties associated with the complexities of the FD system. It permitted a continuous improvement of techniques and methodologies, improved the insight into the dynamics of infestation patterns, rationalised control operations and prepared the ground for further activities aiming at the study and management of populations and vector(s)-host plants-FD disease systems. In particular, the results obtained show that AM actively generated information and appears as management with a plan for learning.

Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture"

Novel tools and new challenges for IPM in viticulture

Oral Session 2

New knowledge and solutions against viruses, phytoplasmas and their vectors: pathogens and interactions with plant and vectors

When a Palearctic bacterium meets a Nearctic insect vector: genetic and ecological insights into the emergence of the grapevine Flavescence dorée epidemics in Europe

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Highlights

- Alders constitute an original reservoir of FD in Europe by hosting FDp isolates propagated by autochthonous or introduced Deltocephalinae leafhoppers
- Such pre-existing isolates are compatible with *S. titanus* and can be responsible for the emergence of FD epidemics
- Variable membrane proteins of FDp, which act as adhesins on insect cells, might play a key role in the adaptation to new vectors

Introduction

Flavescence dorée (FD) epidemics had been associated to the introduction of the leafhopper vector *Scaphoideus titanus*, when Europe imported American *Vitis* rootstocks. However, the geographical and ecological origin of the etiological agent, the FD phytoplasma (FDp), remained unclear despite evidences for a plant host-range not restricted to grapevine. FD-related phytoplasmas of the 16SrV group were described in *Clematis* sp. and *Alnus* sp. in the surroundings of vineyards and it was demonstrated that autochthonous Auchenorryncha feeding on these plants were able to occasionally transmit the phytoplasma to grapevine (Maixner et al., 2000; Filippin et al., 2009). More recently, it was evidenced that the introduced leafhopper *Orientus ishidae* was able to transmit FDp to grapevine (Lessio et al., 2016), but the source plants for acquisition remained to be elucidated. In this study, we bring genetic and ecological insights into the emergence of the grapevine FD epidemics in Europe.

Material and methods

Plant and insect samples were collected in Hungary, France, Germany, Italy and Serbia in the surroundings of FD infected vineyards, known FD-free vineyards and in non-viticultural areas. Plants were *Clematis vitalba* and *Vitis vinifera* exhibiting yellows and *Alnus glutinosa* without typical

symptoms. Leafhoppers were *S. titanus* collected on infected vine stocks and various Cicadellidae collected on alder trees.

For genetic characterisation, the map locus was amplified from DNA of infected samples and sequenced as described in Arnaud et al. (2007). If sequencing revealed a mix of PCR products, they were cloned and 4 clones were sequenced. Primers used for the nPCR amplification and sequencing of vmpA and vmpB genes (Renaudin et al., 2015; Arricau-Bouvery et al., in prep) were defined from the sequences of 16SrV group reference strains. Phylogenetic reconstructions using maximum parsimony were performed by MEGA5. Selection was evaluated by codon-based Fisher's exact test of selection.

For the transmission experiments, alder leafhoppers were sorted by species, placed by groups of 10 to 40 on *Vicia faba* or *A. glutinosa* shoots until death and stored for further testing. Plants were incubated at 25°C constant and regularly tested for symptoms and for the presence of 16SrV phytoplasmas. Infected *V. faba* obtained after transmission were then incubated with *S. titanus* and *Euscelidius variegatus* larvae for phytoplasma acquisition followed by subsequent transmission to new broad beans.

Results and discussion

The map gene sequence was obtained for 600 samples with 130 genotypes identified. Grapevines and *S. titanus* from FD outbreaks hosted 10 genotypes which belong to the genetic clusters map-FD1, FD2 and FD3 (Arnaud et al., 2007). Only 3 genotypes from map-FD1 and FD3 infected clematis. In contrast, a high diversity of 110 genotypes existed in alders, infected at 86 %; half of them with a mixture of genotypes. Interestingly, 23 % of the isolates grouped in map-FD clusters, some identical with grapevine FDp isolates. They could be found in FD-free areas which confirmed that alders constitute an original reservoir of FDp. The rest of the isolates, named Alder Yellows phytoplasma (AldYp), were phylogenically dispersed, some being identical to Palatinate Grapevine Yellows.

Four species of Cicadellidae collected on alders were positive. The Macropsinae *O. alni* was infected at 21 %. Map-FD isolates represented 9 % of the positive, the rest being AldYp. 13 transmissions of AldYp succeeded with *O. alni* but could not be subsequently acquired and transmitted by the Deltocephalinae *S. titanus* and *E. variegatus*. In contrast, the Deltocephalinae *O. ishidae* and *Allygus mixtus*/*modestus* were infected at 52 % and 60 % with map-FD genotypes representing 70 % and 98 % of the positive respectively. *O. ishidae* and *Allygus* spp. transmitted map-FD1/FD2 and map-FD2 genotypes respectively which were subsequently transmitted by *S. titanus* and *E. variegatus*.

VmpA and vmpB genes encoding surface proteins with organisation reminiscent of bacterial proteins required for eukaryotic cell invasion were sequenced. Vmps have a variable number of 234 nt repeats with high sequence diversity between isolates. The topology of phylogenetic trees was similar between vmpA and B but strongly differed from the map gene by clearly discriminating 3 genetic clusters: vmp-I groups all the AldYp and PGY isolates transmitted by the Macropsinae while vmp-II and III group the map-FD isolates from grapevine, alder and transmitted by the Deltocephalinae. Interestingly, in cluster vmpB-I, repeated domains evolved independently, whereas they evolved by duplications in vmpB-II and III. Positive selection was detected on vmpB of cluster II and III when compared to corresponding sequences of cluster I.

In conclusion, we showed that FDp is endemic in European alders. Its emergence as an epidemic pathogen for grapevine is restricted to some genetic variants pre-existing in alder. The compatibility of the phytoplasma to *S. titanus* certainly resulted from the adaptation of Vmps to other Deltocephalinae living on perennial wild plants. We demonstrated that VmpA acts as an adhesin with cells of *E. variegatus* (Arricau-Bouvery et al., in prep). Its organisation similar to adhesion related proteins allows the fast duplication of pre-adapted repeated domains. This suggests a key role of

Vmps in the life-style of woody hosts phytoplasmas that rely on the adaptation to new insect vectors to expand their plant-host range.

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The potential use of endosymbiont/endophytic bacteria to reduce yellows disease symptoms in wine grapes

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Highlights

- *Dyella*-like bacterium (DLB) a cultivable secondary endosymbiont isolated from phytoplasma insect vector was reintroduced successfully into grapevines
- DLB inhibited culture growth of spiroplasma *in vitro*
- DLB reduced yellows symptom under laboratory and field conditions

Introduction

In Israel, yellows disease in grapevines is caused by the obligatory parasitic bacterium *Candidatus* Phytoplasma (stolbur type) resulting in heavy yield loss. The pathogen which resides only in phloem cells is transmitted to grapevines by the planthopper *Hyalesthes obsoletus* (HO). Because conventional control methods against this pathogen are inefficient, and disease management is highly challenging, the use of beneficial bacteria has been suggested as a biocontrol solution (Compant et al., 2013). However, in order to proceed towards practical application, the potential microorganism should be cultivable and able to penetrate and survive within the plant for a reasonable time. A facultative symbiotic bacterium isolated from HO (Xanthomonadacea) was named *Dyella*-like bacterium (DLB; Iasur-Kruh et al., 2016). To test the potential of DLB as a bio control agent, we first confirmed its endophytic life style in grapevines, and then tested its influence on yellows symptoms.

Material and methods

To determine the best way to introduce DLB into grapevines, various plant materials were used: ex-vitro plantlets, one-node cuttings of vernalised grapevine canes, and 3y potted grapevines. Different application methods were investigated including root dip, dip of basal end of stem cutting, injection to the stem and to the berry cluster, leaf-dip, smear of a pricked leaf and leaf spray. The effect of additional surfactant to the solution was also tested. Survival rate was monitored following spray with DLB solution on either ex vitro potted plantlets or field grown vines. The effect of DLB was studied indirectly on the culture growth of *Spiroplasma melliferum*, which served as a model organism (modified from Naor et al., 2015). The effect of DLB on yellows symptoms was studied directly by a. root dip of ex vitro infected plantlets; b. spraying of 20 y old field grown vines every two weeks along the growing season. The presence of the DLB in the plant was confirmed 7-20 days post inoculation (dpi) by PCR analysis using specific primers (Iasur-Kruh et al., 2016).

Results and discussion

DLB penetrated all types of tested plant materials. Penetration rate varied among plant materials and application methods, ranging 25-71 %. Leaf spray was found as the best method of application. Adding a surfactant improved the rate of success by 60 %. DLB migrated up and down the plant ca. 8 cm from point of application and survived in planta up to 20 dpi. DLB inhibited the growth of *S. melliferum* by 9-10 folds compared with the control, and by 2.5 folds compared to oxytetracyclyne (Iasur-Kruh et al., 2016). Under laboratory conditions, inhibition activity of DLB was expressed by improving growth parameters of root dipped plantlets. Following leaf spray under field conditions the improvement was expressed by a reduction in symptom severity and by an increase of recovery rate of infected vines.

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Contrasting susceptibilities to Flavescence Dorée in *Vitis vinifera* rootstocks and wild *Vitis* Species

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Highlights

- An innovative protocol of controlled Flavescence dorée phytoplasma (FDp) transmission by *Schaphoideus titanus* in confined greenhouse allowed the characterisation of the response to FD in *Vitis*
- Contrasting susceptibilities to FD in *Vitis* suggest distinct genetic traits
- Symptom severity and phytoplasma titers were associated in *V. vinifera* cultivars tested.
- Rootstocks and their *Vitis* parents were symptomless with high FDp titers

Introduction

Flavescence dorée (FD) is a quarantine disease of grapevine in Europe, involving interactions between the plant, the leafhopper vector *Scaphoideus titanus* and the Flavescence dorée phytoplasma (FDp). Infected plants cannot be cured; the only way to control FD is to eliminate diseased stocks, to plant healthy material and perform insecticide treatments. Characterising the susceptibility of vine varieties could help in limiting disease propagation.

Here, we characterised the susceptibility of two cultivars, Cabernet Sauvignon (CS) and Merlot (M) growing in vineyards. Then, we developed a protocol to evaluate the susceptibility in controlled conditions by transmission of FDp with the natural vector. Twenty-height cultivars, rootstocks and wild *Vitis* were tested by measuring symptom severity, percentage of infected plants and FDp titers.

Material and methods

Three vineyard plots from Bordeaux area where CS and M were grown side by side and with a significant FD disease outbreak were selected. Mapping of symptomatic plants in the plot was performed and symptom severity was recorded by measuring the percentage of symptomatic branches on each stock. Ten petioles were sampled per plant, homogenised and frozen for further nucleic acid extraction and FDp quantification.

Vitis to be inoculated in controlled conditions were introduced *in vitro* according to Perrin et al. (2004). When necessary, grafting was done *in vitro* with CS or M as rootstock and Chardonnay (Ch) as scion. Plantlets were acclimated in soil in a high confinement greenhouse and grown at 25±2 °C.

S. titanus hatchings were performed as described in Bressan et al. (2005). L3-L5 larvae were transferred onto FD-PEY05 infected broad beans for acquisition of FDp. One week later, insects were transferred onto CS cuttings for a 3-4 weeks latency period.

For the transmission of FDp, 7 infectious *S. titanus* were encaged on each entire plant during 7 days or only on the 4th leaf from the apex for the FDp diffusion experiment. Whole plants were

sampled 5, 10 or 15 weeks post transmission (wpt) and stored at -20 °C. For the diffusion experiment, samples were separated in sections from the base to the apex of the plant. FDp cells were detected and quantified in plants and insects by SYBR Green absolute quantitative real-time PCR of the tuf gene (Eveillard et al., 2016).

Results and discussion

In vineyards, M showed less symptomatic stocks, less symptomatic branches on stock and lower FDp titers, when compared to CS. In greenhouse, FDp titers measured in M remained statistically lower than in CS, thus resembling data obtained in the vineyards.

In the diffusion experiment, all CS sections were infected with a high mean FDp titre. In contrast in M, the section including the leaf that received the infectious *S. titanus* was infected, with low mean FDp titers, whereas the whole plant remained FDp free. These data suggest that FDp circulates within the CS plant, whereas it is limited in M at the site of infection.

To elucidate whether the differential response of CS and M results from plant-insect or FDp-plant interactions, we grafted a scion of Ch, highly susceptible, onto CS and M rootstocks. Insects were encaged exclusively on the Ch scion. At 15wpt, the FDp titers were not statistically different in Ch shoots grafted onto CS or M. All CS rootstocks were infected with a high FDP titer. In contrast, M rootstocks remained FDp free or occasionally with a very low titer. FDp diffused into the plants, through the graft, into CS. Data obtained with M suggest that its resistance to FD can be explained by a plant-FDp specific response.

A collection of 28 *Vitis* accessions was then phenotyped in greenhouse, using CS as a susceptible reference. Results showed a wide range of susceptibility among *V. vinifera* cultivars, rootstock hybrids, and wild *Vitis* spp. which were classified in 3 categories: (1) high FDp titers and high proportion of infected plants, (2) intermediate FDp titers and high proportion of infected plants and (3) intermediate to low FDp titers and low proportion of infected plants.

Vitis vinifera cultivars were distributed in the three categories and their range of susceptibility corresponded to field observations (Boudon-Padieu et al., 1996; Chuche, 2010). Interestingly, reduced symptoms, low FDp titers, and low percentages of infected plants were found to be associated in M and its parent the Magdeleine Noire des Charentes (Magd) whereas CS and its parent Sauvignon showed severe symptoms, high FDp titers and high percentage of infected plants. Rootstocks and their *Vitis* parents, although having high percentages of infected plants and intermediate to high FDp titers, shared a symptomless response. This observation is warning as it illustrates how rootstocks can constitute a silent reservoir of contamination in mother plants fields or when they grow wild, nearby vineyards. Altogether, data suggest distribution of different genetic traits within the *Vitis* genus involved either in insect-mediated phytoplasma transmission, multiplication, circulation, or symptom development.

To consolidate, a cross was performed between the parents of M: Magd (poorly susceptible) and Cabernet Franc (susceptible). Preliminary data after progeny phenotyping suggest segregation in the genetic traits concerning FDp resistance transmitted by Magd.

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New insights into Pinot gris disease and the associated Grapevine Pinot gris Virus

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Highlights

- The main studies carried out by the authors in the last 4 years on the Pinot gris disease and the associated Grapevine Pinot gris Virus (GPGV) are summarised
- The researches included epidemiology and aetiology of the disease, molecular diagnostic and characterisation, and field control strategies aimed to improve the quantitative and qualitative productive parameters

Introduction

Pinot gris disease is a grapevine pathology identified in Northern Italy in 2003 (Giampetruzzi et al., 2012). Symptoms include chlorotic mottling, mosaic and deformation of leaves, shortened internodes, and stunting. Preliminary data on production suggested reduced yields in symptomatic plants, up to 80% (Malossini et al., 2012; Bertazzon et al., 2015).

A new virus, named GPGV (Grapevine Pinot gris Virus), has been identified in 2012 (Giampetruzzi et al., 2012) and then found out in many countries worldwide. However, etiology was not clear, as it occurs also in many asymptomatic plants (Giampetruzzi et al., 2012). Thus involvement of genetically-distinct GPGV isolates in the manifestation of the disease has been suggested (Saldarelli et al., 2015).

The aims of the present work were to study: i) the origin of the new virus, ii) the association between symptoms and virus presence, iii) its spreading in the field, iv) possible field strategies to control the damages.

Material and methods

Vineyard surveys and trials. In 2013-2016 a total of about 300 vineyards were visually surveyed in the Veneto region (Northeast Italy) for the presence of the symptomatology, and each single symptomatic plant was marked.

Eight of these vineyards, showing high symptom occurrence, were chosen for field trials, by applying for two or three years different leaf nutrients and biostimulants. The most important productive parameters were measured at the harvest in symptomatic and asymptomatic plants, treated and untreated.

Four other vineyards with high occurrence of both the virus and the disease were planted in 2014 with 100 healthy vine plantlets, to observe virus and disease spreading to new plants.

Molecular analyses. Molecular analyses for the presence of GPGV were carried out on leaves collected from adults and young, symptomatic and asymptomatic plants. GPGV titre was assessed in each sample by real time quantitative PCR. Sequencing of virus amplicons was carried out on 38 selected vine plants (Bertazzon et al., 2016a).

Moreover, a total of about 200 grapevine samples collected since 2002 from Italy and other European countries, maintained in the CREA-VE collection at -80°C as total RNA extracts, were tested for the presence of GPGV.

Statistical analyses. Statistical tests were performed with the IBM Statistical Package for Social Science (SPSS) program and the CoStat software, using the analysis of variance (ANOVA), the Student-Newman-Keuls and the chi-square tests ($P \leq 0.05$).

Results and discussion

Origin of the new virus. Molecular analyses showed that GPGV was present only in one out of 75 samples collected in 2002-05 from Veneto region, while about 78 % of the newly collected grapevines (2013-14) tested positive. These results revealed a recent appearance of GPGV in Veneto, followed by its rapid and wide spreading. Data obtained from 218 grapevine accessions, collected from all around Europe before 2005, showed the presence of GPGV mainly in Eastern European countries, while samples collected after 2010 showed the presence of the virus in plants coming from all countries considered. These data suggest that GPGV has not been present until 2005 in many grape-growing areas of Europe, and has spread all over Europe only recently (Bertazzon et al., 2016a).

Association between symptoms and virus presence. Results showed that symptomatic vines harboured significantly higher GPGV titre than asymptomatic ones. The phylogenetic tree clearly clustered all the viral isolates into three clades: one clade had mainly isolates from symptomless grapevines, while the other two included principally isolates from symptomatic ones. The overall data confirmed the previously-reported association of the symptoms with different viral variants, but the discrepancies were explained by means of the virus titre. Indeed, in general the viral variants associated with the symptoms displayed higher virus titres in comparison with the latent viral variant (Bertazzon et al., 2016b).

Virus spreading in the field. After one season, already 6 % of the plants were infected, but only one showed symptoms. The occurrence of the virus in the infected plants increased in all vineyards, varying between 20 and 80%, depending on the site. After the third year, 10% of the new plants were symptomatic. Thus, the spreading of the virus seems very fast in field, and it is followed by the symptom appearance only afterwards.

Field strategies to control the damages. The aim of those treatments was to improve the quantitative and qualitative productive parameters in the symptomatic plants. Among all the products tested, up to now the most promising results have been obtained by a mix of 5 products (Chelal Noor, Fructol NF, Chelal B, Chelal AZ, Chelal Alga L) containing NPK, microelements (Mg, S, B, Fe, Mn, Mo, Zn) and sea-weeds, applied 3 or 4 times from the flowering to the berry development. Tendentially significant differences among symptomatic treated and untreated grapevines were observed already in the first year of treatment (2014), when the sugar content was 15.5±1.4 versus 13.6±1.0° Brix, respectively. In 2015, the average grape production was significantly higher in the treated plots (7.4±1.9 kg/plant versus 4.3±1.4), due to an increase both in the bunch weight and in the number of bunches. The results suggest thus an adaptability of the treatment, whose effect acted on the production problems of the year: the sugar in 2014 and the grape quantity in 2015.

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Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture”

Novel tools and new challenges for IPM in viticulture

Oral Session 3

Classical and novel tools against arthropod pests: disruption of insect communications

Mating disruption of vine mealybug, *Planococcus ficus*, using sprayable microensapsulated pheromone in California table grapes

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Highlights

- Field evaluations of foliar applications of microencapsulated vine mealybug pheromone provided significant reduction in the number of male moths captured in pheromone traps in vineyards
- Data collected in 2016 suggests that monthly sprays of pheromone can reduce the number of vine mealybugs on grapevines. Validation of these results is in progress during the summer of 2017

Introduction

Vine mealybug, *Planococcus ficus* (Signoret), is one of the most significant insect pests of California table grapes. Mealybugs reduce crop quality by excreting honeydew, which promotes sooty moulds, and by infesting berry clusters. Producers of fresh-marked table grapes are required to have zero-tolerance policies against mealybug infestations in clusters in order to satisfy market demands, especially for exported fruit.

Over the past decade, researchers have been trying to identify sustainable programs for mealybug management. In 2016, microencapsulated pheromone was registered in California by Suterra under the trade name Checkmate VMB-F. During 2016 and 2017 we conducted trials to evaluate the effects of mating disruption programs on male capture rates in pheromone traps, mealybug populations on grapevines, and the resulting impact on crop quality.

Material and methods

Trials including Checkmate VMB-F were conducted in four table grape vineyards in Kern County, California during 2016 and 2017. Each trial compared mealybug densities in four hectares using a standard chemical control program using Lorsban, Applaud, Admire and Movento to another four hectares, using the same standard chemical control program plus four to five monthly applications of pheromone. During 2017 additional plots were added to the trial to evaluate the effects of sprayable pheromone at 45- compared to 30-day intervals. All applications were made between May and September of each year by cooperating growers at a rate of 12.5 g. a.i./hectare using 473 l/ha of water. During both years of the study we evaluated weekly captures of vine mealybug males, conducted timed searches for mealybugs on the vines, and evaluated impacts on crop quality. During 2016 we also conducted a trial to evaluate the longevity of the impacts of a single application of pheromone.

Results and discussion

Pheromone trap data show that monthly applications of pheromone greatly inhibited the ability of male vine mealybugs to find pheromone traps. At the two northern sites the total male captures in mating disruption plots were 172 per trap from August through mid-November compared to 1966 per trap without mating disruption. This is a reduction in captures of 91.2 %. Similar results were seen at the two southern sites, where captures over the same period of time were reduced by 90.8 %. These reductions are increased to 93.1 % at the northern sites and 92.2 % at the southern sites if traps on the edges of the vineyards are excluded.

Efforts to evaluate the impact of reduced male captures are still underway. Evaluations of mealybug density under the bark in June and of mealybugs in clusters at the start of harvest (late Jun to Aug) in 2016 did not reveal any differences among plots. However, this is not a surprise due to the limited amount of time available for mating disruption to have worked, coupled with the effects of insecticide programs used in the table grape vineyards. Most of the mating disruption in this trial occurred after harvest and impacts of that disruption are currently under evaluation in 2017.

Evaluations of a single application of pheromone resulted in reductions in male captures for periods of time greater than 100 days. However, we know that one application of microencapsulated pheromone does not disrupt mating for 100-day periods. This means that reductions in trapped males for one or two months after application were due to the inability of males to find traps, whereas reductions in males thereafter were because mating disruption reduced the overall mealybug population.

The flowable formulation of mating disruption has several advantages in an integrated pest management program for vine mealybug. Applications can be made with existing equipment used by all grape growers. The active ingredient is exempt from tolerances such that there are no pre-harvest intervals, residues, or international maximum residue limits (MRLS) for exported fruit. Applications can be made as part of a tank mix with other insecticides or fungicides that do not contain products that are oil-based, emulsifiable concentrates (EC) or include organosilicon surfactants. Mating disruption has no known impacts on natural enemies of vine mealybug or other grape pests. The new microencapsulated formulation is also much less expensive than dispenser-based systems used in the past. This is because it is less expensive and allows flexibility on the part of growers as to how many applications they want to make. The key disadvantage of the flowable formulation is that it is not OMRI-approved for organic production due to the microencapsulation process. Organic growers using mating disruption need to maintain the use of Checkmate VMB-XL at a rate of 250 dispensers per acre.

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Year-round mating disruption in vineyards overcomes the vine mealybug (*Planococcus ficus*) population's build-up during the warming winters

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Highlights

- The worming winters, with temperature exceeding the developmental threshold for long duration, call for a change in mealybug pest management to year-round control treatments
- Repeated, year-round applications of mating disruption reduce the pest populations and, with time, may allow for years without treatments

Introduction

Longevity, developmental rate of insects and therefor number of generations per year, are temperature dependent. Accordingly, climate change calls for altering the general approach of pest control.

The vine mealybug, *Planococcus ficus*, is a major pest of vineyards. In southern Israel, calculation using Degrees Day (Varikou et al., 2010) indicates an additional generation, on top of the 9 generations a year, when temperature during winter days exceeds the developmental threshold. Indeed, we observed populations' build-up as early as February. The efficacy of mating disruption (MD) was shown previously, especially on small population. A gradual yearly reduction of infested vines was also observed (Sharon et al., 2016). Commonly, MD is applied once a year, shortly after bud-burst (April-May). Nevertheless, the continuous development of this pest during winter, calls for rethinking.

Here, we suggest year-round MD applications to overcome the pest population's build-up during winter.

Material and methods

The study was conducted in 2015-2017, in three commercial vineyards. Each vineyard was divided into three treatment plots (0.5 ha each): one served as control, in the second MD pheromone was applied in the spring (May) during vine flowering (seasonal MD) and in the third MD pheromone was applied twice a year, before bud-burst (February) and after harvest (August) (Yearly MD).

Pheromone dispensers (CheckMate VBM-XL; Suterra, Bend, OR, USA) loaded with 150 mg of the racemic sex pheromone (+)/(–)-lavandulyl senecioate, were placed in MD-treated plots at a density of 620 dispensers per ha. Dispensers were hung at canopy height, evenly distributed throughout the plots.

Mealybug population level was monitored in all plots in February, just before the early application of "yearly MD" and again in harvest (end of Jul/beginning of August), after which, the second application of the "yearly MD" was applied. To monitor the population, we randomly selected

20 vines per plot, excluding vines on the plot margins. A 5-min search for mealybugs was conducted on each vine (Daane et al., 2006). Male captures in pheromone traps were monitored year-round using three Delta sticky traps, placed in each treatment plot. Traps were baited with 100 mg of the racemic synthetic pheromone (Adama Makhteshim, Beer Sheva, Israel) in rubber septa lures. Sticky plates in traps were replaced every 2 weeks and trapped males were counted under a stereoscope. Pheromone lures were replaced every 6 weeks.

Results and discussion

The challenge presented by climate changes can be demonstrated in the southern areas of Israel where we already face the development of various pests throughout the winter. In this area, the vine mealybugs have nine generations in moderate years and an additional generation when yearly average temperature is higher. The farmers apply mating disruption against the mealybugs during the growing season, but when the pest populations build up earlier and last longer, one application of MD dispensers may not suffice.

In the present study, we demonstrate a higher efficacy in controlling the pest with a second application of MD dispensers. In addition, in the following year, the pest population was smaller at time of first MD application.

At time of harvest, in the study's 1st year, mealybug's population level of the "seasonal MD" plots (MD applied in May) did not differ from the control. The effect of MD on this population was seen in the 2nd year of MD application. This is in accordance with previous studies that showed the need for two consecutive years of MD application when mealybug population is high (Sharon et al., 2016). However, in the current study, applying MD earlier in the season (February, first year of "yearly MD") resulted with a decrease in the pest population level at harvest time, which was lower than that in the control. Furthermore, in the 2nd year of MD application the initial population level in February was lower in the "yearly MD" plots than the control. A further reduction was seen at harvest. At the beginning of the third year we did not find any mealybugs in plots of both MD treatments, whereas, at the same time, the pest populations in the control plots have increased. A similar pattern was observed in the number of inoculated vines; "seasonal MD" had an effect on the number of inoculated vines only in the 2nd year whereas early application of MD dispensers ("yearly MD) had an effect already in the 1st year, with continuous reduction in the initial population every year.

Therefore, when winter's temperatures exceed that of the pest's developmental threshold for many days, the pest population continues to develop. We thus suggest, year-round applications of pest management. We expect that early application of MD combined with repeated applications during the year and continuous yearly applications of mating disruption will reduce the initial pest population and, in time, will allow for years without treatment.

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Future sustainable IPM in viticulture: electrospun mesofibres as alternative approaches for *Lobesia botrana* pest management

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Highlights
- The reduction of pesticide use for increasing human health and for maintaining natural biodiversity is a challenging task for future IPM research
- Plant protection by releasing sensitive, volatile natural products may be an alternative approach towards this goal
- The new electrospinning technology of 2007 was the first of several steps to establish a new paradigm in solving viticultural problems

Introduction

Mating disruption for insect management has been invented some 60 years ago. In a remarkable international effort, various laboratories in France, Germany, UK, US, later also Canada, Japan, Israel, China and Australia contributed to assemble the puzzle of successfully managing pest insect population. Today's perfection is based on many trials and errors in a number of crops. *Lobesia botrana*, *Bombyx mori* and *Pectinophora gossypiella* marked milestones in the development of techniques towards environmentally compatible, nontoxic, "green" procedures of mating disruption (see Hummel et al., 2015/16 for detailed references). These advances would have been unthinkable without developing new experimental ground in areas like analytical chemistry, sensory-/electrophysiology, quantitative behaviour analysis, bioassay guided purification of extracts, organic synthesis, electrospinning of mesofibres as pheromone dispensers, and mechanical deployment of biodegradable fibres effective in cost reduction.

Material and methods

Basis of our field experiments were early reports by Götz in 1939 and 1951. Refinements to the protocol were made by Doye in his PhD-thesis of 2006 with the specific needs of the vineyards managers in mind who were interested to judge the daily results of the disruption efforts from a centrally located screened walk-in bioassay station rather than from dozens of individual test sites distributed at random within the vineyard.

The liquid sex pheromone of *L. botrana*, (E,Z)-7,9-dodecadienylacetate was purchased from Trifolio-M, Lahnau, Germany, and was of higher than 95% purity. For electrospinning of mesofibres,

Ecoflex® (a high volume aromatic-aliphatic copolyester product of BASF, same material as compostable plastic bags) was used.

Electrospinning was performed according to the procedures reviewed by Greiner and Wendorff (2007). Further details can be found in Hein et al. (2009), Hummel et al. (2015) and a subsequent series of reports cited under Hummel et al. (2015/2016) in the bibliography.

The commercial vine cutter cultivator (ERO Co., Niederkumbd, Germany) was modified by attaching a subsystem supplied by German Ropes (Hamburg). This addition can intertwine electrospun mesofibres with the yarn ropes holding the vines in place.

Thus pheromone is dispensed throughout the entire width of the vineyard. During the winter, the mesofibres will completely decompose.

Results and discussion

Given our experiences gained between 2008 to 2010, Hein et al. (2009), Hellmann et al. (2011), as well as Breuer et al. (2012) published various reports on the feasibility and overall success of the field experiments (see Hummel et al., 2015/2016, for detailed references). In a nutshell, we achieved 95 % + mating disruption based on evaluation by the Doye technique with a overall disruptive effect lasting for 7 weeks. "Spaghetti type" dispensers by Shin-Etsu served as positive controls, no treatment as negative control. Results from the mesofibre treatment and the commercial dispenser treatment were statistically indistinguishable. Mating was disrupted in both cases at the 95 % level. Mesofibres used in our experiments provide biodegradable, electrospun pheromone dispensers with some built-in preprogrammed "semi-intelligence". This feature is attractive for releasing sensitive insect pest pheromones in viti- and horticulture. These pheromones are biodegradable, nontoxic natural products, synthesisable from readily available naturally available building blocks without using petroleum precursors. Additionally, the mesofibres dispensers are biodegradable by natural degradation processes and will completely vanish within half a year. The mesofibres used facilitate the adjusted diffusion of pheromone vapours for both attraction and disruption purposes, depending on their concentration. Furthermore, they are easily integrated into schemes for air permeation by continuous rope application. Dispenser efficacy was compared, side by side and throughout the growing season, with conventional Shin Etsu "spaghetti" type pheromone dispensers. With *L. botrana* (Lepidoptera: Tortricidae), we achieved good disruption levels for a period of seven weeks. In future experiments, further refinement and adjustment of experimental parameters might prolong duration of efficacy. Each added week minimises the total number of field applications per year. Due to biodegradability of the fibres, expensive retrieval of spent dispensers is unnecessary. Furthermore, this novel technology is applicable in an environmentally compatible way to any row crop where insect pests have reached damaging status. Our disruption results were consistent with the claim to have access to a sustainable "green" IPM protocol.

So we conclude that:

- Biodegradable Ecoflex® mesofibres (diameters 600 – 1,400 nm), made from renewable non-petroleum based resources, can internally carry up to 33 % w/w of *Lobesia* sex pheromone. By controlled diffusion through the outer walls, its kinetics of volatilisation is precisely adjustable within narrow limits up to an equivalent of 100 g pheromone/ha/6 months.
- Spent mesofibres are deployable mechanically by the modified ERO Co. field cultivators, handling up to 3 independent jobs simultaneously.
- GPS control of the automated fibre deployment process as well as extension of the mesofibre technique to other row crops in horticulture (e.g. apple orchards) seems feasible.

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Exploitation of genetically modified *Vitis vinifera* plants with altered kairomone emission ratio for the control of the European Grapevine Moth *Lobesia botrana*

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Highlights

- *Lobesia botrana* uses olfactory cues to locate its host plants
- We created grapevine transgenic lines, with modified emission of *L. botrana* kairomones, (E)-β-caryophyllene and (E)-β-farnesene
- When transformed plants, extracts and synthetic blends were tested in wind tunnel they were less attractive than controls
- This opens avenues for pest control strategies based on host plant volatile modification

Introduction

In nature plant terpenoids play multiple ecological roles. Many phytophagous insects use them as kairomones to locate their host plants. This is also the case for *Lobesia botrana*, which is the main pest of European vineyards. It was found that a specific blend of the terpenoids (E)-β-caryophyllene, (E)-β-farnesene and the homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene emitted by grapevine was attractive to *L. botrana* females (Anfora et al., 2009), and the attractiveness was shown to be dependent on the kairomone ratio (Tasin et al., 2006). In this work, we generated stable grapevine transgenic lines with altered (E)-β-caryophyllene and (E)-β-farnesene emission compared to natural plants. In the first case we overexpressed a grapevine (E)-β-caryophyllene synthase gene, while in the second case we expressed a sweet wormwood gene that codes for an (E)-β-farnesene synthase. Thus, we modified the ratio between these two kairomones *in vivo*, and tested how it affected *L. botrana* behaviour.

Material and methods

Plant transformation

To modify (E)-β-caryophyllene emission, the terpene synthase gene (*VvGwECar2*) responsible for most of the production of this volatile in the green tissues was overexpressed. Instead, for modifying (E)-β-farnesene emission we could not overexpress any grapevine gene, since apparently there are no known (E)-β-farnesene synthases in the grapevine genome. The small amount of (E)-β-farnesene in the plant's headspace is indeed the by-product of the activity of other terpene synthases. Thus, we decided to insert a gene from *Artemisia annua* which had already been characterised as an (E)-β-farnesene synthase (*Aaβ-FS*). Transformations started from "Brachetto Grappolo Lungo" grapevine variety embryogenic calli, using *A. tumefaciens* carrying either *Aaβ-FS* or *VvGwECar2*

coding sequence inside the pK7WG2D binary vector, and resulting in the regeneration of several independent transgenic lines (Salvagnin et al., 2016).

Plant Headspace extraction and analysis

To check for changes in terpene emission, we extracted and characterised the plant headspace through closed-loop stripping analysis (CLSA) and subsequent gas-chromatography/mass spectrometry (GC-MS).

Wind tunnel

Transformed plants, extracts and synthetic blends were tested for attraction in wind tunnel assays using adult *L. botrana* mated females. Any flight between 50 cm and 170 cm from the odour source was defined as upwind flight, while arrival at any point within 10 cm of the source was defined as landing.

Results and discussion

In this work, we report for the first time on the transformation of a woody plant species of agricultural interest with a modified kairomonal biosynthetic pathway. In particular, we produced stable *V. vinifera* transgenic plants keeping all the kairomones used by *L. botrana* for host-location, but with several degrees of ratio modification between two key components, namely (E)-β-caryophyllene and (E)-β-farnesene, compared to the original plants. Indeed, the headspace extraction and analysis showed grapevine plants with an (E)-β-caryophyllene/(E)-β-farnesene emission ratio significantly divergent from the wild type, with extracts ranging from 0.22:1 to 38:1, while the control plants were in the range of 4:1.

The plants were acclimatised and grown in the greenhouse. Overall, we did not notice any difference in plant phenotypes or growth speed in the different lines or as compared to the controls.

When headspace collections from these plants were tested in wind tunnel behavioural assays, they were less attractive than controls. This result was confirmed by testing synthetic blends imitating the ratio found on natural and transformed plants, as well as by testing the plants themselves. With this evidence, we suggest that a strategy based on volatile ratio modification may also interfere with the host-finding behaviour of *L. botrana* in the field, creating avenues for new pest control methods. The costs of many volatile organic compounds, due to difficulties in their synthesis or isolation from natural sources, is often very high and, in addition, they usually have a short half-life in nature because of the tendency to form isomers and/or become oxidised. Therefore, a genetic engineering approach seems most suitable for studying the effect of kairomone manipulation on insect behaviour and for practical applications.

We conclude that modification of the kairomone ratio within the grape bouquet is sufficient to interfere with the host-finding behaviour of *L. botrana*, and that our findings could form the basis for development of new environmentally friendly approaches for pest and pathogen control.

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Vibrational mating disruption of glassy-winged sharpshooter *Homalodisca vitripennis*, vector of *Xylella fastidiosa* in California

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Highlights

- The mating behavior and associated communication of glass-winged sharpshooter (GWSS) has been described in detail
- GWSS significantly reduced mating when subjected to different playback signals in lab and field
- A new disruptive signal has been created with characteristics specifically designed to disrupt the GWSS

Introduction

The glass-winged sharpshooter (GWSS) is a grapevine pest in California where it represents a serious threat to viticulture due to its ability to transmit *Xylella fastidiosa* that causes Pierce's disease in grapevines (Davis et al., 1978). GWSS reproduces from spring to fall producing at least two generations per year. The objectives of this study were to identify and characterise GWSS vibrational signals and test whether the emission of putative disruptive playback signals could reduce the mating success of the species. Vibrational mating disruption is an innovative method that was successfully applied in laboratory and semi-field conditions to prevent mating in the grapevine leafhopper *Scaphoideus titanus* (Eriksson et al., 2012). Therefore, our aim was to assess whether the same technique could also be applied to disrupt the GWSS mating behaviour.

Material and methods

GWSS was reared on several host plants (*i.e.* okra and sunflower) starting from eggs collected in Bakersfield, CA during springs 2015 and 2016. Nymphs were separated by sex as late instar stages (fourth to fifth) to ensure a virgin status in the adult stage. To describe the mating behaviour (test A), single individuals (either males or females) and pairs were isolated on potted okra plants included in a Plexiglas box and recorded with laser vibrometer (PDV 500, Polytec) for up to 1 hr. Three types of trials were performed using (1) individual, (2) one male and one female, or (3) one female and two males. To test the possibility to disrupt the pair formation process (test B), three types of playback signals were used along with no playback (silence) as the control: white noise, natural female calls, and female noise. Playback signals were emitted through a minishaker (mod. 4810, Bruel and Kjaer). In addition, to disrupt the male calling behaviour (test C), we synthesised and used as playback two other signals with pure frequency band at 80 Hz or 240 Hz and a third signal with frequency bands at 80 and 240 Hz. 80 Hz was chosen because it was the value of the female song fundamental and dominant frequency; 240 Hz was randomly chosen to represent another harmonic. In this case, single

males were stimulated with playback of female calling signal, emitted by one minishaker while a second minishaker transmitted the disruptive frequencies.

Results and discussion

Behavioural analysis showed that GWSS mating communication involved the emission of three male and two female signals, with specific roles in two different phases of mating behaviour, identification and courtship. Females call first and then a vibrational duet between genders is established, which can be temporarily interrupted in the presence of male rivalry. Male rivalry behaviour involved the emission of three distinct rivalry signals. Two rivalry signals mimic female signals and were associated with replacement of the female in the duet by the rival male. The third rivalry signal was emitted by side competing males. Playback of white noise, pre-recorded female signals, or artificial female noise significantly reduced mating of GWSS when compared to silent control mating trials. In response to playback of female signals, females signalled more often than females tested in the absence of playback. After the first playback, almost two-thirds of females signalled a response within 3s. Additionally, one-third of the females signalled within 1s after cessation of white noise, and significantly more in the time periods following noise termination. These results suggest that intermittent noisy habitat conditions elicit GWSS to exploit temporal gaps in the absence of noise for mating communication. Finally, playback bioassays showed that transmission of an 80 Hz pure frequency tone (with and without the 240 Hz harmonic) to plants completely suppressed male signalling to female signal playback. This suggests that an 80 Hz vibrational signal should be tested in laboratory and field experiments to assess its efficacy in disrupting mating of GWSS. Overall, the results of this study are promising as to the possibility of using signal playback for studying the mating system, ecological constraints, and role of intraspecific vibrational signals in promoting dispersal and/or aggregations of wild GWSS populations. In addition, results showed that GWSS mating communication is vulnerable to playback of vibrational signals, suggesting that future mating disruption methods under field conditions may be implemented upon the use of a species-specific disruptive signals (i.e. 80 Hz based signals) that result in little to no non-target effects.

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Open-field vibrational mating disruption: the effect on leafhopper pests and the vineyard ecosystem

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Highlights

- For the first time, the vibrational mating disruption method has been applied to an experimental vineyard in Italy
- The experimental vineyard will be monitored to test the vibrational mating disruption method in an open-field trial through two following season
- The open-field trial will permit to assess the efficacy and potential side effects of the vibrational mating disruption method

Introduction

Recently, the vibrational mating disruption method is taking relevance as a sustainable alternative to pesticides to control populations of pests that rely on vibrations to mate, such as leafhoppers (Polajnar et al., 2015). Since 2012, when the method was first proved to reduce *Scaphoideus titanus* mating in semi-field conditions (Eriksson et al., 2012), other leafhopper pests have been studied to decode and disrupt their signals. Nevertheless, a practical pest management application is still missing.

To definitively test the applicability and efficacy of the method an open-field trial is required. The setup of a 'vibrational vineyard' will enable us to assess the efficacy of the method to reduce two co-occurring leafhopper species populations (*S. titanus* and *Empoasca vitis*), while evaluating the potential side-effects on beneficial arthropods, which are known to use vibrations to detect their preys and hosts (Virant-Doberlet et al., 2011).

Material and methods

A disruptive signal (DS) capable to disrupt the mating communication of both *E. vitis* and *S. titanus* has been synthetised thanks to laboratory trials.

The experimental 'vibrational vineyard' has been set up at the Fondazione Mach, Italy. The chosen vineyard (Cabernet Franc, spurred cordon trained, year of installation 2002-2004) has been divided in two plots of about 1 ha each (i.e. treated and control plot). In the treated plot, the DS has been transmitted to the plants by means of electro-magnetic shakers attached to the poles of each row. Shakers were positioned 25 m from the end of each row and 50 m from the following shaker. The DS was transmitted to the plants throughout summer 2017 (i.e., treatment period). The signal emission quality of each shaker has been tested by means of a laser vibrometer every two week during the treatment period.

To test the efficacy of the mating disruption method leafhoppers were manually sampled by surveying 25 plants (20 leaves per plant) per plot. The sampling was conducted weekly, before applying the DS (from May 9th to July) to assess the initial population density, and continued until the end of September. Females of *E. vitis* and *S. titanus* were isolated to check whether they laid

fertile eggs or not. The potential side effects on beneficial arthropods have been monitored (May 9th - end of September) by assessing the population trend of spiders and parasitoids.

Results and discussion

Until now, the vibrational mating disruption method has been tested only in semi-field conditions. Thus, an appropriate evaluation of the ecological impact was not feasible. We managed to set up a 'vibrational vineyard' (vibrated plants on a continuous surface of about 1 ha) to verify the efficacy of the method on leafhopper populations and its impact on other non-target species, either pests or not. Some beneficial arthropods, such as parasitoids and spiders that are known to contribute in controlling leafhopper populations, use vibrations to detect their preys (Virant-Doberlet et al., 2011), therefore they were also monitored. Vibrational noises, both natural and anthropogenic, can affect spiders prey detection. However, it has never been tested what happens applying to a large surface, such as a vineyard, a DS tuned on the frequencies used by leafhoppers to communicate. If the predatory activity of spiders and parasitoids will be reduced by the DS transmission, the control of leafhopper populations will be totally dependent on the efficacy of the vibrational mating disruption method. Otherwise, the vibrational method could work in synergy with other integrated pest management resources.

The set up will be maintained and activated throughout two following seasons, summer 2017 and 2018. Thus, the efficacy to reduce the population of leafhoppers of the vibrational mating disruption method will be tested during and after one full summer of treatment (i.e., summer 2017-2018 and spring 2018). A first assessment of the method efficacy will be done by measuring the *S. titanus* population density in the spring following the year of treatment.

In conclusion, the 'vibrational vineyard' will enable us to perform open-field studies that will give important answers about the applicability and the ecological impact of the vibrational mating disruption method in a commercial vineyard and its agro-ecosystem.

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First characterisation of herbivore-induced volatiles released by grapevine (cv. Pinot noir) under attack of *Empoasca vitis* (Hemiptera: Cicadellidae)

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Highlights

- When attacked by *Empoasca vitis*, Pinot noir grapevines released several herbivore-induced volatiles not present in the headspace of undamaged or mechanically damaged plants
- Therefore, these volatiles might play a key ecological role as attractants or repellents and could be exploited through novel push & pull, attract & reward, or attract & kill strategies

Introduction

Plants subjected to insect feeding release a subset of volatile organic compounds (VOCs), called herbivore-induced plant volatiles (HIPVs), which are powerful signals involved in both plant-insect and plant-plant communication (Dicke, 2009; Rodriguez-Saona et al., 2013). HIPVs can attract the natural enemies of the attacking herbivore and/or repel the pest insects. Moreover, neighbouring plants may "eavesdrop" on these volatiles to enhance their defences against subsequent attacks. The HIPV composition can be highly specific for each plant species and for the type of plant-herbivore interaction. Because of this potential variability, the HIPVs of each specific plant-herbivore interaction is expected to have its own distinctive traits, which should be experimentally investigated before planning any VOC-based method for controlling pest insects. In this work we characterised and quantified the HIPVs released by grapevines in response to the feeding damage of the leafhopper *E. vitis*.

Material and methods

Experiments were performed on one-year-old grapevines (*Vitis vinifera* L., cv. Pinot noir, grafted on SO4 rootstock). The VOCs were collected from the headspace of grapevines in three time periods of the infestation with *E. vitis*: after 1 h and again 24 and 48 h later in five plant replicates plus a negative control, using the closed-loop-stripping-analysis (CLSA) technique (Kunert et al., 2009). A shoot portion of each plant was enclosed within a plastic bag (Cuki® oven bag, Cofresco, Volpiano, Italy), and a distal shoot portion bearing 4 fully expanded leaves was selected. Air samples were collected using an adsorbent trap loaded with activated charcoal. Each trap was fitted to a vacuum pump that circulated air within the sampling bag. Samples were collected daily from 10 am to 1 pm; at the end of each VOC collection, leaves from the shoots inside each collection bag were excised and their area measured. The collected VOC samples were eluted from the adsorbent traps with 100 µl GC grade dichloromethane and were analysed by gas chromatography-mass spectrometry (GC-MS). The identity of compounds was confirmed by comparing the mass spectra and the retention times with those of authentic standard compounds. The amount of volatiles emitted during the collection period was normalised to the leaf area. For comparison, control experiments were carried out on undamaged and on mechanically damaged plants, using the protocol described above.

Results and discussion

In control experiments on undamaged grapevines ten VOCs were detected in the VOC collections of constitutive emission, including the green-leaf-volatiles (GLVs) (Z)-3-hexenol, (Z)-3-hexenyl acetate and (Z)-3-hexenyl butyrate, the aromatics benzaldehyde, phenylacetonitrile and 2-phenylethanol, and eight terpenes. Infestation with the leafhopper *E. vitis* generally led to a progressive increase in VOC emissions and to the release of VOCs detected neither in constitutive blends nor in mechanically damaged plants. This observation proves that there is emission of HIPV. Terpenoid and aromatic emission in particular changed dramatically. Twenty-three VOCs were detected in the VOC collections from grapevines infested with the leafhopper *E. vitis*, meaning that thirteen VOCs were uniquely induced by the herbivore feeding. These volatiles comprised, among others, the aromatics methyl salicylate and indole, and the terpenes (E,E)-2,6-dimethyl-1,3,5,7-octatetraene, (E)-β-farnesene and nerolidol. To the best of our knowledge, this is the first report of the terpenes (E,E)-2,6-dimethyl-1,3,5,7-octatetraene, neo-allo-ocimene and α-curcumene as HIPVs released by grapevine. This study allowed identifying for the first time potential natural tools to be used in IPM as eco-friendly alternatives to pesticides.

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Novel tools and new challenges for IPM in viticulture

Oral Session 4

Classical and novel tools against arthropod pests: *Drosophila suzukii*

Physical barriers against *Drosophila suzukii* in viticulture

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Highlights

- Kaolin clay applied at the very beginning of oviposition reduces the number of deposited eggs by 50 % and its usage does not affect wine quality
- Insect-proof nets as well as fine-meshed nets against wasps and birds reduce substantially the activity of the fly in the grape zone, decrease oviposition and lower rot disease at harvest
- Anti-hail nets provide, however, an insufficient protection

Introduction

Since 2014, *Drosophila suzukii* has been causing damage in Swiss vineyards. Control relies mainly on prophylactic measures, in particular a good ventilation and lighting of the grape zone. In the case that eggs are observed on grapes, an additional protection with kaolin clay is recommended. However, traditional insecticides should only be applied as a last resort. Exclusion nets could complement these measures, but their benefit is so far sparsely documented in viticulture. Costs, handling, impact on the natural scenery as well as doubts on their efficacy and effect on wine quality have limited the use of nets in this crop until now. This paper presents a synthesis on our recent insights gained on the use of kaolin and nets in Swiss vineyards.

Material and methods

In more than 40 field trials, kaolin clay was applied in the grape zone at a dosage varying from 12 to 32 kg/ha either shortly before the first eggs were laid or at the very beginning of oviposition. Treated blocks were compared to untreated variants by regularly monitoring of the oviposition on at least 50 berries per variant. In one of these trials, the impact on the quality and appreciation of proceeded wines were also assessed.

In 22 field trials, three types of nets were tested: anti-hail nets (medium mesh size 3 × 8 mm), anti-wasp/bird nets (3 × 10 mm) and anti-insect nets (1.2 × 1.2 mm). The nets were mounted in various ways: full cover of several rows, full cover of individual row or limited protection of the grape zone with or without fastening the upper and/or lower parts of the nets. The efficacy of nets was determined by a comparison with uncovered control rows in the same plot. The activity of *D. suzukii* in the grape zone was followed with traps, oviposition was determined by sampling 50 healthy berries once a week and the incidence of rot diseases was evaluated at harvest. In three of these trials, the micro-climatic conditions under the nets were recorded and the chemical and physical properties of the processed musts were measured.

Results and discussion

Overall, the average efficacy of kaolin applications reached 55 % (min. 0 – max. 100 %). Whereas no significant differences could be observed between 12 and 24 kg/ha with 55 % and 47 %

efficacy, respectively, a higher dosage of 32 kg/ha used in three trials increased efficacy to 83 %. Applications conducted shortly before egg laying showed a slightly higher efficacy than the ones applied after the first observation of infestation (61 % vs 49 %). A second application of Kaolin did usually not increase the efficacy; however, it might be justified after heavy rainfall. Bottled wines produced from the red variety Mara treated three times with kaolin at 24 kg/ha shortly before grape harvest could not be distinguished oenologically as well as gustatory from the untreated control. Following these trials, the commercial product Surround® is now registered against *D. suzukii* in Swiss vineyards at a dosage of 24 kg/ha from half-véraison (BBCH 83) to harvest.

All three net types reduced *D. suzukii* captures, the amount of reduction depended, however, on the mesh size. Whereas anti-hail nets reduced captures by 35 %, anti-wasp/bird nets and fine-meshed anti-insect decreased flight activity by 46 % and 83 %, respectively. As a consequence, oviposition under anti-hail nets was as high as in the unprotected controls. Although the mesh size of anti-wasp/bird nets is similar to anti-hail nets, a careful mounting and tightening decreases significantly their mesh size. They therefore provided a better protection and reduced oviposition in berries by 70 % compared to the unprotected controls. With an average efficacy of 93 %, anti-insect nets proved to be the best measure to reduce oviposition by *D. suzukii* on grapes. Moreover, the frequency and intensity of rot diseases under this net type was decreased by 60 % and 78 %, respectively, compared to the unprotected rows. As long as the nets were properly closed and protected well the grape zone, no differences could be noticed between the different mounting systems.

The temperature under white anti-insect nets was on average 0.38°C higher and relative humidity 1.3 % lower than in the unprotected control. Under black nets temperature was, however, lower and humidity higher than in the control. Nevertheless, the analysis of their musts did not indicate any significant differences between the treatments. Nets did therefore not influence grape quality in 2016, a vintage that was characterised by a hot and dry autumn. Complementary observations should be conducted under cooler and wetter conditions in order to confirm these first results knowing that black nets are more suited from a landscape point of view.

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Drosophila suzukii: important differences in the susceptibility of grape cultivars

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Highlights

- The susceptibility of grapes to *Drosophila suzukii* increases with berry maturation
- In particular the red varieties Acolon, Cabernet Dorsa, Cornalin, Divico, Dornfelder, Dunkelfelder, Galotta, Garanoir, Humagne rouge, Mara, Regent and Syrah are at higher risk in Switzerland
- Infestation by *D. suzukii* decreases significantly with the force required to penetrate grape skins

Introduction

The Spotted wing drosophila (*Drosophila suzukii*) is a very polyphagous species that can infest around 100 host plant species (Kenis et al., 2016). Thanks to the serrated ovipositor of females, the insect can attack and develop in a wide range of wild and cultivated fruits, in particular thin-skinned soft fruits and stone fruits. Although past experiences indicate that grapevines have to be considered as a secondary host plant, eggs can be laid in a great variety of cultivars. In general, darker coloured cultivars are at higher risk and infestation increases with the maturation of grapes. As well, early ripening varieties and cultivars with compact grape clusters seem to be more susceptible to oviposition than their counterparts. Finally, Ioriatti et al. (2015) showed that egg infestation decreases with skin hardness. Here we summarise the knowledge we gained on the susceptibility of grape cultivars to *D. suzukii* in Swiss vineyards.

Material and methods

In order to detect eggs deposited by *D. suzukii*, Agroscope and the competent cantonal authorities sampled more than 90,000 berries in 500 parcels in 2015 as well as 150,000 berries in 600 plots in 2016. Usually, 50 healthy berries per plot were collected in a representative way once a week from véraison to harvest. Thereafter the number of berries with eggs was determined.

The evolution of oviposition by *D. suzukii* was also followed on 32 cultivars in the Agroscope collection of grapevine varieties in Pully (Switzerland). Every week 50 berries were sampled and the infestation rate was assessed. We then measured the force required to penetrate the grape skin using a penetrometer.

Results and discussion

The number of infested plots was higher in 2016 than in 2015. However, the first deposited eggs could be found in both years in the second half of August. Infestation increased with berry maturation until harvest and decreased thereafter. In both years, attack was concentrated on red varieties. In particular, the red varieties Acolon, Cabernet Dorsa, Cornalin, Divico, Dornfelder, Dunkelfelder, Galotta, Garanoir, Humagne rouge, Mara, Regent and Syrah were at higher risk. Generally the main varieties Pinot noir, Chasselas, Gamaret, Merlot and Müller-Thurgau were hardly infested. However,

we observed a big variance in the infestation level of plots within the same variety. Overall, the susceptibility of varieties was similar between 2015 and 2016. However, the relation between the proportion of plots infested and the level of infestation did not necessarily correspond. For example, eggs could be found in nearly half of the sampled Merlot plots, but infestation rate stayed low.

Although infestation by *D. suzukii* was lower in the grapevine variety collection in Pully in 2015 than 2016, the susceptibility of cultivars was similar over the two years (R2=0.25, P=0.04). Attacks concentrated on red and rose varieties, in particular Gamay précoce, Chasselas rose, Bondoletta, Kimisch Lutshitsii, Humagne rouge, Cabernet Dorsa and Diolinoir. These cultivars were also characterised by a low force to penetrate the grape skin. The penetration force between 2015 and 2016 was highly correlated (R2=0.42, P=0.004). In both years, the infestation rate decreased with increasing skin hardness (2015: R=-0.40, P=0.04; 2016: R=-0.56, P=0.01).

Our observations confirm that the susceptibility of grapes to *D. suzukii* increases with the maturation of berries, that rose and red varieties are at higher risk than white cultivars and that the risk of infestation decreases with the hardness of the grape skin. Although Rombaut et al. (2017) could demonstrate in the laboratory that *D. suzukii* facilitates *Drosophila melanogaster* infestation and consequently, favours sour rot outbreaks, the exact role of this new pest in the vineyard needs nevertheless to be clarified.

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Influence of field margins containing blackberries on appearance of *Drosophila suzukii* and infestation of grape berries in adjacent vineyards

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Highlights

- Presence of blackberries in the field margins enhances the number of occurring *Drosophila suzukii* in adjacent vineyards and margins
- Abundance gradient from margin into the vineyard
- Occurrence of high numbers of *D. suzukii* in vineyards with pinot noir does not automatically translate to infestation of grapes

Introduction

The invasive spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), was found in Germany for the first time in 2011. SWD causes high economic damage by infesting ripening, intact, soft-skinned fruits (Ioriatti et al., 2015). Although SWD preferentially infests stone fruits and berries, grapes may also serve as hosts and consequently viticulture industry has to deal with potential infestations of grape cultivars (Bellamy et al., 2013).

In order to develop efficient management strategies and to reduce the risk of this pest to emanate, it is necessary to identify the ecological factors that favour the occurrence and infestation of SWD. Arable field margins containing potential wild hosts for SWD are proposed to have an influence on the pest population (Klick et al., 2016). In this study, the influence of blackberry (*Rubus* spp.) hedges adjacent to vineyards on population size and distribution of SWD as well as on berry infestation was investigated.

Material and methods

Experiments were performed in 15 vineyard blocks with pinot noir between July 2016 and March 2017. Study sites are located in the viticulture district Kaiserstuhl in the Baden region of southwestern Germany. Ten sites were adjacent to field margins containing exclusively blackberry (*Rubus* spp.) as host plant (BB) and five sites to margins with non-host grass crops as control sites (C). The sites were not treated with insecticides during the study.

To track the abundance of SWD, three transects with each five traps (two traps in the margin and three traps with 10 m distance into adjacent vineyard; n=15) were installed at each site. The traps contained 150 ml apple vinegar/water (1:1) and a drop of detergent. The traps were controlled weekly and flies were counted by sex. Furthermore, sampling with a modified leaf vacuum was conducted to test a passive trapping method and to compare the data collected from the traps.

Total numbers of flies were compared between BB and C as well as between margins and vineyard within different periods of time to test whether there is a gradient from the margin into the vineyard. Different factors (e.g., transect, surrounding vegetation and weather conditions) potentially affecting the abundance of SWD were considered for each site.

Moreover, infestation of grapes was controlled weekly by inspecting 50 berries per site for oviposition.

Results and discussion

An initial comparison of the total number of SWD collected over the entire duration of the experiment revealed that populations peaked at the same time in both treatments, but the number of flies was considerably higher in BB than C sites. The first population peak occurred in both BB and C at the end of August. After maturation of the blackberries around mid-September, the population size decreased. A second, lower peak occurred at the beginning of October during the grape harvest. In this period of time, the mean sum of captured flies per site was at least twice as high in BB compared to C. After the population had reached an absolute minimum at the beginning of November, the SWD abundance was markedly higher in BB than in C until February 2017.

Comparing the mean total captures of the field margins data showed a more than two times higher abundance of SWD in BB sites. Similar observations were made for flies trapped in the adjacent vineyards. We conclude that the presence of blackberries in the field margin seems to enhance the number of occurring SWD in the whole site.

Overall, the number of flies found in BB margins was higher than in the adjacent vineyard throughout the entire experiment. Furthermore, a gradient from margin into the vineyard was observed. On the contrary, in C sites the number of SWD was more evenly distributed between margin and vineyard.

The samples collected by the leaf vacuum method provide a “snap-shot” of the population. The mean number of flies was generally lower using this method compared to the number of flies collected in the traps. Statistical analysis is currently ongoing to test for the statistical significance of our findings and to correlate our observations to site-specific parameters and weather data.

No successful infestation of grapes was found during the direct monitoring. Just very few dead eggs due to wound healing of the berries were found. This is in line with laboratory choice bioassays, in which we also found that grapes of the cultivar Pinot noir are less suitable for oviposition and egg development than other grape cultivars. In conclusion, the occurrence of high numbers of SWD in vineyards adjacent to margins containing blackberries does not translate to infestation of grapes.

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Biological control of *Drosophila suzukii* by means of the pupal parasitoid *Trichopria drosophilae*: field and semifield experiences

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Highlights

- Released *Trichopria drosophilae* attack *Drosophila suzukii* in multiple open field environments and low the pest eclosion level from the infested fruit
- The augmentorium technique enhances the parasitoid action and represents a valuable low-cost strategy for sustainable crop protection

Introduction

The biological control represents a fundamental part of the final integrated management plan of *Drosophila suzukii*, the spotted wing drosophila (SWD). By regulating SWD populations, the parasitoid action would support the other control strategies, thus improving their efficacy.

Laboratory studies have been carried out on both co-evolved SWD parasitoids (Daane et al., 2016) and parasitoids native of the invaded areas (Chabert et al., 2012; Mazzetto et al., 2016). The firsts have advantages in terms of efficacy and specificity, nonetheless their open field release is still forbidden by restrictive laws preventing the introduction of invasive alien species. The seconds may represent an alternative, since their use does not suffer legal restrictions and some species have shown good potentiality against SWD in laboratory assays. The aim of this study was to verify the ability of the indigenous pupal parasitoid, *Trichopria drosophilae* (Perkins), to attack SWD in open- and semi-field conditions.

Material and methods

Through the creation of a national research network, field trials were carried out in eight different environments, at different altitudes and on different crops. For each site, a control plot and a treated plot were selected. At the border of each plot, a buffer zone hosting wild vegetation was present. Parasitoids were released into the treated plots from a single release point (RP) located within the buffer zone. One thousand adults per week (sex-ratio 50:50) were released during five consecutive weeks. Parasitoid activity was monitored by means of *D. suzukii*-infested traps, placed at various distances from the RP (10, 20 and 40m). Effect of parasitoid releases on fruit infestation was evaluated through fruit sampling both from the plant and from the ground. Greenhouse trials were performed in a high tunnel (30 mx5m) completely isolated by a fine net coverage (50 mesh) and cropped with raspberry (cv. Tulameen and Heritage). An artificial infestation of *D. suzukii* (1 couple/m^2) was performed. The structure was then divided into 3 sectors: the central one was set as control, whereas the other two sectors were treated with parasitoid releases and parasitoid releases plus augmentorium respectively. Fruit samplings were performed in each sector for evaluating fruit infestation levels, *D. suzukii* and *T. drosophilae* eclosions.

Results and discussion

In the field trials, *T. drosophilae* infested the sentinel traps up to 40 m far from the RP. The *D. suzukii* eclosions from the traps were significantly reduced in those ones located at 10 m and a strong tendency to reduction was observed in the ones at 20 m. These results were consistent among seven sites out of eight. The parasitoid releases did not significantly reduced the pest's infestation on the plant-sampled fruit or the level of *D. suzukii* eclosion from the ground-sampled fruit, probably due to the low number of released parasitoid and their consequent dispersion within the environment.

In the greenhouse trials, the parasitoid releases significantly reduced the *D. suzukii* eclosions from the fruit with respect to the control in both the treated sectors. The augmentorium technique enhanced the parasitoid action by increasing the number of parasitoid eclosions. This increment became evident on the third week, corresponding to the parasitoid's first generation eclosion from the augmentoria-collected fruit.

In conclusion, *T. drosophilae* attacks *D. suzukii* in multiple open-field environments and this ability could be exploited for a SWD biological control strategy. Such strategy should be aimed to low the SWD population at the very beginning of the season, when the few SWD adults that survived the winter are looking for alternative food sources (wild vegetation flowering and fruiting, compost, etc.). In this scenario, periodic inoculative releases of *T. drosophilae* over a wide area and in correspondence of SWD feeding hotspots, may contribute to regulate the pest population dynamic before the fruit ripening in the orchards and successively throughout the entire season.

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Field testing of insecticides against spotted wing drosophila (SWD) in viticulture

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Highlights

- Monitoring ovipositions on grapes leads to a correct timing of treatments
- The active compounds spinosad, cyantraniliprole and chlorpyrifos-methyl were tested in field trials against SWD
- Both spinosad and chlorpyrifos-methyl showed a good efficacy in controlling the pest

Introduction

Viticulture represents one of the major agricultural activities in South Tyrol. The most widespread grapevine variety of the province is Vernatsch. This variety is particularly susceptible to *Drosophila suzukii* (Matsumura), because it is characterised by a soft skin, which facilitates females' oviposition. Damages caused by egg laying and the development of larvae in berries can lead to sour rot infestations in vineyards (Barata et al., 2012; Sinn, 2012). Control strategies are being developed and include insecticides applications. Field trials are therefore necessary, in order to characterise the active compounds.

This study compares the efficacy of three different products against spotted wing drosophila and was carried out in three different years in the same vineyard.

Material and methods

Field trials were carried out in a vineyard of the red wine variety Vernatsch, where grapes are grown on a pergola, the traditional training system in South Tyrol. The area covers ca. 2,400 m^2 and was divided into 4 plots of approximately 600 m^2 each. Two plots were treated and the other two were left untreated and acted as control, except in the last field trial in 2016, when only two plots were available. The percentage of berries with eggs was assessed twice a week, starting at the beginning of August. Frequency and intensity of sour rot infestation was assessed before harvesting.

In 2014 the efficacy of the active ingredient spinosad (Laser, Dow AgroSciences Italia S.r.l.) was assessed. Three treatments using 250 ml/ha (120 g/ha of a.i.) were carried out on the following dates: on the 8th and 26th of August and on the 2nd of September. The active ingredient cyantraniliprole (Exirel 2015, DuPont de Nemours Italiana S.r.l.) was evaluated in 2015. Three treatments were performed using 750 ml/ha (75 g/ha of a.i.) on the 26th of August, the 4th and the 9th of September. In 2016 the active ingredient chlorpyrifos-methyl (Reldan LO, Dow AgroSciences Italia S.r.l.) was tested in the field, with treatments performed on the 24th of August, the 2nd and the 12th of September, applying 1,500 ml/ha (337.5 g/ha of a.i.).

All treatments were performed with an axial air-blast sprayer using a water volume of 15 hl/ha.

Results and discussion

Oviposition controls started each year at the beginning of August, when the berries began to change colour. In 2014 eggs were already found in the first sample, the 6th of August, reaching 33 %

of coloured vine berries with at least one egg in one of the control plots. The first treatment was therefore carried out two days later. In the following two weeks the percentage of infested berries decreased not only in the treated plots, but also in the control plots. This is probably due to the increase in ripe berries that attract SWD females. Starting from the end of August, ovipositions in the untreated plots boosted up to 70 %, while they remained under 20 % in the treated plots, after two more spinosad applications. Attacked berries in the last collected control samples were almost twice the amount of infested berries found in treated plots. Average sour rot infestation frequency and intensity remained rather low where spinosad was applied, while they strongly increased in the control. The reduction in both ovipositions and sour rot-damaged grapes in the treated plots showed a good efficacy of the active compound in controlling SWD.

The initial situation in 2015 and 2016 differed from 2014, since the first ovipositions were found on the 21st and 19th of August respectively. In both years, the infestation development was slower compared to 2014, because of the lower pest's pressure of 2015 and 2016 at the beginning of August. The infestation trend in 2015 was similar in the treated plots and the control and only the last two collected samples showed a higher number of infested berries in the untreated plots compared to the treated plots. The results concerning damages caused by sour rot showed a high percentage of affected bunches (between 78 % and 98 %) and variable infestation intensity within the plots. Considerable differences were not evident, since only one of the treated plots showed fewer damages compared to the control. In this trial, cyantraniliprole did not show an adequate efficacy for SWD control.

The field trial of 2016 was carried out without repetitions, due to the strong downy mildew infestation in the vineyard. Except for one of the samples, the number of ovipositions was generally higher in the control plot, reaching 68 % of attacked berries before harvest. The sour rot estimation performed before harvesting showed that grapes of the treated plot were healthier than untreated grapes, barely showing signs of infestation. Despite the simple trial design, these data show that the active ingredient chlorpyrifos-methyl was effective in suppressing SWD infestation.

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Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture”

Novel tools and new challenges for IPM in viticulture

Oral Session 5

Ecology and multiple interactions in pest control

Plant-herbivore interactions in the context of climate change: Effects of elevated CO_2 concentrations on grapevine and European grapevine moth (*Lobesia botrana*)

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Highlights

- Weight of male and female *L. botrana* pupae was significantly higher under elevated compared to ambient CO_2
- Length of *L. botrana* larval development was significantly shorter under elevated CO_2
- Grapevine plants show a CO_2 effect in response to *L. botrana* herbivory at the level of gene expression, with a much stronger transcriptomic response to *L. botrana* feeding under elevated CO_2

Introduction

An increase in atmospheric carbon dioxide (CO_2) concentration and temperature will have impacts on the interaction between plants and herbivorous insects. These impacts are either related to changes in the nutritional quality of the host plant or are due to changes in insect phenology or biology such as lengthened larval developmental time or female fecundity (Zavala et al., 2013). The European grapevine moth *Lobesia botrana* is considered as a key insect pest in European vineyards with an increasing spread to other areas worldwide. Fingerprints of climate change on phenology of this multivoltine species are evident as adult moths emerge earlier from overwintering pupae in spring, which is significantly correlated to an increase in mean winter temperatures (Reineke and Thiéry, 2016). Here, we assessed the effects of elevated CO_2 on both grapevine as a host plant and *L. botrana* as an herbivorous insect using climate chambers as well as a free-air carbon dioxide enrichment (FACE) system.

Material and methods

Lobesia botrana larval developmental time, pupal weight, life span and female fecundity were assessed in climate chambers under two different CO_2 concentrations (400 ppm and 700 ppm). Prior to these experiments, *L. botrana* populations were reared on artificial diet under elevated (eCO_2; 700 ppm) and ambient (aCO_2; 400 ppm) conditions for several generations.

A whole transcriptome analysis was performed with grapevine cv. Riesling plants grown under field conditions in a grapevine FACE system under aCO_2 (395 ppm) and eCO_2 (460 ppm) concentrations. Plants were infested with second instar *L. botrana* larvae at two stages of grapevine development (flowering and berry ripening). After four days of larval feeding, the nearest leaves to a *L. botrana* feeding site were collected both from infested and non-infested control plants. RNAs were extracted from leaf samples and were submitted to transcriptome analysis via RNASeq using the Illumina HiSeq 4,000 system.

Results and discussion

In rearing experiments in climate chambers, no significant differences were obtained for the numbers of eggs laid by *L. botrana* females, number of larvae hatched as well as lifespan of male and female individuals after insects have been reared for three and five generations under aCO_2 and eCO2, respectively. However, after five generations under eCO_2, pupal weight of both male and female pupae was significantly higher compared to pupal weight at aCO_2. In addition, length of larval development was significantly shorter for both sexes under eCO_2.

Of a total of 14,763 genes identified during transcriptome analysis, a substantial number was significantly differentially expressed in grapevine plants as a result of *L. botrana* herbivory at the two time points of sampling (grapevine flowering and berry stage). Altogether, more genes were differentially regulated under eCO_2 compared to aCO_2, indicating that grapevine plants show a CO_2 effect in response to *L. botrana* herbivory at the level of gene expression, with a much stronger transcriptomic response under future eCO_2 conditions. Among the respective genes identified some are known to have a function in processes like “plant-pathogen interaction”, “defence response” or “response to biotic stimuli”. These genes are of particular interest for understanding future grapevine responses to herbivory under elevated CO_2. Accordingly, obtained results will contribute to a better understanding of grapevine-insect interactions in the context of climate change and thus help to identify potential consequences for future pest management strategies.

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Cultural control of *Lobesia botrana* on grapevines

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Highlights

- Bunch-zone leaf removal, during *Lobesia botrana* 2nd flight, reduced infestation by 50 %
- In the lab, from 37-40°C mortality of eggs and newly-hatched larvae increased
- Temperatures of sun-exposed berries exceeded those lethal for eggs and larvae
- On sun-exposed bunches egg and larval mortality increased without egg-laying reduction
- This cultural practice is a valid control tool in IPM context

Introduction

Lobesia botrana (Den. and Schiff.) (Lepidoptera: Tortricidae) is the major pest in European vineyards. In north-eastern Italy the highest damage is associated with second- and third-generation larvae that feed on berries (Pavan et al., 2014).

In the context of IPM, cultural practices can contribute to the control of *L. botrana* carpophagous generations (Villani et al., 1997; Vartholomaiou et al., 2008) in integration to synthetic insecticides, Bacillus thuringiensis Berliner and mating disruption techniques.

In this study, the possibility to control *L. botrana* with either manual or mechanical bunch-zone leaf removal was assessed in the field. Since this practice could affect *L. botrana* infestation by reducing egg laying or increasing egg and larval mortality, these aspects were also investigated under laboratory and field conditions.

Material and methods

During 2007–2016 the effect of bunch-zone leaf removal on *L. botrana* was studied in north-eastern Italian vineyards. Eight randomised block design trials with four replicates were conducted comparing plots with and without bunch-zone leaf removal, applied at the beginning of *L. botrana* second flight. On 50 bunches per plot, the larval nests of the second and third generations of *L. botrana* were counted.

To investigate the mode of action of bunch-zone leaf removal against *L. botrana*, laboratory and field bioassays were conducted during 2015-2016. In the laboratory at 65±5 % RH, the effect of high temperature on eggs and newly-hatched larvae was evaluated comparing the mortality of these development stages at constant 24°C (control) vs. exposures to 37°C, 40°C, 43°C for 4-6 hours. In two vineyards, the effect of bunch-zone leaf removal on berry temperatures on both sides of different-oriented rows was assessed measuring berry temperatures with a portable handheld noncontact infrared thermometer. In another vineyard, *L. botrana* females' egg-laying and egg and larval mortality were evaluated in four assays. At this purpose, on the south side of grapevine rows, fertile females from laboratory rearing were released into transparent tulle cages (Ø 15 cm, L 30 cm) with one sun-exposed and one non-exposed bunches and successively the number of eggs laid, the percentage of egg hatching and larval settlement were recorded on the two types of bunches.

Results and discussion

In all field trials bunch-zone leaf removal significantly reduced *L. botrana* larval infestation of both second- and third generations with an average Abbott efficacy of 51 % (min 33 %, max 74 %) and 57 % (min 50 %, max 63 %), respectively.

In the laboratory, significant increases of *L. botrana* mortality were observed for eggs from 40°C and for newly-hatched larvae from 37°C. The eggs susceptibility increased with embryonic development. Therefore, the susceptibility increases progressively from earliest egg stage to newly-hatched larvae.

In the vineyards where berry temperatures were measured, the berries belonging to sun-exposed bunches on S-, SW- and W-side of grapevine rows, can be at least 10°C above ambient temperature, exceeding 37°C for four or more hours. These temperature levels are consistent with those caused an increase of the mortality of *L. botrana* eggs and newly-hatched larvae in the laboratory.

After sunset, *L. botrana* females confined into cages with bunches did not avoid to lay eggs on berries that some hours before had been exposed to sunlight, indeed females in some cases laid a significantly higher number of eggs on sunny-side of exposed bunches. Moreover, significant increase in egg mortality (23 % on average of four assays) and decrease in larval settlement (59 % of hatched larvae on average of three assays) were observed for sun-exposed bunches in comparison with shaded bunches.

Since in our field investigations sunlight exposure of berries increased *L. botrana* egg and larval mortality and temperatures recorded on berries were higher than those reported as fatal for L. botrana eggs and larvae under laboratory conditions (≥ 37°C) (Kiaeian Moosavi et al., 2017), the efficacy of bunch-zone leaf removal in reducing *L. botrana* infestation in field trials (Pavan et al., 2016) can be associated with an increase in berry temperatures.

Bunch-zone leaf removal can be considered as a valid control tool against *L. botrana* in the context of integrated pest management, particularly in organic viticulture where this practice is the main tool to prevent grey mould, due to the fact that synthetic fungicides are allowed (Pavan et al., 2016).

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Influence of landscape complexity and vineyard management on leafhoppers abundance in North-Italian vineyards

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Highlights

- Landscape complexity and/or vineyard management can affect the abundance of grapevine leafhoppers
- *Empoasca vitis* was more abundant in complex landscapes in early season, later in extremely simplified ones
- *Zygina rhamni* was more abundant in organic than in conventional vineyards
- *Scaphoideus titanus* was frequently detected in vineyards surrounded by complex landscapes and in organic vineyards

Introduction

Grapevine production can be threatened by different species of leafhoppers (Homoptera Cicadellidae). Among these, *Empoasca vit*is (Göthe), *Zygina rhamni* Ferrari and *Scaphoideus titanus* Ball are the most important in vineyards of North-eastern Italy. In particular, *S. titanus* is the key vector of the Flavescence Dorée phytoplasma, a serious disease of grapevine. Insecticide applications are often mandatory to control *S. titanus* populations.

The role of landscape complexity for its impact on the abundance of both pests and natural enemies is often matter of discussion (*e.g.,* Chaplin-Kramer et al., 2011; Veres et al., 2013). At the same time, pest management practices can exert a different impact on pests and their natural enemies (Rush et al., 2015).

The aim of this study was to investigate the effects of landscape complexity and pest management strategies (organic vs. conventional) on the abundance of three species of leafhoppers in vineyards located in the North-eastern Italy.

Material and methods

The presence of E. *vitis*, *Z. rhamni* and *S. titanus* was assessed in 18 vineyards located in the Conegliano-Valdobbiadene DOCG area, the most important area for the Prosecco sparkling wine production. Nine blocks were identified in this area. Within each block, a conventional and an organic (according to EU Reg. 889/2008) managed vineyard were selected. Landscape analysis was performed by assessing the proportion of semi-natural habitats in a radius of 250 m. Three levels of landscape structure were identified according to Tscharntke et al. (2005): a) negligible presence of semi-natural habitats (less than 1 %); b) semi-natural habitats ranging from 1 to 20 % and c) semi-natural habitats abundant (more than 20 %). This survey was carried out from June to September 2016. At each sampling time, 25 leaves were collected from each vineyard and observed under a stereomicroscope in order to assess the abundance of *E. vitis* and *Z. rhamni*. The presence of *S. titanus* was assessed by direct observation in the field of 25 grapevine suckers in each vineyard. Data were analysed using the repeated measures ANOVA with the PROC MIXED procedure of SAS (SAS Institute Inc., 1999).

Results and discussion

The abundance of the three leafhoppers resulted affected by landscape complexity and by vineyard management but these effects sometimes changed across the growing season.

A greater number of *E. vitis* occurred in vineyards within extremely simplified landscapes, but in the first samplings this leafhopper was largely detected in complex scenarios. No differences were found when different pest management strategies were compared. *E. vitis* abundance was influenced by vineyard management across the growing season, showing higher numbers in conventional vineyards in early season, and an opposite situation in late season.

The abundance of *Z. rhamni* was not influenced by landscape complexity while it was affected by management, with a higher number of individuals in organic vineyards.

The abundance of *S. titanus* was influenced both by landscape complexity and management. A greater number of individuals was observed in vineyards within complex landscapes, but with different effects over the time. These differences were observed in the organic vineyards, while in conventional ones the presence of the vector was generally limited. In the first sampling the higher presence of *S. titanus* occurred in vineyards located in complex scenarios compared to the other landscape types; later, no significant differences were found among treatments.

The different amounts of semi-natural habitats that characterised the landscape complexity and pest management strategies can influence leafhoppers populations' densities. In fact, the reduction of *E. vitis* population in complex scenarios over the growing season, and its increase in extremely simplified landscapes could be explained by the higher presence of natural enemies in the former sites. Insecticide applications did not reduce effectively leafhoppers populations in organic vineyards. This effect was clear especially for *S. titanus* in vineyards within complex landscapes. In these cases wild grapes in semi-natural habitats could serve as refuge zone during insecticide applications. The fragmentation of vineyards in landscapes with a high degree of complexity could be an additional factor. In fact, the presence of non-cultivated vegetation around small size vineyards could offer shelters from insecticide treatments and favour a subsequent colonisation of organic vineyards where low persistent insecticides are used. This aspect deserves further investigation since semi-natural areas can also harbour natural enemies like predators and parasitoids.

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Biological control of the mealybug *Planococcus ficus* in vineyards of North-eastern Italy

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Highlights
- *Planococcus ficus* is an important pest of grapevine in North-eastern Italy
- We assessed the occurrence of natural enemies and the effect of the release of coccinellid predators in the control of mealybugs in vineyards
- Among parasitoids a prominent role was found for Anagyrus species
- Releases of coccinellids proved to be successful in the control of *P. ficus* in vineyards

Introduction

The mealybug *Planococcus ficus* is an important pest of grapevine in different grape-growing areas. This pest can cause direct damage as well as indirect damage due to honeydew and sooty mould. *P. ficus* can transmit viruses associated with grapevine leaf roll. This pest has a cryptic behavior posing some limitations for successful chemical control. In the context of reduction of pesticide use in vineyards, alternatives to chemical control are considered with increasing interest. Natural enemies such as parasitoids and predators have been observed in vineyards. Augmentative biological control by using coccinellids can be an option for the control of *P. ficus* in vineyards. Here we present the results obtained in a survey on natural enemies' complex observed in the North-eastern Italy and in a series of experiments to assess the effect of predator releases on *P. ficus* population densities.

Material and methods

A survey on natural enemies complex of *P. ficus* was performed in a number of vineyards located in North-eastern Italy. These vineyards were visited in fall and subsequent spring when motile forms of predators and of mealybugs showing symptoms of parasitism were collected. The emergence of parasitoids was followed in laboratory. Parasitism rate was estimated in different vineyards. Augmentative releases of coccinellids were performed in four vineyards. *Cryptolaemus montrouzieri* Mulsant and Nephus includes Kirsch were used in these experiments. Releases were performed according to the phenology of *P. ficus*, and in particular when second generation ovisacs were observed on plants. The effect of predators release was assessed by comparing the infestation level and the presence of predators observed on release treatments and in the control without predators release. A treatment with insecticide application was compared in two experiments. Data were analysed using ANOVA ($\alpha = 0.05$).

Results and discussion

The presence of parasitoids belonging to the genus *Anagyrus* (Hymenoptera Encyrtidae) was observed in vineyards. Natural occurring predatory larvae (especially coccinellids and lacewings) were also observed in a limited number of vineyards. The parasitisation rate and the occurrence of natural enemies were negatively correlated with the frequency of insecticide applications. Augmentative releases of coccinellids caused a significant reduction of *P. ficus* infestation levels as compared to control. The lower infestation of *P. ficus* was observed on different grape organs, including bunches at harvest. Among coccinellids used in the experiments, the best results were obtained with *C. montrouzieri*. The control of *P. ficus* population obtained by releasing predators was comparable with that obtained by applying insecticide treatments. Results showed that natural enemies of mealybugs, in particular parasitoids can occur in the North-eastern Italy, but they cannot be able to keep pest populations densities below economic thresholds (Duso et al., 1989; Mansur et al., 2011). Biological control can be an effective strategy to keep *P. ficus* under control (Daane et al., 2004). Here we found that the use of coccinellids can be a valuable tactic for the control of mealybugs in vineyards.

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Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture”

Novel tools and new challenges for IPM in viticulture

Oral Session 6

IPM implementation and tools

IPM Implementation benefits from the partnership between scientists and growers: a case study in a Tuscan wine-growing area

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Highlights

- No insurmountable obstacles would prevent substantial increases in IPM implementation in Italian vineyards, if interest among research scientists in promoting and transferring existing knowledge can be cultivated
- Scientists must play a leading role in engaging all groups of stakeholders to work together with a common goal, which probably has been the most important factor in the success achieved in this project

Introduction

Bolgheri district (Tuscany, Italy) is one of the most prestigious areas for the production of top quality wines. The area is characterised by a mild climate, with medium-high rainfall (400-800 mm per year on average) and mostly sandy soil.

The Bolgheri vineyards have historically been affected by heavy infestations of the European Grapevine Moth (EGVM) *Lobesia botrana* (Lepidoptera, Tortricidae) and the Vine Mealy Bug (VMB) *Planococcus ficus* (Hemiptera, Pseudococcidae).

Insecticide strategies generally adopted by growers included 2-3 sprayings per year against EGVM with IGRs or organophosphorates and 1-2 treatments per year against VMB with systemic or neurotoxic insecticides.

In 2014 one famous Winery (Guado Al Tasso – Antinori) located in Bolgheri, province of Livorno (Tuscany), asked for help to manage insect outbreaks.

Insecticides have been showing limited efficacy in the previous years, so that the manager would like to start adopting alternative and more sustainable strategies.

Material and methods

On our proposal the farm applied the Pheromone Mating disruption (MD) against *L. botrana* on one sixth (50 hectares) of the whole farm surface, to be able to compare obtained results with the conventional insecticide strategy. MD was applied with ShinEtsu Isonet L dispensers from the end of March at a rate of about 500 units per hectare.

The strategy for *P. ficus* included the release of two Biological Control Agents (BCAs), the Encyrtid parasitoid *Anagyrus* sp. on *P. ficus* infested tissues in May (1,000 individuals per hectare on a total of 3.5 hectares) and the Coccinellid predator *Cryptolaemus montrouzieri* (about 500 individuals/hectare on a total of 4 hectares) in June and/or July.

From the beginning, the management of the program was in the hands of a technical working group (TWG) composed by University personnel and winery technicians. The monitoring of pest population was carried out with pheromone traps and cluster sampling.

Field assessments were carried out both in MD and conventional vineyards with regard to EGVM and in the plots were BCAs were released.

Results and discussion

In 2014 results were very promising: the farm did not spray against *Lobesia* in MD areas, with low infestation at harvest (less than 5 % of infested bunches), whereas they sprayed 2 times in the conventional areas, with a more limited efficacy. Excellent results were obtained in the control of VMB too, so that in 2015 other local farms joined the project: MD was applied on about 300 hectares, and BCAs were efficiently released in new plots for a total of about 20 hectares.

Hence, in 2016 new farms joined the project; MD was applied on 700 hectares in Bolgheri area and BCAs were released on 400 hectares. The substantial decrease of insecticide applications due to MD and BCAs use was perceived as the first major step forward, which improved the public perception that wine was produced with high environmental safety standards. The action plan drastically reduced insect populations, so that other local farms joined the project in 2017 so that the area managed in IPM is rising further.

Keys of the success: sharing the problem: limited efficacy of insecticides pushed growers to adopt alternative and more sustainable strategies.

Conditions: vineyards were relatively young and well managed; growers and technicians were well trained and open to new experiences; the University's support has been crucial to the success of the program.

Lesson learned: we believe that no insurmountable obstacles would prevent substantial increases in IPM implementation in Italian vineyards, if interest among research scientists in promoting and transferring existing knowledge can be cultivated. Scientists must play a leading role in engaging all groups of stakeholders to work together with a common goal, which probably has been the most important factor in the success achieved in this project.

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How spraying technology could reduce pesticides use in vineyards

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Highlights

- High pesticides dosage reductions were obtained with tunnel sprayers in French Mediterranean vineyards
- An additional dosage reduction was obtained by increasing forward speed
- Despite of the fungicide dosage reduction, downy and powdery mildew control was still efficient

Introduction

Grapevine is one of the major fruit crops worldwide and can be attacked by a large number of pests and pathogens, and therefore many pesticides spraying are done every year for grapevine protection. Nowadays, growing concerns about environmental issues lead winegrowers to reduce the use of pesticides, and more efficient spray application techniques are needed. There is a great variety of sprayers in use in vineyards, and among all sprayers, recycling tunnel sprayers have been identified as combining protection efficiency, people and environment respect. Tunnel sprayers recycle the part of spray that is not intercepted by the crop. Vergès et al. (2017) showed on an artificial grapevine that their high spraying performance could offer the opportunity to reduce pesticide dosage. The aim of this study was to evaluate reductions in doses of fungicides with tunnel sprayers while maintaining an efficient crop protection against downy and powdery mildew in Mediterranean vineyards.

Material and methods

Fields trials with tunnel sprayers were carried out in years 2016 and 2017, in 12 commercial vineyards in the French Mediterranean area. Plots with a reduction of 30 % of the pesticide dose (R30) were compared to reference plots (REF) for downy and powdery mildew control. The 30 % dose reduction with regard to the REF was achieved with an increase in the forward speed of the sprayer, while the concentration of the spray mixture and the liquid flow rate of the sprayer outputs remained constant. Moreover, at early growth stages, the applied dose in both R30 and REF treatments was reduced according to the ratio between the number of open nozzles for the treatment and the number of open nozzles needed in full growth canopy. Treatments were applied as strips with no buffer rows, considering that spraying confinement avoids drift deposit on the adjacent row. Products, concentration and spraying intervals were recorded to calculate the reduction of pesticides use compared to the regional reference. Fungal diseases were regularly assessed in untreated plots. In all treatments, the incidence of powdery and downy mildew was assessed two to three times on leaves and bunches in July and August. In each experimental plot, disease incidence and severity were assessed on 4 placed replicates of 100 randomly selected leaves, and 50 bunches. We calculated the

difference in disease incidence and severity between R30 and REF treatments for each plots. All data analyses were made with R software.

Results and discussion

The recycling rate was highly variable according to the grapevine phenological stage and the tunnel sprayer. The recycling rate decreased as the grapevine canopy developed: at the beginning of the growing season, the recycling rate was above 50 % and collapsed to 20-30 % at full growth stage. The most efficient tunnel sprayer had an average recycling rate over the season of around 40-50 % against less than 15 % for the less efficient.

The mean average number of sprays applied for downy and powdery mildew control in 2016 was 5.3 and 5.5 respectively. Compared to the registered dose, which is a fixed dose per hectare in France, the mean average reduction of fungicide dose achieved in REF treatments was 54 %. This reduction was obtained thanks to both the dosage adjustment to the development of the grapevine canopy and to the recycling capacity of tunnel sprayers. For the R30 treatments, an additive reduction of around 30 % was obtained by increasing forward speed, and the mean fungicide dosage reduction was 68 %. The French national action plan Ecophyto aims to cut the nationwide use of pesticides by 50 %, and set a regionalised monitoring by crop type, based on the “IFT” or treatment frequency indicator. The fungicide reference IFT for grapevine is of 11.8 in the Mediterranean area. Regarding the REF treatment in 2016 only one out of the six experimental fields reached the 50 % reduction level set by the Ecophyto plan, whereas all plots with R30 treatment reached it. Despite of these pesticide dosage reductions, downy and powdery mildew control in the R30 plots was still efficient. Moreover, differences between incidence and severity in R30 and REF were not significant, despite a slightly higher incidence of powdery mildew in R30 treatment.

Tunnel sprayer is a powerful spraying technology to reduce pesticide use in vineyards and avoid pesticides losses in the environment, but we observed great differences in the recycling rates of the four tunnel sprayers monitored. A standardised methodology for assessing recycling rate of tunnel sprayers will be helpful for both grape growers and authorities for selecting the more efficient sprayers. Increasing tunnel sprayer forward speed is an easy way to adjust pesticides dosage, and we showed, that despite a 30 % increase in forward speed, the grapevine protection against powdery and downy mildew was not affected. This could therefore offer new opportunities for precision spraying, in order to adapt pesticide dosage to the grapevine needs at plot scale. In suitable plots (i.e. regular soil, no stones or excessive slopes), a way to improve production could also be the reduction of working time per hectare cost, by increasing forward speed.

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New tools to improve the sustainability of the vineyards: scouting with 4GRAPES app, cloud processing and real-time visualisation on CARTO

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Highlights

- 4GRAPES app to collect and Perleuve CARTO to view vineyard data allow the user to optimise and enhance the work for monitoring and greatly reduce the time spent on data collection and processing
- real-time data sharing can increase efficiency of the pest management strategies, maximising the yields and grape quality and sanity and reducing costs and impact of pesticides

Introduction

The European legislation 2009/128/EC deals with "the sustainable use of pesticides" and emphasised the importance of the field monitoring of insects and diseases harmful to plants growth. Nowadays a systematic and objective method of monitoring is required to define areas at high or low infection risks, to evaluate the incidence of the infection and amount of inoculum yielded during the season.

Usually the advisors inspect the vineyards with the aim to assess the occurrence of diseases or pests, but a quick and objective numerical evaluation is often difficult to assess. In addition, the information is never available in real-time to other users (*i.e.* other advisors or phytosanitary services) because the data are often recorded on notes and analysed only at the end of the season, thus they are useless for any decision during the season. A user-friendly tool for data collection was actually needed.

Material and methods

4GRAPES is an application for smartphones and tablets that can be used Android and iOS environment. The application allows the collection of geo-referred data in the vineyard, such as surveys of plant diseases, phenology and production parameters. There are two ways to insert the monitored data: a. the users can manually enter a rough visual estimation of the percentage of disease incidence and severity, or b. using a counter, the user can enter single precise assessments of disease intensity, thus incidence and severity are calculated automatically. Based on your GPS location, the collected information can automatically be coupled with the information of the vineyards monitored (*i.e.* grape variety, rootstock, planting date, etc.), by loading the "vineyard" layer in the system. In any case, if the layer is not available, we can insert manually the information related to the vineyard at the time of the first observation. All the observations are stored in a cloud in real-time and are ready to be processed by the users. Together with the numerical observations, photos can be added and automatically associated with the observation.

The data stored in the cloud is immediately visualised in the "Perleuve Carto" dashboard over maps. There are many possibilities to display the data, from the most simple and intuitive distribution of the monitored points, to more sophisticated processing together with other GIS tools.

Results and discussion

The advantages of this kind of approach are described as follows:
a. a detailed description of the situation of all the vineyards owned by a farm or a viticultural area;
b. a real-time knowledge of the phytosanitary status of the vineyards;
c. objective and quantitative evaluation of pest/disease incidence and severity;
d. quality of data checked when entering the database, minimising possible mistakes of typing;
e. possibility to access both the raw data and the processed data.

The users can decide what information to share and with whom, so there are many possible customisations of data sharing. By this way, different users are part of the same workgroup, can share the data collected among them in real-time. The same data, averaged within an area, can be used at a higher level such as a consortium of wine producers or at provincial/regional/interregional scale. The averaged data can be viewed by all users.

The aggregated data can help as real-time support for local warning systems, useful to evaluate the development of a certain disease/pest in a vineyard/area and so optimise the control strategy.

When aggregated at territorial level, data can be used by technicians and inspectors of the Regional Phytosanitary Services (as institutional services), to answer the obligations that derive from the application of the European Directive 2009/128/EC, and by research centres or universities for further more complex processing.

On a regional scale, the availability of processed data might improve the advices of integrated and organic pest management, identifying the areas with different disease/pest pressures thus customising protection strategies. By this way it is possible to understand more details as regard a certain pathogen/pest and, depending on the time of season, recommend particular interventions only on some plots or areas (i.e. based on the fly of berry moth).

The benefits of this approach are:

- reduction of inputs for the pest management: sprayings are applied only where and when needed;
- less environmental impact of pesticides;
- reduction of the costs for the farm;
- less risk for vineyard operators and users of the landscape;
- increased efficiency of the pest management strategies, maximising the yields and grape quality and sanity.

The possibilities that the "4GRAPES" app and "Perleuve CARTO" dashboard offers in terms of versatility, management and data processing are many. Relying on cost-effectiveness and user-friendly use of the "4GRAPES" app to collect and "Perleuve CARTO" to view the data, the user can optimise and enhance the monitoring work and greatly reduce the time needed for data collection and processing.

We will show some practical examples and applications in different environments during 2016 and 2017 seasons of potential benefits and outcomes obtained by using 4Grapes and Carto tools.

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BugMap, a citizen science approach to monitor the spread of the invasive Brown Marmorated Stink Bug *Halyomorpha halys* (Hemiptera: Pentatomidae)

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Highlights

- Understanding the dynamics of invasive alien pests in a newly invaded region can help limit their spread and prevent overwhelming outbreaks
- The involvement of volunteering citizens in this scientific inquiry allows the efficient gathering of a large amount of data and the spatio-temporal modeling of *H. halys* in recently invaded region

Introduction

The invasive Brown Marmorated Stink Bug *Halyomorpha halys* is becoming one of the most alarming alien pests worldwide. The title is earned through polyphagy, multi-voltinism and a capacity of long distance dissemination, causing enormous agricultural damages (Leskey et al., 2012). A promotion to public nuisance pest was also due to its tendency to overwinter in man-made structures.

The presence of *H. halys* in the Province of Trento was first reported in 2016. The close association of overwintering *H. halys* with humans prompted volunteer citizens to help track the invasion. Therefore, we designed a mobile application, BugMap, to capitalise on this aspect, involving citizens in a process that would allow the efficient gathering of a large amount of data, and a faster understanding and reaction to the invasion.

The presence of suitable hosts in Northern Italy such as apples, grapes, small fruits and others renders the situation even more critical. Thus, spatial analysis and distribution modelling are employed to better understand the invasion dynamics of this pest.

Material and methods

BugMap reports are received as a citizen filled form on the number of specimens, the detection site (building, means of transport, garden or agricultural cultivation), along with photographs that allow the validation of the sightings. These reports were coupled with data from traps deployed in various locations in Trento, to account for presence and absence of *H. halys* (http://appmeteo.fmach.it/bugMap/). As described by Capinha and Anastácio (2011), environmental features with potential effect on *H. halys* distribution were selected i.e. Digital Elevation Model with a resolution of 10 meters, land-use, hydrography and forest tracks; all available at the Autonomous Province of Trento cartographic portal (http://www.territorio.provincia.tn.it/portal/server.pt/community/cartografia_di_base/260/cartografia_di_base/19024).

From the Digital Terrain Model (DTM), the slope was derived using GRASS GIS version 7. All data have been resampled at 100 meters resolution to increase the speed of calculation in Maxent using the jackknife test for assessing variable contribution.

Multivariate plotting in R was also done to test for correlation among the above-mentioned GIS layers and other variables including finding method (visual or trap), distance from railways, lakes, rivers and main streets, as well as the detection location in buildings or gardens.

Results and discussion

Understanding landscape factors -both natural and anthropogenic- that facilitate the spread and establishment of alien pests is a critical element in invasion dynamics and biology. The fresh infiltration of *H. halys* in Trentino offers an excellent opportunity to determine factors affecting the colonisation process, and provides insight into developing novel control strategies against this pest.

Maxent model showed an accuracy of 0.97, estimated with the ROC curve. The variables that contributed the most to the distribution of this species were elevation and distance from houses, accounting for 49.29 % and 42 % respectively. These results illustrate the suitability of Trento for hosting *H. halys*, particularly in terms of altitude, ranging from 190-300 metres above sea level. Additionally, there is a strong association with urban development, whereby this bug finds numerous overwintering sites and green refuge areas surrounding houses and buildings, further facilitating population build-up.

Moreover, plotting the distance from main streets and rivers with respect to the finding frequency, displays that most sightings were reported at a distance usually not exceeding 200 metres. These results corroborate the findings of Maistrello et al. (2016), verifying the "Hitchhiking" behaviour exhibited by *H. halys*, which could be enhancing the colonisation process.

Furthermore, our observations indicate that the number of BugMap reports was higher during autumn, the period when *H. halys* aggregates in buildings to overwinter, and is therefore in close association with humans. Whereas in April-May, fewer reports were recorded, this is probably due to the dispersal of the bug away from watchful citizens, onto host plants where it mates and develops through 1 or 2 generations. These observations are in agreement with the described biology of the species in several states in the USA (Leskey et al., 2012).

Although still young and in its early stages, BugMap has proven to be a resourceful tool, capable of involving citizens in the scientific inquiry. More importantly, it allows the gathering of a large amount of data in a cost and time effective manner, which is readily helping scientists understand and better react to this alien invasion.

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VitiMeteo in Europe - supporting IPM for practitioners

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Highlights
- VitiMeteo supports IPM on more than 150.000 ha throughout Europe
- VitiMeteo is the product of a close collaboration between viticultural research and practise

Introduction

VitiMeteo is a decision support system that aids winegrowers with Integrated Pest Management (IPM). It was founded in 2002 by Agroscope (Switzerland) and the State Institute of Viticulture and Enology (Germany) and is based on extensive scientific research. Over the past 14 years, it has grown into a successful system that is currently used on more than 400 weather stations in 8 countries, covering approximately 150,000 ha of vineyard.

Both research institutes own the copyright of the software and brands. Together with GEOsens, a private company that is responsible for the development, maintenance and operation of the software, they form the VitiMeteo Consortium.

This unconventional partnership between research institutions and practice ensures the implementation of scientific research into viticultural practice is one of the key success factors of VitiMeteo.

Material and methods

Fifteen years ago the State Institute of Viticulture, (Germany) and Agroscope (Switzerland) decided to found VitiMeteo in order to improve plant disease models that are based on their scientific research and to make them accessible to winegrowers. It governs the future development of VitiMeteo.

The two research institutes provide the scientific knowledge and data, whereas GEOsens, as a private company, is responsible for the practical parts such as the development, distribution and maintenance of the software.

Results and discussion

VitiMeteo currently hosts models of the most predominant and economically relevant grape diseases and has made a fundamental impact on the practice of IPM in Viticulture throughout Europe. In 2017, VitiMeteo is a platform that provides the technical and organisational basis to implement models from the scientific community, thereby implementing science into practice in a most effective way.

The VitiMeteo Consortium works at the interface between research and practical advice. We will show some of the specific problems and requirements of that position, and present some suggestions on how to improve this process.

Our second focus lies on monitoring systems. These are internet-based systems that allow winegrowers to enter observations regarding plants and diseases. The monitoring system takes the different, local needs in Switzerland and Germany into account. We want to present the current state of these systems, future plans and how we aim to integrate the monitoring system into VitiMeteo. We will further discuss the potential for its application in science.

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Forecasting flight activity of *Lobesia botrana*, in the Northeast of Portugal, using a degree-day model

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Highlights

- DD models are useful tools for predicting timing of intervention against pests
- A nonlinear model that predicts flight phenology of L. botrana was developed
- In this model, first catches of overwintering males were used as Biofix
- The use of this DD model can improve IPM tactics against *L. botrana*

Introduction

The grape berry moth, *Lobesia botrana*, is among the most economically important insect pests in Europe and has recently been found in vineyards in Chile, California and Argentina. Prediction of flight activity during the growing season is critical to improve IPM tactics through better timing of sampling or control operations. The aim of this study was to develop a degree-day (DD) model for predict flights occurrence of *L. botrana* in Douro Demarcated Region (DDR), based on data on male captures in sex pheromone traps recorded over a 19-year period.

Material and methods

Data collected were investigated in terms of degree day accumulation and results compared with thermal constants for *L. botrana* development. A nonlinear model based on Boltzmann regression equations was developed using the percentage of accumulated male catches and degree day accumulation.

Results and discussion

Accumulation was counted from the date on which the emerging adult males were captured in pheromone traps, using a lower threshold temperature of 7.3ºC. It was found that the emergence of *L. botrana* adults in DDR occurs at 763.6 ± 9.4ºDD for the second flight, 1500.2 ± 14.8ºDD for the third and 2,285.6 ± 31.4ºDD for the fourth. The model predicted that 50 % of the cumulative catches occurs at 155.1ºDD for the first flight, 932.8ºDD for the second and 1759.0ºDD for the third. Most values predicted were found to be reasonable and the biased ones are discussed. The results obtained could be useful in timing *L. botrana* control measures, especially biorational pesticides application that requires accurate information on insect phenology to be effective.

Acknowledgements

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VIVA indicators: the global approach to assess, improve and communicate grape-wine sustainability in Italy

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Highlights

- There is an increased need of sector-specific standardised methodologies to assess wine sustainability
- Four sustainability indicators were developed and applied to 28 wine product, in the framework of VIVA Sustainability and Culture project
- The indicators allow to assess greenhouse gasses emission, water footprint, agronomic, economic and social impact of wine products or wine companies

Introduction

Italian wine sector is recognised as one of the agricultural components, which better identifies Italian culture and Italians ability to manage and protect rural environment and agrarian landscape.

Increasing awareness for sustainability has pushed the wine sector towards the need of a more controlled system of production and the monitoring of wine production sustainability. Therefore there is an increasing need to determine standardised and sector-specific methodologies and tools in order to evaluate and assess environmental, social and economic burdens of grape cultivation and wine production. This paper presents the indicators and the ongoing results of the global approach to wine sustainability, which was developed in the framework of VIVA Sustainability and Culture project, promoted by the Italian Ministry for the Environment, Land and Sea.

Material and methods

VIVA is able to assess the sustainability performance of a wine product (functional unit: a wine bottle of 0.75 l) or a wine company (functional unit: the organisation) towards the application of four wine-specific sustainability indicators (AIR, WATER, VINEYARD and TERRITORY), developed on the basis of international sustainability standards, which addresses the triple bottom line of sustainability. AIR evaluates the greenhouse gas emissions directly and indirectly attributed to the life cycle of a product or to an organisation. WATER analyses the total volume of fresh consumed and polluted water (both in field and in cellar), and could be applied both to a wine bottle (0.75 l) and to company activities. VINEYARD takes into account the agronomic management practices in the vineyards; the functional unit could be a wine bottle (in this case only the vines used for the specific product are sampled) or the entire company's surface. TERRITORY represents a set of qualitative and quantitative indicators, to evaluate the socio-economic impact of company's activities on the territory and the local community, the functional unit is the estate where the specific wine is produced, or the set of estates ascribed to the studied company. The application of the indicators is guided by technical specifications and is applicable towards online software (www.viticolturasostenibile.org).

Results and discussion

The results of the indicators analysis are verified by a third party and are confirmed by the Italian Ministry for the Environment. After this process, the wine product (product certification) or the company (organisation certification) obtain the VIVA label (in case of product certification the label is black and it is applicable on the bottle, while in case of organisation certification the label is white and it could be affixed on organisation promotional materials and website). The detailed results are displayed through the virtual part of VIVA label, freely consultable in the viticolturasostenibile.org website.

Nowadays 28 Italian wine products (for the total of 175.1 Ha) have obtained the VIVA label, while other 10 wine products and 11 companies are computing the analysis of VIVA indicators. The 28 VIVA labelled wines are produced by 17 different winegrowing farms, located on the whole Italian territory.

All of the products satisfy TERRITORY indicator (this is the only prerequisite which determine the entry criteria for each wine and wine company). High variability was found in overall Water Footprint (WF - computed through WATER indicator) and Carbon Footprint (CF - computed through AIR indicator) result. The 28 wines are representative of Italian wine production and they include still and sparkling red wine, still and sparkling white wine. Results refer to a 0.75 l wine bottle of wine at the winery gate. CF is on average equal to 1.435 g CO_2eq/0.75 l (min 831 g CO_2eq/0.75 l and max 2,070 g CO_2eq/0.75 l; SD 394). The high variability in CF is mainly due to differences in vineyards and cellar operations and distribution. WF is on average equal to 920 l/0.75 l (min 350 l/0.75 l and max 1773 l/0.75 l; SD 403). The high variabilities in WF is due to grape yields and climate zones of production. VINEYARDS results are expressed with an index that goes from A (minimum agronomic impact) to E (maximum agronomic impact). All companies performed well, 11 of them got A, and 13 of them got B (for 4 to 28 products VINEYARD indicator was not computed). These results are probably linked to TERRITORY performance: a good management of biodiversity and rural area (already satisfied with TERRITORY indicator) is often a good predictor of virtuous agronomic practices.

The application of VIVA global approach to wine products and companies can improve and standardise sustainability analysis of wine sector; furthermore it is a load towards farm and winery improvements.

The results of VIVA indicators can support decisions and can help in identifying corporate strength and weakness regarding social, environmental, economic and cultural issues. VIVA labels could be also helpful in communicating sustainability implementation as driver of corporate social responsibility and wine sector renovation.

Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture”

Novel tools and new challenges for IPM in viticulture

Oral Session 7

Classical and novel tools about pathogens

Analysis of the predominant factors generating various epidemic profiles on resistant varieties

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Highlights

- Growers are constrained to significantly reduce the use of fungicides by UE directives and the French government currently enforces a action plan for pesticide reduction which aims at halving pesticide use over the next 8-year period
- Then, low-pesticide systems based on the development and integration of innovative control methods need to be developed and their performance to be evaluated

Introduction

Among the innovative methods, resistant varieties show, up to now, the highest potential for controlling diseases, especially for the pathogen that are the most problematic in Europe and around the world, powdery and downy mildews. Resistant varieties are on the way to be authorised for cultivation in France in 2017. However, some of the resistance seems to have already lost their effectiveness. In a context of plant resistance durability, it seems important to better understand the part of variation of epidemic profiles in the vineyard which is due either to 1) the variations in traits of life of the fungus when interacting with the plant or 2) to the global evolution of the landscape of susceptibility of the crop or 3) to cultural practices. Increase knowledge of the period of greatest vulnerability to the diseases of resistant varieties in the field, will help to use the most appropriate strategy to control the disease.

Material and methods

In the study presented we have compared five varieties (one susceptible and 4 resistant) for their performance against powdery mildew. Life traits of the pathogen (infection efficiency and sporulation) are compared on leaves depending on leaf age. Epidemics were created in the field, and factors such as date of pruning, growth development of the vinestock, bunches phenology, and the susceptibility of the variety are measured and their effects on disease development was assessed by multidimensional analysis (partial least square path modelling).

Results and discussion

It appears that depending on the variety, the factors that have the main effect on the disease on leaves and on bunches are different.

Anti-fungal capacities of ozone dissolved into water against fungi associated with Grapevine Trunk Diseases

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Highlights

- Ozone dissolved into water is an efficient anti-fungal solution against major fungi associated with Grapevine Trunk Diseases
- This efficiency is verified on a phytopathosystem model

Introduction

Grapevine Trunk Diseases (GTD) are some of the major concern for the vineyard today. After exhibiting chronical foliar symptoms, grapevines can die by apoplexy within only few days (Bertsch et al., 2013). A range species of fungi was described to be associated with the apparition of early symptoms GTD. Two major places of contamination are described. Contaminations can occur in nurseries or in vineyard (i.e. pruning wounds). Currently, disease management in nursery mainly uses fungicides in order to sanitise propagation material. However pesticide use represents both an environmental and a social concern. In vineyard, since banishment of sodium arsenite, no fully efficient treatment is proposed to farmers. It is well known that ozone dissolved into water is a powerful disinfectant with no remanence (Khadre et al., 2004). The main goal of this study was to test the efficiency of this process on different fungal species associated with GTD.

Material and methods

***In vitro* tests**

Eighteen strains of four different species associated with GTD were selected (*Phaeomoniella chlamydospora*, *Botryosphaeria obtusa*, *Phaeoacremonium aleophilum* and *Botryosphaeria parva*). Fungal species were maintained on Malt Extract Agar (26 °C in dark) for at least three weeks. Spore or mycelium suspensions were prepared. Ozone dissolved into water or autoclaved demineralised water (control modality) was applied on spore/mycelium suspensions. Suspensions were then plated on agar medium. Germinating spores or developing mycelia were observed after incubation at room temperature for five days.

***In vivo* tests**

We tested the anti-fungal property of ozone dissolved into water on grapevine plants previously inoculated with fungal spore suspensions. Cuttings of *Vitis vinifera* Cabernet-Sauvignon clone 15 were grown under greenhouse conditions for two months prior to inoculation. Then, the plants were drilled until the vascular channels. In each injury, plants received 20 µl of spore suspension (105 spores/ml) of *P. aleophilum*, *P. chlamydospora* or a mixed suspension containing 105 spores/ml of each fungus. Immediately after inoculation, infected wounded damages were treated with 20 µl of

ozone dissolved into water (4.5 $g.m^{-3}$ according the Henry's law). The fungal development was evaluated 4, 6 and 9 weeks after inoculation by q-PCR.

Results and discussion

***In vitro* tests**.

The anti-fungal properties of ozone dissolved into water were firstly verified by the level of fungal development on media agar. Solution of ozone dissolved into water presented a complete sporicide effect. Indeed, no spore germinated in ozonated treatments whereas water treated controls normally developed. We observed a different impact of ozonated water on spores compared to mycelia. The anti-fungal property on *B. obtusa* and *B. parva* mycelia did not show black/white type of data. Results revealed anti-fungal efficiency on mycelia regeneration after treatment with ozone dissolved into water. However, we saw differences of efficiency between strains.

***In vivo* tests**.

The anti-fungal abilities of ozone treatment were secondly assessed by quantification of *P. aleophilum* DNA in woody tissues (via qPCR). Four weeks after inoculation, the difference between control and ozonated modality was not statistically significant. However nine weeks post-inoculation, we quantified 50 % less fungal DNA in plant treated with ozone dissolved into water compared to the water treatment. Indeed, ozone treatment strongly reduced the source inoculum present in the injury, resulting in a 50 % decrease of the number of *P. aleophilum* copies per ng of total DNA quantified nine weeks post treatment (p-value < 0.05) (Pierron et al., 2015). Regarding the experiments with *P. chlamydospora* and with *P. aleophilum* and *P. chlamydospora* mixed, like the experiments are still running, the results should be discussed during the congress.

In conclusion, ozone treatment has an anti-fungal effect against spores of fungi associated with GTD we tested. On mycelia, the capabilities of ozone dissolved into water should be optimised. These results suggest that this proposition can be considered like a part of innovative management practices to reduce the fungal pressure. Finally, we consider that ozone dissolved into water may be a tool among an anti-fungal IPM in vineyard.

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The FEM grapevine breeding program for pathogen resistances: towards a sustainable viticulture

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Highlights

- Since 2011, the FEM grapevine breeding activity follows two main motives: the introgression of novel resistance to the pathogens of primary importance into vinifera background; the pyramiding of characterised resistance-associated (R) loci into unique superior genotypes, donors of durable resistances

Introduction

Downy mildew (DM) and powdery mildew (PM) are the two most important plagues affecting viticulture. Both reduce fruit quality and yield, either by direct infection of berries or as negative results derived from leaf infections. Their control is based on the massive use of fungicides, leading to problems such as environmental pollution and pathogen resistance development. The use of grapevine varieties showing durable resistance to DM and PM is a promising strategy.

Within the FEM grapevine breeding program, the selection process has been based on the major need for innovation raised by grapevine growers. During the past years, this request has been addressed to increase the complexity of wines, while in the last decade the need for new varieties (mid-) resistant to pathogens has emerged. Lately, the FEM germplasm collection has increased its number of acquisitions in order to employ unknown and to valorise known genotypes as parents into the disease resistance (pre-) breeding program.

Material and methods

Genetic material. A total of 264 grapevine accessions acquired from (non-)European breeding programs, an Italian private breeding platform and wild-collected in north-eastern America during 2011 were studied. Most were phenotyped for DM and PM resistance, while all were genetically characterised at 190 microsatellite markers well-scattered across the grapevine genome.

Genotyping. Firstly, nine reference markers were used for the true-to-type identification through international and private databases, where feasible. Secondly, in order to validate the available pedigree information and to infer new relationships, the 50 most informative microsatellite markers were selected and analysed with Cervus v.3.0.7 software (Kalinowski et al., 2007). Finally, 12 R-loci, as well as the flower sex and the monoterpen content loci, were screened in the entire sample set (VIVC, 2016).

Phenotyping. Within the overall genetic material, 100 accessions present at FEM were evaluated for their degree of resistance against DM and PM, through *in vitro* leaf disc bioassay and *in vivo*

pathogen inoculation on potted plants respectively. DM symptom annotation was performed based on three parameters: Severity (percentage of the disc area showing symptoms of sporulation), Incidence (number of discs with sporulation/total number of discs), according to OEPP/EPPO (OEPP/EPPO, 2001), and the OIV 452-1 descriptor (OIV, 2009). PM symptoms were evaluated based on the foliar OIV 455 descriptor.

Results and discussion

The overall genetic material resulted divisible into five classes: I. known and related (38 %); II. unknown and related (21 %); III. known and unrelated (10 %); IV. unknown and unrelated (23 %); and V. redundant (8 %). Within these classes, the most important resulted to be II and IV. The unknown and related genetic materials holds a great potential for the breeding activities of FEM and other institutes, since they provide indications about already known or unidentified R-loci that can be traced in their future progeny. The unknown and unrelated genotypes, obtained from abandoned breeding programs or derived from wild-collections, play a relevant role as exclusive genetic resources; in fact, once characterised through the identification of novel Quantitative Trait Loci (QTLs) associated to features of interests, they will provide molecular information for the ongoing Marker-Assisted Breeding, first in the introgression and thus in the pyramiding sub-programs.The 100 accessions analysed at phenotypic level are representative of all classes, except for V. (redundant). The *in vitro* evaluation demonstrated a wide phenotypic variability in terms of DM resistance. In particular, 41 accessions resulted highly resistant and will be employed as a direct source of resistance in breeding sub-programs with the objective of releasing novel sustainable grapevine cultivars presenting also good fruit/wine quality. Regarding PM, only 18 out of the 100 *in vivo* tested accessions resulted resistant. This demonstrates that PM is rarer than DM resistance and highlights the importance of discovering new genetic sources.

In conclusion, novel and exclusive genetic resources were identified, providing peculiar and preparatory information to ongoing and forthcoming Marker-Assisted (pre-) breeding programs. This specific genetic material - *in vitro* healthy maintained and propagated - contributed to enrich the FEM breeder Golden Book, a dynamic collection of parental genotypes suitable for crossing combinations with different purposes. In the next coming few years, FEM is going to launch eight new varieties which are mid-resistant to DM and PM.

Acknowledgements

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Microbial ecology of resistant grapevine genotypes sheds light on biocontrol prospects of *Plasmopara viticola*

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Highlights

- Distinct microbial communities, in terms of composition and diversity in accordance to their host's level of resistance, developed out of soil influenced by unspecific populations
- Bacteria isolated from moderately resistant host plants showed efficacy up to 70 % in reduction of *P. viticola* symptoms

Introduction

Economically important grapevine cultivars classified as *Vitis vinifera* subsp. *vinifera* are susceptible to downy mildew. However, *Vitis* species endemic in North America are more resistant, most likely because of their longer coevolution with the obligate biotrophic oomycete *Plasmopara viticola* causing the disease. Leaf surfaces are complex micro-ecosystems harbouring pathogens but also a majority of commensal or mutualistic bacteria that positively affect the health and growth of their hosts.

Material and methods

By assessing differences in microbial ecology between susceptible and resistant *Vitis* species the current work aims in gaining a better understanding of plant genotype effects on associated phyllosphere microbiomes. The alphaproteobacterial leaf microbiome of seven different *Vitis* accessions during spring and autumn samplings was examined by Illumina sequencing of 16S rRNA gene amplicons and processed by bioinformatics tools. Simultaneously, we tested 700 bacterial antagonistic candidates isolated from all *Vitis* genotypes for their antagonistic potential against *P. viticola* and visualised specific strains ad planta by fluorescence in situ hybridisation in combination with confocal laser scanning microscopy.

Results and discussion

Alphaproteobacterial community composition was dynamic from spring to autumn with richness of species and diversity significantly higher in spring and two dominant families prevailing at the end of the growing season: Sphingomonadaceae accounting for 56 % and Methylobacteriaceae for 35 % of the core microbiome were shared by all samples. Despite the overall decreasing microbial diversity, distinct communities in accordance to the *P. viticola* resistance levels of their hosts were shaped during the vegetation period as shown by beta diversity metrics. Clustering of significantly co-occurring taxa revealed a microbial network that was denser and more centralised in autumn. While in spring 13 % of the taxa represented in the network were identified to prevail on a specific

grape genotype, 58 % were closely related to one particular host plant in autumn. This is in accordance to our findings that indicate seasonal succession towards differential community structures dependent on the host plant. From highly diverse, soil influenced but unspecific communities in spring we observed an alteration towards distinct alphaproteobacterial populations specialised to their host plant's phyllosphere. Most promising bacterial candidates isolated from moderately resistant *V. vinifera* subsp. *sylvestris* showed efficacies of up to 70 % in reducing disease severity on susceptible leaf discs. Fluorescence in situ hybridisation in combination with confocal laser scanning microscopy revealed inter-kingdom interactions between inoculated bacteria and encysted *P. viticola* zoospores on stomata. We demonstrate that resistant *Vitis* species are a potential source for microbial antagonists against grape downy mildew and that a better understanding of host-specific effects on their respective phyllosphere communities may contribute to find sustainable plant protection strategies for viticulture.

Ontogenetic resistance of grapes: A chance to reduce fungicides in vineyard and residues in wine?

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Highlights

- Scientific studies have shown that the susceptibility of grapes against powdery and downy mildew decreases when berries reach pea size
- Trials were carried to investigate the effects of the last fungicide treatments
- The main finding is, that it is possible to spray only the upper canopy zone at the last application, without the grape zone
- This method offers many sustainable benefits

Introduction

Investigations of Stark-Urnau and Kast (1999) and Gadoury et al. (2003) indicated that growing grapevine bunches are highly susceptible to infections of *Erysiphe necator* from one week before flowering time to the date when the berries reach pea size. No functional stomata are present at pea size. Only a few functional stomata are situated on the rachises, which could be damaged by a high number of infectious spores (Hill, 2012). Considering the mentioned findings the following research questions were examined in detail:

- Is it possible to reduce pest control residues if only the upper leaf wall (canopy zone without grapes) is treated?
- Are there differences between plant protection products, e.g. between copper or organic fungicides against powdery mildew?
- How strong are the biological effects of the last two treatments?
- Are the last two treatments necessary at all?
- Is it possible to reduce the amount of fungicides without any risk, if only the upper leaf wall is treated?

Material and methods

A total of 29 experiments from 2009 to 2016 were performed and evaluated as field trials in Weinsberg and Freiburg in order to answer these questions and offer new solutions to the winegrowers. The experiments in Weinsberg were made with a tunnel sprayer and evaluated according to EPPO- Guidelines. The trials in Freiburg were carried with plant protection sprayers, which are used in practice and with a tunnel sprayer. Planning, implementation and evaluation was done according to the EPPO- Guidelines.

Results and discussion

No residues of all applied fungicides could be found in wines in 2009, by spraying only the upper leaf zone (and not the grape zone). In 2010 residues on grapes were investigated. Only the spraying

of the fungicides on grapes at the last application led to residues in extremely low values on grapes (Bleyer and Kast, 2013).

- The different fungicides had no practical relevance on the infestation of downy mildew (Bleyer et. al., 2016).
- The omission of the last treatment caused no relevant attack on grapes by downy and powdery mildew! But leaf infestation was significantly higher in some years, but this did not lead to any measurable loss of quality!
- In all trials no significant differences of infestation were found between the variant “treatment of the whole canopy zone” and the “treatment of the upper canopy zone without grapes”.

Currently the recommendation is not to omit the last applications! But the suggestion is to spray only the upper canopy zone in the last spraying.

Winegrowers should first test this method in low-risk vineyards. Low-risk areas are not particularly vulnerable to downy and powdery mildew. If this system proves to be successful, it can be extended to other vineyards.

The advantages of this method are obvious in economic and ecological terms:

- Lower residues with plant protection products on the grapes
- Reduced consumption of plant protection products
- Reduced time requirements (set-up times)
- Lower raw material consumption (fuel, water, etc.)
- Lower costs
- Better image

These results proved that the importance of the last treatment is often overestimated in practice. It is still more important to prevent the spread of diseases in the early stages of the epidemic.

Further experiments are planned for the next years, in order to elaborate further findings on this important question.

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Biomarkers identification in 'Bianca' grapevine leaves after *Plasmopara viticola* infection

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Highlights

- Identification of early stage biomarkers of infection in Bianca grapevine after *Plasmopara viticola* infection
- Vitis-*Plasmopara viticola* interaction causes change on primary metabolism within the first 48 hours and thereafter a modulation of secondary metabolism at 48, 96 hours post infection

Introduction

Downy mildew (*Plasmopara viticola*) is one of the most destructive diseases of the cultivated species *Vitis vinifera*. Due to their levels of resistance, *Vitis* spp. from North America have been crossed with *V. vinifera* to introgress resistance. The use of grapevine varieties showing durable resistance to downy mildew is a promising strategy to control the disease (Bisson et al., 2002). *Vitis-P. viticola* interaction is still poorly understood from metabolic point of view therefore applying a metabolomic approach can extended on how knowledge how the plant system is disrupted after stress and let us known which metabolites more affected, and are probably involved in resistance mechanisms. In this work we evaluated metabolic perturbation in Bianca leaf discs after infection with a suspension of *P. viticola*; the aim was to discover new early stage biomarkers.

Material and methods

Plant pathogen interaction was evaluated using grapevine plants from the 'Bianca' cultivar. Grapevine cultivar Bianca is an hybrid between Villard Blanc and Bouvier, that shows a good resistance to downy and powdery mildew (Bellin et al., 2009) For each plant, the third, fourth and fifth fully expanded leaf was detached, rinsed with ultrapure water and 1.1 cm diameter discs were excided from each leaf with a cork borer and placed randomly onto wet paper in Petri dishes with the abaxial side up. Discs were sprayed with *P. viticola* inoculum suspension at 1x106 sporangia/ml.

We investigated primary and secondary metabolism at 12, 24, 48 and 96 hours post infection (hpi) using methods of identification and quantification for lipid (LC-MS/MS), phenols (LC-MS/MS), primary compounds from acids, amino acids, amines/others, sugars (GC-MS), and semi-quantification for volatiles compounds (GC-MS).

Results and discussion

We were able to identify and quantify or semi-quantify 176 compounds belonging to acids, amino acids, amine and other and sugars, carnitines, sterols, fatty acids, glycerolipids, glycerophospholipids, sphingolipids and prenols; benzoic acid derivates, coumarins, phenylpropanoids, dihydrochalcones, flavones, flavan-3-ols, flavonols, stilbenes+stilbenoids and other phenolics, volatile acids, alcohols, aldehydes, benzenoids, ketones, terpenoids, other VOCs and unknown VOCs.

Starting from the construction of the general Principal Component Analysis (PCA) we noticed changes in the second dimension at 24, 48, and 96 hpi based on the two different conditions (infected and control). Focusing on each class of compounds we found changes during the first 24-48 hpi regarding the primary metabolism in response to the pathogen infection with a modulation of some metabolites belonging from lipids, amino acids, acids and sugars. Afterwards, the secondary metabolism was affect more strongly by the pathogen with a change in volatile compounds at 48-96 hpi.

The classes of compounds more affected after the infection turns out to be phenylpropanoids, flavonols, stilbenes and stilbenoids at 96 hpi. The time course of appearance of viniferins after the infection in our study is in full agreement with the sequential mechanism, extensively discussed by Bavaresco et al. (2012), with the progress from the initial synthesis of resveratrol, towards the formation of dimers and then of the higher oligomers. The importance of the oligomers of viniferin is further confirmed by the values of concentration required to induce inhibition of mildew development recently reported by Gabaston et al. (2017).

Based on our results we can assume that all these compounds significantly differentiate in infected samples have a role in Bianca-*P. viticola* interaction some of them we know as biomarker of resistance from previous studies and some of them might have a role as putative biomarkers in Bianca leaf discs after *P. viticola* infection. It is up to our knowledge the first time that an extensive metabolites study has been undertaken using a hybrid grape variety inoculated with *P. viticola*, with the aim to find early stage biomarkers. This work offers some important aspects in the study of grapevine *P. viticola* interaction in discovering new ever reported biomarkers. These results can be a starting point to better understand the grapevine resistance and can lead to the discoveries of new mechanisms of plant-pathogen interaction between grapevine and *P. viticola*.

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Impact of elevated CO_2 concentration on interactions between *Vitis vinifera* L. and *Plasmopara viticola*, the causal agent of downy mildew

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Highlights

- *Vitis vinifera-Plasmopara viticola* interaction was studied under elevated CO_2 using a FACE system
- Stomatal density was increased under elevated CO_2 concentration in cv. Riesling leaves
- No change in disease severity on cv. Riesling leaf discs under elevated CO_2 was evident
- *P. viticola* significantly affected the grapevine transcriptome under elevated eCO_2

Introduction

Increasing evidences from the literature are proving the importance of elevated CO_2 concentrations (eCO_2) on plant-pathogen interaction. The impact can be indirect through changes in the plant's physiology/anatomy or direct through changes in the pathogen's virulence. Different impacts of eCO_2 on the severity of plant diseases were reported; however, these impacts differ between pathosystems. Here, we report on the impact of eCO_2 on the *Vitis vinifera-Plasmopara viticola* pathosystem. *P. viticola* is the causal agent of grapevine downy mildew that may cause severe economic losses in cool climate viticulture.

Material and methods

A grapevine free-air carbon dioxide enrichment (FACE) system (480 ppm; investigations on mature acclimated vines) as well as climate chambers equipped with CO_2 (750 ppm; investigations on leaf discs from acclimated vines) were established to study the effects of eCO_2 on *V. vinifera-P. viticola* pathosystem. Two varieties were used (cv. Riesling and cv. Cabernet Sauvignon). From the fungal side, disease severity was assessed, while other parameters such as zoospore vitality and oospore germination are currently studied. On the plant side, a pathogen-relevant morphological characteristic (stomatal density) was investigated. In addition, transcriptomes of *P. viticola* inoculated vines (24 hpi and 72 hpi) grown under eCO_2 were analyzed in order to understand grapevine response to infection with *P. viticola* under anticipated increasing eCO_2.

Results and discussion

There was no significant increase or decrease in disease severity on leaf discs between elevated and non-elevated CO_2 rings except from one eCO_2 ring. There was a significant increase in the number of stomata on the abaxial side of cv. Riesling leaves under eCO_2, while stomatal density did not change in cv. Cabernet Sauvignon leaves. Transcriptomes of inoculated vines under eCO_2 revealed a change in transcription. Grapevine showed an overall different transcriptional response to infection with *P. viticola* under elevated and non-elevated CO_2 as well as at 24 hpi and 72 hpi. A total of 17,421 genes were transcribed of which 2,731 genes, corresponding to 15 %, were differentially

expressed under both infection and eCO_2. Genes coding for enzymes involved in secondary metabolism (e.g. stilbene synthase and jasmonate O-methyltransferase) were up-regulated – however, this is not enough to suppress infection at 72 hpi. Genes involved in cell wall metabolism, among others, were strongly down regulated at 72 hpi. These results can potentially explain the anticipated higher susceptibility of cv. Riesling to *P. viticola* under eCO_2. Results obtained from the grapevine-*P. viticola* interaction under eCO_2 can be used to refine/update the existing downy mildew forecast model by integrating a CO_2 sub model.

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Botrytis bunch rot control in South-West France vineyards using a novel bacterial biological control agent and two biocontrol registered products

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Highlights

- Two products, commercialised against *Botrytis cinerea*, were tested during three seasons. Both achieved disease reductions, although highly variable. The product based on potassium bicarbonate showed better performance than the one based on *Aureobasidium pullulans*
- The field application of the INRA bacterial strain *Bacillus ginsenghiumi* S38 during two seasons showed promising efficacy results

Introduction

The grey mould or Botrytis bunch rot (BBR), caused by *Botrytis cinerea*, is one of the main fungal diseases of grapevine, causing remarkable damage both in terms of yield and quality in temperate regions. The current constraints for using synthetic fungicides in agriculture and consumer preferences for more environmentally friendly practices are fostering research in alternative control strategies since a few decades. Biological control using microbial antagonists is regarded as one of the most promising strategies. A few products are commercially available for use in viticulture against BBR, whereas researchers continue to develop biocontrol yeast, fungal or bacterial strains. The aim of this study was to test the efficacy of different alternative strategies to synthetic fungicides, based on commercialised products or novel biological control agents (BCAs) in developmental stages, for the control of BBR in Bordeaux vineyards.

Material and methods

During three growing seasons, efficacy of two commercial products based on potassium bicarbonate (Armicarb – de Sangosse) and the yeast-like fungus BCA *Aerobasidium pullulans* (Botector – de Sangosse) were evaluated in the frame of the Aquitaine region organic viticulture network ("Resaq VitiBio"). During the 2014, 2015 and 2016 growing seasons, these products were tested in a whole of 20 (Armicarb) or 25 (Botector) experimental plot*year combinations (cv. Merlot). Treatments consisted of three applications of Botector (pre-bunch closure, véraison and 21 days before harvest) or two applications of Armicarb (véraison and 21 days before harvest). Applications were carried out by vineyard manager, as well as the *Lobesia botrana* (grape berry moth) control and leaf removal. Similarly, during two seasons (2015 and 2016), the antagonistic strain *Bacillus ginsenghiumi* S38, developed at INRA Bordeaux, was tested in two experimental field sites (GF site, cv. Merlot; CHS cv. Semillon) in order to observe BBR reductions. Treatment consisted of five spray applications at key phenological stages of the bacterial strain, along with the additives Sticman (CHS

site in 2015 and 2016) or Fungicover (GF site in 2016). During both seasons, efficacy of the *B. ginsenghiumi* S38 was compared with other bacterial strains in developmental stages as BCAs.

Results and discussion

The field experiments conducted in the 2014, 2015 and 2016 seasons with the two commercialised products evidenced significant BBR incidence reductions in 10 % (Armicarb) and 12 % (Botector) of the studied cases (plot*year). Moreover, a trend in incidence reduction was observed in 60 % and 44 % of the cases, for Armicarb and Botector, respectively. Concerning BBR severity, only the Armicarb treatments significantly reduced BBR severity in 20 % of cases, whereas a trend of BBR severity reduction by Botector treatments was observed in 32 % of the studied cases. The application of both products represented a short advantage compared to the application of cultural practices alone. Thus, the results showed a variable efficacy of both products, although it may be linked to the low BBR pressure during the three seasons (5-10 % maximum overall severity). The Armicarb product showed a better performance compared to Botector in similar experimental conditions and with one spray application less during the season. This product might represent a good alternative to synthetic fungicides, notably for late season applications against BBR, in the climatic and cultivar conditions of South-West France.

The five applications *B. ginsenghiumi* S38 significantly reduced BBR incidence at harvest in 2015 by 72 % in one of the experimental sites (GF) but not in the other (CHS). Severity was reduced by 71 %, although this effect was not significant. Inversely, at harvest in 2016, no significant differences were detected between the control and treatments in the GF site, whereas, in the CHS site, incidence was significantly reduced by 13 % in the treatments including *B. ginsenghiumi* S38, compared to the untreated control. The partial efficacy demonstrated by this bacterial strain confirmed its potential as a new BCA, encouraging further research in field control of BBR with this bacterial antagonist.

Overall, results also highlighted the variability in biological control of BBR, including commercial formulations already available in the market. These products may represent an advantage in certain situations; however, more research is needed in order to find the optimal application strategy of each product to maximise its efficacy, as well as to develop new BCAs, like the *B. ginsenghiumi* S38.

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Endophytic yeasts in shoots of healthy and esca diseased grapevines

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Highlights
- Little differences in yeast community between healthy and esca diseased plants.
- *Aureobasidium pullulans*, a yeast with known antagonistic effects against several fungi, was one of the most frequently detected yeasts

Introduction

Esca disease of grapevine is one of the most important diseases in winegrowing regions of Germany, Spain, France, and Italy. The incidence of esca depends on several factors like environmental conditions, the age of the plants, and the cultivar. Until now, no efficient control agents are available (Fischer, 2002). Endophytic fungi moved towards the centre of research in plant pathology, because of their ability to protect host plants from biotic and abiotic environmental stress, and also to increase tolerance and resistance to pathogens, insects, herbivores and nematodes. However only few works are dealing with yeast-plant associations and even less is known about fungi in grapevine.

The aim of this work was to isolate, identify and analyse endophytic yeast communities of grapevine and to estimate their differences between healthy and esca diseased plants

Material and methods

Shoots from the grape variety Riesling, were collected in two vineyards (“Wehlen” and “Bekond”) in Germany. From each trial site 6 (“Bekond”) or 10 plants (“Wehlen”) of each diseased a healthy vines were sampled. From each plant three shoots were cut and each shoot was divided into three subsamples (4-7 cm long internodes). The subsamples were sealed with barrel wax, surface sterilised with sodium hypochlorite (12 % active chloride) for 15 minutes and washed twice with sterile water. To extract the endophytic yeasts from the vascular system the subsamples were cut at both ends, the vascular system was rinsed with ¼ ringer solution. This liquid was collected in sterile 15 ml tubes and 100 ml of the liquid were plated in triplicates onto PDA plates. Yeast colonies were counted and the colony forming units (CFU) cm³ xylem was determined. To identify the yeasts, the colonies were grouped into different morphotypes based on color and structure of the colony (surface, texture, size and shape of the cells). The D1/D2 region of the 26s rDNA from representative pure cultures of each morphotype was sequenced (Kurtzman and Robnett, 1998). Sequences were blasted against GenBank at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and a phylogenetic placement with an appropriate selection of reference sequences of yeast strains was generated.

Results and discussion

Comparing healthy and diseased plants, the mean average of the total CFU counts [CFU cm³ xylem] showed no significant differences: 2.93 x 10^4 CFU cm³ in healthy plants (n=42) and 3.54 x 10^4 CFU cm^3 in diseased plants (n=42).

Across all samples a total of 16 morphotypes were defined, and 229 representative isolates were sequenced. After phylogenetic placement these 16 morphotypes were placed into 29 different taxa. Three of the identified taxa belonged to Ascomycota and the other 26 species belong to Basidiomycota. The morphotype NMT32, identified as *Filobasidium* sp. was only found in the trial site Bekond but here with very high CFU counts. Other morphotypes were very rare like morphotype NMT28/30. Sequences of this morphotype belonged to two taxa: *Rhodotorula* cf. *nothofagi* (40 %) and *Sporobolomyces* sp. (60 %). Morphotype NMT 5A was identified as *Aureobasidium pullulans* and was the most abundant morphotype. *Aureobasidium pullulans* was found in both, healthy and esca diseased plants. The antagonistic role of this yeast is well studied (Bencheqroun et al., 2007), but in this study no significant correlation of this yeast with the sanitary status of the plant was observed. However, the abundance of this yeast was higher in the diseased plants. Other commonly isolated yeasts were *Rhodotorula* spp., *Sporobolomyces* spp., and *Cryoptococcus* spp. There were no significant differences between healthy and diseased plants in the total CFU counts. When comparing healthy and diseased shoots there were significant differences between the endophytic yeasts communities, but only few species were responsible for these differences. The basidiomycete yeast *Auriculibuller fuscus*, and the ascomycetes *Tetracladium* sp. 1 and *Tetracladium* sp. 2 were found only in diseased samples. However, they were only detected rarely.

Many of the detected yeasts are well known as phylloplane inhabiting epiphytes but can, as endophytes, also invade the vascular system. Some of the detected yeasts can promote plant growth. For example, *Sporobolomyces roseus* – one of the most abundant yeasts in our study – is known to enhance wheat yield by 30 %, and also a special strain of *Rhodotorula* increased the growth of tomato and fruit yield (El-Tarabily and Sivasithamparam, 2006).

To understand the ecological pattern of these endophytes and their interactions with the host is of high importance. Understanding the interactions of endophytic fungi with plant-fungal pathosystem will be a giant leap towards an effective control of grapevine trunk diseases. The attention being focused on endophytic yeasts is increasing, although more investigations are necessary to understand the ecological role of these yeasts.

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Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture"

Novel tools and new challenges for IPM in viticulture

Poster Session 1

New knowledge and solutions against pathogens and their vectors

Distribution of symptoms associated to GPGV and its potential vector *Colomerus vitis* in North-eastern Italy

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Highlights

- The distribution of GPGV symptomatic vines and of its potential vector *Colomerus vitis* was studied in two vineyards
- The distribution of GPGV grapes was aggregated in both vineyards while that of *C. vitis* was partially correlated with that of GPGV symptomatic vines

Introduction

Grapevine Pinot Gris Virus (GPGV) has been identified for the first time in Italy in 2012 (Giampietruzzi et al., 2012). Later, it has been detected in several geographic areas and for a number of varieties representing a potential threat for grapevine production in Europe and elsewhere (e.g., Fan et al., 2015; Al Rwahnih et al., 2016). Transmission trials showed that eriophyoid mites (*Colomerus vitis*) collected from infected grapes were able to transmit GPGV to healthy grapevines (Malagnini et al., 2017). Observations were conducted in Trentino vineyards to identify trends in GPGV epidemiology. In particular, the spatio-temporal distribution of GPGV symptomatic grapes was studied in two vineyards for two subsequent growing seasons. Later, these observations were coupled with those on *C. vitis* assuming the existence of a potential relationship between eriophyoid mites and GPGV spread.

Material and methods

The spatial distribution of GPGV symptomatic grapes has been studied in three subsequent growing seasons (2013, 2014 and 2015) in two vineyards located in the Trento Province, North-eastern Italy. In 2015 we also analysed the spatial distribution of *C. vitis*. Tests of non-randomness ($\alpha = 0.05$) based on the overall index of aggregation was performed on *C. vitis* and GPGV symptoms data. Indices of local aggregation were interpolated using kriging and mapped. We also quantified the degree of spatial association among the different variables considered.

Results and discussion

The distribution of GPGV symptomatic grapes was aggregated in both vineyards while that of *C. vitis* in one vineyard. The distribution of GPGV symptomatic grapes in subsequent growing seasons were associated each other's. In one of these vineyards the distributions of *C. vitis* and of grapes showing fresh GPGV symptoms were associated. Spatial distributions of GPGV symptomatic grapes over the seasons were substantially stable showing a slight increase of symptoms. However, *C. vitis* distribution was only partially associated with that of GPGV symptomatic grapes. Further studies are requested to improve our knowledge on relationships between vectors and virus spread.

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Grapevine virus diseases in South Tyrol – a survey

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Highlights

- Infectious degeneration caused by the Grapevine fanleaf virus (GFLV) is the major virus disease in the Girlan/Cornaiano viticultural area
- The dagger nematode *Xiphinema index* was detected in many of the shallow soils of the affected vineyards
- Preliminary work for a field trial with the goal to apply control measures against the vector nematodes has been started

Introduction

Grapevine diseases caused by viruses have increased over the past years and growers in South Tyrol – in particular in Girlan/Cornaiano – suffer severely from yield loss. To estimate the impact of various viruses and to find reasons for their recent expansion, a survey in three representative areas in the Überetsch/Oltreadige viticultural district (South Tyrol, Northern Italy) was conducted. The main goals of this survey were to estimate disease incidence, to find possible vectors, and to explain the recent expansion of the problem.

Material and methods

Disease incidence was assessed in summer 2014 and 2015 by estimating the percentage of symptomatic plants in each of the 116 vineyards in our three test areas. Single grapevines with symptoms of viral diseases were marked, later sampled, and tested in the laboratory for the presence of virus applying the well-established ELISA method. Soil samples have been tested for the presence of vector nematodes that are known to transmit viruses. Additionally, growers owning the vineyards in the test areas have been interviewed to gather information on rootstocks, date of planting, history of the vineyard, soil type, management practices, and their felt problem with virus diseases.

Results and discussion

We found a relatively high number of vineyards with a disease incidence of more than 10 %. Symptoms could be associated mainly with infectious degeneration. The most severely affected varieties were Gewürztraminer and Sauvignon blanc. Only a few plants showed symptoms associated to the recently described Pinot gris disease. ELISA tests confirmed the presence of the causal agents Grapevine fanleaf virus (GFLV) and Arabis mosaic virus (ArMV) for the infectious degeneration and Grapevine Pinot gris virus (GPGV) for the Pinot gris disease. Other viruses were detected only sporadically and are not economically important. Soil sampling and subsequent analysis revealed the presence of the dagger nematode *Xiphinema index*, known to be an efficient vector of GFLV. The soils in the most severely affected vineyards are characterised as shallow with low pH and low humus content.

Taken together, our data demonstrate the following: vineyards where white wine varieties were planted around the year 2,000 after uprooting older Vernatsch vines in shallow soils containing dagger

nematodes suffer severely from infectious degeneration. This might be explained as follows: (i) the current varieties are more sensitive than it was the variety Vernatsch, (ii) the focus on quality is nowadays more important than it was 30 years ago, (iii) due to climate change drought stress has become more important recently, (iv) training system and management practices such as irrigation and soil working have changed during the last years.

Future actions are directed towards identifying those control measures, which are most promising to minimise the problems with infectious degeneration in South Tyrolean vineyards. For this reason, a long-term field trial where different measures are tested will be starting soon.

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New acquisition about the role of *Colomerus vitis* in the transmission of Grapevine Pinot gris virus

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Highlights
- *Colomerus vitis* could trasmit symptom and symptomless strains of GPGV

Introduction

Grapevine Pinot Gris Virus (GPGV) is a new Trichovirus associated with symptoms of chlorotic mottling and leaf deformations in grapevine (*Vitis vinifera*).

GPGV was reported in several Italian regions and in other countries around the world. Different studies conducted to associate GPGV with symptoms showed the existence of different strains of the virus responsible for eliciting or not the symptoms. Preliminary studies indicate that GPGV can be transmitted from vine to vine by the eriophyid mite *Colomerus vitis* (Pagenstecher). Acquisition and transmission by an arthropod vector is central to the infection cycle of the majority of plant pathogenic viruses. Filling the gap of information of epidemiological aspects of GPGV strais/*C. vitis* interactions would help implementing efficient strategies of control of the associated disease on grapevine. In this work we carried out transmission trials and analysed the virus to detect which strains of the GPGV is transmitted by *C. vitis*.

Material and methods

Specimens of *C. vitis* were collected in GPGV symptomatic vineyards and mites were extracted from infested buds and leaf erinea. A pool of 10 mites for sample was subjected to RT-PCR to ascertain the presence of GPGV. Total RNAs of *C. vitis* were extracted and GPGV detection was carried out by RT-PCR and real-time PCR (RT-qPCR) in order to amplify distinct GPGV genomic regions. To detect GPGV strains, PCR products were both sequenced and compared with GenBank, and digested by BAM HI.

Transmission trials were carried out under controlled conditions (22°C, 70 % relative humidity, 16:8 L:D) using GPGV infected buds and leaf erinea infested by *C. vitis*. Buds and leaf erinea were placed onto GPGV free vines. After transmission trials the appearance of erinea was observed and each plant was analysed with the above described methods to assess the presence of GPGV and qualify the strain

Results and discussion

Both strains of GPGV were found in *C. vitis* as well as in the GPGV positive vines obtained by transmission trials.

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Three years experience in France using PreDiVine DSS analysing 25 vineyards for *Scaphoideus titanus* monitoring

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Highlights

- This work presents a three-year project where 25 vineyards have been monitored in France, by means of the Decision Support System (DSS) called PreDiVine, and discusses the criteria used to evaluate the monitoring, the results and the benefits of its usage

Introduction

This work presents a three-year project where 25 vineyards have been monitored in France, by means of the Decision Support System (DSS) called PreDiVine, and discusses the criteria used to evaluate the monitoring, the results and the benefits of its usage. PreDiVine (Predicting Diseases of Vine) is a web-based and self-adaptive DSS able to predict the evolution of vineyard's pests and diseases and suggests just in time targeted treatments. In particular PreDiVine provides real-time forecast of the life stages of *Scaphoideus titanus*, vector of flavescence dorée (FD). The benefit of using PreDiVine is to decide the timing of insecticide application and the planning of in-field monitoring tasks. Phytosanitary services are able to decide, with about 2-3 weeks in advance, when wine growers have to perform the chemical treatments.

Material and methods

During the three-year project (2014-2016) we analysed the field observations of 25 vineyards associated with 17 reference meteorological stations. We conducted a preliminary site analysis to understand whether the optimal conditions to perform computations with PreDiVine were met. PreDiVine performed the computations with a calibration based on previous years where observations of *S. titanus* and meteorological data were given. The goal was to understand whether between the three seasons there have been improvements in Predivine forecast.

Results and discussion

In particular, we identified some criteria in order to evaluate the vineyards (by means of distance from the weather station, historical *S. titanus* observation and whether the vineyard was under treatment or not), the weather stations (by means of historical data and quality of the data), and the feedback reported by the users/scouts.

Finally we assessed the PreDiVine performances by analysing the results in order to identify those factors that had an impact in the life stages forecast in comparison with the observed samples. Some of these factors include the distance of the weather station from the site, the sample protocol frequency, the *S. titanus* population and the vineyard was already under treatment.

We observed that, during those three years, we had a continuous increase in the accuracy of PreDiVine predictions with some minor exceptions in some sites between 2015 and 2016.

PreDiVine proved to be a robust DSS able to provide accurate predictions already starting from a zero-knowledge condition of the site and, at the same time, being flexible by re-using the data collected every year to self-improve the system calibration for future real-time predictions.

Acknowledgements

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Longevity and reproductive profile of *Scaphoideus titanus* Ball adults reared under controlled conditions

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Highlights

- Despite the economic importance of *S. titanus* many aspects of its biology are poorly known. These data are important for a correct pest management
- Our study improved the knowledge on the repruductive biology of *S. titanus*
- The results suggest that *S. titanus* prefers relatively mild climates and they could contribute to explain its distribution area in Europe and undestand its invasive behavior

Introduction

The invasive leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) was introduced into Europe in the past century and has become one of the most important pests in the European viticulture since when its role as epidemic vector of Flavescence dorée (FD) was ascertained. It has colonised many of the most important European viticultural areas from Portugal to the Black Sea and it is subjected to mandatory insecticide treatments wherever FD is present. Despite its economic importance, many aspects of its biology, in particular the biology of the adults, are still poorly known. These data are important for a correct pest management, and are crucial for the development of physiologically based demographic models, an important tool to predict the phenology, age structured dynamic, and distribution of the species across wide geographic areas.

Material and methods

In order to study biology of *S. titanus* adults, couples were formed a few days after adult emergence. The couples were caged and reared on vine seedlings under controlled conditions, at constant temperatures ranging from 16±1 °C to 30±1 °C. The longevity, fecundity, egg oviposition duration and rate were recorded.

Results and discussion

As expected the maximum average longevity, 112 days, was registered at the lowest temperature. Adult lifespan was greater than one month up to 24 °C, but it dropped to 12 days at 30 °C. The results confirmed that the data reported in the literature underestimated both the fecundity and the duration of the egg laying period. The females passed through a long pre oviposition period, began laying eggs two to four weeks after emergence, and continued laying eggs until death. The maximum fecundity was registered at 21°C, with an average of 50.8 eggs per female. The number of eggs decreased abruptly above 24 °C to almost zero (0.24 eggs/female) at 30 °C. Conversely, at 15 °C the fecundity was greater than 20 eggs female^{-1}. At all temperatures, except at 30 °C, the oviposition rate was between 0.5 and 1 egg/day/female. These data suggest that *S. titanus* prefers relatively mild climates and they could contribute to explain its distribution area in Europe.

Comparative transcriptome profiling of two *Vitis vinifera* varieties with different level of resistance/susceptibility to Flavescence dorèe

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Highlights

- The comparison of the transcriptomic response of two grapevine varieties with different levels of susceptibility to Flavescence dorèe highlighted both passive and active defence mechanisms against the vector and/or the pathogen in the partially resistant variety, as well as the repression of a defence reaction against the insect in the susceptible variety in the presence of the phytoplasma

Introduction

Flavescence dorée (FD) is a very serious grapevine disease, caused by phytoplasmas transmitted by the leafhopper *Scaphoideus titanus* (Bertaccini, 2015). The disease and its vector are included in the quarantine pest list in Europe. Despite many efforts, FD is still expanding and causing major economic losses in grapevine production. Variability in grapevine susceptibility to FD is well known from field observations and laboratory trials (Eveillard et al., 2016). These epidemiological data suggest that specific genetic features are present in the different grapevine cultivars, which are associated to resistance to the phytoplasma and possibly its vector.

The present research aims to identify the components of the molecular response activated in grapevine in the early stages of phytoplasma infection, by means of the comparison of the transcriptomic changes during the interaction "grapevine – vector – FD phytoplasma" in two vine varieties with opposite behaviour to FD infection.

Material and methods

Two grapevine cultivars were used: Chardonnay, very susceptible to FD, and Tocai friulano, partially resistant. *In vitro* micropropagated plantlets of the two varieties were experimentally infected with FD phytoplasma using insect vectors reared in controlled conditions and experimentally infected by feeding on FD-infected grapevine leaves. Three theses per variety were compared: healthy plantlets (mock), healthy plantlets with healthy vectors, plantlets with infected vectors.

Sequencing of the whole transcriptome (RNAseq) of the two varieties was carried out by means of NGS technology (Illumina). Two to three biological replicates for each genotype/condition/timing combination were used. Read counts were generated from Bam alignment files with HTSeq software. Data normalisation and call of differentially expressed genes was implemented with DESeq2 version 1.2.8 Bioconductor (R) package by setting to local, False Discovery Rate (FDR) threshold to 0.05 and enabling independent filtering.

GO enrichment analyses were conducted with the GOseq Bioconductor package. Data preparation for GOseq analysis was as previously reported (Biselli et al., 2015), apart from the use of an FDR cutoff of 0.1 for GO enrichments.

Results and discussion

The constitutive transcriptomic profiles of Chardonnay and Tocai friulano were largely distinct; indeed 6,233 differentially expressed genes were identified in the comparison between the mock theses. Functional analysis showed a constitutive higher expression in Tocai friulano of many defence related genes compared to Chardonnay. In details, significant differences were related to genes involved in signalling transduction pathways, in production of secondary metabolites and pathogen-related (PR) proteins, some of them with a possible role in the attraction/repulsion towards insects (War et al., 2012). These results suggest the existence of constitutively expressed passive defence mechanisms against the insect and/or the pathogen owned by Tocai friulano.

The feeding of the healthy vector produced robust transcriptomic changes especially in Chardonnay, where the expression of many defence-related genes increased significantly. The most relevant classes of genes modulated in Chardonnay were related to: signalling pathways governed by protein kinases and calcium, hormone-related pathways, in particular jasmonic acid, ethylene and abscisis acid, transcription factors, and genes involved in the cell wall metabolism, all genes known to be involved in the resistance against insects (Bodenhausen and Reymond, 2007).

In the early stage of the FD-infection, obtained by means of infectious vectors, the partially resistant variety (Tocai friulano) showed high transcriptomic changes in respect to the other conditions, due to the interaction among plant, phytoplasma and insect: an active defence reaction was registered, mainly based on the modulation of several genes involved in the cell wall modification, as known in resistant hosts (Guest and Brown, 1997). On the opposite, in Chardonnay, the most susceptible variety, the response was delayed and mainly based on the modulation of the phenolic metabolism, with an increased expression of many genes coding for stilbene synthase. The jasmonic acid- and ethylene-mediated defence reactions activated by Chardonnay following the healthy insect feeding were not detected in presence of the phytoplasma-infected vector. Thus, it can be suggested that FD phytoplasma enhances its success on Chardonnay plants by suppressing the effectual jasmonic acid- and ethylene-mediated defence responses induced by vector feeding.

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Development of a new strategy for the control of Flavescence dorée disease based on a multiannual model of *Scaphoideus titanus* and a landscape analysis

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Highlights

- FD appeared for the first time in Switzerland. Despite the 13 years application due to mandatory control, FD was consistently diagnosed in the 12.7 % of the infected vineyards without the presence of populations of its epidemic vector
- The aim of the work is to test if the FD complex could be considered as an open system by investigating the presence of putative vectors and host plants

Introduction

Flavescence dorée (FD), a quarantine disease caused by "*Candidatus* Phytoplasma vitis", belonging to the Elm Yellows or 16Sr-V group, is transmitted epidemically by *Scaphoideus titanus*. FD appeared for the first time in Switzerland in 2004 in the vineyards located in the South of the Alps. Despite the 13 years application due to mandatory control, FD was consistently diagnosed during a time interval between 4 and 13 years in the 12.7 % of the infected vineyards without the presence of populations of its epidemic vector.

Some studies let suppose that the FD epidemiological cycle could be more complex than the simple transmission from grapevine to grapevine by *S. titanus*. *Ailanthus*, *Alnus* and *Clematis* are known to be FD plant hosts. It was also demonstrated the capacity of *Dictyophara europaea* to transmit the FD from *Clematis vitalba* to grapevine and, recently, the capability of the leafhopper *Orientus ishidae* to transmit 16SrV group phytoplasmas to grapevine (Lessio et al., 2016).

Material and methods

A study was carried out during 2013-2015. In 2013 and 2014 we worked in a test vineyard of Chardonnay located in Stabio and, on the basis of the results, we extended in 2015 the analysis to six other vineyards distributed in the different winegrowing area of Ticino and cultivated with Chardonnay, Pinot noir and Cabernet franc. The survey of the leafhoppers population was carried out using yellow sticky traps and the beating tray method. Symptomatic grapevine plants were found between July and September. Furthermore, leaf samples were also collected from symptomless plants as well as woody and shrubby plants that showed symptoms, possibly caused by the presence of phytoplasma.

Results and discussion

Six leafhoppers were found positive to the FD: *Hyalesthes obsoletus*, *Thamnotettix dilutior*, *Graphocephala fennahi*, *Thamnotettix* sp.*, Japananus hyalinus* and *Orientus ishidae*. *O. ishidae* was the most abundant in all studied vineyards, but with varying population densities, followed by *J. hyalinus*. Contrary to *O. ishidae*, which transmits 6SrV group phytoplasmas to grapevine (Lessio et al., 2016), the ability of the other leafhopper species to transfer the FD to grapevines is still to be demonstrated. Due to the scarce population density of *S. titanus*, the epidemic level of the FD in the infected vineyards remained low. *Corylus avellana* and *Salix* sp. are the preferred host plants of O. ishidae and they were also found positive to FD (Casati et al., 2017). *C. avellana* is particularly important because of the wide distribution in Ticino and positive individuals were present in all analysed vineyards.

It is reasonable to hypothesise that the ecological cycle of FD could be related not exclusively to the grapevine-specific feeding diet of *S. titanus*, but it could include other insect vector(s) and/or plant host(s). Therefore, the FD ecology appears to be a much more opened system than previously thought (Casati et al., 2017). The landscape surrounding the vineyards plays an important role determining which alternative plant host(s) and potential alternative leafhopper vector(s) are present. The current control strategy, based on the eradication of FD, is challenged and must be reassessed with the aim to find a long-term coexistence with this disease.

In order to deal with complex problems high in uncertainty like the FD-system, an adaptive management (AM) strategy was developed (Jermini et al., 2013). A multiannual infestation model for *S. titanus* is the basis of this strategy and it will be associated with a landscape analysis to quantify the secondary factors affecting the FD spread. The aim is to rationalise pest management and to learn how the pest population system works.

We started in 2016 a project in three vineyards cultivated with Merlot with the aim to develop a strategy for a coexistence with the FD. The first step was to stop the mandatory control against *S. titanus* to assess the annual increase of its populations. These data will be used to validate the multiannual model. Simultaneously, a landscape analysis method coupled with a survey of leafhopper population was developed to observe the presence of FD symptoms in the plots and in the woody and shrubby plants surrounding the vineyards.

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Mating behavior and vibrational communication of the meadow spittlebug *Philaenus spumarius*

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Highlights
- Intra-specific communication in *P. spumarius* is mediated by substrate-borne signals
- Males use a call and fly strategy to enlarge their search of mates

Introduction

Xylella fastidiosa is a xylem-limited bacterium that causes economically important diseases, including Pierce's disease of grapevine (Janse and Obradovic, 2010). In southern Italy, the *X. fastidiosa* subsp *pauca* strain is associated with the quick decline syndrome of olive (QDSO). The meadow spittlebug *Philaenus spumarius* has been identified as a vector of *X. fastidiosa* in southern Italy (Cornara et al., 2016). Our aim is to provide essential information on the reproductive behaviour of *P. spumarius* which could be basic to develop, in the future, more environmentally friendly control practices. Mate recognition and localisation in 'Auchenorryncha' are mediated via vibrational signals transmitted through the substrate (Virant-Doberlet and Čokl, 2004). We investigated the vibrational communication of the spittlebug to determine the role of substrate-borne vibrational signals in intra-specific communication and pair formation of this species.

Material and methods

Nymphs of *P. spumarius* were collected in April 2017 from fields in Valenzano (Apulia, Southern Italy) and then transported to a climatic chamber at the Fondazione Edmund Mach (Trentino, Northern Italy). All nymphs were reared in Plexiglas cages at 25+1°C, 65+5 % relative humidity, and 16:8 (L:D) photoperiod. The cages contained plants of *Trifolium repens* and *Convolvulus arvensis*. Rearing cages were checked every day and adult males and females were removed from the nymphal culture on the day of eclosion and kept separated by gender and age to obtain virgin individuals. To describe the mating behaviour, single males and pairs were isolated on potted *Agropyrum repens* plants included in a Plexiglas box and recorded with laser vibrometer (Ometron VQ-500-D-V). Trials were performed using (i) individuals and (ii) pairs of one male and one female. To study the daily pattern of male calling activity, the trials were performed at: early morning (08:00–10:00 h), late morning (10:00–12:00 h), afternoon (13:00–15:00 h), early evening (15:00–17:00 h) and late evening (17:00–19:00 h). The emission of vibrational signals and the behavior of the insects were observed for a maximum of 30 minutes.

Results and discussion

21.4 % of *P. spumarius* virgin males emitted calling signals spontaneously within 10-30 minutes of being placed on a plant. The male activity is not influenced by photoperiod, being the percentage

of calling males not different across the day periods. In the absence of a female response, males either remained stationary or jumped off the plant thus showing a typical call & fly behaviour. When a male arrived at a short distance from the female, long and rapid pulse trains were emitted, characterised by a temporal pattern different from the calling signal. Virgin females did not respond to male signals until they reached sexual maturity; however, they were never observed to emit vibrational signals. The non-receptive female rejected the male by displaying a distressed behaviour (i.e. quick wing fluttering) or jumping off the plant. Further researches are needed to understand the processes that lead to pair formation in *P. spumarius*. As already demonstrated in the case of the leafhopper *Scaphoideus titanus* (Eriksson et al., 2012), vibrational signals can be used to disrupt the mating behaviour hoppers, however, the polyphagy of *P. spumarius* would suggest a scarce applicability against this species. Rather, it would be interesting to find attractive/repelling signals to be used as lure & kill/push & pull stimuli.

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Management of Downy Mildew (*Plasmopara viticola* Berk. & Curtis, Berk. & De Toni) by reducing copper applications through BCAs treatments and new copper formulations

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Highlights
- Strategies with forecast model improve crop protection
- Modulation of copper amount per treatment improves protection against downy mildew: higher copper and better is the result
- Some alternative products showed good results in downy mildew management
- To reduce the use of copper in vineyards strategies based on DDS, the use of different types of copper coupled with BCAs should be applied

Introduction

Copper is the only fungicide that can be used against downy mildew in organic farming, and the European Regulation EC 473/2002 limited the maximum rate in 6 kg/ha/year. Another opportunity that was recently explored, deals with the use of BCAs somehow stimulating the response of the plant. Plant reactions can be triggered in few seconds, few hours and even after weeks, and sometimes the resistance mechanism is too slow to cope the evolution of disease. Based on the possible future scenarios of reduction of copper in organic farming and probable disappearance of active substances for integrated pest management, a field trial was set out to evaluate alternative solutions to reduce the amount of copper used in vineyards. The strategies tested includes the adoption of the forecasting model vite.net® (Horta srl) and were not set up by a scheduled program. The present work aims to show the results obtained in the two seasons, although trial were carried out since 2013 (Mosetti et al., 2016).

Material and methods

The trial was carried out since 2013 in a vineyard of Merlot, Guyot trained, in Cormons, Friuli Venezia Giulia, North Eastern Italy. A randomised block experimental design with 4 replicates was set up. The products under investigation were distributed with a portable sprayer using a water volume of 1000 l/ha at full vegetation. To evaluate the efficacy of the tested items, periodical assessment of incidence and severity of downy mildew on 100 leaves and 100 clusters per plot was undertaken according to the standards guidelines EPPO (1997).

Rates were set according to the vineyard density. Tested products in 2016 are listed in the table below:

Treatment	Product type
T0	
T1	Copper hydroxide (3kg) and plant extracts
T2	Copper hydroxide (3kg) and plant extracts Clay

T3 Copper hydroxide (3kg)
T4 Copper hydroxide (6kg)
T5 Copper hydroxide (3kg)
Copper sulphate
T6 Copper hydroxide (3kg) and therpens
T7 Copper hydroxide (6kg)
Clay
T8 Copper hydroxide (3kg)
Yeast extracts, microelements and copper sulphate
T9 Copper hydroxide (3kg)
Mycorrhizae and microorganisms
Mycorrhizae and microorganisms

In 2016 and 2017, applications were imposed to not overcome overall 3 or 6 kg of Cu/ha. In each thesis, alternative products were coupled with copper based pesticides to check their efficacy.

ANOVA was applied to incidence and severity on leaf and cluster and mean comparison was performed applying Student Newman Keuls ($p \leq 0.05$).

Results and discussion

High severity of Downy mildew on both leaves and clusters was recorded in the different treatments in 2016. T0 severity was 100 % on clusters. Strategies with higher copper ensured a better protection against downy mildew (T3 and T7) especially on clusters. Some interesting results were shown for BCAs. In particular, yeast extract in 2016 (T8) showed a better cluster protection with a low copper level, if compared to T4 (only copper application), even if there is also copper sulfate in the commercial preparation. The best low copper strategy has been T5 (copper hydroxide coupled with copper sulfate in liquid formulation) that resulted effective on both leaves and clusters. As opposite, T1 and T2 showed results similar to T0, possibly related with problems of formulation. The use of clays like zeolite and kaolin to control downy mildew seemed to be ineffective (T2 and T7). Also the application of copper hydroxide added with terpenes to improve persistence did not show any difference, probably because of the high number of treatments and close to each other needed in 2016.

Some alternative products showed good results in Downy mildew management, especially some copper based products used as foliar fertilisers. Modulation of copper amount per treatment improves protection against downy mildew by using more copper with high risk of infections and by setting strategies with forecast model. To reduce the use of copper in vineyards strategies based on DDS, the use of different typologies of copper coupled with alternative copper products should be applied according to downy mildew pressure.

In the season 2017, the same trial as in the previous season has been set up, with the aim to check the same strategies that showed good results in previous years, but also adding some new strategies.

At the end of 2017 trial, statistics will be carried out and comparison with 2016 will be performed.

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Insights into the genetic bases of downy mildew resistance and polyphenol induction in a grapevine inter-specific segregating population

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Highlights

- DM resistance in the Merzling × Teroldego segregating population is mainly mediated by the Rpv3-3 locus
- Novel shared as well as specific QTLs were associated to 33 different phenolics-related parameters upon *Plasmopara viticola* inoculation

Introduction

All grapevine cultivars (*Vitis vinifera* L.) traditionally grown in Europe are susceptible to downy mildew (DM) caused by *Plasmopara viticola* (Berk. & Curt.), an oomycete able to attack any grapevine green tissue. DM control mainly relies on the use of synthetic fungicides which are costly and have negative environmental impact. The exploitation of *Vitis* genetic resources for the development of new DM-resistant varieties is a promising alternative.

Studies conducted at E. Mach Foundation, taking advantage of a segregating population derived from Merzling (M, a mid-resistant hybrid) and Teroldego (T, a susceptible landrace), pointed to the importance of the stilbenoids in conferring DM resistance (Malacarne et al., 2011).

With the aim to elucidate the genetic bases of DM resistance and polyphenol production upon *P. viticola* inoculation in this population, 136 F1 individuals were characterised by an integrative approach combining genetic, phenotypic and gene expression data.

Material and methods

In 2012 season, 136 F1 individuals derived from the M×T cross were grown as potted plants in greenhouse. They were characterised at genotypic level by means of 190 microsatellite markers to build the M×T linkage map using JoinMap v.4.1 software.

They were *P. viticola*-inoculated both on potted plants (P) and on leaf disks (LD) (Vezzulli et al., submitted). The DM response was evaluated at 8 days post-inoculation (dpi) on P and at 4, 5, and 6 dpi on LD by means of three parameters: Severity, Incidence, and OIV 452 (for P) or OIV452-1 (for LD) descriptor. The 2th-3rd leaves from the P apex were collected at 6 dpi (828 samples) and analysed for the content of 42 phenolics (18 different stilbenoids) by targeted metabolomics (Vhrovsek et al., 2012); additional 22 sum/ratio parameters were calculated.

Quantitative Trait Loci (QTL) analysis was performed using MapQTL v.6 software. QTLs were declared significant if the maximum LOD exceeded the LG-wide LOD threshold (1000 permutations) and mean error rate was <0.05.

Candidate genes (CGs) included in the confidence interval of reliable QTLs were selected from the PN4002412Xv2 reference genome based on proximity to LOD peak offset, involvement in trait regulation based on literature, assignment to over-represented functional categories, and involvement

in functional categories of interest. Finally, expression analysis of some CGs of interest in 12 F1 individuals with divergent features was performed by quantitative RT-PCR.

Results and discussion

Out of the 190 scored, 181 markers were actually mapped into the M×T linkage map that was built at LOD=8; this genetic tool represents an advanced version in terms of marker number/order and progeny individuals compared to the map by Salmaso et al. (2008). The phenotypic analysis indicated an approximately normal distribution of the DM resistance parameters, and a significant induction of different polymeric stilbenoids upon *P. viticola* inoculation; the latter occurred in a subset of F1 individuals which are characterised by a high degree of resistance.

The association between genotypic and phenotypic data allowed to:

i) identify a major QTL on LG 18 for all the DM resistance parameters, which explained up to 23 % of phenotypic variance; although this region corresponds to the Rpv3 locus previously discovered in other resistant genotypes (Di Gaspero et al., 2012), the present study led to the characterisation and definition of the Rpv3-3 haplotype as associated to DM resistance in Merzling;

ii) define a number of novel QTLs on 12 different LGs associated to 33 out of the 64 phenolics-related parameters. In some cases more than one region was associated to a certain parameter, as well as in most of the cases - except for a QTL on LG 12 specifically associated to gallic acid - the identified regions showed pleiotropic effects on several parameters and explained different percentages of variance.

No QTL associated to phenolics-related parameter fell into to the major DM resistance QTL.

The number of genes within the identified QTL regions varied from a minimum of 5 (LG 18) to a maximum of 984 (LG 16). Due to the high number of gene predictions underlying the QTL regions we adopted four criteria to identify CGs putatively associated to the traits under investigation. Candidate functions of particular interest were signalling, regulation of transcription, response to abiotic and biotic stimuli, secondary metabolism and transport. Focusing on these categories, a refined list of 57 selected CGs was generated. Of these, 24 were yet associated in literature to the regulation of the traits under investigation, while the remaining 33 were novel CGs identified in the present study.

Interestingly, many Rpv3-dependent resistance genes (Casagrande et al., 2011), such as signal transduction genes, genes encoding pathogenesis-related proteins, WRKY transcription factors and HR-associated genes, fell within regions associated to phenolics known to be involved in defence response like stilbenoids, hydrossicinnamic acids and flavan-3-ol monomers and dimers.

We are currently further corroborating these findings by gene expression analysis, of some strong CGs on F1 individuals with a divergent phenotype in terms of both DM resistance and phenolics induction. Our final goal would be to understand if a cross-talk exists between the Rpv3-3-dependent resistance and the phenolics metabolism during DM response at grapevine leaf level.

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Effect of weather on appearance of grape downy mildew in Israel

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Highlights

- Excessive fungicide applications against downy mildew (DM) on vines can be reduced by evaluating: 1) spring DM prediction with a decision support system (DSS); 2) DM sporulation suppression associated with khamsin or drought stress
- We show that the Vite-net DSS can enable great reduction of chemical treatment against DM, and that khamsin and drought stress can reduce DM infection

Introduction

Grape DM is caused by the oomycete *Plasmopara viticola*, against which Israeli farmers apply fungicides in spring according to the 10:10:10 'rule of thumb' – rainfall ≥ 10 mm; temperatures ≥ 10ºC, shoot length > 10 cm. In Italy and Canada vite-net DSS systems successfully predicted primary infection in differing geographic regions (Caffi et al., 2011; Rossi et al., 2014). DM sporulation depends on high humidity whereas Israel's spring features random rainfall, and khamsin periods with high temperatures and dry conditions. Soil drought stress, achieved by lower irrigation, can promote grapevine secondary metabolite production, which can affect plant resistance (Langcake and Pryce, 1977). The present long-term objective was to reduce fungicide use against DM. Therefore, we tested the Vite-net DSS as a primary infection predictor, studied the effect of the khamsin on DM sporulation, and tested leaf susceptibility to DM secondary infection during water stress.

Material and methods

DM monitoring: In 2013-2016 we monitored 25 untreated plots, each with 100-300 vines: Barbara, Cabernet Sauvignon, Carignan, Crimson, Emerald Riesling, Gewurztraminer, Merlot, Muscat Canneli, Pinot-Noir, and Sangiovese. We recorded rainfall, leaf wetness, temperature, relative humidity, wind speed and direction and radiation hourly, and monitored plots once or twice weekly for 3 weeks after each rainfall, to determine phonological stages and 1st appearance of DM lesions. We uploaded weather data and plot details into a Vite.net DSS (Horta Co.), and recorded DM warnings.

DM sporulation during and after khamsin: We monitored naturally infected leaves in 2 vineyards: (i) cv. Syrah in Avny Aitan, with 4 khamsin days – May 17-20, 2015; (ii) cv. Malbek in Yonatan, with 8 khamsin days – June 18-25, 2016. We monitored sporulation (no. of sporulating lesions/leaf) on all vines during and 1 d after khamsin, and on cv. Malbek also 5 d after it. Also, we took leaves from the same vineyards to the lab and estimated sporulation after 48-h incubation in a humidity chamber.

DM tolerance after water stress: We tested mature Cabernet Sauvignon leaves for DM sensitivity: 5-6 leaves above the cluster, on north side of the row, under high and low irrigation, i.e., stem water

potential of -8 and -16 atm, respectively. We inoculated each leaf disk with ca. 350 *P. viticola* sporangia – 20 µl of *P. viticola* suspension with 1.0 × 104 sporangia/ml and estimated sporangia coverage 5 d post infection.

Results and discussion

DM monitoring: In spring DM appeared in 8 of 25 plots, in all cases after continuous rain; severe damage developed in only 3 plots. DM appeared after 9 of 38 rain events, 6-19 days after the rain, and significantly correlated with minimum temperature (logistic test: Chi2 = 7.04, P = 0.008); infection occurred only at ≥ 8.2oC. There were no correlations between 1st DM appearance and rain amount (Chi2 = 1.05, P = 0.3053) or hours of 100 % leaf wetness (Chi2 = 0.12, P = 0.7308) on the infection day. However, there were significantly more DM inoculations when rain continued more than 1 day, with more than 10 mm on the 2nd day with rain falling on wet soil, than with 1 day of rain or 2 days of which the 2nd day had less than 10 mm (Chi2 = 4.374, P = 0.0365). Our results agree with Magarey's (2010) finding that oospores in the upper 1-2 cm of soil need wetting for ≥ 16 h at temperature ≥ 8°C for primary infection. Interestingly, in our study, in 1 plot 1 day of rain sufficed for DM inoculation but on that day there was light rain in the morning that wetted the soil and was followed after 7 and 8 h by two strong rain events of 16.8 and 9.8 mm.

Vite-net DSS evaluation after rain: In 11 % of rain events lesions were predicted and observed (true positive); lesions not predicted and not occurring (true negative) happened in 77 % of rain events. In 6 % of rain events predicted lesions did not occur (false positive), and in another 6 % unpredicted lesions did occur (false negative). Vite-net DSS evaluations in diverse growing regions in Italy (Rossi et al., 2014) and in Quebec, Canada (Caffi et al., 2011) encouraged possible use to improve fungicide spray timing against *P. viticola* in the north of Israel.

DM sporulation during and post khamsin: In completely dry air with maximum temperature ≥ 33.5oC, no leaves with DM lesions sporulated in the vineyards. However, 6 out of 7 leaves and 76 ± 14.5 % of the lesions in Malbec, and all leaves and 36 ± 12.8 % of lesions in Shiraz sporulated after 48 h incubation in laboratory wet chamber. When weather conditions returned to normal, one night with wet leaves (1 or 2 h in Malbec and Shiraz respectively) were not enough for sporulation in the vineyard, but 50 % of leaves in the Malbec vineyard (n = 8) and 38 ± 15.5 % of the lesions resumed sporulation 3 days later, with 2-6 h of wet leaves each night.

DM tolerance after water stress: DM sporulated significantly less on mature leaves collected from water-stressed (-16 Mpa) than from well-watered (-8 Mpa) vines.

To conclude: DM incidence in northern Israel was low in 2013-2016. Fungicide application can be significantly reduced by using the Vite-net DSS. Hot spells can delay DM development, but cannot prevent re-sporulation. Water stress reduced grapevine DM sensitivity.

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Insight into the mechanism of conidial germination of the powdery mildew mycoparasite *Ampelomyces quisqualis*

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Highlights

- Conidia of *Ampelomyces quisqualis* are able to infect powdery mildew but they must be supplied with exogenous nutrients before they are able to germinate
- Different nutritional supplements seem to overcome the self-inhibition of conidia and trigger the conidial germination
- Mycoparasitism associated pathways seem to be regulated only by fungal host

Introduction

Ampelomyces quisqualis is a mycoparasitic fungus infecting the causal agents of powdery mildew (Erysiphales) and it is widely exploited to control powdery mildew of various crops. (Kiss et al., 2004) Conidia of *A. quisqualis* are typically nutrient-dependent and do not readily germinate in sterile water. They usually require an exogenous input of nutrients for germination. Studies on the host-parasite interaction of the mycoparasite and its powdery mildew host showed that water-soluble substances released from powdery mildew can stimulate the germination of *A. quisqualis* conidia *in vitro* (Gu and Ko, 1997). Moreover, their conidia may use germination-stimulating compounds from a host plant as an alternative chemical cue when nutrient concentrations are too low for conidial germination (Sundheim and Krekling, 1982). Thus, the present study focused on the existence of external sources affecting physiological and molecular processes of the conidial germination of the mycoparasitic fungus.

Material and methods

Conidia of *A. quisqualis* (1 × 105 conidia/ml) were mixed with substances composed by different carbon and nitrogen which were homogenised into sterile distilled water at different concentration. Four drops (10µl each) per combination (four replicates) were put onto a glass slide, which was placed in a Petri dish (RH = 100 %) and stored in a dark incubator kept at 25°C. The germination rates and germ-tube elongation of 200 conidia (four replicates, 50 conidia per replicate) were evaluated under a light microscope after 12 hours. Secondly, we investigated on some regulatory pathways activating conidial germination and mycoparasitic activity of *A. quisqualis*. Six putative genes potentially related to germination and mycoparasitism were selected for expression analysis by quantitative real-time (Siozios et al., 2015). Lyophilised conidia of *A. quisqualis* were dissolved in 100 ml flask at the concentration of 1 × 105 conidia/ml and 105 conidia/ml of *Podosphaera xanthii* and 1 g/ml of shrimp shell were added. Then suspensions were shaken at 120 rpm at 25°C and 0, 30, 60 minutes after inoculation, 2 ml aliquot was sucked and total RNA was extracted. Then, qPCR reaction was carried out to calculate the relative expression ratio value for each gene at each time point.

Results and discussion

We identified some nutritional supplements which seem to easily overcome the self-inhibition of germination of *A. quisqualis* conidia. As previously demonstrated the mildew host stimulates the conidial germination and fungal growth of *A. quisqualis* (Gu and Ko, 1997) but supplementation with chitin-based compounds, simple carbohydrates or aminoacids even had a stronger positive effect. Germination rate of *A. quisqualis* was very poor in the absence of any supplements. In sterile, distilled water only a small fraction (7 %) of the conidia had germinated at the end of 24 hrs incubation and the length of their germ tubes was less than 20 µm. interestingly, conidial germination and germ tube elongation were strongly stimulated when different amount of exogenous supplements were added to the distilled water. However, significant differences in the induction of germination were detected among the different substances: chitin polymers (N-acetil-glucosamine) and the three tested chitin based compounds (*P. xanthii* conidia, mushroom and shrimp shell) induced germination in the first 24 hrs after inoculation. After 24 hs the germination rate and tube elongation of conidia were stimulated most by N-acetil-glucosamine (32–35 % and 36–60 um) but also shrimp shell (15–27 % and 48–65 um), mushroom (14–24 % and 42–58 um) and fungal host *P. xanthii* (8–33 % and 41–51 um) were able to stimulate conidia. Germination was less, but still significantly induced by glucose, asparagine and yeast. Results showed that germination rates and tube elongation of conidia of *A. quisqualis* increased with increasing substance concentration. Generally, the highest germination rate was recorded at the concentration of 10 mg/ml. However, chitin polymers and mushroom treatments at the highest dosage strongly inhibited conidial germination of *A. quisqualis*. The investigation on the molecular pathways involved the host recognition phase of the parasitism revealed that several signalling pathways allow *A. quisqualis* to perceive the substances released from powdery mildew conidia and shrimp shell and initiate a transcriptional response. All of genes playing a role in the early signal-transduction events were up-regulated during the early germination process in the presence of the two substances. However, different expression patterns between shrimp shell and powdery mildew was observed after 30 minutes. Our results suggest that shrimp shell triggers the conidial germination but mycoparasitism associated pathways seem to be regulated only by fungal host.

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Are Swiss fungal resistant varieties appropriate for growth under the soil and climatic conditions of Quebec, Canada?

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Highlights

- Winter injuries greatly impact grapevine bud survival and yield
- Varieties showed different susceptibilities to diseases under field conditions and the most sensitive variety was Gamaret
- First two harvests showed that higher yields were obtained for IRAC 2060 and Garanoir
- At harvest, TSS was higher in IRAC 2060 and TA higher in Divico followed by Gamaret, IRAC 2060 and Garanoir

Introduction

Research in viticulture must favour the development of sustainable, economically viable and environmentally friendly production. Implantation of disease-resistant (DR) varieties adapted to regional soil and climatic conditions is essential to the development of the wine industry but also to reduce the use of pesticides (Pedneault and Provost, 2016). Optimal variety selection is a key factor for successful implementation of organic grape production but also under conventional practice (Sivcev et al., 2010). Resistance to major diseases significantly reduces the need for pesticides and thus represents a major advantage in organic farming, especially in humid areas. Under Québec conditions, several constraints limit grapevine growth and the evaluation of new varieties must consider cold hardiness and grape maturity. The main objective is to evaluate agronomic and oenological parameters of DR varieties developed by Agroscope Changins-Wädenswil under the climatic conditions of Quebec, Canada.

Material and methods

An experimental plot was implanted in 2013 in the experimental vineyard of the CRAM, Quebec, Canada (45°29'35.0"N 74°01'46.3"W). Four Swiss disease-resistant grapes varieties were selected: Gamaret (tolerant to Botrytis), Garanoir (tolerant to Botrytis), Divico (IRAC 2091) (tolerant to downy mildew) and IRAC 2060 (tolerant to downy mildew) (Dupraz et al., 2010; Gindro et al., 2012). The rows are oriented southwest and the soil is a gravelly loam. The spacing between the vines is 1.22 m and 2.44 m between the rows. Each grape variety has four repetitions of ten vines distributed into randomised blocks. Training system is Gobelet. A similar cluster thinning is applied for all vines. The following agronomic parameters were noted between the 2014 and 2016 growing seasons: diseases tolerance, cold hardiness, vegetative development, lignification and yield. In order to follow berry maturation, weekly chemical analyses (sugars, pH, total acidity) were carried out from the beginning of August until harvest (September). Wines were produced separately for each variety according to standard winemaking protocols. The first significant yield was obtained in 2016. Chemical analyses were carried out on the must, at the end of the alcoholic and malolactic fermentations.

Results and discussion

Preliminary results show that the four varieties have different sensitivities to the diseases found in North America. In the field, the most sensitive varieties to downy mildew, *Plasmopara viticola*, are Gamaret and Garanoir. Powdery mildew, *Uncinula necator*, occurrence was noted only during the 2014 season and Gamaret and Garanoir were most the sensitive. Black rot, *Guignardia bidwelli*, was observed during the 2015 and 2016 seasons and Gamaret was the most sensitive variety. Winter frost tolerance is an important aspect of the adoption of these varieties in Canada. Following the winters of 2014 and 2015, the rate of survival of vine buds was higher for Gamaret and Garanoir in comparison to Divico and IRAC 2060. However, winter in 2016 was devastating and cold injuries reached 80 to 100 % of the buds for all varieties. The first small harvest was obtained in 2015 and another one in 2016 but was still very low given the winter frosts. Preliminary results for the yield showed differences in grape production. Higher yield by vine and cluster weight were obtained for IRAC 2060 and Garanoir. At harvest, total soluble solids content was higher for IRAC 2060 than for other grape varieties. Divico had the highest total acidity followed by Gamaret, IRAC 2060 and Garanoir. Currently, the main constraint to use these disease-resistant grape varieties under Québec (Canada) conditions is winter temperature. Despite applying a winter protection (geotextile), high cold injuries were observed and the vines were greatly affected. Observations in the coming years will be more representative and will provide an overall picture for the production of Swiss varieties in Quebec, Canada.

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How vineyard features and application time may affect the phosphonates residues in the bunches

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Highlights

- The good antifungal activity of phosphonates and their good tox and eco-tox profile increased their use in plant protection
- Time of application is the main factor that affects the level of residues of phosphonates in bunches at harvest, but the role of weather conditions and vineyard vigour cannot be underestimate

Introduction

Phosphonates are increasingly used against *Plasmopara viticola*. They are highly effective against Oomycetes and have a good tox and eco-tox profile. They have been often use as fertilizers, taking also advantage of their fungicide effect. In Europe the use of phosponates as fungicides is allowed only if registered according Reg. 1107/2009 and it si not allowed in organic agriculture..

The antifungal activity and the behavior of phosphonates inside the tissues was studied in some crops (Anil Kumar et al., 2009; Deliopoulos et al., 2010), but is not fully known in the grapevine. This inorganic salt, unlike phosphate, is not metabolised by the plant cells as phosphorus source with consequently progressive accumulation, especially in the bunches (Speiser et al., 2000; Kauer, 2010). As reported in literature, we found that the time of application is crucial on the residue amount at harvest time, but vineyard vigour and the agronomic practices are not highly relevant.

Material and methods

In 2015 and 2016, potassium phosphonate (Century®SL, BASF, Germany) was applied in two vineyards located in San Michele all'Adige (TN, Italy) with different characteristics (variety, vigour). The experimental set up included various application times (pre and/or post bloom) in combination with canopy management (hedging in 2015 and leaf removal in 2016).

The experimental set up was randomized complete block design. Blocks (33 m^2, 8-9 plants) were treated with Century®SL (12 l/ha per year) and compared with traditional fungicides and untreated control. Each treatment had four blocks (replicates). The treatments were applied with a motorised backpack mistblower (Solo 450®, Germany) using a spray volume of 550 l/ha. Some treatements were complemented with copper hydroxide. Timing was adjusted based on weather forecast and the experimental design. Plant material removed with the summer pruning was weighed and the vegetation weight estimated in three times (leaf removal, hedging and harvest).

In 2015 samples of bunches (1 kg) were collected in each replicate for phosphorous acid (PA) analysis (QuPPe method) at harvest. In 2016 the sampling was carried out four times, between last treatment and harvest, in order to draw a residue (expressed as fosethyl) trend, evaluating also the volume and weight growth of the berry. The disease efficacy was assessed on 60 leaves and 40 bunches for each replicate at the end of the season.

Results and discussion

In 2015 the disease was much less destructive than in 2016 with a disease severity on the untreated, respectively of 7 % and 50 % on leaves and 23 % and 80 % on bunches. In aggreement with previious literature, the efficacy of phosphonates was confirmed, both in 2015 and in 2016, with a better protection of leaves than bunches, respectively with an efficacy of 97 % - 88 % and 88 % - 71 %.

The main question was related to the residues of PA at harvest (expressed as fosetyl) and, in particular, on how it could be affected by application time, vineyard vigour and canopy management.

In 2015 the bunches collected in one vineyard, named SD (Pinot gris), showed higher content of PA than the other one, named FAC (Schiava). Moreover, the analysis highlighted the importance of the time of treatments. In the case of early harvest (vineyard SD), application before the blooming decreased the residue level (26 ppm) considerably more than application after blooming (77 ppm). Timely treatments, before and after the blooming, showed intermediate residue levels (44 ppm). In vineyard FAC, with late harvest, the PA values in the different treatments were similar (below 20 ppm). Hedging, used as the test factor of the canopy management, did not affect the PA residue. Another difference of vineyard FAC respect to SD was the vigour: higher in FAC than the SD. For this reason, in 2016, two areas of the same vineyards with high and low vigoury were selected to evaluate this aspect. The levels of PA in the two vineyards at harvest were all between 5 and 15 ppm, with the higher values always in correspondence of the late treatments. The effect of vineyard vigour was not significant in the PA accumulation in the bunches. Canopy management, in terms of leaf removal as test treatment, caused a slight reduction of the residues only in the SD vineyard. By analyzing the data of the four samplings over time, the PA concentration in the bunches decreased from the last treatment till the harvest, but in parallel with berry size increase. The contents of PA (µg) in the berry increased in the first three weeks, then they stabilised or slightly decreased.

An open question is ‘why the residual values of vineyard SD in 2015 were higher than in 2016?’. One factor was not considered: the weather conditions, and in particular the rains. Between the last treatment and the harvest, precipitations were 25 mm and 129.8 mm, respectively in 2015 and in 2016. Part of phosphonates, also if they are moved into the plant, remain probably outside and thus they are subjected to the weather conditions.

This work was done to understand the accumulation of phosphonates in the bunches, commonly used in vineyard against grapevine downy mildew. In light of the results, the main factor that affects the PA accumulation in the grape is the application moment of phosphonate. Vineyard vigour, variety and canopy management have only a slight effect on the residues. Further studies are desirable on how the weather conditions could affect the PA residues.

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VITIFUTUR – A transnational platform for applied research and further education in viticulture

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Highlights

- Viticulture in the Upper Rhine region faces new challenges due to global climate change and consumers' increasing demand for sustainable agricultural practices
- Viticultural research institutions joined forces to tackle challenges of viticulture in the transnational project VITIFUTUR
- Close collaboration with local winegrowers ensures incorporation of new innovations into practice

Introduction

Global climate change facilitates the transmission of previously unknown diseases of the grapevine in the Upper Rhine area, which has both ecological as well as economic implications for the region. At the same time, consumers are increasingly demanding more sustainable agricultural practices, i.e. a reduced use of pesticides. To bridge the gap between the winegrowers' needs and the consumers' demands, novel innovations are needed. It is equally important to subsequently transfer these novel solutions into agricultural practices and to educate the public on sustainable winegrowing practices.

Material and methods

VITIFUTUR is a transnational platform for applied research and further education in viticulture that is co-financed by the EU programme INTERREG V Upper Rhine. In this project, all viticultural research institutions and universities in the German, French and Swiss parts of the Upper Rhine region have joined forces with local winegrowers to address current and future challenges in viticulture.

VITIFUTUR focusses on three scientific research topics: Evaluation of the resistance of Piwi-varieties, sensitivity of different grape varieties to wood decaying fungi that cause the Esca-syndrome, and development of strategies to prevent the spread of viral diseases in the region.

To ensure that the innovations developed in the research labs meet the needs of the winegrowers, partners from the wine industry are involved in the project right from the start. For example, surveys are performed among winegrowers to evaluate their specific needs and to gather information on the acceptance of novel practices such as the use of resistant grape varieties. The winegrowers are furthermore involved in conducting field experiments.

Results and discussion

Research on the underlying mechanisms of resistance against fungal diseases in Piwi-varieties is currently ongoing. A particular focus of the research is to gain a better understanding of the stability of resistance as well as accessing measures to maintain this resistance over time. Furthermore, surveys on the acceptance of resistant grape varieties are conducted among winegrowers, which will help to

introduce these varieties into viticultural practices. Overall, this research endeavour will result in practical strategies and recommendations for the sustainable cultivation of Piwi-varieties in the Upper Rhine region.

The second scientific focus lies in gaining novel insights into wood decaying fungi that cause the Esca syndrome, a disease that is difficult to combat. The response of different grape varieties to infection with wood decaying fungi is retrieved in order to gain knowledge on resistance factors. This information can then be used to improve plant breeding efforts.

The vector based transmission of viral diseases is increased through global warming and additionally new, previously unknown viruses are spreading in the region. The third research focus therefore lies in the development of novel strategies to prevent the spread of viral diseases in the Upper Rhine region. The studies range from field experiments to access the local occurrence of known viruses to testing virus-resistance of new root stocks with the aim of developing new strategies to prevent the transmission of viral diseases.

VITIFUTUR is not only a research project, but also a think tank for sustainable solutions to current and future challenges in viticulture in the Upper Rhine region. The scientists are in continuous dialogue with partners from the winegrowing industry to ensure the transfer of knowledge and implementation of innovations into the viticultural practices. Seminars for winegrowers are organised on a regular basis and also young scientists have the opportunity to learn about viticultural practices through practical training in the vineyards.

Another aspect of VITIFUTUR is educating the public about innovations in sustainable viticulture. Research results and innovations are therefore made available to the general public in regular events that convey the potential and significance of viticultural research for the Upper Rhine region to a broad public.

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Grapevine protection: from proof of concepts to pre-industrial biofungicides

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Highlights
- New low-impact molecules or BCAs are continually analyzed, but only few of them can become registered bio-fungicides after a formulation study that promise a good efficacy over time
- Modus operandi with lab, greenhouse and field trials in order to validate a potential biofungicide

Introduction

The reduction of synthetic chemicals use in agriculture in favour of low-impact pesticides is a priority for the safeguard of the environment and the consumers. The increasing public awareness pushes to substitute the indicted chemicals, therefore the searching of new tools in the pests control aim to provide low-impact molecules having similar features and efficacy/price ratio.

Several plant and animal extracts, fungi, bacteria and others emerge from the research as novel possible bio-fungicides, but only few of them become an efficient product usable in professional agriculture. This transformation often requests a long and hidden process with the creation of a formulation that guarantee a good efficacy under field conditions.

In the last years our group followed the development of several plant and animal extracts, BCAs and natural molecule as biofungicide on grapevine with several test from the lab to the field, through the greenhouse until the pre-registration stage.

Material and methods

Lab proofs. Five leaf disks (283 mm^2), placed in 90 mm-Petri dishes on four-layer wet absorbent paper were treated with the different products through Potter spray tower or manual sprayer (Cabús et al., 2017). After drying under chemical hood, the pathogen was inoculated in drop, in spray or only dry conidia.

An artificial rain through eyedroppers, located above the leaf disks, was applied before the inoculum in order to evaluate the rain fastness.

Greenhouse proofs. 2.5-liters potted grapevine plants with 12-15 leaves, grown in greenhouse conditions, were treated with 15 ml/plant through air compressed sprayer (0.4 Mpa). The products effectiveness was tested inoculing the pathogen on the plants when they were dry. Through the use of a rain simulator (53 mm/h), 20, 40, 60 mm of washoff rains was applied on treated plants and assay the formulations rain fastness. Moreover, increasing the period between treatment and inoculation (6, 3, 2, 1 days), it allowed to estimate the persistence of the products (Dagostin et al., 2010).

Field trials. In two experimental vineyards, sited in San Michele all'Adige (TN, Italy), randomised blocks (33 m^2, 8-9 plants) were treated with test and control products. Each treatment

was composed by four replicates (blocks). According to weather forecast, the probable pathogen infections were identified and treatments were applied. Products were sprayed with a motorised backpack mistblower (Solo 450, Germany) using a spray volume of 550 l/ha.

Results and discussion

The main requested features of an active ingredient or a formulated biofungicide are the direct or indirect activity against the target disease (effectiveness trial), the efficacy over the time (persistence trial) and the ability to persist on the tissues after a rain (rainfastness trial).

Starting from a natural molecule or BCA, we developed several products/formulations in order to individuate the most effective. The screening of them and the identification of the minimum effective concentration was carried out under laboratory conditions with a bio-assay on grapevine leaf disks. The disease severity was assessed when the sporulation was developed and it was used to discerner the efficacy of the product in comparison with the controls.

The satisfactory formulations were tested in planta under greenhouse conditions and disease incidence and severity were assessed after the incubation period. This kind of test allowed understanding the behaviour of the products on plant, approaching the field concept in controlled conditions.

The last step was the proof of the resulting good formulations of greenhouse proofs under field conditions. In according to EPPO Standards, the activity against the pathogens was assessed based on the presence of disease symptoms on leaves (oil spots or sporulation) and bunches (sporulation or necrosis). Disease severity and incidence was evaluated at intervals of 7-12 days in each replicate on 60 leaves and 40 bunches in each plot. (Giovannini and Pertot, 2016). The phytotoxicity assessment, by checking for discoloration of leaf or berry and necrosis on leaves and bunches, was another important evaluation. In the case of BCAs, the cultivable microorganism population was assessed on grapevine leaves, bunches and soil collected from each replicate of all treatments (Segarra et al., 2015). All data are necessary to complete a pre-registration dossier of the product.

In the last years, using this procedure, our group tested more than 200 formulations coming from internal lines of research, European and Italian projects, companies etc. and four of them are become commercial products.

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Residents non-dietary pesticide exposure risk perception survey: knowledge gaps and challenges for targeted awareness-raising material development

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Highlights

- Non-dietary exposure to agricultural pesticides is an emerging issue in rural areas with high population density
- Risk communication should be realised on the basis of context specifics risk perception analysis
- Awareness-raising material should take into account different age and cultural targets
- Perception analysis should guide the training of professionals who provide info on the subject

Introduction

Pesticide risk evaluation as currently run is a deterministic and quantitative process that does not address socio behavioural aspects. Most pesticides, however, in the use phase can lead to environmental and health risks, if not used in compliance with the Good Agricultural Practices (GAP). The exclusion of the analysis of behaviours and perceptions could be responsible for a reduced compliance to GAP and for a society science-hostile trend (Calliera, 2016; OEDC, 2015). There is the need of an improvement of measuring context specific risk perception to sensitise on the importance of observation of standards and best practices and increase trust in the society risk evaluation process. The object of this pilot study is to evaluate citizens risk perception of non-dietary exposure to pesticide, produce guidelines that can assist policy-makers and risk communicators in the development of targeted awareness-raising material for residents and bystanders.

Material and methods

We explored, through a household survey, knowledge of, health risk perceptions of, and information sources related to non-dietary exposure to agricultural pesticides in residents indoor and outdoor environment. The survey, designed within the project of the Catholic University ANAPNOI, was conducted in the rural area of the Piacenza province, in Emilia-Romagna region, north Italy. The selected area is characterised by high population density, with an intensive agricultural activities, which have a significant impact on national agricultural production. Survey operators conducted a face-to-face questionnaire. Specific questions were designed including the evaluation of the relation between residential air quality and health status; habits; cultural interest and consumptions and their influence on the awareness of the problem (with particular reference to pesticides as air pollutants); the level of knowledge of respondents with respect to environmental problems in general and pesticides and the factors that nudge individuals to give importance to several possible sources of pollution; mitigation measure or precautions that residents take to protect themselves from possible sources of outside-inside pollution.

Results and discussion

The objective was to understand the social perception of air contamination by pesticides, which are the determinants, and to what extent individuals think they can control the problem and feel safe in the domestic environment, in order to explore knowledge gaps and challenges that might be considered to prevent exposure. In addition, farmers have been included in the survey, since they could be considered both operators (or workers) and residents.

The survey is ongoing but preliminary results show that also if the air quality of the residential area is not judged negatively, pesticides are perceived as air pollutants that could lead to an effective exposure and are correlated to the health status, especially in particular periods of the year when treatments are perceived intense. The perception of risk, however, seems not to be dependent only on the distance from home to field. The interpretative hypothesis that the perception of the relationship between air quality and health is influenced by the cultural issue and by psycho-sensory factors and not supported by proper information, also if with some differences by the age groups, seems to be confirmed. Media, television and internet are for the interviewed the main information source, also if the majority feels to be not sufficient informed on the issue (except for farmers). Doctors (mainly) and researchers are recognised as professionals that can provide the correct information on the relation between health and pesticides exposure, while journalists and politicians are not trusted. There is an awareness of the potential of the pesticide to pollute the indoor environment, but mitigation measures adopted to protect the air quality of the domestic environment are poor.

The preliminary results also confirm that perception is also linked to age and cultural interest. To this reasons awareness-raising material should also include a proper problem definition; should be different in accordance to target age their information sources and habits; should be targeted according to different public categories (farmers should be sensitised on the importance of observation of standards and best practices). Furthermore citizens should be informed on the correct mitigation measures (structural and behavioural) to limit the risks of exposure, in this contest the training of professionals (such as doctors) to which people rely for correct information has a strategic relevance.

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The SUDOE "VINOVERT" project: potential of pesticide use reduction in three South-West European vineyards regions

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Highlights

- Transdisciplinary EU project evaluating possibilities to reduce pesticide use in viticulture and oenology
- In 2017-2018, 32 commercial vineyard plots will be tested in France and Spain to compare low- vs. high-input pesticide strategies
- Multi-pests incidences, pesticide application rates, and other indicators will be assessed, to analyse effects on wine quality and consumer willingness to pay

Introduction

Consumer preferences may tend to select wines characterised by lower rates of chemical use (organic wines, zero-residue wines or wines without added sulphites). Meanwhile, regulations for pesticide use are becoming more restrictive. These changes represent a challenge for European wine producers for their practices and competitiveness in a global market, regarding long-term prospects.

VINOVERT (http://vinovert.eu/en/) is an innovative EU project integrating several disciplines: Economy, Sociology, Agronomy, Chemistry and Pathology. It involves wine and/or viticulture companies and institutions, in order to investigate potential solutions with a real feasibility. The target includes some long-term solutions for improving the control of major pests and diseases (resistant grape varieties; Work Package 1), mid-term solutions for the pesticide use reduction (viticulture practices; Work Package 2) and short-term solutions for the reduction of oenological sulphites (Work Package 3)

Material and methods

The VINOVERT WP2 evaluates the technical possibilities to reduce pesticide use at the plot scale. Thus, a network of 32 commercial vineyards plots is being monitored in 2017 and 2018. The plots are distributed in 3 grapevine growing regions in South West Europe: i) Rias Baixas (Galicia, Spain) with Atlantic humid climate conditions with high disease(s) pressure; ii) Penedés (Catalonia, Spain) with typical Mediterranean climate, and iii) Bordeaux region (Nouvelle Aquitaine, France), under Atlantic climatic conditions. Per region, only one grapevine cv. will be tested, i.e. Albariño, Tempranillo and Merlot noir for Rias Baixas, Penedés and Bordeaux regions, respectively.

Vineyards are managed by the local growers without any specific recommendation from VINOVERT scientists. The vineyard network is composed of plot couples, in which 2 plots are very close and have similar features: slope, age, training and pruning systems. However, the 2 plots in a pair differ by pesticide use, e.g. high use (conventional) vs. low use (IPM" or "organic). The grower practices, diseases/pests rates and yield parameters will be recorded and quantified with appropriated indicators. Standardised micro-vinifications will be made from selected plot pairs. The wines will be checked for chemical/oenological quality, pesticide residue levels. Moreover, experimental-economy experiments will be carried out in order to reveal the willingness to pay of consumers, for the different wines produced.

Results and discussion

The 2017 and 2018 seasons will represent case studies, in which a high amount of data associated with each plot is being collected through various indicators. Some of them have been specifically developed: "AIDB+" indicator: based on the AIDB indicator (Assessment Index of Damage in Bunches), developed in a previous published study (Fermaud et al., 2016). "AIDB+" will integrate the cumulative damage in grapevine fruit of the main pests and diseases, i.e. powdery mildew, downy mildew, Botrytis bunch rot, gape berry moth, black rot and grape trunk diseases (GTDs). Three evaluations of diseases/pests rates are conducted during the season, at flowering, pre-véraison and pre harvest stages.

Technical capacity index: This index quantifies the technical capacities of vineyard managers, regarding spraying machinery and associated plant protection knowledge: precision on dose and timing for applications, training or access to decision tools and/or local advisors (private or public).

Production Cost Index: a general cost analysis of the vineyard production will be carried out, based on the FADN guidelines for the evaluation of production costs at the farm level (Farm Accountancy Data Network; http://ec.europa.eu/agriculture/rica/).

In addition, other indicators, currently validated, will be also used. Treatment Frequency Index (TFI): This indicator is designed to record the use of phytosanitary products at the plot, farm, or farm group scale (http://agriculture.gouv.fr/sites/minagri/files/ift_manuel_v1_octobre_2015.pdf). It integrates the number of reference doses per hectare during a growing season, including four main groups of pesticides: herbicides, fungicides, insecticides and acaricides, other products.

In 2017, the extensive network of commercial field plots has been developed, by a collaborative work between local partners and INRA Bordeaux-Aquitaine researchers. Measurement protocols are standardised and field evaluations are currently being conducted. Significant differences between plots in the same pair, according to some of the indicators, are expected at the end of the season. The main results comparing regions and vineyard plot indices will be presented in this work.

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Roll-out of IPM in Belgian viticulture

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Highlights

- Viticulture is the fastest growing branch of the agricultural sector in Belgium. Unfortunately, most winegrowers are newcomers to agriculture and therefore not familiar with current farming trends
- The need to demystify-teach-implement IPM to this new branch was urgent but also of great importance as 'eye-opener' to other future new winegrowers of this new agricultural booming business-unit

Introduction

Viticulture is the fastest growing branch of the agricultural sector in Belgium. Improving climate-conditions, success-stories and good wine-quality creates high interest in Belgian viticulture. During the last years, the yearly growth even increased and currently counts approx. 100 semi- or full-professional winegrowers. Unfortunately, most winegrowers are newcomers to agriculture and therefore not familiar with current farming trends. The need to demystify-teach-implement IPM to this new branch was urgent but also of great importance as 'eye-opener' to other future new winegrowers of this new agricultural booming business-unit.

Material and methods

Starting in mapping the situation, a taskforce of winegrowers, scientists and crop advisors formed a targetplan to fullfill the shortcomings and needs for the Belgian winegrowers. By means of demonstrations and lectures on the different IPM topics the winegrowers were educated and demonstrated how to make the necessary steps towards implementing IPM in their wine business. The winegrowers of the taskforce were monitored and steered to fullfill a guidance-roll to their colleague-winegrowers in each specific area. During the project, prediction models for Oidium and Peronospora were implemented and a nation-wide 'warning-system' (Vitimeteo-based) was installed. The project was concluded by publishing monitoring guidelines and a step-by-step manual for the winegrowers.

Results and discussion

We noticed that winegrowers, although sceptical at first, very easily were transformed and enthousiastic about this approach towards sustainable techniques and methods. Within 3 years time, IPM has grown from 'unknown' to the common practice in Belgian winegrowing.

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Grapevine trunk diseases: the relevance of disinfection of propagation material

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Highlights

- Disinfection of propagation material using low impact products and biocontrol methods is important for the limitation of grapevine trunk diseases

Grapevine trunk diseases (GTDs) are a major threat for viticulture, in all grape-growing countries. The main diseases affecting vineyards in Europe are the Esca complex: grapevine leaf stripe disease, black wood streaking, Petri disease and white rot. Cankers caused by Botryosphaeriaceae are also found with increasing frequency, associated with the death of grapevine cordons and spurs. Nursery production has a major role in producing plants strong enough to withstand aggressive wood pathogens, once they are planted in the field. At the same time, they must be as free as possible from pathogen infections at early life stages. Many years of trials have been carried out to evaluate strategies for reducing early nursery infections, comparing different new with established methods. Plant material infections by *Phaeomoniella chlamydospora, Phaeoacremonium minimum*, and species of Botryosphaeriaceae were assessed in either non-inoculated or artificially inoculated graftings, treated with different products. Promising results were obtained in the control/limitation of GTDs pathogen infections by treatment with innovative, low impact products (e.g. electrolysed water, ozone) and biological control methods. The benefits and relevance of superior quality planting stock are only realized when subsequent agricultural activities follow well-planned and balanced vineyard management practices.

Meeting of the IOBC-WPRS Working Group "Integrated protection in viticulture"

Novel tools and new challenges for IPM in viticulture

Poster Session 2

IPM implementation and tools against arthropod pests

Emulpar' 940 EC as a mechanical natural product in two-spotted spider mite (*Tetranychus urticae* Koch.) control on berry crops

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Highlights

- Emulpar' 940 EC is a natural product, contains oil from plants of camelina (*Camelina sativa* L.). It has been tested in the laboratory and field conditions against two-spotted spider mite (*Tetranychus urticae* Koch) occurring on blackcurrant, strawberry and raspberry crops
- Its efficacy was varied from 63-100 %. No visual symptoms of phytotoxicity were observed on treated plants

Introduction

Blackcurrant, strawberry and raspberry are important crops in Poland. Serious damages to plants are often caused by the two-spotted spider mite (*Tetranychus urticae* Koch). All pest motile forms feed on the lower surface of leaves and cause distinct bronzing of the foliage and sometimes lead to premature leaf fall.

Over last few years the list of acaricides to control *T. urticae* on fruit bushes is very short (few active substances are registered). Therefore, there is a constant need to search for new plant protection products to control mentioned pest, including non-chemical methods. The aim of this work was to estimate the efficacy of new natural product Emulpar' 940 EC, containing oil from plants of camelina (*Camelina sativa* L.) in the control two-spotted spider mite on berry crops, in field and in the laboratory conditions.

Material and methods

Field tests. The experiments were carried out in years 2014-2015 on blackcurrant, strawberry and raspberry plantations. Emulpar' 940 EC was used at the concentration 0.9-1.6 %. The treatments were applied using the motorised knapsack sprayer Stihl SR 420 with spray volume of 500 l/ha on blackcurrant and 750 l/ha on strawberry and raspberry plantations. The two-spotted spider mite population densities were estimated just before pest control application, and then after one, two and three weeks from the treatment. At any sampling date, 25-30 leaves were randomly taken from each experimental plot, and motile forms and eggs were counted.

Laboratory tests: Effectiveness of Emulpar' 940 EC in the control of *T. urticae* eggs. Females of *T. urticae* were placed onto discs cut from bean leaves (*Phaseolus* sp.). After 24 hours (time needed for oviposition) the pest females were removed from leaves. The eggs present on leaf surface were counted and then sprayed with Emulpar' 940 EC (0.9 and 1.2 %) under the Potter Spray tower. During the next 7 days, the number of hatched mites was daily counted to assess the treatment efficacy.

Effectiveness of Emulpar' 940 EC in controlling *T. urticae* motile forms. *T. urticae* motile forms were placed onto discs cut from bean leaves and sprayed with Emulpar' 940 EC (0.9 and 1.2 %) under

the Potter Spray tower. Daily, during next 7 days the number of mites alive was calculated and the efficacy of treatment was assessed.

Results and discussion

On blackcurrant cv. 'Ruben' the procedure of pest control was performed after bloom (28th of May). The effectiveness of Emulpar' 940 EC at the rate 6 l/ha in 500 l of water (concentration 1.2 %) used in the control of mite mobile forms was 90–98 %, at one to three weeks after treatment. The efficacy of eggs control was similar or slightly lower in comparison to the control of motile forms. On strawberry cv. 'Honeoye' the treatments were carried out after fruit harvest (1st of July). The efficacy obtained with Emulpar' 940 EC at the concentration 0.9-1.6 % (at the rate 6.75–12.0 l/ha), in 750 l of water on strawberry at one and two weeks after treatment was 94.7–100 %. On raspberry cv. 'Laszka', fruiting on the two-year-old shoots, the control treatments were applied after harvest (22th of July). The efficacy obtained with Emulpar' 940 EC at the concentration 0.9-1.6 % (at the rate 6.75–12.0 l/ha), in 750 l of water on raspberry ranged from 45.5 to 97.5 % at one week after treatment and from 75.0 to 100 % at two weeks after treatment, depending on the rate of product per ha. In all above experiments good efficacy of Emulpar' 940 EC was also proved against eggs of *T. urticae*. No visual symptoms of phytotoxicity after pest control treatment were observed on any of the plants at either assessment.

In the laboratory the effectiveness of Emulpar' 940 EC at the concentration 0.9 and 1.2 % in the control of mite eggs ranged from 63.0 to 85.0 % respectively, and for motile forms from 78.0 to 95.0 %, at seven days after treatment.

The results obtained with Emulpar' 940 EC were similar to those obtained with the tested reference products – fenpiroximate as Ortus 05 SC at the rate 1.25 l/ha and Siltac EC (silicon compounds) at the rate 1.5 l/ha (Łabanowska et al., 2014). Emulpar' 940 EC is effective not only in the control of spider mites, but also of other pests present on treated plants such as aphids and eriophyid mites. Emulpar' 940 EC has a mechanical effect on pests - prevents their gas exchange and lowers the mobility, leading to death. It can be a useful tool in the anti-immune strategy. It is also safe for the consumer and the environment - the grace period for this product is not specified (Łabanowska, 2015).

Conclusions:

1. Emulpar' 940 EC can be used, even just before harvesting, because it doesn't leave residues on fruits and doesn't have any pre-harvest period.
2. Emulpar' 940 EC gave high effectiveness in the control of mite motile forms (75.0-100 %) and eggs (63.0-85.0 %) of T. urticae in fields and laboratory experiments.
3. The results obtained with Emulpar' 940 EC in control motile forms of *T. urticae* were similar to those obtained with Ortus 05 SC (fenpiroximate) and Siltac EC (silicon compounds).
4. Emulpar' 940 EC did not show any phytotoxic symptoms on treated plants.
5. Mentioned product is a good tool in rotation with others plant protection products and can be used in organic and integrated plant protection programmes.

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Impact of leaf removal, copper and kaolin on grape skin thickness in order to reduce *Drosophila suzukii* infestation

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Highlights

- Different vineyard treatments might have the ability to thicken the berry skin and consequently decrease their susceptible to *Drosophila suzukii* infestation
- Here we present the impact of leaf removal, copper and kaolin on the force to penetrate berry skins and *D. suzukii* infestation

Introduction

The Spotted wing drosophila (*Drosophila suzukii*) has become an economically important, major pest throughout Europe and the Americas in recent years. It is particularly worrying due to its ability to lay eggs in healthy, soft skinned fruits using its serrated ovipositor. *D. suzukii* is a very polyphagous species and can therefore infest roughly 100 host plant species, both wild and cultivated. The high number of host plants available makes it very difficult to manage. Therefore, techniques focused on strengthening a fruits resistance to *D. suzukii* might prove more successful than techniques focused on managing populations of the fly. In grape berries it has been shown that infestation rates drop as the penetration force required to break the grape skin increases (Ioriatti et al., 2015).

The aim of this study was to explore if there are any vineyard practices that can strengthen grape berry skin thickness and therefore make the fruit less susceptible to infestation by *D. suzukii*.

Material and methods

The experiment will take place between August and October 2017 in the experimental vineyard of Agroscope at Changins (Switzerland). Four rows of Mara grapes will be put into a three factorial random block design. The three factors included are an early leaf removal (100 % of fruit zone leaves removed post bloom), a copper foliar spray (1.5 kg/ha rate applied three times) and a kaolin application (applied every two weeks).

The four rows will be sampled every two weeks. Grape berries will be analysed for *D. suzukii* infestation levels by visual observation under a microscope and for penetration force with a texture analyser.

At the end of the growing season each of the eight treatments will be harvested individually and the resulting must from each treatment will be analysed for Brix, TA, pH and undergo a sensory evaluation for quality.

Results and discussion

Since the data collection will take place between August and October 2017, no results of the study can be presented at this stage. However, any results that show positive signs in increasing the

berry skin thickness could ultimately prove critical in helping to control *D. suzukii* infestation rates in vineyards.

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Entomopathogenic fungi as control agents for *Drosophila suzukii*

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Highlights

- An integrated strategic approach to the control can be the most effective and ecologically sustainable solution against *Drosophila suzukii* infestation
- Biopesticides, based on entomopathogenic fungi, adopted in appropriate timing plans and at suitable climatic condition, in integrated pest management strategy, can represent a good tool in *D. suzukii* control method

Introduction

Last few years, the infestations of *Drosophila suzukii*, the Spotted Wing Drosophila (SWD), have created many problems to fruit growers in Italy, in terms of crop damage and yield losses, especially small fruits, sweet cherries and, during years characterised by cool and rainy summers, as the one occurred in 2014, wine grapes (Gargani et al., 2015). To date, no decisive SWD control methods have been characterised. In the present study, results obtained in laboratory bioassays, performed using commercial products, based on entomopathogenic fungi registered in Italy as bioinsecticides, are reported.

Material and methods

The products investigated, commercially available and tested in the different lab trials, were: Naturalis: a registered bioinsecticide, based on living spores of naturally occurring strain of the entomopathogenic fungus *Beauveria bassiana* (strain ATTC74040, g 7.16 Equal to 2.3x107 viable spores/ml); MET52: a product based on a strain of the fungus *Metarhizium anisopliae* (strain F52, containing 2 g with 9 x 10^8 CFU)/gram); NoFly: a commercial bioinsecticide based on the fungus *Paecilomyces fumosoroseus* (strain FE 9901, it contains 2 x 10^9 CFU/gram). Five lab trials were performed, using *D. suzukii* individuals coming from the rearing maintained at CREA DC laboratories, at 25°C, 65 % R.H. 16:8 hr L:D climatic conditions. Three trials to test the direct toxicity of MET52 on *D. suzukii* adults, preimaginal stages and crossing treated and non-treated adults were performed. As regards Naturalis and NoFly, two trials were set to test direct toxicity on *D. suzukii* preimaginal stages and side effects of the products.

Results and discussion

None of the commercial bioinsecticides tested in these laboratory trials caused the whole mortality in *D. suzukii*: entomopathogenic fungi determined reductions in fly population numbers but were unlikely to completely control/eradicate the SWD populations.

The application of MET52 in granular formulation, determined an adult mortality over 80 % just with two hours after contact, showing that the fungus *M. anisopliae* can infect adults through contact among treated and untreated individuals; no effect was detected on fly fecundity as other authors

verified (Woltz et al., 2015). The direct toxicity of MET52 on preimaginal SWD stages gave a lower efficacy, even if the calculated mortality (58 %) was quite similar to the value (61.5 %) registered by Woltz et al. (2015) and higher than 40 % obtained by Cuthbertson et al. (2016) with a 1 % solution of the fungus. At now, in Italy, the granular MET52 formulation is the only available, limiting so the application of the product at soil level in the fruit crops and making less efficient its use as the bioethological habits of SWD (Razinger et al., 2017).

The other two fungi, *B. bassiana* as Naturalis and *P. fumoseroseus* as NoFly, applied singly, with the addition of sucrose or in mixed solution, gave different results but, on the whole, higher than those obtained by Cuthbertson et al. (2016). In particular, the mixed solution of the two fungi and the addition of sucrose to Naturalis were efficient in suppressing SWD population determining in both cases mortality over 75 %. Other fungus strains of *M. anisopliae* and *P. fumoseroseus* (=*Isaria fumoserosea*) (Naranjo-Lazaro et al., 2014), *in vitro* bioassays showed variable results in term of mortality.

The use of entomopathogenic fungi in controlling *D. suzukii* can therefore make sense in integrated pest management strategies, by timely applications of these products, when there is not a too much high density of SWD and climatic conditions are suitable.

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Management of *Linepithema micans* and *Eurhizococcus brasiliensis* in new vineyards

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Highlights

- Management of *Linepithema micans* (Hymenoptera: Formicidae) and *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) in new vineyards using hydrogel baits

Introduction

Linepithema micans is the main ant species responsible for the spread of *Eurhizococcus brasiliensis*, the main soil scale associated with decline and death of grapevine plants in southern Brazil (Botton et al., 2012). *E. brasiliensis* has been controlled with neonicotinoid insecticides (imidacloprid and thiamethoxam) applied to the soil in November, targeting first-instar nymphs (Botton et al., 2010). This management practice has been effective for many years, although with some limitations, particularly the presence of toxic residues in the fruits and the risk of environmental contamination.

One strategy to reduce scale infestation in vineyards may be controlling *L. micans* with hydrogel. This technology has been used for the control of *Linepithema humile* in South Africa and California (Boser et al., 2014; Buczkowski et al., 2014).

In this study, we evaluated the effectiveness of hydrogel toxic baits in controlling *L. micans* and *E. brasiliensis* populations in newly planted vineyards.

Material and methods

The experiments were installed in vineyards with natural infestations of *E. brasiliensis* and *L. micans* located in Caxias do Sul, Rio Grande do Sul, Brazil (S 29º 14' 9" W 051º 14' 3"). The vineyard was divided into three blocks, and in each block, 27 own-rooted Paulsen 1103 (*Vitis berlandieri* × *Vitis rupestris*) vines were planted in August 2016. In one block we applied hydrogel, in another thiamethoxam applied via drench, and the control block we left untreated.

To prepare the hydrogel we immersed hydrogel crystals in a sucrose solution (25 %), using 0.007 % thiamethoxam as the toxicant. The proportions used were 25 % sucrose solution, 20 g hydrogel and 0.28 g Actara 250 WG (25 % thiamethoxam; Syngenta Crop Protection Inc.). The hydrogel crystals were left in the solution for at least 1 h to allow them to become saturated. Before applying the baits, we estimated the initial population of L. micans in the plots, using ground pitfall traps (20 per area) (Nondillo et al., 2016).

Hydrogel baits was applied by hand every 45 days (1st application in November 2016) using 3 Kg (dry) or 210 l (saturated) of hydrogels per treatment/ha/application. Thiamethoxam was applied via drench around the trunk in a concentration of 0.24 g a.i./plant. After 6 months the plants were pulled in order to count the number of scales per plant (Botton et al., 2010). The data for the number of cysts in the treated and control areas were compared using the Tukey test ($p<0.05$).

Results and discussion

In the area where hydrogel was applied the population of *L. micans* was significantly lower than in the control (P<000.1). As a consequence, the population of *E. brasiliensis* on the roots of vines was much larger, 25.37 ± 6.28 insects per plant in the control area, compared to 2.92 ± 0.78 and 4.59 ± 1.51 in the plots treated with the hydrogel-thiamethoxam baits and the thiamethoxam drench, respectively These results showed that hydrogel baits containing 0.007 % thiamethoxam effectively reduced these *L. micans* and *E. brasiliensis* populations in new vineyards. Similar results were observed for the same technique used to control a *L. humile* population in a plum orchard in South Africa (Buczkowski et al., 2014).

Taking into consideration that *L. micans* is the predominant species of ant in areas where *E. brasiliensis* is present in southern Brazil, we showed that using 3 applications of thiamethoxam, in an effective delivery system such as hydrogel, can reduce the amount of active ingredient used in the field to control *E. brasiliensis*.

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Evaluation of arthropod biodiversity in the vineyards of the Centre-Val de Loire region

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Highlights

- Specimen identifications with morphological and genetic approaches revealed an important biodiversity within the 6 studied vineyards (Chinon, Vouvray, Montlouis, Touraine, Touraine-Amboise and Menetou-Salon)
- The presence of numerous carnivorous species of Carabidae suggests that they could play a significant role in pest control

Introduction

Since several years, the preservation of biodiversity and the improvement of ecosystem services represent a worldwide issue. Biodiversity is a key factor allowing agricultural sustainability (Altieri et al., 1999). In France, the "Wine Country", viticulture represents 15 % of the value of the national agricultural production and progressively integrates a sustainable grape production respecting biodiversity. To study biodiversity, Arthropods, such as Insects, Arachnids, Myriapods and Crustacea, are known as reliable indicators of environmental quality. In this context, the BioVAL project (2016-2018) aims (1) to realise the first inventory in Arthropods in the vineyards of the region Centre-Val de Loire, from Sancerre to Chinon considering cultural practices and landscape structure; and (2) to determine characteristics suitable to functional diversity.

Material and methods

The Arthropod biodiversity of 6 vineyards (6 Appellation of Controlled Origin wines: Chinon, Vouvray, Montlouis, Touraine, Touraine-Amboise and Menetou-Salon) of the Centre-Val de Loire region is measure by following 16 vine plots. For each plot, insecticide use, landscape heterogeneity (open or closed) and the percentage of grass cover were determined. Arthropods were collected from May to July over two years (2016-2017) with pitfall traps and combi traps placed at the centre of each plot. Identification of Arthropod species was realised by a morphological approach with binocular loupe and identification keys (Jeannel, 1941). Unidentified species have been then directed to molecular biology analyses for genetic determinations using the DNA barcoding technic. DNA extractions were performed using Promega© (Madison, USA) protocol. A portion of the cytochrome oxidase I (*COI*) gene (648 pb) of the mitochondrial DNA was amplified and sequenced using the following couples of primers LepF1/LepR1 and LCOI- 1490/HCOI-2198. PCR amplification was performed with Qiagen kit (Hilden, Germany) and PCR products were sent to sequencing (GATC Biotech, Germany). COI sequences were then corrected and blasted on the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2007).

Results and discussion

Preliminary results show an important arthropod diversity in the different vineyards of the Centre-Val de Loire region with already more than 200 species identified. In particular, rare species in the region have been identified such as several species of Coleoptera (Tenebrionidae: *Myrmechixenus subterraneus*, Apionidae: *Apion ulicis* and Anthicidae *Anthicus ater*) or Arachnida species (Theridiosomatidae: *Theridiosoma gemmosum*, Gnaphosidae: *Scotophaeus quadripunctatus* and Phalangidae: *Lophopilio palpinalis*). Interestingly, vines may indeed represent a suitable habitat for these uncommon species.

Numerous Cicadellidae individuals have been collected, belonging to five different species: *Euscelis incisus*, *Euscelis* sp., *Doratura homophyla*, *Anaceratagallia lithuanica* and *Anaceratagallia* sp. These leafhoppers can be important vine pests because they are both phytophagous and potential vectors of pathogens. Interestingly, Bressan et al. (2006) showed that after infection with the flavescence dorée phytoplasma, individuals of *Euscelis incisus* could transmit them to plants.

The Carabidae family, known to be excellent indicators of biodiversity and important natural enemies, has been more precisely examined. Among the 41 species identified in the vineyards of the region Centre-Val de Loire, 16 were dominant and three of them largely prevailed over the samplings: *Nebria salina* (24 %), *Nebria brevicollis* (15.5 %) and *Harpalus affinis* (10.2 %). These three pioneer species are known to be the first species colonising disturbed environments such as crop fields. Several uncommon herbivorous and carnivorous species have been determined as *Bradycellus harpalinus*, *Poecilus lepidus*, *Brachinus explodens* and *Philochthus mannerheim*. Nevertheless, within all the studied AOC, a majority of carnivorous carabidae has been detected. These occurrences could be due to the presence of preferential preys in the vineyards. Assessment of biodiversity indexes for each plot shows a high species richness (18 species) in the Montlouis vineyard in comparison with all studied vineyards (mean of species richness 7.71 ± 2.76). Analyses of cultural practices are running to understand this trend.

These preliminary results allowed to better understanding pest and natural enemy communities present in the vineyards of the Centre-Val de Loire. In the long term, the project results will connect taxonomic and functional biodiversity to vineyard characteristics and will be the basis of recommendations in term of agro-ecological management.

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Efficacy of kaolin against *Empoasca vitis* in vineyards

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Highlights
- In the laboratory, kaolin caused a significant increase in *Empoasca vitis* nymphs' mortality
- In the field, kaolin applications decreased leafhopper nymphs' populations both in preventive and curative trials
- To control of *E. vitis*, kaolin could be of profitable use as an alternative to synthetic insecticides in conventional vineyards and to natural pyrethrins in organic vineyards

Introduction

Empoasca vitis (Göthe) (Hemiptera: Cicadellidae) is the most widespread leafhopper in European vineyards. The leafhopper feeds on leaf veins causing leaf injury that can lead to yield and quality losses (Fornasiero et al., 2016).

The control of leafhoppers in conventional vineyards is based on broad-spectrum synthetic insecticides often causing detrimental effects on beneficials (Pozzebon et al., 2015). In organic vineyards, leafhopper control relies on natural pyrethrins, which often have suboptimal efficacy (Mori et al., 2004). Therefore, effort should be made to develop alternative tools. Kaolin particle film technology affects pest populations on different crops, mostly by reducing oviposition and feeding activity (Glenn et al., 1999), and could be of profitable use also in vineyards.

In this study, the activity of kaolin against *E. vitis* was evaluated under laboratory and field conditions.

Material and methods

In the laboratory, the effect of kaolin (Surround WP, Tessenderlo Kerley Inc., Phoenix, Arizona, USA, 4 % W:V) on *E. vitis* was evaluated on 1st- and 2nd-instar nymphs (1st experiment) and 3rd- to 5th-instar nymphs (2nd experiment) placed on treated and untreated leaves (40 replicates per treatment). Leaves and nymphs were taken from an insecticide-free vineyard. Mortality was daily recorded as long as nymphs alive were observed in both treatments.

In four vineyards of north-eastern Italy, a treatment with two kaolin (2 % W:V) applications, spaced out of 5-6 days, was compared with an untreated control in a randomised block design with 4 replicates per treatment. In two vineyards kaolin was applied at the beginning of *E. vitis* 2nd-generation (preventive trials), and in other two at the peak of the third generation, when many nymphs older than the second instar were present (curative trials). Nymphs on 50 leaves per replicate were counted (i) weekly for six times, from the day before the first kaolin application, in the preventive trials, and (ii) just before each of the two applications and 4-d after the second application in the curative trials. In the preventive trials, *E. vitis* adults were monitored with yellow sticky traps replaced

weekly for more than two months from 2 weeks before the first kaolin application. In a preventive-trial vineyard, the percentage of leaf surface with symptoms by *E. vitis* was recorded at harvest time.

Data were analysed as reported in the Results.

Results and discussion

In both laboratory experiments kaolin significantly increased the mortality of *E. vitis* nymphs (Abbott efficacy > 90 %) with a greater effect on younger (first experiment) than on older instars (second experiment) ($P < 0.0001$ and $P < 0.05$ at Fisher's exact test, respectively, at 1-d after kaolin application).

In the two preventive field trials, kaolin significantly affected *E. vitis* nymphs on leaves and adult captures with yellow sticky traps on grapevines ($P < 0.001$ at repeated-measures ANOVA). The nymph infestation expressed as cumulative nymph-days was reduced by around 90 % and the adult captures on average by 45 %. The effect of the two kaolin applications was persistent and the differences from the control remained for up to four weeks. The reduction of the infestation was associated with a reduced symptom expression on leaf surface by over 90 %.

In the two curative field trials, kaolin significantly affected *E. vitis* nymphs ($P < 0.01$ at repeated-measures ANOVA). The nymph infestation between the first kaolin application and harvest time, expressed as cumulative nymph-days, was reduced by almost 80 %. Efficacy was lower compared to the preventive trials in agreement with the lower efficacy observed in the laboratory on *E. vitis* older instars.

Based on these results, two kaolin applications against *E. vitis* appear to be a valid alternative to synthetic insecticides in conventional vineyards and should allow a higher efficacy than natural pyrethrins in organic vineyards (Tacoli et al., 2017).

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Berry skin resistance explains oviposition preferences of *Drosophila suzukii* best in different pre-damaged grapevine cultivars

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Highlights
- Susceptibility to damage by *Drosophila suzukii* varies greatly between grapevine cultivars
- In a vineyard with mechanically pre-damaged berries, penetration resistance of the berry skin explained oviposition better than chemical parameters

Introduction

Drosophila suzukii (Matsumura), also known as spotted wing drosophila (SWD) is a pest insect native to Asia, which has recently spread to Europe. Females of SWD have a serrated ovipositor allowing oviposition in intact fruits. The developing larvae damage the fruit. SWD has a wide range of fruit hosts including blueberries, strawberries, cherries, as well as grapevine. Considerable damages in viticulture were associated with SWD in Northern Italy in 2012 and in Southwest Germany in 2014. To identify the need of early harvest or insecticide sprayings growers need parameters that are simple to measure and allow them to determine the moment in which grapes in the field switch from “not susceptible” to “susceptible”.

The aim of this study was to identify parameters influencing SWD oviposition. Beside other ripening parameters (e.g. sugar content), we included penetration resistance as explanatory variable, which has previously shown to be an important factor for SWD (Ioratti et al., 2015).

Material and methods

The study took place 2015 in Siebeldingen, Germany in a collection of different grapevine cultivars (one row of 25 plants per cultivar) experiencing the same soil tillage und plant protection treatments. For each cultivar, oviposition of SWD and several physiological parameters were assessed weekly from 21 September until end of October. Oviposition was estimated for 50 randomly collected berries per cultivar and date. Berries were checked for eggs under the stereo microscope. In the study year, the whole vineyard was pre-damaged by wasp feeding and by Powdery Mildew (*Erisyphe necator*). Several physiological parameters that are related to berry ripening, like sugar content, acidity etc. were assessed by analysing the must from 50 berries randomly sampled per cultivar and date. Moreover, penetration resistance was measured (N) on 25 randomly collected berries per cultivar and date (Letaief et al., 2008). We used linear models with oviposition as dependent variable (log transformed) and the physiological parameters as independent variables.

Results and discussion

Model comparison revealed penetration resistance as most important factor explaining oviposition of SWD: berries with a low penetration resistance (soft skin) had significantly more eggs then berries with a high penetration resistance (hard skin). Investigating this pattern within the cultivars, within the different dates (between the cultivars) and between the cultivars (with the dates summed up), shows that it is dominated by differences in the penetration resistance between the different grapevine cultivars. Soft skinned cultivars had higher infestation of SWD than hard skinned cultivars.

Among the cultivars examined we could distinguish between three different types:

1. No oviposition at all (no susceptible?);
2. Oviposition negatively correlated to penetration resistance (susceptible under preconditions?);
3. Oviposition possible, but no such correlation;

Further research is necessary to find out if there are varieties that are susceptible without preconditions and if penetration resistance is a useful parameter for decision support (earlier harvest or insecticide treatment). Moreover, the investigated vineyard was pre-damaged, and to our experience, only pre-damaged grapes are notably susceptible to SWD (Jarausch et al., 2017). Therefore it would be interesting, if our results still hold under “undamaged” conditions.

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Compensation effects induced by grape phylloxera on root growth, leaf respiration and sink activity in *Vitis* ssp.

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Highlights
- Biotrophic grape phylloxera increases belowground root biomass and aboveground CO2 fixation rates as part of a compensation strategy to maintain host fitness
- Elevated levels of carbon and nitrogen detected in nodosities underlined their physiological sink strength for the host's primary metabolism

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae*) induces root galls (nodosities) on tips of susceptible and partially resistant rootstock cultivars. The biotrophic parasite affects the host by altering the water and mineral uptake, carbohydrate translocation and manipulation of root growth. Phylloxera based damage depends on phylloxera biotype, vineyard management, climate and soil conditions and may range from growth decline to vine death. Nodosities were shown to contain elevated levels of carbohydrates as well as amino acids withdrawn from the plant primary metabolism. Within this study we aim to analyse changes induced by phylloxera nodosity formation on the primary metabolism of its *Vitis* host.We hypothesise that phylloxerated rootstocks respond with a compensation strategy comprising increased root growth (H1), leaf respiration and photosynthetic activity (H2) as well as elevated amounts of carbon and nitrogen compounds in the root system of phylloxerated plants (H3).

Material and methods

Experiment 1: Two year old grafted grapevines (*V. vinifera* L. 'Pinot Noir' on SO4 (Teleki 4A (*V. berlandieri* Resseguier x *V. riparia*)) were cultivated in 3L pots containing a peat:perlite substrate (9:1) located in two isolated climate chambers (26°C, 45 % rH, 16 h photoperiod). Phylloxera inoculation was done with 150 phylloxera eggs per plant from an identical phylloxera strain (biotype C). Photosynthetic activity, gas exchange (CIRAS II), vegetative and phenological parameters were assessed.

Experiment 2: Double eye cuttings of the rootstock Teleki 5C (*V. berlandieri* x *V. riparia*) were cultivated in 3 l pots containing a peat:perlite:sand substrate (8:1:1) in greenhouse conditions (20-25°C, 50-60 % rH, 16 h photoperiod). Fertilisation was done weekly with two nutrient solutions: solution A applied to control vines contained sufficient macro and micronutrients to ensure plant growth; solution B for N-deficient vines excluded nitrogen and was pH corrected with NaOH. Phylloxera inoculation was done with of 300 eggs per plant of a field population from Burgenland Austria. Phenological and plant growth parameters as well as nutrient levels of C, N, P, K were assessed.

Results and discussion

Root dry mass, but neither vegetative growth nor phenological developmental parameters, was significantly increased upon phylloxeration supporting a phylloxera induced compensation hypothesis belowground. Significantly increased leaf transpiration rates 35 dai indicated the promotion of CO_2 fixation in source leaves of grafted phylloxerated vines. Nutrient analyses revealed significantly increased amounts of carbon and nitrogen in root galls and non-infested tips of phylloxerated vines. Amounts of phosphorous and potassium were significantly decreased in root galls, potentially due to the dysfunctionality of the otherwise nutrient absorbing root tips.

Symplastic transport of carbohydrates between sink and source organs is known to be driven by the concentration gradient in the phloem. We showed that root phylloxeration resulted in an increased CO_2 fixation rate in the leaves representing photosynthetically active source organs. Former experiments demonstrated the functional phloem connectivity to phylloxera root galls (Wieczorek et al., 2013) as well as upregulated gene expression patterns of sugar transporters mediating carbohydrate import into root galls (Griesser et al., 2015). As a consequence carbohydrates, specifically starch (Griesser et al., 2015; Du et al., 2008; Forneck et al., 2015), and amino acids (Du et al., 2008; Kellow et al., 2004) were shown to be accumulated in phylloxera root galls. Our results confirm these findings and underline the strong sink activity of root galls even under N-deficiency conditions. Furthermore significantly elevated carbon levels in non-infested root tips demonstrated the sink strength of the entire phylloxerated root system including not only root galls but also non-infested root tips.

Summarising we provide evidence that phylloxeration of rootstocks results in a partial compensation effect by increasing belowground biomass without affecting other aboveground sink organs. One explanation might be a phylloxera induced promotion of the photosynthetic CO_2 fixation in leaves. However a functional proof focusing on quantitative root tip growth comparing phylloxerated versus non infested vines remains to be done.

Acknowledgements

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Preliminary analysis of arthropod aggregation overwintering in vineyards with different management

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Highlights

- The arthropod community found beneath the bark is strongly influenced by the type of vineyard and different management system
- The bark often represents the only arthropod shelter in vineyard
- The structure analysis of arthropod community may characterise the level of potential resilience reached in the vineyard

Introduction

The pests and weeds control system plays a prevalent role in the environmental sustainability of a vineyard. In addition, the overall impact of such practices on arthropod community can influence the persistence of beneficial organisms within the vineyard, and thus its resilience. Potential refuging areas in specialised vineyards are scarce and the bark is often the only arthropod shelter. Aim of this preliminary study is to compare the aggregation of overwintering arthropod community under the bark in different vineyards management to characterise the level of resilience reached.

Material and methods

The observations were carried out during winter 2017 in four Apulian vineyards (South Italy) of different management systems. Two vineyards are organic experimental for table (A-TG) and wine (A-WG) grape production respectively, located at the CIHEAM -IAMB in Valenzano (BA). The other two vineyards (B and C) are for wine grape production, contiguous and managed according to the principles of Integrated Pest Management with a "Tendone" trellis system. The latter are located near Barletta (BT) (northern part of Apulia Region) and differ only in the weed management, with the presence of spontaneous winter weeds in vineyard B and chemically weeded out in vineyard C.

In each vineyard in February, 10 plants along the central transect were decorticated and arthropods collected were identified in the laboratory. The data were submitted to ANOVA and a matrix was built after removing taxa with a low value of F ($F < 1.5$). The matrix was subjected to multivariate statistical analysis with the R software (R Core Team, 2017). Species assemblages were compared between vineyards by means Principal Component Analysis (PCA), whereas differences between vineyards were tested using the Multiple Response Permutation procedures (MRPP). Associations between arthropods and vineyards were defined by using an Indicator Species Analysis (ISA), with 10,000 permutations.

Results and discussion

The four investigated vineyards showed wide variability in number of arthropods found beneath the bark. The lowest value was detected in the vineyard A-TG with 3.2 individuals/plant and the greatest value in the vineyard B with 30.7 individuals/plant. The data ordination in space defined by the first two PC explains 44.2 % of total variance.

Results of PCA showed that the vineyard B tends to separate on the PC1, while vineyards A-TG and A-WG tend to differentiate each other on the PC2.

The Multiple Response Permutation Procedures showed a non-random distribution of data but with an aggregation in four assigned groups (vineyards).

Results of ISA showed only Nitidulidae taxon associated to the vineyard A-TG ($p < 0.001$); the vineyard A-WG shows associated Collembola ($p = 0.001$) and Psocoptera ($p = 0.028$) taxa; Vineyard B shows associated Dermaptera ($p < 0.001$), larvae of Raphidioptera ($p = 0.003$) and Opiliones ($p = 0.025$) taxa; the vineyard C shows no associated taxon.

Preliminary results of Indicator Species Analysis describe well the characteristics of the investigated vineyards. The vineyard A-TG, although organic, does not show beneficial species associated beneath the bark, since it has been subjected to decortication with localised treatment for several years to reduce infestations of *Planococcus ficus* (Signoret); in contrast the long presence of ripened clusters on the vine last autumn, has favoured the associated Nitidulidae taxon.

Wine vineyard A-WG, organic managed too, presents a better situation with the associated detritivores/omnivores taxa at the base of the trophic chain. Vineyard B has reached the highest levels of potential resilience since the arthropods assemblage is more complex and with associated taxa having mixed diet regime (detritivores/omnivores/predators) such as Dermaptera and Opiliones and presence of Raphidioptera, that is an important cortical predator. This complexity is probably attributable to the sustainable management of weeds, because the contiguous vineyard C differs only by the application of weeding and did not show any associated taxon.

In conclusion, preliminary results have shown that the analysis of the overwintering arthropods assemblage beneath the bark could be an interesting method to assess the level of potential resilience reached.

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Drosophila suzukii oviposition behavior on wine clusters

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Highlights

- *Drosophila suzukii* females significantly lay higher number of eggs on more mature grape berries
- Organoleptic parameters that more correlate with the number of laid eggs are sugar contents and acidity
- Female choosiness is particularly evident at early stages of vineyard infestation, 20 days before harvest, and gradually decreases while the cluster ripens

Introduction

The Spotted Wing Drosophila (SWD), *Drosophila suzukii*, is an invasive pest of soft fruits and cherries in several Western Palearctic areas. It is not yet clear whether the fly can be considered a commercial pest of grapes. Despite slower larval development and higher mortality rate compared to other host plants (Ioriatti et al., 2015), SWD can elicit indirect damage to grape clusters by favouring sour rot derived from oviposition. In Trentino (Northern Italy) the cv. Schiava is highly susceptible to oviposition primarily because of the berry skin penetration force that is comparatively lower than in other regional varieties (Ioriatti et al., 2015). This indicates that females tend to select the oviposition sites by testing the clusters that they visit. Here we investigated the SWD female accuracy in selecting berries suitable for oviposition once confined on a Schiava grape cluster by analysing organoleptic parameters of berries and comparing between those with and without laid eggs.

Material and methods

We studied the role of seven organoleptic parameters (i.e. sugar contents, relative density, potassium, pH, total, malic and tartaric acidity) associated to the berry maturity. We randomly selected 45 plants from three vineyards (Rotaliana Plain, Trento, Italy), and from each plant we chose three grape clusters. We artificially infested the grape clusters with SWD individuals for 4 days (cv Schiava) in three different decades of September 2016 (the last one coincided with the harvest time) and then afterwards we collected and separated berries with (g+) and without (g-) eggs with stereomicroscope. We quantified the organoleptic parameters and then we run a principal component analysis (PCA) to provide a relative value of their contribution in terms of susceptibility to SWD oviposition. The factor coordinates of PCA cases were used to form two datasets (g+ and g-) that were compared with the Wilcoxon signed-rank test to assess significant different positions of the centroids given by g+ and g-, respectively. A significant difference of centroids would have indicated a significant difference of the studied parameters along any of the principal components. A Wilcoxon

signed-rank test followed to assess significant differences in the contents of each organoleptic parameter between g+ and g-.

Results and discussion

Our results indicate that SWD females once landed on a cluster explore and probably test the berries to choose those more suitable (i.e. more ripe) for oviposition. The PCA accounted for the 83.6 % of variance. The analysis of factor coordinate cases showed that the centroid g+ has significant higher value than the centroid g- at the first ($p < 0.01$) and second ($p < 0.05$) sampling date, while they do not differ ($p > 0.05$) at the third sampling period. This indicates first of all a significant contribution of the investigated parameters in determining a significant difference between g+ and g- berries. In addition, we found a progressive reduction of difference between the two classes of berries during the maturation. Taken each sampling period separately, we measured a significant difference in all organoleptic parameters (except for tartaric acid) at the first period; grapes sampled at the second period showed significant difference only in terms of sugar content ($p < 0.01$) and relative density ($p < 0.01$), which were higher in g+, while malic acid was significantly higher ($p < 0.01$) in g-. Finally, grapes collected in the third sampling ($n = 19$) period differed between g+ and g- for total acidity and acid tartaric values, which were higher (respectively, $p < 0.01$ and $p < 0.05$) in g- and pH, which was higher in g+ ($p < 0.05$). Taken together these data show that SWD females are rather choosy at early infestation (20 days before harvest) when the clusters are still maturing and there is still high variability of organoleptic parameters between berries of the same cluster. Instead, at the harvest time the cluster is more homogeneous and thus we did not find any difference between berries with and without eggs. Therefore we assume that, even if SWD females lay eggs on cv Schiava because it has skin softer than other grapevine varieties, however also the berry organoleptic components play an important role. Females showed remarkable accuracy in choosing the proper oviposition sites which could be driven by inputs arising from labellum and tarsi where are likely located most of the gustatory sensilla able to identify the preferred sucrose rich substrate (Montell, 2009). On the contrary, there is not yet evidence of the use of the ovipositor for explorative piercing however it is known that each valva of the SWD ovipositor presents 5 putative gustatory sensilla (Biolchini, 2015). Further research is warranted to understand how the females acquire the information of fruit maturity (i.e. sugar contents and acidity) that means to clear further up about the reproductive behaviour and biology of this important fruit pest.

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Does *Drosophila suzukii* represent an additional factor of risk of sour rot disease development in wine grape?

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Highlights

- The development of sour rot on grape berries is strongly favored by wounds on the berry surface
- SWD can be vector of microorganisms, also including causal agent of sour rot, therefore SWD infestations can quickly spread sour rot on the cluster
- Intact berries are much less susceptible to sour rot, even in case of SWD infestation

Introduction

Spotted wing drosophila (SWD) *Drosophila suzukii* (Matsumura) is a global pest attacking various berry crops included grapevine. SWD can lay eggs on unwounded ripening wine grape berries. Although wine grapes are in most cases not ideally suited for SWD population development, the fly can feed, oviposit and develop to adults on wine grapes (Asplen et al., 2015). Oviposition intensity increases during the ripening period and is correlated to physiological changes occurring during grape berry development, i.e. increase in sugar content, decrease of acidity levels and skin penetration force (Ioriatti et al., 2015). High populations of *D. suzukii* potentially present on wine grapes during the harvest period may result in a significant risk considering the vectoring of spoilage bacteria. To assess this hypothesis, laboratory bioassays were performed to assess the actual ability of SWD to vector spoilage bacteria to intact wine grape and to trigger sour rot disease development.

Material and methods

Three varieties, Pinot noir, Schiava and Cabernet sauvignon either intact or damage sterilised berries were offered as oviposition substrate to SWD adults from a lab-colony. SWD flies have been previously kept for two days in a cage in contact with cotton impregnated with a suspension of bacteria to let them carrying a bacterial load similar to that present on SWD trapped in vineyard. After 4 days, SWD adult survival and oviposition on berries were assessed, the flies were removed while the grape barriers have been further kept in the same plastic boxes under controlled climatic conditions for 10 days. Berries were then crashed and the homogenised materials were deposited onto sterile Petri plates containing a proper synthetic growth media to perform qualitative and quantitative characterisation of microflora developed on the grape berries. Volatile acidity values of the assembled grape samples of each variety were assessed and considered as an indicator of sour rot incipient development. To confirm laboratory results, an artificial SWD infestation of bagged grape clusters of

the cultivar Schiava were set up in three vineyards in the Trento Province. Oviposition and subsequent larval development was evaluated and their relationship with production of acetic acid volatile considered.

Results and discussion

Results demonstrated that SWD can vector spoilage bacteria through contact and/or feeding on damaged berries. Sour rot developed because of contamination of wounded berries by a microbiota transported by contaminated SWD, which disseminated spoilage bacteria from damaged or infected berries. Moreover, while oviposition on intact sound berries was not a sufficient condition for triggering sour rot development, the successive larval development constituted an additional way for spreading spoilage bacteria such as *Acetobacter* spp. in wine grapes during the harvest period. We also demonstrated both under controlled laboratory and field conditions, that inoculative infestation with SWD and consecutive larval development within grape berries can promote the increase of acetic volatiles, as an indicator of increased sour rot levels. However, we think that the presence of SWD eggs in the grape berries is not “per se” an issue for the wine growers, provided egg laying and successive larval development do not cause skin ruptures that promote microorganism infection. On the other hand, since sound grape berries are less susceptible to the development of microbiota associated with sour rot and spoilage, a number of factors affecting skin integrity (rainfall, wind, temperature, diseases, insect pests, viticultural practises, etc.) can strongly influence grape microbiota that are primarily responsible for development of grape sour rot.

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Oviposition deterrent effects of particle films and entomopathogenic fungal strains against *Drosophila suzukii*: preliminary laboratory assays and field trials

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Highlights

- Entomopathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*), kaoline, and potassium silicate are effective in reducing the *D. suzukii* oviposition activity in laboratory condition
- The different compounds showed different persistence levels in laboratory assays
- Preliminary field trials confirmed the laboratory performances

Introduction

Drosophila suzukii is an alien, polyphagous, invasive pest of soft skinned fruits (Rota-Stabelli et al., 2013). Currently, broad-spectrum insecticides are used several times during the harvest season to prevent the fruits loss. This approach has multiple challenges such as affecting natural enemies, increasing the risk of other pest outbreaks, managing pre-harvest and restricted entry intervals (PHI and REI), and the high risk of resistance developing (Klick et al., 2016). Several control techniques are being developed, but the field application may require more than one approach to be implemented and combined (mass trapping during early season, letting the natural enemies work during the season, applying sanitation and netting protection, use of entomopathogens, use of deterrents of oviposition). The integrated approach may reduce pesticide amounts, the risk of exceeding MRLs or the exposure of humans to pesticide residues.

Material and methods

The deterrent effect on *D. suzukii* oviposition of three entomopathogenic fungal strains (ATCC74040, MET15 and OF13), kaolin (35g/l) and potassium silicate (5ml/l) was evaluated in no-choice (1) and double-choice (2) conditions. 30 sane and uninfested grapes (cv Red Globe) were immersed in each suspension for 5 seconds. Treated grapes were then put in plastic box (15x10 cm) containing sterile sand and placed in an experimental arena. In each arena, 30 *D. suzukii* adults (sex ratio 50:50) were introduced, 1, 3, 5 or 7 days after the treatments, providing food and water ad libitum. Fruits were exposed to *D. suzukii* for 5 days, incubating boxes in climatic chamber (24 ± 2°C, 62 ± 4 % RH, 14:10 photoperiod). After 5 days of exposure, the grapes were observed under the binocular microscope in order to register the number of *D. suzukii* eggs laid. The double-choice

experiment was carried out introducing in the same arena two plastic boxes containing 15 treated and untreated fruits. Treatments were conducted as in the no-choice test.

Preliminary field trials were performed on cherry orchard using potassium silicate, alone and in combination with *M. anisopliae*, during the harvest period. Samples (100 fruits) were collected 5 days after the treatments and observed under the binocular microscope in order to record the infestation rate.

Results and discussion

The statistical analysis revealed a significant influence of the variable "Treatment" and "Time" on the *D. suzukii* oviposition. All the treatments reduced the average number of eggs laid into the fruits with respect to the untreated control. The different compounds showed different persistence levels in laboratory assays.

Results of the preliminary field trials confirmed the laboratory performances: potassium silicate, alone and in combination with *M. anisopliae*, reduced significantly the infestation rate and therefore show interesting perspectives for the integrated management of *D. suzukii*.

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Morphological study of the antennal sensilla of the invasive *Halyomorpha halys* Stål (Hemiptera: Pentatomidae)

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Highlights

- We characterised and determined the abundance and distribution of the antennal sensilla of *H. halys* using scanning and transmission electron microscopy
- Five morphological sensillum types were recognised: basiconica, coeloconica, chaetica, trichodea and asparagus-like
- Sexual dimorphism was not observed

Introduction

Halyomorpha halys (Stål), commonly known as the Brown Marmorated Stink Bug, is an invasive agricultural and domestic pest in North America and Europe. It originates from East Asia and it was first recorded in Italy in the province of Modena in 2012 (Maistrello et al., 2014). It is highly polyphagous and capable of causing significant damage to many economically important crops. Moreover, *H. halys* is considered an urban nuisance pest due to its overwintering behaviour, as it often aggregates in anthropogenic structures.

Antennae of insects are indispensable sensory organs bearing various sensilla, utilised in different adult behaviours such as host location, mating and aggregation. To support our ongoing research on its communication mechanisms, we investigated the diversity of antennal sensilla of *H. halys* using scanning and transmission electron microscopy (SEM, TEM). The results of this work will provide a solid base for future chemical and behavioural ecology studies.

Material and methods

Halyomorpha halys individuals were obtained from a laboratory reared colony (24 ± 2 °C; 70 ± 5 % RH; 16h:8h L:D), originally collected from Trento and Modena, Italy.

Antennae of adults and 5th instars were dissected, gradually dehydrated through a series of ethanol, dried, mounted on aluminium stubs, sputtered with gold, and then observed using scanning electron microscopes SEM (FEI Nova Nano).

For transmission electron microscopy, adults were fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.1M, pH 7.2) + 5 % sucrose at 4°C for 24 h, and then rinsed in the same buffer. Subsequently, antennomers were detached and fixed in 1 % osmium tetroxide and left at 4°C for 1 h. After rinsing in the same buffer, the specimens were gradually dehydrated in ethanol of ascending concentrations and then embedded in Epoxy-Araldite with propylene oxide as bridging solvent. The samples were

left in the oven to polymerise for 60 h at 65 °C. Ultrathin (~80 nm) sections were cut with a diamond knife on an ultramicrotome "Nova", mounted on formvar-coated 50-mesh grids, stained with uranyl acetate, and finally examined with a TEM (Philips® EM 208).

The different types of sensilla were determined based on the classifications made by other researchers on different species of Hemiptera (Kim et al., 2016). To determine any significant sexual differences in terms of length of antennomers, abundance and distribution of the antennal sensilla, data obtained were analysed using ANOVA post-hoc SNK and T-tests.

Results and discussion

In this study, we examined the number, morphology and ultrastructure of antennal sensilla of *H. halys*. The antenna of adults consists of five antennomers: scape, pedicel 1, pedicel 2, flagellum 1 and flagellum 2, whereas that of the fifth instars consists of four (single pedicel segment). The antenna was about 9 mm in length. Five major sensillum types were identified by SEM analysis: basiconica, coeloconica, chaetica, trichodea and asparagus-like. The latter was observed for the first time in pentatomids, exclusively in fifth instars. No differences were found between dorsal and ventral surfaces of the antennae with respect to the distribution of sensilla. The density of sensilla increased and their types differed from the proximal to distal ends of the antennae, with the dominant type being sensilla trichodea. Sexual dimorphism was not observed in terms of length of the antennal segments and the total number of the sensilla present. However, fifth instars had fewer sensilla compared to adults in general.

The probable function of each sensillum type can be deduced on the basis of their structure. For instance, the absence of a multiporous cuticular wall makes an olfactory function very unlikely, whereas the presence of a single pore suggests that a gustatory role is plausible (Zhang et al., 2014). Results and analysis of the observations using TEM are still in progress in order to elucidate the functions of the identified sensillum types on the antennae of *H. halys*.

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The use of biorational compounds for vineyards protection against pests

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Highlights

- Pests in vineyards *Lobesia botrana* Den.&Schiff., *Eotetranychus pruni* Oudmns. and *Colomerus vitis* Pgst. were studied in Krasnodar and Stavropol territories of Russian Federation.
- Assessment of biological effectiveness of biorational compounds for vineyards protection has been done for products based on a.i. abamectin, fenoxycarb and combined product of a.i. spiromesifen and abamectin.

Introduction

Grapevine (*Vitis vinifera*) is a valuable source of food and one of fruit crops grown worldwide. On the territory of Russia, the first vineyard was planted in 1613 in Astrakhan. Tsar Aleksey planted in the 17th century a vineyard in the vicinity of Moscow. His son Peter the Great supported viticulture and ordered grapevine cultivation in 1706 along the river Don. Nowadays in Russia vineyards are grown in North Caucasus, the Krasnodar Territory, Central Russia, the Central Black Earth Belt, in the non – Chernozem Belt and in Siberia (Gachkova, 2008). In Krasnodar and Stavropol territories of Russian Federation among the most important pests, from economic point of view, there are *L. botrana*, *E. pruni* and *C. vitis*. For IPM in vineyards of Rostov region and Krasnodar territory pheromone mating disruption against *L. botrana* was tested (Dolzhenko et al., 2017).

Material and methods

All experiments were carried out in field conditions in Krasnodar and Stavropol territories. Additionally tasks of evaluation were done in laboratories of Sankt-Petersburg State Agrarian University and FSBSI VIZR. Assessment of biological efficacy of biorational compounds was done following EPPO Standards (PP1) - Efficacy evaluation of plant protection products with minor corrections.

Results and discussion

Colomerus vitis (syn. *Schizotetranychus pruni*) (Acari: Eriophyidae), grape erineum mite, is vermiform with soft body, about 0.15 mm long, visible only with lens. On grapevine it develops biological races of this mite, which are distinguishable by the type of damage caused. One race causes characteristic swellings (erinea), the second rolls up on the leaves and the third attacks the buds and it is called "bud mite". It develops about 7 generations annually. Damage is caused by bites on the young leaves, which induce the appearance of swellings on leaves, the petioles, and the stems. Growth of plant could be impaired.

Eotetranychus pruni (Acari: Tetranychidae) is a pest of vine, apple, pears, plums, cherry-plum and apricot. In infested leaves chlorophyll is destroyed, and premature leaves fall off. Damaged leaves become yellowish on white varieties of the vine and reddish-brown on black varieties. *E. pruni* damage causes the loss of grape yield by 15-60 % and decreases of sugar content in grapes. Population density depends on predators *Typhlodromus reticulates* Oud., *Phytoseius spoofi* Oud., *Scolothrips acariphagus* Sakh., *Scymnus (Stethorus) punctillum* W.S., and larvae of *Chrysopa carnea* Steph.

Lobesia botrana (Lepidoptera: Tortricidae), grape berry moth is regarded as a potentially serious pest on a worldwide scale for all the vine-growing areas. Initially larvae damage buds and ovaries separately from surface, and then spin neighbouring ovaries into a web. Each larva is capable of causing damage to 40-60 buds, flower or ovaries. Crop losses from the pest may reach up to 80 %

Research on evaluation of insecticides on beneficial arthropods confirmed that insecticides on the base of a.i. imidacloprid, thiacloprid, thiamethoxam and chlorantraniliprole have more or less adverse effect on Anthocoridae, Cecidomyiidae and Coccinellidae (Dolzhenko and Dolzhenko, 2016). With a goal to minimise the use of chemicals the method of pheromone mating disruption was tested against *L. botrana*. It was found out that 500 dispensers per hectare ensured a high protection during the whole vegetation period (Dolzhenko et al., 2017).

Research has been conducted on assessment of biological effectiveness of biorational compounds for vineyards protection against *L. botrana*, *E. pruni* and *E. vitis* in Krasnodar and Stavropol territories of Russian Federation. Plant protection products based on a.i. abamectin (Vertimec EC, 18 g/l), fenoxycarb (Fasis WP, 250 g/kg), (Fora, WP, 250 g/kg) as well as a combined product, Oberon Rapid, SC (228.6 g/l of spiromesifen + 11.4 g/l of abamectin) were studied. The effectiveness of these products was sufficient to decrease populations of phytophagous arthropods below economic damage threshold. In IPM in vineyards there is the need to use control measures which would save populations of native antagonists of insect and mite pests.

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Is *Drosophila suzukii* ovipositor involved in chemoreception?

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Highlights

- Chemosensory-related genes have been found expressed in the ovipositor of *D. suzukii* suggesting that this species may use this appendix for perceiving chemical stimuli related to oviposition behavior
- Transcription of chemosensory-related genes in ovipositor seems to be conserved along Drosophila phylogeny and it is present also in species that oviposit in rotten substrates

Introduction

Drosophila suzukii Matsumura is a serious pest of soft fruits and grapes. It belongs to the well-studied genus Drosophila, whose members mainly show rotten substrate feeding and oviposition behaviour. In contrast, gravid *D. suzukii* females lay eggs on undamaged ripening fruits, thanks to a serrated ovipositor that is typical of *D. suzukii* and of its sister species *Drosophila subpulchrella*. Drosophila, and more in general insects, select suitable oviposition sites mainly exploiting chemical stimuli from the environment, hence chemoreception is fundamental in driving female choice (Karageorgi et al., 2017). Here we analyse the potential of the ovipositor of *D. suzukii* to act as a chemosensory organ by identifying chemosensory-related genes on a whole-transcriptome level. We also test if ovipositor chemoreception is related to the innovative oviposition behaviour of *D. suzukii* by checking presence of chemosensory-related transcripts in Drosophila species with different oviposition guilds.

Material and methods

Ovipositors were manually excised from gravid females belonging to four Drosophila species: *Drosophila melanogaster* and *Drosophila biarmipes*, which oviposit on fermenting substrates, and *D. suzukii* and the sister species *D. subpulchrella*, which oviposit on undamaged ripening fruits. RNA was extracted with RNeasy purification kit and treated with DNaseI. Sequencing was carried out at Beckman Coulter Genomics as following: cDNA libraries were prepared using Ilumina TruSEQ library preparation kit and paired-end sequenced on an Ilumina Hiseq platform. 100 bp reads were later cleaned and trimmed using FastQC. A de novo assembled transcriptome was generated for each Drosophila species using Trinity software. Transcriptomes were functionally annotated using Blast2GO. Transcripts of chemosensory-related genes (Crava et al., 2016; Ramasamy et al., 2016), particularly those coding for olfactory receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs), odorant-binding proteins (OBPs), and chemosensory-binding proteins (CSPs) were manually searched. Transcription of chemosensory-related genes in the *D. suzukii* ovipositor was confirmed by RT-PCR. For this purpose, RNA was extracted as described above, treated with DNAse I, and retrotranscribed to cDNA using SuperScript III reverse Transcriptase. PCR was carried out

with GreenTaq DNA polymerase and results visualised on agarose gel 1 % stained with ethidium bromide.

Results and discussion

De novo assembled transcriptomes generated for the ovipositor of four Drosophila species contained around 22,500 transcripts each (from 22,192 transcripts for *D. subpulchrella* to 23,605 transcripts for *D. biarmipes*). The N50 size was also comparable among the four species (it ranged from 2,146 in *D. suzukii* to 3,224 in *D. melanogaster*) and around 70 % of the contigs in each transcriptome had a blast hit against public databases. In total, around 60 % of the contigs were functionally annotated in every species. Functional annotation revealed that transcription was quite comparable in tested Drosophila since the most enriched GO terms were the same for all species. Most represented GO terms categorised for biological process were "cellular process", "metabolic process", and "multicellular organismal process", whereas most represented GO terms categorised for molecular function were "cell killing", "catalytic activity", and "nucleic acid binding transcription factors".

D. suzukii transcriptome data were manually mined for transcripts encoding chemosensory-related genes. We found the presence of several transcripts encoding OBPs, CSPs, and some chemoreceptors. In particular, we found transcription of one OR, three GRs, and six IRs. Transcription was confirmed by RT-PCR using primers for the odorant co-receptor Orco and the GR64a as negative control, since these two genes were not present in the ovipositor transcriptome. Manual searching of chemosensory-related transcripts in transcriptomes of the other three Drosophila species rendered from seven to twenty transcripts encoding ORs, GRs, and IRs, revealing that transcription of genes coding for chemoreceptors is common along Drosophila phylogeny. Species that oviposit in fermenting substrates had a greater number of transcripts encoding chemoreceptors (20 in *D. melanogaster* and 17 in *D. biarmipes*) than fresh-fruit ovipositing species (7 transcripts in *D. subpulchrella* and 10 in *D. suzukii*). We identified a core of three transcripts shared among the four species, whereas other seven were shared among three or two species. Transcripts encoding chemoreceptors specific for the *D. suzukii* ovipositor were five, namely two GRs and three IRs.

Overall, our results reveal a conserved transcriptional activity of chemosensory-related genes in the ovipositor of Drosophila species, which may be correlated to the oviposition site choice of gravid females. We anticipate that oviposition behaviour may be modulated in different species by the expression of species-specific receptors or binding proteins, which we suggest are involved with adaptation to specific ecological niches. Hence, identification of chemoreceptors expressed in the *D. suzukii* ovipositor represents a crucial step in understanding the basis of its detrimental oviposition behaviour.

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Assessment of capture efficacy of *Drosophila suzukii* (Matsumura) trapping devices in mass trapping and release recapture trials

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Highlights

- A modified Droso-trap™ baited with DroskiDrink was evaluated for its efficacy in capturing *Drosophila suzukii*
- The capture rate assessed in release recapture trials was low, 3.7% on average
- Mass trapping carried out in spring in a *D. suzukii* high populated overwintering site was not effective in reducing the population density in the neighbouring areas

Introduction

Drosophila suzukii in South Tyrol has caused damages since 2011 with attacks directed on the most widespread grapevine variety "Vernatsch". The species is now considered a dangerous pest for the south Tyrolean viticulture (Sinn, 2015). The species overwinters as adult fly mainly in woody landscape and it starts to reproduce in spring on the first available soft fleshed fruits; it develops new generations in succession on different host plants and in autumn reaches very high population densities. Winter is considered a neck bottle for the pest population development. Therefore it was taken into consideration the possibility to reduce the overwintering population by the use of attractive traps, in order to retard the population development in the orchards. A trapping device and bait commercially available were tested in release recapture and in mass trapping trials in a *D. suzukii* overwintering site, to exploit the potentiality of this approach and develop a targeted control strategy.

Material and methods

Release recapture trials. Modified Droso-trap™ (Biobest) devices were filled with 200 ml Droski Drink (Grassi et al., 2014) and hung in a vineyard, locality Piglon (BZ, Italy) at 1.5 m height into concentric circles. In 2015, 8 traps were hung at a distance of 50 m from the centre of the circle in the 8 cardinal and intermediate directions. At 35 m radius distance a second concentric circle was set up with 16 traps. In 2016 2 additionally concentric circles were set up at 20 m and 40 m radius with 8 traps each. During May 2015 and May 2016 3-7 days old males and females of *D. suzukii* originated from the Laimburg rearing were released in the centre of the circle in groups of 3,400-5,000 insects for 7 releases totally. Traps were checked for captures at day 7 and 14 after release in 2015 and at day 1, 3 and 7 in 2016.

Mass trapping trials. In 2015, in a *D. suzukii* overwintering site 30 Droso-traps™ baited with Droski Drink were collocated at 6-10 m distance from each other in a 1,000 m² forest area adjacent to a vineyard. Traps were checked weekly from March until May. Additionally two monitoring traps, placed at distances 15 m (SL-Forest) and 60 m (SL-Hedge) respectively from the mass trapping area, were also controlled. In 2016, the same area was split into two areas to check for differences in capture efficacy in relation to the trap densities. For this purpose six traps were hung at 10 m distance from each other and 12 traps were hung at 5 m distance.

Results and discussion

Release recaptures trial. The capture rates varied from 2.7 % to 5.4 %. Despite the higher amount of traps present in 2016 over the same area of 2015, recaptures were not significantly higher (Pearson Correlation 0.51; p = 0.38). Raining periods and rain quantity negatively influenced the captures, but not significantly (rain minutes Pearson correlation -0.22, p = 0.36; rain mm Pearson correlation -0.24, p = 0.48). The capture efficacy was very low and could not be improved by incrementing the traps number inside the 50 m radius circle.

The comparison of captures/trap between traps collocated at 35, 40 and 50 m distance from release point did not show significant differences (ANOVA F = 0.21; df = 8, p = 0.81). Several insects flew up to the 50 m distance trap circle, overpassing the 35 m and 40 m traps circles. This indicates an attraction range which seems to be lower than 10 m in those experimental conditions. Wind speed after releases was most of the time below 5 km/h. In one case wind speed was constantly higher than 10km/h, and *D. suzukii* adults were captured in all directions, despite the main wind speed direction. That could be an indication that *D. suzukii* moves forward by short flights.

Mass trapping trial. The mass trapping carried out from 11th March until 8th May 2015 captured 12,564 males and 35,384 females, on average 1,598 insects/trap. During the same time interval the monitoring traps SL-Forest and SL-Hedge captured respectively 2,135 and 9,298 individuals. After the begin of mass trapping the catches in SL-Forest showed a conspicuous decrease, while no influence on captures was observed for the SL-Hedge trap. The authors hypothesise an attractive influence of the mass trapping "odour cloud" only for a striking distance, as the SL-Hedge trap collocated at 60 m distance did not compete with the mass trapping.

In 2016, captures of traps collocated at 5 m distance from each other were compared with captures of traps collocated at 10 m distance of each other. The traps were checked weekly for a period of 4 weeks. Traps set at 5 m distance did not catch weekly significantly more individuals than the traps set at 10 m distance despite the twofold number of traps into an area of the same size (t-test-0.51; df = 6; p = 0.63).

The trial was carried out in a high populated *D. suzukii* overwintering site (Zerulla et al., 2015). Results showed limits in improving the capture efficacy by incrementing the number of traps into the target area. Low recapture rates and short attraction range indicate that the overwintering population can unlikely be reduced by this control method in a significant way.

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Rearing *Campoplex capitator* in Italy and in Chile: preliminary achievements

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Highlights

- We report the salient features that have been recently addressed to obtain an established breeding of *Campoplex capitator* in Italy and in Chile

Among insect parasitoids, the complex of species associated with the European Grapevine Moth (EGVM) *Lobesia botrana* (Lepidoptera, Tortricidae) in Europe includes mainly Hymenoptera Ichneumonoidea (Ichneumonidae and Braconidae), Chalcidoidea (Chalcididae, Pteromalidae, Eulophidae, Elasmidae, Trichogrammatidae), and two species belonging to Diptera Tachinidae. As for most natural enemies of EGVM, the role played in natural control by each species of parasitoid is greatly variable in space and time. Typically, the frequency of egg and larval parasitoids is high in the first two generations and decreases drastically in the overwintering generation, which is mainly affected by larval–pupal and pupal parasitoids.

Regardless of the extensive scientific effort to apply biological control against EGVM, an effective method for a practical use in the field is still lacking.

Egg parasitoids of the genus *Trichogramma* have been artificially released against EGVM according to the inundative strategy but with inconsistent results. The Pteromalid *Dibrachys affinis* Masi was employed 15 years ago in the Russian vineyards. This species and *Dibrachys cavus* (Walker) are gregarious generalist larval–pupal parasitoids of Lepidoptera, Diptera, and Hymenoptera and are quite easily raised in the laboratory. Because they have low host selectivity and are prone to act as hyperparasites, applied entomologists have not attempted to use them for biological control of EGVM.

The most active, frequent, and efficient parasitoid in European vineyards is the larval parasitoid *Campoplex capitator* Aubert (Hymenoptera: Ichneumonidae).In a 2-year study in French vineyards, this species showed an average parasitism rate of about 40 %. *C. capitator* is still regarded as the best candidate for EGVM biological control but its application in the open field has been prevented by difficulties in its mass rearing.

In 2016, a mass rearing of the wasp was started in a common research project between the University of Pisa and SAG (Servicio Agricola y Ganadero, Chile).

Here we report the salient features that have been recently addressed to obtain an established breeding of *C. capitator*, aimed at its future release in the open field against EGVM.

This is the abstract of a video which will be shown at coffee breaks

Five-year analysis of population dynamics in *Drosophila suzukii*: usefulness of monitoring traps and their relevance for viticulture

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Highlights

- Five years of monitoring data in different habitats showed preferences in the occurrence of *Drosophila suzukii*, with greater numbers of flies found in more heterogenous habitats
- Local vegetation as an indicator for *Drosophila suzukii* population dynamics in combination with weather data including temperature and relative humidity

Introduction

The Asian fruit fly *Drosophila suzukii* is a mayor polyphagous invasive fruit pest, which occurred in Germany for the first time in 2011. It emerged as a pest for domesticated berries and stone fruits but also infests wild growing fruits (Gutierrez et al., 2016). A monitoring system using vinegar traps has been established by the State Institute for Viticulture and Oenology in 2012.

The aim of the study was to validate the monitoring system in different habitats, such as vineyards, orchards, and forests and to assess whether the traps can be used to predict population sizes and dynamics throughout the year.

Material and methods

A total of 55 traps were installed in four different habitats: vineyards, orchards, at the edge and in the middle of forests including ground and treetop traps. The traps consisted of plastic cups with 10 holes at the sides, through which the fruit flies could enter. They were filled with 125 ml of a vinegar/water solution and one drop of dish soap. The number of trapped flies and the sex of the flies was determined once per week. Furthermore, weather data such as temperature, precipitation and relative humidity was recorded. The number of collected flies in each trap was correlated to the occurrence of alternative hosts and refuges.

Additionally, “cage experiments” were performed to investigate whether the flies found in the traps are representative for the total population size. This was done by setting up 3 aluminium cages covered with fabric, each of a volume of 4.5 m³ with a monitoring trap placed into the centre of the cage. One hundred flies, originating from a permanent breeding of the State Institute for Viticulture and Oenology, were released into each cage. Additional experiments were performed in which branches and fruits were placed inside the cage supplementary to the traps as alternative attractant and refuge. The aim was to quantify the attractiveness of the traps under different habitat conditions and to evaluate the impact on the behaviour of the flies. The traps were controlled and changed every 12 hours and the number and sex of the flies was determined.

Results and discussion

The number of flies collected in the traps differed between the habitats. The number of flies peaked around the same time every year between 2012 and 2017 in all habitats. The number of trapped flies also varies between the years during the five-year experiment, with more/less flies being trapped in warm/dry/moist/etc. years. Over the course of the years, different periods in population dynamics can be defined. For example, the number of flies found in the traps peaked in late autumn in all habitats. Overall, more flies were detected in more heterogeneous habitats compared to homogenous habitats. The highest number of trapped flies occurred on the edge of the forests and the lowest number of flies was found in vineyards.

In contrast to our expectations, gender ratios were not homogenous distributed, with more males in comparison to the females.

Preliminary data from the cage experiments revealed that just a proportion of flies were trapped in cages with only traps. Further investigations with additional branches or fruits compared to cages with only traps were carried out over summertime. A possible result could be that the availability of branches and fruit increases the lifespan of the flies resulting in a greater number of trapped flies.

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Beneficial insects associated with *Lobesia botrana* in vineyards

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Highlights

- *Lobesia botrana* Den.&Schiff. is a major pest in vineyards. Natural enemies of *L. botrana* are 6 species of predators and 111 species of parasitoids belonging to Ichneumonoidea (80 species) and Chalcidoidea (31 species)
- Plants provide a nectar source for parasitoids and contribute to quantitative and qualitative diversity of insects associated with grape berry moth in vineyards

Introduction

Beneficial insects are recorded on grapevine (*Vitis vinifera*) following their host the grape berry moth *Lobesia botrana* Den.&Schiff. (Lepidoptera: Tortricidae). In the IPM, in vineyards one key point is enhancing functional biodiversity of natural enemies for pest suppression. Maintenance and management of ecological infrastructures (weedy field margins, undisturbed habitats in or adjacent to crop fields) are considered important aspect of sustainable agriculture (Landis et al., 2000). Natural vegetation is used to improve fecundity and longevity of natural enemies and for that reason is increasing floristic diversity within farming systems in many countries. There is evidence that habitat fragmentation and landscape structure can affect beneficial insects. Ground cover plants management can significantly influence arthropod fauna, including beneficial groups providing increased efficacy of biological control agents (Burgio et al., 2016).

Material and methods

Based on the literature review it was prepared a list of beneficial insects associated with *L. botrana* belonging to following taxa: Coleoptera, Dermaptera, Diptera superfamily Ichneumonoidea and Chalcidoidea, (Kasparyan, 1981; Yu, 2012; CABI, 2017).

Results and discussion

The research on beneficial insects in vineyards confirmed predators and parasitoids associated with grape berry moth. There are reported the following predators: *Chrysoperla carnea, Forficula auricularia, Malachius sardous, M. spinipennis, Stethorus punctillum* and *Xanthandrus comtus*. In complex of parasitoids of grape berry moth are recorded five species from family Braconidae. The most diverse is fauna of Ichneumonidae with 80 species recorded worldwide on this host. The majority of species are parasitoids of many Lepidoptera and Coleoptera widely distributed in Palaearctic region. From superfamily Chalcidoidea there are recorded 31 species related to grape berry moth. They belong to families: Eulophidae, Pteromalidae, Elasmidae and the most of them are from family Trichogrammatidae. In Italy, France and Germany on grapes the following biological

control agents are recorded: *Dicaelotus resplendens, Ichnus alternator, Itoplectis alternator, I. alternans, I. tunetana, Pimpla apricaria, P. contemplator, Pristomerus vulnerator, Scambys elegans, Theroscopus hemipterus, Trichogramma agrotidis, T. daumalae, T. maidis, T. principium, T. rhenanum* and *T. semblidis* and *Triclistus lativentris*.

Adults of Ichneumonidae are rapidly flying cautiously and fearful. They attend flowers of perennial plants, especially with open nectarines (e.g. Apiaceae, Euphorbiaceae, Umbelliferae, Lamiaceae). They visit trees and shrubs along margins of cultivated crops. Adults have a need for supplementary feeding with carbohydrates and proteins. That is why they sting the host with ovipositor or bite with mandibles more host individuals, than they lay eggs in them. Due to this activity, host mortality is increasing.

Habitat management is important in reduction and regulation of pests because it can enhance functional biodiversity and biological control as one of ecosystem services. Burgio at al. (2016) gave evidence of the role of habitat management of organic vineyard, where they studied the role of cover plants management on arthropod functional biodiversity. Cover plants used in the experiment were *Lobularia maritima, Phacelia tanacetifolia, Fagopyrum esculentum, Vicia faba, Vicia villosa* and *Avena sativa.* All Hymenoptera parasitoids represented 72.3 % of the specimens collected by sweeping net and 92.7 % by vacuum sampling. Ground cover management significantly affected insect fauna, including beneficial groups, which can provide ecosystem services in vineyard. Plots cultivated with flowering plants attracted Chalcidoidea. The only response that was missing in the experimental plot was with *Phacelia*. There is also evidence that flowering plants are attractive for predators like Syrphidae and Coccinellidae.

According to qualitative composition of beneficial insects in vineyards a mixture of cover crops and diversity of plants in field margins will be suggested.

Acknowledgements

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Circadian densities of mites on vine leaves in Montalcino area (Tuscany, Italy)

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Highlights

- The biological control of *Eotetranychus carpini*, the yellow spider mite, is performed by releases of phytoseiids in vineyards located in Central Italy

Introduction

Generally, densities of phytophagous and predatory mites inhabiting crops and vineyards are evaluated in different sampling periods by sampling at the daylight hours. Weekly-monthly-seasonal variation is a key parameter to assess mite communities' structure and population dynamics. There is scarce knowledge of a possible variation in presence of the mites during day periods, like the night time, generally unusual times to carry on screening on mites' population parameters. To this end, in vineyard areas with infestation of yellow spider mite and release of phytoseiids to control the pest (Castagnoli et al., 2009), the study of the circadian densities of phytophagous and predator mites has been prepared with day and night surveys in order to evaluate the optimisation and validity of the control strategies adopted.

Material and methods

The abundance of phytophagous and predator mites was recorded in different vineyards of two farms ('Sante Marie' and 'Case Basse - Soldera') near Montalcino, in 2009. The vine variety was 'Sangiovese Grosso'; in both farms, four years earlier, phytoseiids were introduced to achieve the control of the infestation of the yellow spider mite. For each sampling, at least 25 vine leaves were sampled. Four day rounds of samplings (monthly, on June, July, August, and October) were performed. All over the 24 hours, five samplings/day were performed, every 4-5 hours (4:00-5:00am, 8:00-9:00am, 12:00am-1:00pm, 7:00-8:00pm, 0:00pm-1:00am), to record the densities of motile stages of phytophagous and predatory mites. Simultaneously with the mites' monitoring, the temperature and relative humidity of environmental air and the temperature of the leaf surface were acquired. All mites were determined in laboratory at the species level. Analysis was performed to evaluate: the correlation between the densities of phytophagous and predatory mites; the effect of monthly and/or day period sampling by Anova; the effect on mite densities of previous releases of phytoseiids in the vineyards was considered.

Results and discussion

In all samplings, the presence of the phytoseiids species, both in vineyards where they were released in previous years and where not, was recorded in more than 90 % of the leaves sampled. A positive correlation was registered between prey and predator densities (Pearson correlation, $P < 0.01$, $N = 1{,}160$). The numbers of motile mites, registered at the same day time, was similar in each monthly

sampling. The highest phytoseiid number/leaf was registered in the last two monthly samplings (August and October). The density of phytoseiids/leaf was higher in night periods ranging from sunset to early morning. By interpolation of data, the peak of phytoseiids' presence was around 11pm. Variability in the density of phytoseiids was registered in samples taken in the late afternoon and at the beginning of the night. Significant differences were not found (ANOVA, Tukey Test, $P < 0.05$) with respect to the other surveys. Considering the density of tetranychids, the density of yellow spider mite *Eotetranychus carpini* (Oudemans), the highly represented species, showed some difference between monthly samplings but just little oscillations at the various day periods, confirming the limited or not noticeable mobility of this phytophagous mite within the 24 hours (F-test, $P = 0.103$). In the two farms, considering all samples, the presence of phytoseiids was higher in the vineyards where predators had been released ('Case Basse Soldera' farm: t-test=14.3, N=598, $P<0.001$, 'Sante Marie' farm: t-test=3.81, N=558, $P<0.001$). Among the mite predators recorded, the phytoseiids *Kampimodromus aberrans* (Oudemans) was the most represented species and able to control yellow spider mite infestations. It is likely that day time may play an important role in the activity of predator species, but not in phytophagous species. Our results show that phytophagous mites, although more sensitive to the monthly/seasonal fluctuations, remain on leaves during all the day, whereas predators seem to 'choice' different micro habitats, by showing variable presence on leaves during the day (Parecis-Silva et al., 2016). The dispersion and recording of *K. aberrans* has confirmed its ability to successfully settle and remain on vineyards even after some years from release. Furthermore, by considering the data acquired, it is to remark that its behavior may allow good chances in facing phytosanitary strategy.

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Assessment of new trap designs and liquid baits improved with lactic acid bacteria for capturing *Drosophila suzukii* Matsumura

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Highlights

- Improvement of the attractiveness of *D. suzukii* commercial baits with the addition of different lactic acid bacterial strains thanks to the volatiles released during the fermentation process
- Combined effect of trap design and liquid baits to enhance the trap performance in field conditions

Introduction

The spotted-wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), native of Eastern Asia, is an invasive alien species in Europe and the Americas and is one of the main emerging pests of valuable crops, including soft fruits and wine grapes (Cini et al., 2012).

The conventional approach to handle infestations of SWD involves the use of commercially available insecticides, but these do not seem able to ensure effective results. Consequently, alternative strategies are strongly required. Monitoring and control methods based on effective traps combined with attractive lures are among the most promising for the integrated management of *D. suzukii*. Consequently, an improvement in the bait composition is beneficial to guarantee reliable attractivity to SWD. This study was therefore aimed to investigate and improve upon one of the best commercial liquid baits for SWD, the "Droskidrink" (DD) (Tonina et al., 2016).

The experiments were focused on the exploitation of lactic acid bacteria as a biological catalyst in the production of organic volatile molecules attractive to SWD, thanks to the fermentation of sugars and organic acids present in the liquid bait.

Material and methods

We chose strains of lactic acid bacteria usually involved in the fermentation of wine and vinegar, the most attractive fermenting liquids for drosophilids. A series of preliminary field trials was coupled with laboratory tests to describe the behaviour and performance of various preparations

of lactic acid bacteria.

Afterwards, we focused our analysis on different biotypes of the bacterium *Oenococcus oeni* (Garvie) Dicks et al. that revealed a high resistance to stressful environmental conditions of the liquid bait and, at the same time, the emission during malolactic fermentation of volatiles known for their olfactory activity to SWD.

Subsequently a new model of trap able to produce a combined effect with the attractive mixture was evaluated in open field.

Results and discussion

Our results showed that the attractiveness of DD was greatly increased by the addition of *O. oeni* to the standard mixture. The new trap-bait combination provided excellent results, increasing the number of catches, especially with regard to female individuals and particularly during the cold seasons, when SWD has low population density. In addition, this new trap design is simpler and faster to service, compared to the traps used previously.

The long-term goal is to accelerate research and technology transfer toward the development of mass trapping and attract-and-kill strategies based on the use of traps baited with this new attractant. Catching a large number of insects is crucial to obtaining a reduction in the damage caused by SWD to the fruits (Rossi-Stacconi et al., 2016).

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On the importance of the sister-species comparison in agricultural pest science and IPM: *Drosophila subpulchrella* as a case study

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Highlights

- Current genomic studies in agricultural entomology focus only on the insect pest
- Adding a sister-species to the research agenda highly increases our understanding of the pest
- The genome of *Drosophila subpulchrella* promises to ameliorate the Integrated Management of its pest sister species *Drosophila suzukii*
- We advocate that a sister species approach should be extended to other insect pests.

Introduction

Genomic studies are often used to clarify the biology of agricultural pests and to define management strategies against them. There is however one crucial aspect so far neglected: the comparison between a pest and its closest non-pest sister species. In the absence of a sister species, genome comparisons cannot discriminate characters that are unique to the pest from those shared with other members of its clade. While a sister species approach is common in microbiology and has been used for example to understand the evolution of grapevine downy mildew (Rouxel et al., 2012), it has never been applied to insect pests of agriculture.

Material and methods

To highlight the benefit of a sister species comparison, we sequenced a draft genome and transcriptome of *Drosophila subpulchrella*, the closest known putative non-pest sister species of model fruit pest *Drosophila suzukii*. Raw data were generated using Illumina Hiseq2000 technology and processed following protocols as in Ometto et al. (2013). We assembled the draft genome and transcriptome of *D. subpulchrella*, as well as its mitogenome and the draft genome of its harboured symbiont *Wolbachia, wSpc*. We used a blast approach to select a set of 2133 orthologs for evolutionary (dNdS and phylogenetic) analyses, and a set of highly conserved 91 orthologs for molecular clock analyses. We manually annotated various families of chemosensory genes and

studied their evolution on the species phylogeny. We finally performed some physiological experiments to put some of our evolutionary analyses into a phenotypic context.

Results and discussion

Comparison of divergences among nuclear, mitochondrial and *Wolbachia* genomes reveals a complex evolutionary history characterised by an initial speciation no older than 2 million years followed by likely more recent hybridisation events, and a recent *Wolbachia* transfer, something relevant for the actual use of bio-control *Wolbachia*. Inter specific mating experiments indicate that the two species can actually interbreed, in accordance with a similar courtship behaviour. Our evolutionary analyses indicate that *D. subuplchrella* is also characterised by reduced evolutionary rate as D. suzukii, indicating that both species reduced their generations per year as a likely adaptation to temperate environments (Ometto. et al. 2013).

We could polarize various genomic events on the species tree, and revealed a set of key chemosensory genes unique of *D. suzukii* that will ease the development of specific trapping strategies based on lures. The two species share however most of their novelties in the chemosensory gene repertoire indicating a strong progressive modification in the suzukii subgroup toward the peculiar *D. suzukii* feeding and oviposition behaviour (Karageorgi et al., 2017).

Overall, our results reveal both differences and similarities between *D. suzukii* and its sister species *D. subpulchrella*. While differences will aid the development of targeted management tools based on new lures and proper use of *Wolbachia*, the similarities alert us on the yet unexplored pest capability of *D. subpulchrella* and/or the chances of invasive inbred *D. suzukii* - *D. subpulchrella* strains. From a broad methodological point of view, our results show that applying a sister species concept to applied entomology research agenda can increase our knowledge of the pest, enhance the forecasting of new invasive species/strains and help defining new management strategies.

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